FACTORS AFFECTING BULL SEMEN QUALITY AT BREEDING STATION

A THESIS

BY

SHARABAN TOHURA
REGISTRATION NO.: 1605506
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MASTER OF SCIENCE (MS)

IN

THERIOGENOLOGY

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2018

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Dedicated to My Beloved Mother

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ABSTRACT

The knowledge of factors affecting sperm production and semen quality is important with regard to reproductive efficiency and thus genetic improvement, hence, this study aimed to investigate the effects of breed, age, scrotal circumference, BCS, season and nutrition on bull semen quality at breeding bull station of Ejab Alliance Limited, Thakurgaon, Bangladesh. A total of 777 ejaculates were collected from 25 bulls (Friesian=10; Sahiwal=15) using an artificial vagina. Ejaculate volume, concentration, mass activity, individual motility, morphology significantly ($P<0.05$) differed with the factors. All semen parameters were significantly ($P<0.05$) higher in Sahiwal bulls than in Friesian bulls except concentration which vary non-significantly ($P>0.05$) between the two breeds. No significant ($P>0.05$) effects of breed, age, BCS and nutrition were found on the concentration of semen. The volume, individual motility, mass activity and sperm morphology were significantly ($P<0.05$) higher in adult age group (>3.5-4.5 years) than in younger (2.5-3.5 years) and older groups (>4.5years-above). Scrotal circumference significantly ($P<0.05$) affected all the parameters regarding to semen quality. The highest values were observed in bulls with scrotal circumference of 31.1-33 cm and the lowest values were observed in bulls with scrotal circumference of 33.1 cm to above. The volume, individual motility, mass activity and sperm morphology were higher ($P<0.05$) in bull group with BCS of 4 to 4.5 than in bull group with BCS of >4.5 to 5. Significantly ($P<0.05$) highest values regarding to semen quality were found in winter and the lowest in summer but the effect of season on volume was non-significant ($P>0.05$). During the study, Vit. ADE supplementation significantly ($P<0.05$) improved the semen quality. In conclusion, Sahiwal breeds should be promoted at the bull station in the subtropical condition like Bangladesh. Most semen should be collected during winter. Bulls of >3.5 to 4.5 years of age with moderate scrotal circumference and BCS should be the choice of priority. This conclusion, however, is based only on one year study, a long duration trial need to be necessary to confirm the effects of all related factors and their interactions with the reproductive physiology and management of bulls in a breeding station.

ABBREVIATIONS & SYMBOLS

ACTH	: Adrenocorticotropic Hormone
AI	: Artificial Insemination
ANOVA	: Analysis of Variance
AV	: Artificial Vagina
BCS	: Body Condition Score
BSE	: Breeding Soundness Evaluation
^{o}C	: Degree Celsius
cm	: Centimeter
DCP	: Distal Cytoplasmic Droplet
et al.	: And his associates
etc	: Etcetera
F	: Friesian
Fig.	: Figure
FSH	: Follicle Stimulating Hormone
gm	: Gram
GnRH	: Gonadotropin Releasing Hormone
HPG	: Hypothalamo Pituitary Gonadal
HSTU	: Hajee Mohammad Danesh Science and Technology University
Inj.	: Injection
IU	: International Unit
Kg	: Kilogram
L	: Local
LH	: Luteinizing Hormone
Ltd.	: Limited
ml	: Milliliter
MSO	: Medicine, Surgery and Obstetrics
No.	: Number
NS	: Non Significant
NST	: National Science and Technology
PCD	: Proximal Cytoplasmic Droplet
PUFA	: Polyunsaturated Fatty Acid

R	: Receptor
ROS	: Reactive Oxygen Species
SC	: Scrotal Circumference
SD	: Standard Deviation
Se	: Selenium
Sec	: Second
Sig.	: Significant
SL	: Sahiwal
THI	: Temperature Humidity Index
TVC	: Testicular Vascular Cone
µg	: Microgram
µl	: Microliter
µm	: Micrometer
Vs	: Versus
&	: And
%	: Percentage
<	: Smaller than
>	: Greater than
±	: Plus-minus

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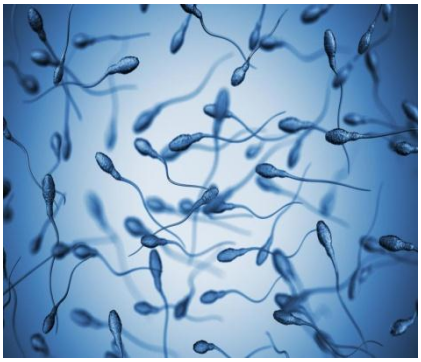


Chapter I
Introduction

CHAPTER I
INTRODUCTION

Bangladesh is a densely-populated, low-lying, mainly riverine country. It is predominantly an agrarian country. Agriculture is the largest employment sector in Bangladesh. It is worth mentioning that the economy of Bangladesh depends largely on the broad agriculture sector, which includes agriculture, livestock, fisheries and forestry. Livestock plays an important role in the agricultural sector in Bangladesh. It constitutes an important part of the economy of the country. The livestock population in Bangladesh is continuously increasing, and has been estimated to be about 3839.45 lakh of which the population of cattle is about 239.35 lakh, buffalo is about 14.78 lakh, sheep is about 34.01 lakh, goat is about 259.31 lakh and poultry is about 3292.00 lakh. The GDP contribution of this sub-sector has been a modest 1.6% annually with a remarkable livestock product growth rate of 3.32%. It provides full time employment for 20% of the total population and part-time employment for another 50%.The production of milk, meat and eggs was 92.83 lakh metric tons, 71.54 lakh metric tons and 1493.31 crore, respectively, in 2016-17 (Hossan, 2017).

Cattle among other livestock species available might be the best choice in relation to existing integrated agricultural farming system in Bangladesh. It plays a prominent role in rural livestock production providing the milk, meat, manure and draught power for crop production and fuel for domestic use. Despite the remarkable livestock product growth rate, the shortage accounts for 55.85 lakh metric tons, 0.19 lakh metric tons for milk and meat respectively (Hossan, 2017). The low productivity is often attributed to a number of factors among which are qualitative deficiency in feed resource base, high prevalence of disease and poor animal performance (Plaizier, 1993). Poor genetic quality of cattle is the main cause of acute shortage of milk and meat. The production potential of livestock can be increased by genetic improvement using one of the modern ways of breed improvement, as for example: Artificial Insemination (AI). The cattle improvement program in Bangladesh aims to improve local cattle for milk and meat production by the incorporation of both tropical breeds (Red-Sindhi, Sahiwal) and temperate breeds (Holstein-Friesian and Jersey). Insemination is acknowledged as a breeding method that contributes to improve farm animal populations, particularly of cattle. Artificial Insemination allows for maximum use of the most valuable breeders and at the same time,

for significant increase of breeding advancement. Moreover, using semen of improved quality reduces the spread of sexually transmitted diseases. This is a basic tool for rapid genetic improvement of local cattle. The major advantages of AI being genetic improvement, controls of venereal diseases, improved record keeping, more economical than natural service when genetic merit is considered, safe to eliminating injury during natural service and availing geographical restrictions (Bearden *et al.,* 2004). Selection of sound bull is one of the most important decisions of sound breeding program. Breeding soundness refers to a bull's ability to get cows pregnant (Berry *et al.,* 2011). Breeding Soundness of a bull implies a complex interaction of factors such as physical and reproductive health, libido and mating ability, semen quality and interactions among animals in the breeding herd (Chacon *et al.,* 1999). Among the reproductive traits, quality of semen plays a major role in determining the fertility and reproductive efficiency of any livestock production. Semen is the secretion of male accessory reproductive glands and contains the male gametes (spermatozoa) produced in the testis (Parkinson, 2004). To select breeding bulls, evaluation of semen is of prime importance.

Quality semen is the key indicator towards the successful breeding program (Dasinaa and Pagthinathan, 2015). A fertile ejaculate must meet certain semen parameter quality standards, such as: progressive motility, normal morphology, active energy metabolism, structural integrity and functionality of the membrane, penetration capacity and optimum transfer of genetic material. A crucial component of efficient calf production is high quality bull semen which is also necessary for successful fertilization and improved herd health (Swanson and Herman, 1940). Poor quality semen may affect herd fertility and productivity of the farm by lengthening calving intervals. Semen volume, sperm concentration, sperm motility, mass activity, sperm morphology and seminal pH per ejaculate are the common criteria for evaluating semen quality at most AI stations (Den Daas, 1992). The quality of semen in relation to fertility is determined largely by the motility of sperm. The quality of semen again depends on concentration of spermatozoa, proportion of live and morphologically normal spermatozoa, seminal pH and optimum metabolic features of individual sperm (Hoque, 1998). Some factors such as breed, age, season, nutrition, scrotal circumference, body condition have a direct effect on the quality of semen that the bull will produce at ejaculation (Ahmed *et al.,* 2014). Age is one of the major contributing factors to semen characteristics with testicular size being closely related to total sperm output (Oldham *et al.,* 1978; Ahmad and Noakes, 1995). David *et*

al. (2007) found that motility, concentration and number of spermatozoa were higher up to the age between 2-3 years and then declined chronologically. Scrotal circumference is a very important trait in bull reproduction and positively correlated with age (Hoogenboezem and Swanwpoel, 2000). Several experiments have found a positive relationship between scrotal circumference and sperm production and semen quality in growing bulls (Mamabolo, 1999; Brito *et al.*, 2002b). BCS is an important predictor of sperm quality (Sitali *et al.*, 2017). Change in body condition score over time reflects both body composition and energy balance, which in turn are critical for metabolic stability, health and fertility (Hossein-zadehand and Akbarian, 2015). The major contribution to the semen variation is environment. Environmental temperature is one of the factors affecting reproduction. High ambient temperature leads to abnormal scrotal thermograms and testicular degeneration, which result in an increased percentage of abnormal spermatozoa and a lower percentage of normal sperm in the ejaculate. Nutritional restriction is less evident in male fertility, but nutritional deficiencies depress the production characteristic of semen such as the increase in the frequency of abnormal spermatozoa, reduction in spermatozoa motility and fertilization capacity of the sperm. Season of semen collection affected all semen quality parameters. Where there is marked seasonal variation in temperature, humidity and nutrition, semen quality tends to be lower during summer months (Curtis, 1993). The determination of those factors that cause variation in semen quality and the extents of the variation are of vital importance.

The knowledge of factors affecting sperm production and semen quality is important with regard to reproductive efficiency and thus genetic improvement as well as for the productivity and profitability of AI centers. Therefore the objectives of the study were to observe -

- The factors affecting the quality of fresh semen at breeding bull station.
- The interactions among the various factors on semen quality in bull.

Chapter II
Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Fertility in breeding bulls is a complex trait that is made up of several physiological processes such as development of the reproductive system from birth to puberty, spermatogenesis, ejaculation etc. For optimum semen quality, all these physiological processes should be coordinated.

2.1 Puberty in bull:

The attainment of puberty in bulls is determined by using two criteria. The first marker is the ability of an animal to produce an ejaculate containing 5.0×10^7 sperms with 10% motility, and the second is the attainment of a scrotal circumference (SC) of 28 cm (Lunstra and Echternkamp, 1982; Wolf *et al.,* 1965). Often, the attainment of 28 cm SC precedes the animal's ability to produce the required amount of sperm (Wolf *et al.,* 1965). SC serves as a valuable tool for estimating bull fertility because it is known that testicular size, and therefore size of the scrotum, is highly correlated to the amount of semen a bull is able to produce (Almquist *et al.,* 1976). The age at which puberty begins for conventionally raised bulls varies by breed. For example, previous studies reported puberty at 326 days in Hereford bulls, 295 days in Angus bulls, and between 273 and 287 days of age for Holstein bulls (Lunstra and Echternkamp, 1982; Lunstra *et al.,* 1978).

Bearden *et al.* (2004) reported that some changes occur before the spermatozoa are present in the ejaculate, prior to puberty. As for example, 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. Al-Gedawy and 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 gonadotropin, LH and FSH, which have been suggested to play a major role in the regulation of sexual development and semen production. At the onset of puberty, gonocytes migrate to the periphery of the seminiferous tubules and produce two types of cells (King, 1993). One is stem cells, which give rise to spermatogonia and another one is reversed stem cells, which contribute to the increase in population of stem cells. Hafez and Hafez (2000) 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. The two previously described criteria for puberty serve as useful tools for researchers in terms of comparisons for experimental endpoints.

2.2 Age at puberty:

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 behavioral estrus (Bearden *et al.,* 2004). Dairy bulls reach puberty at the age of 10-12 months, at a body weight of 160-270 kg (Bearden *et al.,* 2004). It is much more difficult to determine the exact time of puberty in males because the first differentiation of spermatogenic cells precedes the release of the first spermatozoa from the seminiferous tubules by a month or more (Hafez and Hafez, 2000). Size and weight at puberty is more important than age in determining the onset of puberty. Mamabolo (1999) found that the testis weight, which is correlated with sperm production and quality, is also positively correlated with body weight. He also reported that approximately 80% of the variation in testicular weight occurs 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.

2.3 Endocrine and testicular physiology in mature bulls:

An understanding of post-pubertal male reproductive endocrinology and physiology aids in the understanding of the events occurring before puberty. The Hypothalamo Pituitary Gonadal axis (HPG axis) controls the pre-pubertal maturation and the post-pubertal function of the male reproductive tract and spermatogenesis. (Schwanzel-Fukuda and Pfaff, 1989).

2.3.1 GnRH, LH, leydig cells, and testosterone:

Hypothalamus plays major role in males by secreting the decapeptide hormone GnRH. GnRH is synthesized within the arcuate nuclei of the hypothalamus and released in a pulsatile manner from specific GnRH-releasing neurons in mature males. These GnRH-releasing neuron's axons originate in the surge and tonic centers of the hypothalamus and release GnRH where they terminate upon blood vessels of the hypothalamo-hypophyseal portal system in the median eminence of the hypothalamus. (Schwanzel-Fukuda and Pfaff, 1989). Upon release from the portal vasculature, GnRH reaches its cellular target tissue, the gonadotropes of the anterior pituitary. Here, the binding of GnRH to GnRH receptors

(GnRH-R) in the anterior pituitary stimulates these cells to produce and release the hormones Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH; Campbell *et al.,* 2009). The steps involving the release of LH from the anterior pituitary in response to GnRH are dynamic (Schanbacher and Echternkamp, 1978). The main target tissue of LH is the testicular leydig cells, which reside in the interstitial space of the testis outside of the seminiferous tubules.

Mature leydig cells function in producing testosterone when LH binds to LH-R. Testosterone is then able to regulate hypothalamic hormone release as well as spermatogenesis in mature bulls most notably by feeding back negatively on the anterior pituitary and hypothalamus (Schanbacher, 1982). However, testosterone's major role in the adult involves the maintenance of spermatogenesis. Testosterone concentrations will begin to reach to the levels similar to those seen in adulthood around 6 months of age in bulls (Miyamoto *et al.,* 1989), and concentrations within the testes will also increase drastically at this time. The concentration of testosterone within the testes is high due to binding with androgen binding protein produced by mature sertoli cells (Dadoune and Demoulin, 1993). High concentrations of testosterone within the testis are believed to be crucial for prevention of degradation of maturing spermatocytes (Walker and Cheng, 2005).

2.4 Attainment of functional spermatogenesis:

The main hormonal constituents required for spermatogenesis and their pre-pubertal development have been highlighted in the previous sections. The combination of these endocrine changes and physiological development culminate in the bull's ability to produce and ejaculate viable sperm. The early stages of spermatogenesis begin once spermatogonia occupy the spaces along the basement membrane of the seminiferous tubules and this can be visualized in testis histological sections beginning between 3 to 4 months of age in the bull (Chandolia *et al.,* 1997a). Spermatocytes can be seen by 6 months of age, and elongated spermatids by 8 months of age (Barth, 2004; Chandolia *et al.,* 1997a). Early ejaculations may contain sperm with visual abnormalities. It occurs when puberty has not been finalized and testicular components necessary for sperm maturation may not be fully developed (Evans *et al.,* 1995). These abnormalities may include the presence of proximal droplets or compromised motility, but there is a marked decrease in sperm abnormalities just prior to puberty (Evans *et al.,* 1995). With the attainment of puberty testosterone concentrations in the gonad are increasing which decrease visible sperm abnormalities and induce maturation of sperm in the epididymis

(Martig and Almquist, 1969). The testes experience rapid growth beginning between 25 and 28 weeks of age (Lunstra *et al.*, 1978), and this is most attributable to the changes in individual functions and numbers of somatic and germ cells (Bagu *et al.*, 2004). Overall composition of the testis is changing as the tubules begin to rapidly develop and fill with sperm cell-types of varying maturity. From 12 to 32 weeks of age the percentage of testis comprised of seminiferous tubules increases from 44 to 81% (Curtis and Amann, 1981), and the testes will continue to grow in size before they will be 90% of their final size by 24 months of age (Coulter, 1986).

The accessory sex glands must develop in synchrony with the gonads, so that the vesicular glands and prostate can begin to increase in size rapidly after 34 weeks of age (Chandolia *et al.*, 1997b). Sperm collection attempts in bulls at 40 weeks of age, or at 28 to 30 cm scrotal circumference for some high genomic bulls. Few bulls will mount a teaser at this age, but as time progresses most will gain the ability to mount and ejaculate into an artificial vagina by 12 months of age, and it is estimated that 95% of bulls have gained the ability to mount and ejaculate sperm of acceptable quality by 12-13 months of age (DeJarnette *et al.*, 2004). Sperm numbers per collection will also continue to increase during the initial year of collection, and most bulls will reach a peak of semen production around 48 months of age (Monke, 2002).

2.5 Ejaculation:

Semen ejaculation may be defined as the ejection of semen from the body. The semen includes spermatozoa from the vas deferens and epididymis and fluid from the accessory sex gland. Ejaculation varies between the species in a number of aspects. Bearden *et al.* (2004) indicated that the ejaculation time is less than one second in bulls, rams and bucks and more than 10-20 minutes in boars. The volume of the ejaculate is also higher in boars (200 ml) compared to bulls (6 ml), rams (1.5 ml) and stallions (75 ml). Bearden *et al.* (2004) found that the concentration of sperm per ejaculate for bulls, rams and stallions was 1.2 billion/ml, 2.0 billion/ml and 150 billion/ml, respectively.

2.6 Collection of semen:

Ejaculated semen is the combination of the product of testicles and excurrent ducts and the secretion of the accessory glands (Van Denmark and Free, 1970). Collection of semen involves the proper scheduling and sexual preparation of the male as well as the proper use of the collection technique. Cole and Cupps (1977) reported that during semen collection

many sources of sperm losses occur including losses before and during collection and during handling. It was also indicated that the efficiency of semen collection depends mostly on the daily sperm output, effect of sexual preparation, increasing sperm output and also decreasing the sources of sperm losses (Van Denmark and Free, 1970). If semen collection is to be accomplished within a reasonable period of time, it should be well organized (Cole and Cupps, 1977).

2.7 Analysis of fresh semen:

Immediately after ejaculation samples should be viewed with naked eyes and the density and color noted. Bearden *et al.* (2004), Colenbrander *et al.* (1993) and Den Daas (1992) reported that the percentage of normal sperm, motility, sperm concentration and volume per ejaculate are common criteria for evaluating semen quality at most AI stations. Analysis of sperm morphology is an important part of routine spermiogram, 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.

2.7.1 Chemical composition of bull semen:

The semen is composed of spermatozoa and a liquid part called seminal plasma. The final composition of the semen depends on the level of development of accessory reproductive glands. Finally the share of secretions from reproductive organs and the volume of spermatozoa in total make the semen. In the bull these secretions are the following: 50% secretion from alveolar glands, 25% secretion from bulbourethral glands, 7% secretion from the epididymis, 5% secretion from the prostate gland and 14% spermatozoa (Karolina *et al.,* 2012).

One ml of fresh bull semen is composed of sodium (230 mg), potassium (140 mg), calcium (144 mg), magnesium (9 mg), chlorine (180 mg), fructose (530 mg), citric acid (720 mg), glyceryl phosphoryl choline (35×10^4 mg), protein (6.8 gm) and sorbitol (75 mg) (Bearden *et al.,* 2004). Hossain *et al.* (2012) reported that incase of Local bulls of Bangladesh the volume, concentration, motility and pH of fresh semen were 9.3 ml, 1654.8 million/ml, 68.1% and 6.4, respectively. They also found that the volume, concentration, motility and pH of fresh semen were 11.5 ml, 1355.3 million/ml, 64% and 6.4 in Friesian bulls and 9.8 ml, 1858.4 million/ml, 68.8% and 6.4 in Sahiwal bulls in the subtropical condition of Bangladesh ($P<0.05$).

2.7.2 Properties of bull semen:

2.7.2.1 Color:

The color of normal bull semen has the appearance of whole milk, and its variations are due to the sperm concentration and contamination during the ejaculatory process. Pathological colors are described as the following according to Perry *et al.* (1947):

- Pink or red – Suggesting the presence of blood (which appears as the result of penis abrasion, fistulas of cavernous bodies, urinary stones),
- Green – Suggesting the presence of pus,
- Yellow – Suggesting the presence of urine,
- Watery white – Suggesting lower quantity of spermatozoa or water that got to the semen when collected from the artificial vagina.

(Sitali *et al.* (2017) reported that the semen color was affected by BCS ($P<0.05$). Bulls with a BCS of 2.0 were more likely to have a watery semen sample relative to a creamy sample compared to bulls with a BCS of 3.5 ($P<0.05$).

2.7.2.2 Volume:

The volume of semen is influenced by the number of spermatozoa and the secretion of accessory glands. Medhin *et al.* (2007) stated that differences in semen volume could be due to differences in the nutritional status of the animals, geographical locations, month of semen collection, collection method and bull handling during collection procedure and the frequency of ejaculation.

Hirwa *et al.* (2017) reported that Friesian bulls had superior ($P<0.05$) semen volume (5.76±0.08 ml) to that of Jersey (4.29±0.09 ml) and Inyambo (3.37±0.1 ml). Dasinaa and Pagthinathan (2015) studied with four stud bulls. The mean volume (ml) of Friesian, Sahiwal, AFS and Murrah were 4.1±0.87, 6.0±1.78, 8.3±1.96 and 4.5±2.09, respectively. Higher mean volume was found in Sahiwal bulls than in Friesian bulls ($P<0.01$). Atiqur *et al*. (2014) found the highest (7.19±0.19 ml) volume of semen ($P<0.01$) in Holstein-Friesian×Zebu bulls and lowest (4.41±0.2 ml) in Red Chittagong bulls. Nasrin *et al.* (2008) found highest ($P<0.01$) volume per ejaculate (4.01±0.14 ml) in Holstein cross bull and the lowest volume (2.58±0.07 ml) was found in Red Chittagong bull.

Lepard *et al.* (1941) found out that mature bulls produced a greater (P<0.05) volume than young bulls. Herman and Swanson (1941) stated that the age of the bull did not have as much influence on production of spermatozoa as did the size. Ruttle *et al.* (1975) found no significant age effect on the volume of semen. Differences in volume are sometimes due to the vigor of the thrust at the time of collection (Perry *et al.*, 1947). Ahmad *et al.* (2003) studied with 21 Sahiwal bulls divided into 3 age groups: group-I (upto 3 years), group-II (3-5 years) and group-III (above 5 years). The result indicated that the ejaculatory volume produced by bulls of group-II were significantly higher (P<0.05) than that of bulls of group-I and III. They also grouped months of the year into 4 seasons: winter (November-January), spring (February-April), summer (May-July) and autumn (August-October) depending on photoperiod. High ejaculatory volume was recorded during spring and low during summer (P<0.05). Fiaz *et al.* (2010) reported that Friesian bulls produced lower (P<0.05) ejaculatory volume during dry summer season (April-June). Sitali *et al*. (2017) studied sperm quality from 365 bulls raised on commercial farms in Zambia. Bulls of BCS 3.5 had the highest semen volume while those of BCS 2.0 had the lowest (P<0.05).

2.7.2.3 Concentration:

According to Hafez and Hafez (2000) the concentration of bull sperm ranges from 800-2000 million/mm^3. Concentration was positively correlated with the motility rating and negatively correlated with the percentage of live and abnormal spermatozoa, (Dasinaa and Pagthinathan, 2015). These results partially agree with the study of the semen concentration of Sahiwal and AFS stud bull having the positive (P<0.05) relationship with its motility (Mathevon *et al.*, 1998). Dasinaa and Pagthinathan (2015) also found the negative correlation of semen volume with concentration. The similar responses has been reported by Hossain *et al.* (2012) as while the volume of the semen increasing, concentration of the semen decreased. Friesian (r=0.4924) showed the increasing trend in an acceptable way.

Dasinaa and Pagthinathan (2015) reported that the mean value of semen concentration was 1785.3, 1411.5, 438.1 and 735.0 million/ml for Friesian, Sahiwal, AFS and Murrah respectively. Atiqur *et al.* (2014) found the highest (1257.34±30.46 million/mm^3) value of concentration of semen (P<0.01) in Holstein-Friesian×Zebu breeds of bulls and the lowest (1115.20±30.43 million/mm^3) in Red Chittagong breed of bulls. Ahmad *et al.* (2003) found no significant difference in sperm concentration of bulls in different age groups and during four seasons of the year. However, they reported highest sperm concentration

(1.00±0.01) in adult Sahiwal bulls (3-5 years) and lowest (0.96±0.01) in the young bulls (≤3 years) of the same breed. They also found highest sperm concentration during spring (1.04±0.01) and lowest in summer (0.90±0.01). Salah *et al.* (1992) reported that ejaculates collected from Friesian bulls during hot summer season had significantly (P<0.05) lower sperm concentration than those collected during winter time. Lasley and Bogart (1943) and Erb *et al.* (1942) found that the average concentration of dairy bull semen and total sperm per ejaculate was maximum during April, May, and June. Swanson and Herman (1944) did not find any statistically significant monthly variation in volume, concentration, and percentage of total abnormal spermatozoa.

2.7.2.4 Semen pH:

The pH of seminal plasma ranges from 6.7 to 7.4, which is common in the domestic species (Roberts *et al.,* 1986) and has the potential to neutralize vaginal acid. Lowering of pH with lactic acid was demonstrated to immobilize bull sperm (Carro *et al.,* 1985; Acott and Carr, 1984). It was reported by Anderson (1942) that the mean pH of normal bull semen was 6.73±0.02. Significant (P<0.05) differences were noted among bulls tested. Atiqur *et al*. (2014) studied with four different bulls of cross breed (Holstein-Friesian×Zebu, Sahiwal×Zebu, Sindhi×Zebu and Red Chittagong bull). Significantly (P<0.05) the highest pH was found in Holstein-Friesian×Zebu and lowest in Red Chittagong breed of bulls. Cannon (1947) observed a positive correlation between the pH readings taken from the semen of individual bulls within the different breeds.

A definite seasonal fluctuation, with a higher (P<0.05) pH in February and a lower pH in May, was recognized. Ahmad *et al.* (2003) reported that effect of age and season on pH of semen was non-significant. Terezenha *et al.* (1991) recorded non-significant variations in pH of semen from bulls of young, adult and old age groups and within each age group. Highly significant (P<0.05) negative correlation was found between pH and the concentration of spermatozoa. The more acid the pH of semen on collection, the better was the motility retained on storage. Raps and Reid *et al.* (1948) found a significant relationship of the pH change in incubated semen to a number of different semen characteristics, such as motility and viability, and indicated its value as an indirect measure of overall semen metabolism.

2.7.2.5 Mass activity:

Atiqur *et al.* (2014) studied with four different bulls of cross breed. Significantly ($P<0.01$) the highest mass activity was found in Holstein cross and lowest in Red Chittagong breed of bulls. Ahmad *et al.* (2003) found that age and season had no significant effect on the mass activity of the ejaculate. However the result indicated highest mass activity value (2.91±0.04) in bulls of group-II (3-5 years) and lowest (2.35±0.01) in group-III (above 5 years). High mass activity (3.38±0.03) was recorded during spring and low (2.05±0.02) during summer ($P<0.05$).

The mass activity of semen of rams with concentrate supplementation tended to be higher than the control rams (Golam *et al.*, 2013). Cunha *et al.* (2012) found that a diet containing cottonseed did not influence the mass activity of ram semen. Susan (2010) studied with reproductive performance of boars fed a diet supplemented with organic selenium (Se) and vitamin E. Mass activity produced by bulls supplemented with 0.3 ppm organic Se and tocopherol significantly higher ($P<0.05$) than that of bulls supplemented with basal diet. On the other hand the mass activity increased significantly in semen of buffalo fed ammoniated rice straw (El-Khadrawy, 1991).

2.7.2.6 Motility:

Perry *et al.* (1947) stated that initial motility was believed to be the best evidence of viability of sperm of a fertile male, although it is not necessarily a sign that the sperm possesses normal fertilizing power. Nasrin *et al.* (2008) reported that the average mass motility (%) in fresh ejaculate was ranged between 52 and 65. Pal *et al.* (2006) and Bratton *et al.* (1954) reported average mass motility of bovine fresh semen as 63.3% and the range was 50-80%. This variation could be caused by the age of animals, climate and management. Dasinaa and Pagthinathan (2015) reported that the mass motility of the fresh semen was 83.2±2.5%, 80.5±1.46%, 80.3±1.28% and 80.0±0.00% for Friesian, Sahiwal, AFS and Murrah respectively. Atiqur *et al.* (2014) found the highest (69.40±0.67%) value of motility of semen ($P<0.01$) in Sahiwal×Zebu breed of bulls and lowest (62.12±0.97%) in Red Chittagong breed of bulls.

Ulfina Galmessa *et al.* (2014) selected twelve Sahiwal bulls and grouped into two on the basis of age (<50 months; >50 months). Significantly ($P<0.05$) higher mean motility was observed for the younger (76.40±3.07) than the older bull (65.00±3.50). They also observed higher mean motility for bulls having scrotal circumference <33cm than the bulls

having scrotal circumference >33cm (P<0.05). Ahmad *et al.* (2003) studied with 21 Sahiwal bulls divided into 3 age groups: group-I (upto 3 years), group-II (3-5 years) and group-III (above 5 years). The result indicated that the motility percentage produced by bulls of group-II were significantly higher (P<0.05) than that of bulls of group I and III. They also grouped months of the year into 4 seasons: winter (November-January), spring (February-April), summer (May-July) and autumn (August-October) depending on photoperiod. High motility percentage was recorded during spring and low during summer (P<0.05). Fiaz *et al.* (2010) reported that the mass motility of semen in Friesian breed was significantly (P<0.05) lower during wet summer (July-September). Salah *et al.* (1992) reported that ejaculates collected from Friesian bulls during hot summer season had significantly (P<0.05) lower sperm motility than those collected during winter time.

Rowe *et al.* (2014) reported that at collection, motile (69.1 vs. 55.2%) and progressive (50.3 vs. 38.5%) sperm were greater (P<0.05) for bulls receiving the organic trace mineral supplementation compared with bulls receiving the inorganic trace mineral supplementation. Likewise, progressive sperm were improved (P=0.004) for bulls receiving organic (70.0%) compared with inorganic (55.4%) trace mineral supplementation. The percentage of motile sperm with rapid motility (path velocity >50 μm/sec) was also greater (P=0.002) for bull supplemented with organic compared with inorganic trace mineral (50.7 and 38.0%, respectively). Susan (2010) studied with reproductive performance of boars fed a diet supplemented with organic selenium (Se) and vitamin E. Motility percentage produced by bulls supplemented with 0.3 ppm organic Se and tocopherol significantly higher (P<0.05) than that of bulls supplemented with basal diet.

2.7.2.7 Morphology:

2.7.2.7.1 Percentage of live spermatozoa:

Nasrin *et al.* (2008) conducted an experiment with bulls of different genetic groups. Analogical with other characteristics live sperm percentage was lower (P<0.01) in Red Chittagong bull than in any bulls of other breed. Hahn *et al.* (1969) observed the average live sperm percentage for Holstein bull (83.5%) and with a range from 70-90%. Stone *et al.* (1950) obtained a multiple co-efficient of correlation of +0.69 of fertility with spermatozoa concentration and the percentage of spermatozoa surviving at 0ºC cold shocks. Madden *et al.* (1947) found, using 1% significance level, that the correlation

between initial motility and initial percentage of live sperm was highly significant with a coefficient of correlation of 0.69. A close relationship between the percentage of live sperm and the motility of sperm in storage was shown. No significant differences were noted in motility, longevity, or the percentage of live sperm between semen which resulted in conception and semen which did not produce conception. They also stated, except in extreme cases, the percentage of live sperm in semen did not seem to be a satisfactory test for estimating the value of semen to be used for inseminating purposes. A significant correlation between the percentage of live spermatozoa at ejaculation and the percentage of spermatozoa surviving a cold shock after dilution of semen was found by Lasley and Bogart (1943). They did not find any correlation with the percentage of live spermatozoa in non-diluted semen. They stated that semen which contained less than 50 per cent live spermatozoa is of questionable fertility.

2.7.2.7.2 Percentage of normal and abnormal spermatozoa:

Sitali *et al.* (2017) studied sperm morphology from 365 bulls raised on commercial farms in Zambia. The most common defects were the simple bent tails (4.42±0.31%), normal dead (7.51±0.40%), proximal cytoplasmic droplets (PCD) (3.50±0.32%), loose dead heads (1.65±0.20%) and the distal cytoplasmic droplets (DCD) (1.36±0.16%). Tail defects were the most predominant with (9.00±0.43%), followed by the mid piece defects (4.50 ±0.35%) and head abnormalities (0.78±0.07%), respectively. Breeding bulls with a BCS of 2.0 had the highest percentage of dead sperm (11.20±1.40%) and the highest percent of major defects (10.17±1.43%) while those of BCS 3.5 had the lowest percent dead sperm (5.1±0.66%) and major defects (4.27±0.78%), ($P<0.05$). The highest percent PCD (5.35 ±1.13%) and lowest percent PCD (2.26±0.44%) was found in bulls of BCS 2.0 and 3.5, respectively ($P<0.05$).

Sarder (2008) found non-significant association of sperm abnormalities with the body conditions of the breeding bulls. Notably the highest head abnormalities were recorded in bulls with excellent body condition, followed by average, very good and good body conditions. They also found significant effect ($P<0.05$) of scrotal circumference on all the sperm abnormalities. The highest head abnormalities (6.04%) were recorded in <34 cm and the lowest (5.29%) in >38 cm scrotal circumference groups. The highest proportion of mid piece abnormalities was observed in 36-<38 cm and the lowest in <34 cm groups. The highest percentage of tail abnormalities was observed in >38 cm (6.64%) and the lowest in <34 cm (5.96%) groups. The highest percentage of total sperm abnormalities (17.00%)

was recorded in >38 cm groups followed by <34 cm (16.76%), 34-<36 cm (16.39%) and 36-<38 cm (15.73%) groups. Sitali *et al.* (2017) found that the *Sussex* breed had the highest head abnormalities (2.05±1.05%) and highest major defects (14.60±4.90%) (P<0.05) while the *Santagetrud* breed had the lowest head abnormalities of (0.50±0.18%) (P<0.05) compared with other breeds.

Vilakazi (2003) studied with 329 bulls. The Least square means (LSM±SE) for the percentage normal sperm, major defects and minor defects in Friesland and Jersey bulls were 80.6±1.06% versus 78.9±2.31%, 14.8±0.90% versus 15.0±2.62% and 5.1±0.43% versus 7.6±0.94%, respectively. The results of the study indicated that Friesland had better sperm morphology than Jersey. This was due to the higher percentage normal sperm and lower incidence of abnormal sperm that was observed in Friesland bulls compared to Jersey bulls. The study demonstrated seasonal variation on semen morphology. Least square means for semen defects (P<0.01) were significantly higher during summer and autumn compared to spring and winter. Least square means for percentage normal sperm (P<0.05) was significantly higher during spring and winter compared to summer and autumn. Higher percentage of normal sperm and less incidence of sperm defects was observed in bulls aged 36-48 months of age, compared to the bulls younger than 36 months and older than 72 months of age. Younger bulls (less than 36 months) had better semen morphology than their older counterparts (older than 72 months).

Nasrin *et al.* (2008) conducted an experiment with bulls of different genetic groups. There was a significant (P<0.01) bull variation for head length and total length of spermatozoa. In this study, significantly highest (P<0.01) head length of sperm was found in Holstein cross bulls followed by Red Chittagong Sindhi cross and Sahiwal cross bulls. But total spermatozoal length was significantly (P<0.01) lower in Red Chittagong bull than in other bulls. Head breadth of spermatozoa in bulls of all breeds/types behaved alike genetic group of bulls showed uniformity in spermatozoal measurement. Susan (2010) studied with reproductive performance of boars fed a diet supplemented with organic selenium (Se) and vitamin E. Percentage of abnormal sperm produced by bulls supplemented with 0.3 ppm organic Se and tocopherol significantly lower (P<0.05) than that of bulls supplemented with basal diet.

2.8 Factors affecting the quality of fresh semen:

2.8.1 Breed:

Wide variations exist in properties of semen among different breeds of bull as well as different ejaculates of the same breed. Alemayehu and Tena (2015) evaluated semen quality and fertility of 45 bulls (36 breeding and 11 pre-service young bulls). The overall mean (±SD) of libido, volume, concentration, mass motility, pre-freezing and post-freezing motilities of the breeding bulls were 3±0.0, 7.71±2.4 ml, 1.03±0.3 billion/ml, 3.47±0.5, 78.35±4.2% and 55.98±5.9%, respectively. Borana bulls produced small volume but highly concentrated semen with the highest mass motility of sperm ($P<0.001$) whereas Holstein-Friesian bulls produced the largest volume. Pre-freeze motility of sperm was lowest in crosses ($P<0.05$) than other breeds. The mean (±SD) scrotal circumference was 34.5±1.7 cm with a relatively higher percentage of dead sperm (34.2±19.5%) in the pre-service young bulls.

Atiqur *et al.* (2014) performed a study with different bulls of cross breed. The mean values of semen ejaculate volume, sperm motility, mass activity, sperm concentration, percentage of live sperm and dead sperm, pH and fertility varied significantly ($P<0.01$) among breeds. Significantly ($P<0.01$) the highest volume per ejaculation, sperm concentration, mass activity were found in Holstein cross and lowest in Red Chittagong breed of bulls. Significantly ($P<0.05$) the highest live spermatozoa percentage, pH, fertility rate were found in Holstein-Friesian×Zebu and lowest in Red Chittagong breed of bulls.

Hossain *et al.* (2012) conducted an experiment on 97 breeding bulls to find out the physical and chemical properties of different bull semen. It was observed that the maximum average ejaculate was obtained from SL×F and the mean value was 12.9 ml. However, the minimum average ejaculate was obtained from LF×F and the mean value was 7.4 ml. Before freezing, the maximum average sperm concentration was obtained from SL and the mean value was 1858.4 million/ml. The minimum average sperm concentration was obtained from LF_1×F and the mean value was 1286.6 million/ml. The maximum average pH was obtained from LF_2×F and the mean value was 6.5. The maximum average motility was obtained from SL and the mean value was 68.8%. However, the minimum average motility was obtained from L×F and the mean value was 63.7%.

Fiaz *et al.* (2010) collected semen production data of Holstein-Friesian and Jersey bulls over a period of three years. Friesian bulls produced lower ejaculatory volume during dry summer season, whereas Jersey bulls produced higher ($P<0.05$) volume during wet summer compared to other seasons. Seasonal pattern of mass motility and individual motility of semen was different between two breeds. However, individual motility in the semen of Friesian bulls did not differ among seasons ($P<0.05$) but in Jersey bulls it was lower during wet summer than other seasons.

Nasrin *et al.* (2008) conducted an experiment with bulls of different genetic groups. Individual bull effect was found to be significant ($P<0.01$) on volume per ejaculate, mass motility, sperm concentration, live sperm and normal sperm percentage. The significantly ($P<0.01$) highest volume per ejaculate, sperm concentration, live sperm and normal percentage were found in Holstein cross and lowest in Red Chittagong Bull. Non-return rate was highest in Holstein cross bull and lowest in Red Chittagong Bull. Significant ($P<0.01$) positive correlation was observed in mass motility, live sperm and normal sperm percentage with non-return rate.

2.8.2 Age:

Almquist (1982), Everett and Bean (1982) and Almquist and Amann (1976) reported that considerable attention should be paid to the evaluation of changes in semen quality due to variation in the age of bulls. Coe (1999) found that age was one of major causes in the variation of semen quality among the bulls, due to physiological changes that occur as bulls grows to sexual maturity. Salisbury *et al.* (1978) found that semen production and quality increase beyond the first year after puberty. Salisbury *et al.* (1978) found a correlation of 0.51 between sperm production and the age at first pubertal life in Holstein bulls.

Almquist (1978) reported that the main factor determining the total number of sperms produced was the size of the testis. Size of the testis is correlated with age of the bull (Brito *et al.*, 2002b). Testis size increases for at least 5 years after puberty (Amann and Almquist, 1976). In dairy bulls, Bearden *et al.* (2004) reported that the characteristic of the ejaculates differed according to the size of the breed. It was found that large dairy bulls had semen volume, motility and concentration of 7-8 ml, 50-80% and $1\text{-}1.5\times10^6$ per ml respectively, while small breeds have 5-6 ml, 50-80% and $1\text{-}1.5\times10^6$ per ml respectively.

Several researches such as Mathevon *et al.* (1998), Gamer *et al.* (1996), Shannon and Vishwanath (1995), Everret and Bean (1982) and Almquist and Amann (1976) reported that the volume of the ejaculate, sperm concentration and semen motility improved with an advance in age of the bull. This however, contradicted with Brito *et al.* (2002a) who found no significant effect of age on sperm concentration and motility. Mathevon *et al.* (1998) found that the volume, concentration, motility, total sperm and motile sperm were 5.48 cm^3, 1296×10^5/ejaculate, 51%, 7090×10^4/ejaculate and 3757×10^4/ejaculate in young bull (<3 years) and 6.73 cm^3, 1380×10^5/ejaculate, 57%, 9310×10^4/ejaculate and 5339×10^4/ejaculate in mature bull (>3 years) respectively. Serrano (1984) found that bulls from 2-5 years of age produce larger volumes of ejaculate compared to bulls less than 2 years of age and bulls older than 5 years of age ($P<0.05$).

Van Denmark and Free (1970) also reported that the semen volume increased with age and body weight. It was also indicated that young bulls just coming into service produce as little as 1-2 million sperm per ejaculate, while fully matured bulls produces 10-15 million sperm per ejaculate. This is also in line with the results of a study done by Mamabolo (1999) where a high correlation of 0.90 between the body size and semen production was recorded. On the other hand, Kumi-Diaka *et al.* (1981) indicated that irrespective of the breed type, either exogenous or endogenous sperm cell concentration was significantly ($P<0.05$) higher in the younger matured bulls of 3-7 years as compared to bulls of 7.5-10 years of age.

Spermatozoa morphology is often used as one of the important criteria in the evaluation of semen quality in domestic animals (Howard *et al.,* 1983). An increase in the frequency of abnormal sperm cell has been observed with advancing age in bulls (Rao and Rao, 1979). This is also similar to the results reported by Wolfe *et al.* (2000) where it was reported that males at the age of 6 and 7 years produce fewer normal structural sperm than their younger counterparts. Even in the human, increase in semen volume of 3-22%, sperm motility of 3-37% and number of normal sperm of 4-18% are associated with an increase in age (Kidd *et al.,* 2001). Gustafasson and Sekoni (1980) reported that old bulls (5-8 years) had higher incidence of sperm abnormalities than young bulls (1.5-2 years) and younger bulls less than 13 months had higher incidence of head abnormalities and proximal cytoplasmic droplets than bulls of 1.5-2 years of age ($P<0.05$).

Dowsett and Knott (1996) reported that all semen characteristics with the exception of color and urethral pulsation in the stallion vary significantly ($P<0.05$) with age. It was further indicated that semen quality characteristic such as volume, sperm concentration, total sperm number and sperm abnormalities were poorest in a stallion under the age of 3 years and over 11 years of age. Salisbury *et al.* (1978) reported the incidence of some defects such as abnormal mid pieces and pseudo-droplets which were associated with advancing age of the bull.

2.8.3 Scrotal circumference:

Scrotal circumference (SC) is a very important trait in bull reproduction, which can be used as a measurement of fertility. It is also an important component in examining bulls for breeding soundness (Hoogenboezem and Swanepoel, 2000). SC has a heritability estimation of 50% (Lasley, 1978), which means that it is influenced to a large extent by genetic rather than environmental factors.

Brito *et al.* (2002a) found that scrotal circumference had a positive relationship with ejaculate volume and negative correlation with sperm motility and major sperm defects. Furthermore, it was also reported that scrotal shape had a negative correlation with minor sperm defects. Brito *et al.* (2002b) also reported that the scrotal surface temperature had a negative correlation with sperm motility and scrotal surface temperature gradient had a positive correlation with sperm concentration. Scrotal circumference is positively correlated with the age of the animal (Brito *et al.*, 2002a). Several experiments have reported a high positive relationship between scrotal circumference and sperm production and semen quality in growing bulls (Hoogenboezem and Swanepoel, 2000; Vale Filho *et al.*, 1997; Palasz *et al.*, 1994; Godfrey *et al.*, 1990; Neville *et al.*, 1988; Gipsom *et al.*, 1985; Hanks *et al.*, 1981). This also agrees with the results in the study of Coe (1999) where scrotal circumference and age was associated with approximately 11% variation in semen quality.

Hoogenboezem and Swanepoel (2000) indicated that scrotal circumference is positively correlated with the overall potential breeding efficiency and seminal characteristics (such as percentage of live sperm, sperm quantity, motility and sperm concentration). Hoogenboezem and Swanepoel (2000) suggested that semen quality and scrotal circumference are affected by factors related to underdevelopment of the testes and testicular degeneration. These factors may be related to certain management practices such as feeding excessive energy which leads to fat deposition in the scrotum and reduced body

condition of the bull (Cupps, 1991; Coulter and Kozub, 1984). Small scrotal size may also result from the lack of adequate testicular development (King, 1993) due to thermal and nutritional factors which interfere with the endocrine regulation of reproduction (Bearden *et al.,* 2004). The temperature of a scrotum also plays a major role in determining the production and quality of semen. Coulter and Kastelic (1994) and Kastelic *et al.* (1997;1996;1995) reported that the scrotum and testicular vascular cone (TYC) participate in the testicular thermo-regulation ability and therefore variation in these structures among bulls could affect the quantity and quality of semen produced. Coulter and Kastelic (1994) and Coulter (1988) indicated that a pendulous scrotum may improve the testicular thermo-regulation by moving the testis away from the body and thus facilitating heat loss.

To successfully complete a breeding soundness evaluation, a bull must have at least 30% sperm motility, 70% normal sperm morphology, and a minimum scrotal circumference based on age (Chenoweth *et al.,* 1992). Chenoweth *et al.* (1992) reported the minimum scrotal circumference requirements for bulls to successfully pass a BSE based on the age of bulls. According to Chenoweth *et al.* (1992) bulls of ≤15 months aged should have SC of 30 cm, >15≤18 months 31 cm, >15≤18 months 32 cm, >21≤24 months 33 cm and ≥24 months 34 cm, respectively.

2.8.4 BCS:

Body Condition Scoring (BCS) is a method for determining the relative fatness in breeding bulls. Body condition scoring is a management tool that can be used to evaluate the semen quality in bulls. Herd and Sprott, (1986) rated body condition on a scale from 1 to 9, with 1 being severely emaciated and 9 extremely obese. Animals are judged by fat thickness in areas such as the spine (vertebrae), ribs, hooks and pins, tailhead, brisket, and muscling in the round and shoulder. Body condition scoring can be done using visual indicators.

Fat in the scrotum can negatively affect semen quality. Semen quality is reduced for bulls in a body condition of 7 or greater or those with body condition scores less than 4 (Barth and waldner, 2002). Bulls with a BCS of 2-4 have semen of satisfactory quality relative to those of 2.5-3.5. Fat deposition around the testicles in bulls of BCS >4 affects thermo-regulation, influences sperm motility and morphology. Bulls of poorer BCS or those losing excessive condition show testicular degeneration resulting in poor reproductive parameters (Barth and waldner, 2002).

2.8.5 Season:

Season can include many factors, such as temperature, photoperiod and humidity. Differences in environmental temperature, humidity and seasonal variation could affect semen output (Siratskii, 1990; Castillo *et al.,* 1987; McDowell, 1972). Seasonal fluctuation in the production of spermatozoa has been demonstrated in a number of animals. Mathevon *et al.* (1998), Ibrahim (1997) and Ahmad and Noakes (1996) reported that the month and season of the year had a significant effect on semen quality parameters. In the area where there is marked seasonal variation in environmental temperature, bull semen quality tends to be lower during summer months (Curtis, 1993). This occurs due to thermal stress which causes testicular degeneration and abnormal scrotal thermogram and hence lowers the semen output (Curtis, 1993; McDowell, 1972). When considering the effect of season on semen quality, ambient temperature and humidity has to be interpreted carefully.

Curtis (1993) reported that when bulls exposed to temperature humidity index (THI) of 37°C, the sperm concentration and total sperm output decreased markedly. Everett and Bean (1982) associated the increase in semen output with the humidity below 50%. Mathevon *et al.* (1998) observed no significant variation in the volume of ejaculates and sperm motility with season in matured bulls. These results agree with Afiefy *et al.* (1984) where no variation in the volume of the ejaculate with season was observed in Friesian and Buffalo bulls. Regardless of the age and breed of the bull, the semen concentration and total number of cells were higher ($P<0.05$) during winter and spring than summer and fall (Mathevon *et al.,* 1998).

Abi Saab and Hamadeh (1984) reported that in rams, volume of the ejaculate was affected by the season of semen collection and it was observed to be higher ($P<0.05$) during August to October and lower during winter and early summer. Schwab *et al.* (1987) reported that the season of collection did not affect the percentage of motile spermatozoa in mature bulls. The results found by Schwab *et al.* (1987) were contradictory to those reported by other researches such as Stalhammar *et al.* (1989) who found that the percentage of motile spermatozoa was poor during spring and winter. This could be occurred due to climate, which determines the nutritional status of the bull. Soderquist *et al.* (1996) and Parkinson (1987; 1985) reported that significant seasonal variation occurred in the incidence of sperm head abnormalities and total sperm abnormalities and the least square means for sperm abnormalities were significantly higher during the warmer season (spring and summer) compared to the colder season (autumn and winter). On the other

hand, Trudeau and Sanford (1986) found that ejaculated protein and citric acid were higher ($P<0.05$) in winter and lower in spring.

2.8.6 Nutrition:

Semen quality and quantity are the major factors of concern in reproduction and can be adversely affected by malnutrition (Brown, 1984). Reduction in feed intake in animals exposed to high temperatures may lead to a deficient protein and energy intake. Severe under-nutrition or over-feeding and deficiencies of specific nutrients are the most common causes of impaired reproductive capability of the bull in terms of semen production and quality (McDowell, 1972). Brown (1984) found that the reproductive function in the young bull was more susceptible to dietary restriction of energy and protein than in adult. This was also similar to Cupps (1991) who indicated that the negative effect of restricted nutrition was more abundant in young than in old animals.

There is evidence that the effect of nutrition on semen quality is mediated via the effect of dietary constituents on the hypothalamic-pituitary axis, although there are also some indications that dietary changes affect the testis growth indirectly (Brown, 1984). A restricted diet is associated with a decrease in the release of LH and FSH, which affects semen production and quality through the effect on testicular size (King, 1993). Similar results have been also reported by Martin *et al.* (1987) and Oldham *et al.* (1978) where the level of nutrition was associated with a reduction in testicular size, which in turn affects the production and quality of spermatozoa.

A low plane of nutrition suppresses the production of gonadotropins by the pituitary gland and the secondary sex hormones. As a result, atrophy of the prostrate and seminal vesicles occurs thereby affecting semen quality in terms of fluid volume and concentration (Alkas *et al.*, 1982). During the periods of nutritional stress, the animal body secretes adrenocorticotropic hormone (ACTH), which in turn stimulates the secretion of glucocorticoids, which lower the circulation or secretion of FSH and LH and results inefficient spermatogenesis and poor semen quality (Bearden *et al.*, 2004). The interaction of diet and breed have been shown by researchers to affect reproductive parameters such as semen volume (Field *et al.*, 1979), epididymal sperm reserves (Buhr *et al.*, 1993) and sperm reserves (Coulter and Bailey, 1988) which may potentially affect fertility in dairy bulls. Diet has also been shown to affect the membranes associated with the salivary gland (Alam and Alam, 1986) or enzymes in the liver (Pugh and Kate, 1980) and the protein

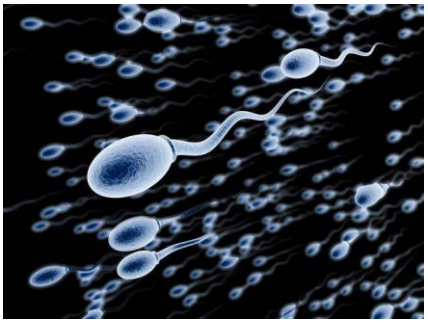
receptors (Knazek and Lui, 1979). Buhr *et al.* (1993) claimed that dietary fatty acids also exert a direct effect on these membranes. It was also indicated that dietary changes alter not only the composition but also molecular interaction within the cellular sperm membranes.

Cupps (1991) reported that deficient nutrient intake, as for example iodine, zinc, cobalt, vitamin A, E and minerals are associated with a reduction in semen quality in terms of morphology, concentration and motility. Scott *et al.* (1998) reported that in many species, a selenium deficiency affects the morphology and motility of the spermatozoa and may be linked to the sub-fertility in many domestic animals. Spermatozoal membrane, being rich in polyunsaturated fatty acids (PUFA), is easily damaged by reactive oxygen species (ROS) or membrane lipid peroxides which lower the viability and fertility of the spermatozoa (Silva, 2006). Selenium increases the formation of anti-oxidant glutathione peroxide activity, which decreases the reactive oxygen species and hence increase in spermatozoa viability and fertility (Bray *et al.,* 1997).

Underwood and Sommers (1969) reported that zinc is responsible for larger semen production as it is involved in the nucleic acid and protein metabolism for production of the sex hormones, including testosterone and GnRH (Hambidge *et al.,* 1986). Underwood and Sommers (1969) indicated that zinc requirements for spermatogenesis are greater than that the requirements for body growth, so a deficiency may alter spermatogenesis and lead to a high proportion of abnormal spermatozoa. Saaranen *et al.* (1987) found that zinc deficiency affects the morphology and abnormalities in semen since it is associated with the attachment of the head to the tail. Bray *et al.* (1997) reported that zinc deficiency may also lead to an increase in reactive oxygen species, which affect sperm viability. Zinc also contains anti-oxidant properties that act to reduce reactive oxygen species leading to an increase in semen viability.

Abdel-Rahman *et al.* (2000) reported that in small ruminants the percentage of motile spermatozoa decreases as the content of potassium and calcium increases and sodium, chloride, phosphorus and magnesium decreases. This is in line with Bearden *et al.* (2004) who reported that high concentrations of potassium and calcium were detrimental to semen metabolism of the bull spermatozoa, which determines the motility of spermatozoa. On the other hand Abdel-Rahman *et al.* (2000) also reported that the percentage of live spermatozoa correlated positively with potassium and calcium and negatively correlated with phosphorus.

Prolonged dietary vitamin A deficiency impaired semen quality and semen production. Vitamin A deficiency lowered the spermatozoa concentration, semen storage capacity and also delayed sexual maturity and suppressed spermatogenesis in young bulls (Cupps, 1991). Rode *et al.* (1995) reported that the proportion of spermatozoa with morphological defects was greater in bulls with vitamin A deficiency. Vitamin B complex has a marked effect on appetite and its deficiency may cause inanition and adversely affect semen production through a reduction in testicular and body weight. Phillip and Lardy (1940) claimed that vitamin C deficiency leads to a reduction in sex drive and semen quality in manner similar to vitamin A. The distribution of major ions between sperm fractions and seminal plasma could provide the basis for variation in semen quality and should be considered in the interpretation of the result obtained in the evaluation of semen.



Chapter III
Materials And Methods

CHAPTER III
MATERIALS AND METHODS

3.1 Study area and duration:

The study was conducted at breeding bull station of Ejab Alliance Limited situated at Thakurgaon district of Bangladesh from March, 2017 to February, 2018. Thakurgaon is a barinda district, situated on the northern part of Bangladesh.

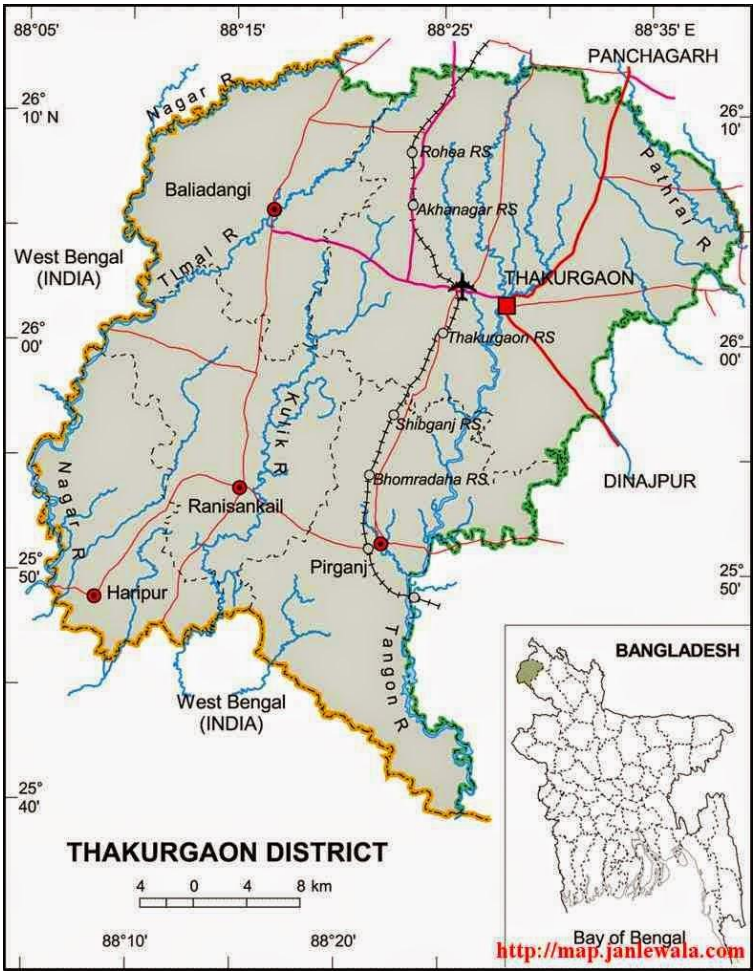


Fig.1. Map of Thakurgaon District

3.2 Study animals:

In this study, 25 bulls from two breeds namely Friesian (87.5%) and Sahiwal (75%) were used. Sahiwal bulls were dominant in the study (n =15) while only 10 Friesian bulls were available.

Fig.2. Farm province of Ejab Alliance Ltd.

Fig.3. Friesian bull

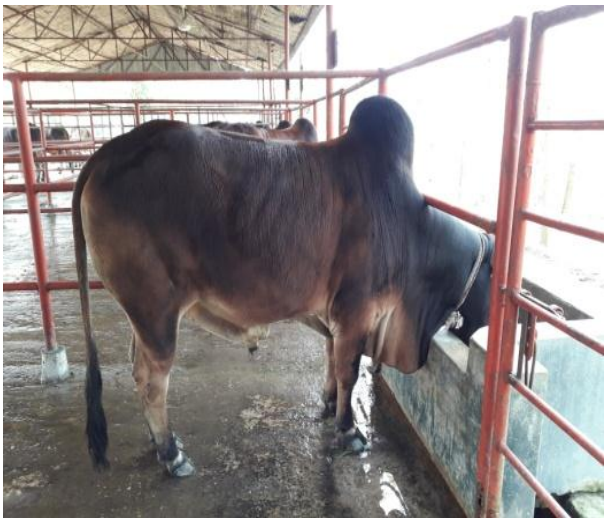
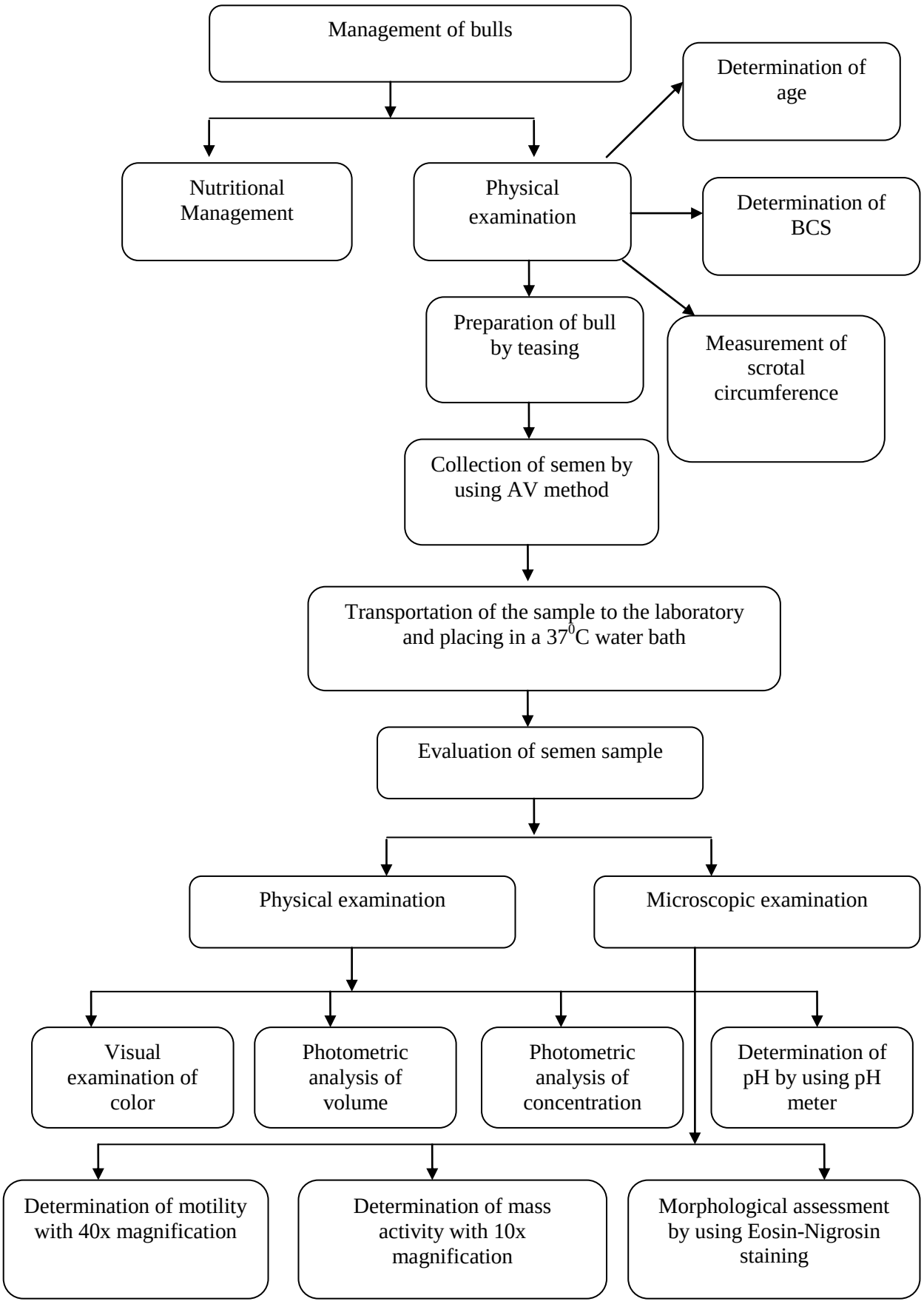


Fig.4. Sahiwal bull

3.3 Study design:



3.4 Physical examination of the bulls:

3.4.1 Determination of age:

The age of the bull was determined by using dental formula according to Johnson (1999).

3.4.2 Determination of BCS:

To properly evaluate body condition for bull's major emphasis gave on skeletal structures, muscle and fat positioning. The tailhead area was scored by feeling the amount of fatness. The loin area was scored in a similar way, using the same hand, when the bull is relaxed. This manuscript was focused on the commonly used scale of 1 to 5, with 1 being thin and 5 being optimum conditioned according to Herd and Sprott (1986).

3.4.3 Measurement of scrotal circumference:

Scrotal circumference was measured by pulling the testes firmly down into the lower part of the scrotum and placing a measuring tape (60 Inch tape) around the widest point. Measurements were recorded in centimeters (cm).

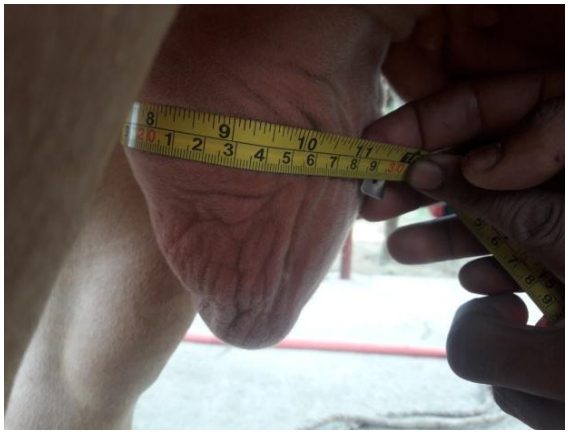


Fig.5. Measurement of scrotal circumference

3.5 Grouping of the study animals:

Bulls were grouped according to

Breed: Two groups were made based on breed, namely-

- Friesian (n=10)
- Sahiwal (n=15)

Age: An attempt was made to group the bulls into three sub-groups according to age, namely-

- G I: 2.5 to 3.5 years (n=6)
- G II: >3.5 to 4.5 years (n=10)
- G III : >4.5 years to above (n=9)

Scrotal Circumference: The animals were grouped into four sub-groups based on scrotal circumference, namely-

- G I: 27-29 cm (n=2)
- G II: 29.1-31 cm (n=6)
- G III: 31.1-33 cm (n=5)
- G IV: 33.1 cm-above (n=12)

BCS: The animals were grouped into two sub-groups according to BCS, namely-

- G I: 4 to 4.5 (n=16)
- G II: >4.5 to 5 (n=9)

3.6 Nutritional management of bulls:

Two types of feed supplementation were supplied to the two different groups of the studied bulls namely type I (Normal feed+Vit. ADE supplementation) and type II (Normal feed+extra protein supplementation).

Drugs were induced as Vit. ADE supplementation. At first each bull was injected with Inj. Acivit-ADE (100 ml vial) @ 10 ml intramuscularly once in a week. Then the bull was injected with Inj. E-Vet plus (100 ml vial) @ 10 ml intramuscularly once in a week. Extra amount of protein supplementation was added in type II ration formulation where mustard oil cake was replaced by sesame oil cake.

Table 1: Feed supplementation for bulls

Type I:

Ingredients	Amount (per day per animal)
Wheat bran	4 kg
Rice polish	2 kg
Molasses	100 gm
Mustard oil cake	200 gm
Gram (Germinated)	300 gm
Pulse	200 gm
Salt	100 gm
Straw	1.5 kg
Green grass	12-15 kg
Vit. ADE supplementation	Drug induced

Type II:

Ingredients	Amount (per day per animal)
Wheat bran	4 kg
Rice polish	2 kg
Molasses	100 gm
Sesame oil cake	300 gm
Gram (Germinated)	400 gm
Pulse	200 gm
Salt	100 gm
Straw	1.5 kg
Green grass	12-15 kg

3.7 Collection of semen:

3.7.1 Preparation of bulls and sexual stimulation:

During the study, no artificial hormones were used to sexually stimulate the bulls. Sexual excitement prior to semen collection was induced by parading of the dummy or teaser bulls around the bull. At least three false mounts were allowed to enhance the degree of sexual excitement.

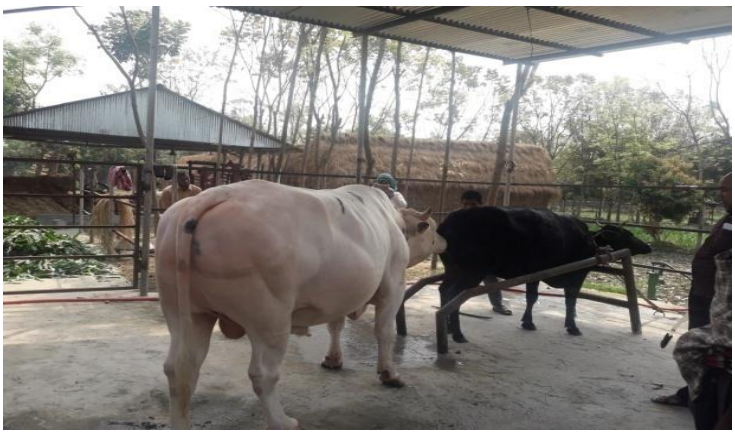


Fig.6. Teasing of bull

3.7.2 Schedule of collection:

Semen collection was done once in a week (Thursday or Friday). The collection was scheduled during three seasons of the year, namely summer (March-June), rainy (July-October) and winter (November-February). One ejaculate was collected from each bull during each collection session. Collection was always performed in the morning between 7 am to 9 am.

Fig.7. AV apparatus

Fig.8. Preparation of AV

3.7.3 Collection procedure:

The semen was collected by using artificial vagina. It consisted of (i) an outer heavy rubber cylinder, (ii) inner sleeves of rubber, (iii) the semen receiving cone, (iv) semen collecting vial made of glass or plastic which was graduated. Prior to collection all these parts were cleaned, sterilized and assembled into artificial vagina. The inner sleeve was put into the outer cylinder and both the ends of inner sleeve were reflected over the cylinder forming a water tight space between them and water at 40-50ºC was filled in there.

Fig.9. Collection of semen by AV

The cone along with the attached vial was then slipped over one of the ends of that water jacketed barrel and then tightly secured with the help of rubber bands. The vial and cone were then invaginated into the artificial vagina and 3" to 4" at the open end were lubricated with sterilized jelly. When the bull mounted, the penis was quickly guided into the artificial vagina with the operator. After the bull dismounted, the artificial vagina was taken off the penis. Then it was kept in an upright condition after releasing the pressure by draining water and air out of it. During the collection of semen mature bulls required an outer rubber casing of 40 cm in length and 6.4 cm in diameter, while yearling bulls required a casing of 30 to 35 cm long and 5.1 to 5.7 cm in diameter.

3.8 Transportation of semen samples to the laboratory:

The vial was then detached from the cone and immediately taken to the laboratory for examination. After taking to the laboratory the samples were placed in a 37°C water bath.

3.9 Evaluation of semen:

3.9.1 Physical examination of semen:

3.9.1.1 Color:

The color was recorded with necked eye as thick-watery, creamy-milky white. The density of the fresh ejaculate was recorded and scored in 5 scales according to Nath (1988) -

- Thick creamy - Excellent
- Thin creamy - Very good
- Thick milky - Good
- Thin milky - Fair
- Watery - Extremely poor.

Fig.10. Examination of color of semen

3.9.1.2 Volume:

The volume of semen (ml) was evaluated by photometric analysis (imv photometer). A sample of the semen (0.02 ml) was diluted with a physiological solution (buffered formal saline) at the ratio of 1:200 and placed in a macro-cuvette. The result of the analysis was visualized on the digital screen.

Fig.11. Evaluation of semen volume & concentration

Fig.12. Reading of imv Photometer

3.9.1.3 Concentration:

The concentration of sperm (million/ml) was also evaluated by using imv photometer immediately after taking the reading of volume of semen.

3.9.1.4 Semen pH:

pH meter (HI 2211 pH/ORP Meter) was used to measure the pH of the collected fresh semen. At first, the pH meter was calibrated at pH 7.00 and then at pH 4.00 before taking the reading. About 8-10 ml of semen sample was placed in a clean beaker. Then the pH probe was placed in the beaker and the measuring button was pressed. Reading was visualized within 1-2 minutes on the pH meter digital screen and was recorded. (Hanna HI 2211 pH meter manual)

Fig.13. Determination of pH of semen

3.9.2 Microscopic examination of semen:

Fig.14. Slide preparation for direct Microscopic examination

Fig.15. Direct microscopic examination

3.9.2.1 Mass activity:

A small drop of fresh undiluted semen was placed on a pre-warmed (37°C) grease free slide and observed under microscope at 10x magnification without cover slip. Score (0-4) of mass activity was recorded according to the criteria of Nath (1988) -

- 0 = No mass activity,
- +1 = Slow moving without forming waves,
- +2 = Slow waves,
- +3 = Quick waves,
- +4 = Waves, churning of whirls and eddies.

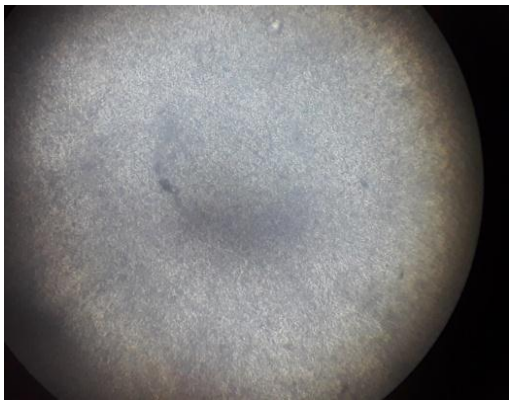


Fig.16. Mass activity evaluation

3.9.2.2 Motility:

The motility was determined by eye estimation by placing a small drop of semen on a pre-warmed (37°C) slide and covered with a cover slip. Then it was examined under a microscope with 40x magnification. The motility was expressed as percent of spermatozoa showing a progressive forward movement.

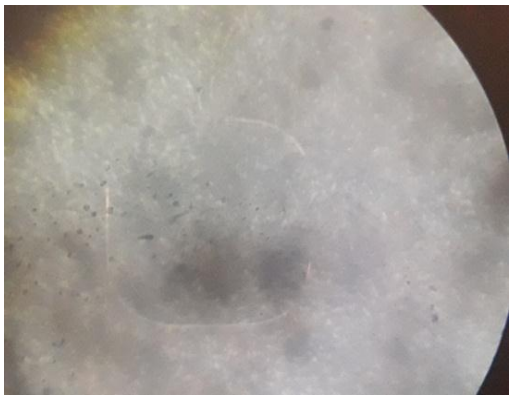


Fig.17. Motility evaluation

3.9.2.3 Morphology:

The morphology of spermatozoa was assessed after staining with Eosin-Nigrosin stain. At least 100 sperm cells were examined under microscope at 100x objective.

3.9.2.3.1 Staining procedure:

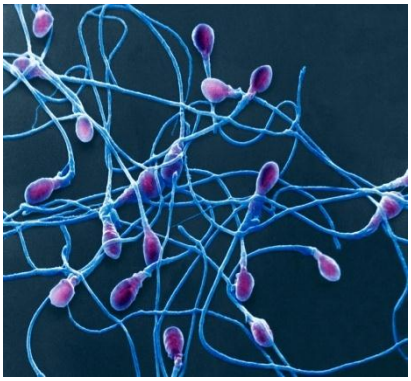
Staining of semen samples were done according to Bjorndahl *et al.* (2003) –

- Fresh semen sample was mixed with thorough swirling before staining and evaluation.
- One drop of fresh semen was placed on a slide and mixed with two drops of Eosin stain (0.5%, aqueous). Then waited for 30 seconds.
- After 30 seconds, three drops of Nigrosin stain (10%, aqueous) was added and gently swirled to mix.
- A blood film type smear was prepared with a focus on uniform distribution and then the slide was allowed to air-dry.
- Air-dried slide was coverslipped with compatible mounting medium and examined under oil immersion with 100x objective.

Fig.18. Eosin-Nigrosin staining

3.10 Statistical analysis:

- One Way Analysis of Variance (ANOVA) was used to evaluate semen quality and results were expressed as Mean ± Standard Deviation using SPSS program (version 17, SPSS).
- Difference between means was considered statistically significant with at least P value<0.05.



Chapter IV
Results

CHAPTER IV

RESULTS

The data on quality of semen was collected from 25 bulls of two breeds namely Friesian (n=10) and Sahiwal (n=15). In order to investigate the quality of semen from different bulls 777 ejaculates were collected and randomly evaluated throughout the total duration of the study. The effects of breed, age, scrotal circumference, BCS, season and nutrition on semen quality of breeding bulls were studied.

4.1 Effects of breed on the quality of bull semen:

Effect of breed on the quality of semen in breeding bulls is presented in table 2. The mean value of volume was 11.07±3.55 ml in Friesian bulls and 13.05±4.07 ml in Sahiwal bulls. The mean value of volume of semen was higher in Sahiwal bulls than in Friesian bulls. The mean values of volume of ejaculate differed between the two breeds which was statistically significant ($P<0.05$). The mean value of sperm concentration was 558.37±23.45 million/ml in Friesian bulls and 587.63±33.06 million/ml in Sahiwal bulls. Higher mean value of sperm concentration was observed in Sahiwal bulls as compared to Friesian bulls. However, the values did not differ significantly ($P>0.05$) between the two breeds.

The mean value of mass activity of sperm of Sahiwal bulls (3.39±0.14) differed from that of Friesian bulls (2.77±0.17) which was statistically significant ($P<0.05$). The value was higher in Sahiwal bulls as compared to Friesian bulls. As regards to the motility of sperm, the mean values differed between the two breeds which was statistically significant ($P<0.05$). Higher (77.43±2.60%) value of motility was found in Sahiwal bulls than in Friesian bulls (73.08±2.25%).

The average percentage of normal sperm was 82.41±1.84% in Friesian bulls and 85.07±2.15% in Sahiwal bulls. Higher mean value was found in Sahiwal bulls than in Friesian bulls. The variation in values between the two breeds was statistically significant ($P<0.05$). The average percentage of abnormal sperm was 17.59±1.84% in Friesian bulls and 14.93±2.15% in Sahiwal bulls. The percentage of abnormal sperm was lower in Sahiwal bulls as compared to Friesian bulls. The difference in values between the two breeds was statistically significant ($P<0.05$).

Table 2: Characteristics of fresh semen in different breeds of bull (Mean ± SD)

Breed	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
Friesian	11.07±3.55	558.37±23.45	73.08±2.25	2.77±0.17	82.41±1.84	17.59±1.84
Sahiwal	13.05±4.07	587.63±33.06	77.43±2.60	3.39±0.14	85.07±2.15	14.93±2.15
Sig. level	*	NS	*	*	*	*

(Abnormal sperms: Narrow head, pear shaped head, head narrow at the base, abnormal acrosome, head detached, simple bent, abaxial, double folded, broken at the neck, coiled mid piece, tail coiled around the head, tail coiled toward mid piece, tail coiled in lower part, tailless mid piece, free loose, swollen tail etc.)

* = Significantly differ (P<0.05); NS = Non Significant

4.2 Effects of age on the quality of bull semen:

Effect of age on the quality of semen in breeding bulls is presented in table 3. The bulls were grouped into three sub-groups according to age, namely G I (2.5-3.5 years), G II (>3.5-4.5 years) and G III (>4.5 years-above).

The mean values of volume were 10.61±3.60 ml, 13.33±3.48 ml and 10.55±3.90 ml in G I, G II and G III, respectively. The highest mean volume per ejaculate was found in G II and the lowest volume was found in G III. The mean values of volume of ejaculate differed among the three age groups which was statistically significant (P<0.05). The average values of sperm concentration were 585.45±29.47 million/ml, 592.22±32.45 million/ml and 549.32±27.51 million/ml in G I, G II and G III, respectively. There was no significant (P>0.05) difference in average sperm concentration of bulls in different age groups, although it was the highest for ejaculates collected from bulls in G II and the lowest in G III.

The average values of mass activity of sperm differed among the three age groups which was statistically significant (P<0.05). The mean values were 3.15±0.34, 3.26±0.29 and 3.07±0.35 in G I, G II and G III, respectively. The highest value was recorded in bulls of G II and the lowest in bulls of G III. Significant effects (P<0.05) of age on sperm motility percentage were observed during this study. The average values were 75.87±3.12%, 76.51±3.12% and 75.17±3.32% for G I, G II and G III, respectively. The average values differed among the three age groups which was statistically significant (P<0.05). The highest value was observed in G II and the lowest value was observed in G III.

Higher average percentage of normal sperm was recorded in G II (84.42±2.40%) as compared to G I (84.14±2.33%) and G III (83.71±2.44%). The lowest value was observed in G III. The variations in values among the three age groups were statistically significant (P<0.05). The average percentage of abnormal sperm for G I, G II and G III were 15.86±2.33%, 15.58±2.40% and 16.29±2.44%, respectively. The lowest value was found in G II and the highest value was found in G III. The differences in values among the three age groups were statistically significant (P<0.05).

Table 3: age wise variation of fresh semen characteristics in bull (Mean ± SD)

Age group	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
G I (2.5-3.5 Years)	10.61±3.60[b]	585.45±29.47[a]	75.87±3.12[b]	3.15±0.34[a]	84.14±2.33[a]	15.86±2.33[a]
G II (>3.5-4.5 years)	13.33±3.48[a]	592.22±32.45[a]	76.51±3.12[a]	3.26±0.29[a]	84.42±2.40[a]	15.58±2.40[a]
G III (>4.5 years -above)	10.55±3.90[b]	549.32±27.51[b]	75.17±3.32[b]	3.07±0.35[b]	83.71±2.44[b]	16.29±2.44[b]
Sig. level	*	NS	*	*	*	*

[a,b] Superscript letters in same column differ significantly (P<0.05), NS = Non Significant

4.3 Effects of scrotal circumference on the quality of bull semen:

Effect of scrotal circumference on the quality of semen in breeding bulls is presented in table 4. The bulls were grouped into four sub-groups based on scrotal circumference namely G I (27-29 cm), G II (29.1-31 cm), G III (31.1-33 cm), G IV (33.1 cm-above).

The mean values of volume were 11.10±4.18 ml, 11.65±3.60 ml, 12.89±3.63 ml and 9.26±3.14 ml in G I, G II, G III and G IV, respectively. The highest mean volume per ejaculate was found in G III and the lowest volume was found in G IV. The mean values of volume of ejaculate differed among the three age groups which was statistically significant (P<0.05). The average values of sperm concentration were 571.35±27.62 million/ml, 640.00±33.37 million/ml, 696.26±35.24 million/ml and 469.90±25.44 million/ml in G I, G II, G III and G IV, respectively. The average value of sperm concentration was highest in G III and lowest in G IV. The differences in values among the three age group were statistically significant (P<0.05).

The average values of mass activity of sperm differed among the four groups which was statistically significant (P<0.05). The mean values were 3.22±0.33, 3.36±0.14, 3.37±0.14 and 3.01±0.34 in G I, G II, G III and G IV, respectively. The highest value was recorded in bulls of G III and the lowest in bulls of G IV. The average values of sperm motility percentage were 76.64±3.22%, 77.01±2.61%, 77.07±2.82% and 74.81±3.23% for G I, G II, G III and G IV, respectively. The average values differed among the four groups which was statistically significant (P<0.05). The highest value was observed in G III and the lowest value was observed in G IV.

Higher average percentage of normal sperm was recorded in G III (84.65±2.34%) as compared to G I (84.72±2.31%), G II (84.74±2.17%) and G IV (83.50±2.38%). The lowest value was observed in G IV. The variations in values among the four groups were statistically significant (P<0.05). The average percentage of abnormal sperm for G I, G II, G III and G IV were 15.35±2.34%, 15.28±2.31%, 15.26±2.17% and 16.50±2.38%, respectively. The lowest value was found in G III and the highest value was found in G IV. The differences in values among the four groups were statistically significant (P<0.05).

Table 4: Characteristics of fresh semen based on scrotal circumference of bulls (Mean ± SD)

Bull Group	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
G I (27-29 cm)	11.10±4.18 [b]	571.35±27.62 [c]	76.64±3.22 [b]	3.22±0.33 [a]	84.65±2.34 [a]	15.35±2.34 [a]
G II (29.1-31 cm)	11.65±3.60 [b]	640.00±33.37 [b]	77.01±2.61 [a]	3.36±0.14 [a]	84.72±2.31 [a]	15.28±2.31 [a]
G III (31.1-33 cm)	12.89±3.63 [a]	696.26±35.24 [a]	77.07±2.82 [a]	3.37±0.14 [a]	84.74±2.17 [a]	15.26±2.17 [a]
G IV (31.1 cm- above)	9.26±3.14 [c]	469.90±25.44 [d]	74.81±3.23 [c]	3.01±0.34 [b]	83.50±2.38 [b]	16.50±2.38 [b]
Sig. level	*	*	*	*	*	*

[a,b,c,d] Superscript letters in same column differ significantly (P<0.05), NS = Non Significant)

4.4 Effects of BCS on the quality of bull semen:

Effect of BCS on the quality of semen in breeding bulls is presented in table 5. The bulls were grouped into two sub groups according to BCS namely G I (4-4.5) and G II (>4.5-5). The mean value of volume was 13.33±3.48 ml in G I and 10.58±3.77 ml in G II. The mean value of volume of semen was higher in G I than in G II. The mean values of volume of ejaculate differed between the two groups which was statistically significant (P<0.05). The mean value of sperm concentration was 585.45±29.47 million/ml in G I and 567.56±29.75 million/ml in G II. Higher mean value of sperm concentration was observed in G I as compared to G II. However, the values did not differ significantly (P>0.05) between the two groups.

The mean value of mass activity of sperm of G I (3.20±0.32) differed from that of G II (3.07±0.35) which was statistically significant (P<0.05). The value was higher in G I as compared to G II. As regards to the motility of sperm, the mean values differed between the two groups which was statistically significant (P<0.05). Higher (76.14±3.13%) value of motility was found in G I than in G II (75.17±3.32%).

The average percentage of normal sperm was 84.26±2.36% in G I and 83.71±2.44% in G II. Higher mean value was found in G I than in G II. The variation in values between the two groups was statistically significant (P<0.05). The average percentage of abnormal sperm was 15.74±2.36% in G I and 16.29±2.44% in G II. The percentage of abnormal sperm was lower in G I as compared to G II. The difference in values between the two groups was statistically significant (P<0.05).

Table 5: Characteristics of fresh semen in different bull groups based on BCS (Mean ± SD)

Bull Group	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
G I (4-4.5)	13.33±3.48	585.45±29.47	76.14±3.13	3.20±0.32	84.26±2.36	15.74±2.36
G II (>4.5-5)	10.58±3.77	567.56±29.75	75.17±3.32	3.07±0.35	83.71±2.44	16.29±2.44
Sig. level	*	NS	*	*	*	*

* = Significantly differ (P<0.05); NS = Non Significant

4.5 Effects of season on the quality of bull semen:

Effect of season on the quality of semen in breeding bulls is presented in table 6. During the study, the collection of semen from the bulls was scheduled for three seasons of the year namely summer (March-June), rainy (July-October) and winter (November-February).

The mean values of volume were 11.48±4.09 ml, 11.95±3.91 ml and 12.23±3.59 ml in summer, rainy season and winter, respectively. The highest mean volume per ejaculate was found in winter and the lowest volume was found in summer. However, the values did not differ significantly ($P>0.05$) among three seasons of the year. The average values of sperm concentration were 538.60±30.80 million/ml, 582.02±27.71 million/ml and 613.36±29.69 million/ml in summer, rainy season and winter, respectively. The mean value was highest in winter and lowest in summer. The mean values of sperm concentration differed among the three seasons which was statistically significant ($P<0.05$).

The average values of mass activity of sperm differed among the three seasons which was statistically significant ($P<0.05$). The mean values were 3.00±0.33, 3.12±0.32 and 3.31±0.31 in summer, rainy season and winter, respectively. The highest value was recorded in winter and the lowest in summer. Significant seasonal effects on sperm motility percentage were observed during this study. It averaged 72.98±2.20%, 75.90±2.11% and 78.45±2.63%, respectively for summer, rainy season and winter. The average values differed among the three seasons which was statistically significant ($P<0.05$). The highest value was observed in winter and the lowest value was observed in summer.

Higher average percentage of normal sperm was recorded during winter (86.18±1.66%) as compared to rainy season (84.28±1.67%) and summer (81.77±1.35%). The lowest value was observed during summer. The variations in values among the three seasons were statistically significant ($P<0.05$). The average percentage of abnormal sperm for summer, rainy season and winter were 18.23±1.35%, 15.72±1.67% and 13.82±1.66%, respectively. The lowest value was found in winter and the highest value was found during summer. The differences in values among the three seasons were statistically significant ($P<0.05$).

Table 6: Characteristics of fresh semen in different seasons of the year (Mean ± SD)

Season	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
Summer	11.48±4.09[b]	538.60±30.80[c]	72.98±2.20[c]	3.00±0.33[b]	81.77±1.35[c]	18.23±1.35[c]
Rainy	11.95±3.91[b]	582.02±27.71[b]	75.90±2.11[b]	3.12±0.32[b]	84.28±1.67[b]	15.72±1.67[b]
Winter	12.23±3.59[a]	613.36±29.69[a]	78.45±2.63[a]	3.31±0.31[a]	86.18±1.66[a]	13.82±1.66[a]
Sig. level	NS	*	*	*	*	*

[a,b,c] Superscript letters in same column differ significantly (P<0.05), NS = Non Significant)

4.6 Effects of nutrition on the quality of bull semen:

Effect of nutrition on the quality of semen in breeding bulls is presented in table 7. During the study period, two types of feed supplementation were supplied to the two different groups of the bulls, namely type I (Normal feed+Vit. ADE supplementation) and type II (Normal feed+extra protein supplementation).

The mean value of volume was 12.23±3.75 ml in bulls fed with type I feed supplementation and 11.50±3.99 ml in bulls fed with type II feed supplementation. The mean value of volume of semen was higher with type I supplementation than with type II. The mean values of volume of ejaculate differed with the two types of feed supplementation which was statistically significant (P<0.05). The mean value of sperm concentration was 592.44±27.95 million/ml in bulls fed with type I feed supplementation and 569.69±31.11 million/ml in bulls fed with type II feed supplementation. Higher mean value of sperm concentration was observed with type I supplementation as compared with type II. However, the values did not differ significantly (P>0.05) with the two types of feed supplementation.

The mean values of mass activity of sperm differed with the two types of feed supplementation which was statistically significant (P<0.05). The value was higher (3.18±0.33) in bulls fed with type I feed supplementation and lower (3.10±0.34) in bulls fed with type II feed supplementation. As regards to the motility of sperm, the mean values differed with the two types of feed supplementation which was statistically significant (P<0.05). Higher (76.79±2.56%) value of motility was found with type I supplementation than with type II (74.60±3.50%).

The average percentage of normal sperm was 84.95±1.84% in bulls fed with type I feed supplementation and 83.07±2.54% in bulls fed with type II feed supplementation. Higher mean value was found with type I supplementation than with type II. The variation in values due to two types of feed supplementation was statistically significant (P<0.05). The average percentage of abnormal sperm was 15.05±1.84% in bulls fed with type I feed supplementation and 16.93±2.54% in bulls fed with type II feed supplementation. The percentage of abnormal sperm was lower with type I supplementation than with type II. The difference in values due to two types of feed supplementation was statistically significant (P<0.05).

Table 7: Characteristics of fresh semen in different types of feed supplementation (Mean ± SD)

Feed Supplementation	Volume (ml)	Concentration (million/ml)	Motility (%)	Mass activity (0-4)	Morphology (%)	
					Normal	Abnormal
Type I	12.23±3.75	592.44±27.95	76.79±2.56	3.18±0.33	84.95±1.84	15.05±1.84
Type II	11.50±3.99	569.69±31.11	74.60±3.50	3.10±0.34	83.07±2.54	16.93±2.54
Sig. level	*	NS	*	*	*	*

* = Significantly differ (P<0.05); NS = Non Significant

4.7 Color of bull semen:

During the study, the color was recorded with necked eye as thick-watery, creamy-milky white. Color of semen did not vary between the two breeds. However, adult bulls (>3.5-4.5 years) were more likely to have creamy color semen relative to a watery color compared to younger bulls (2.5-3.5 years). The variation in color may be due to the higher sperm concentration in adult bulls (Perry *et al.,* 1947). Color of the semen did not alter due to seasonal variation.

4.8 pH of bull semen:

In present study, pH of fresh semen was found within the range of 6.4-6.7. It differed between the two breeds ranging from 6.4-6.5 in Friesian bulls and 6.5-6.7 in Sahiwal bulls. During the study, pH did not alter with age of bulls and seasons of the year.

4.9 Abnormalities of spermatozoa:

The following abnormal sperms were evaluated during staining-

- **Head abnormalities:** Pear shaped, enlarged at base, joined head, narrow, narrow at base, abnormal acrosome, head detached, undeveloped, abaxial etc.
- **Mid piece abnormalities:** Coiled, double folded, broken at the neck, broken at the middle, broken at the junction with tail, undeveloped etc.
- **Tail abnormalities:** Simple bent, coiled toward mid piece, coiled in lower part, coiled around head, tailless (end piece), knot tail, swollen tail etc.

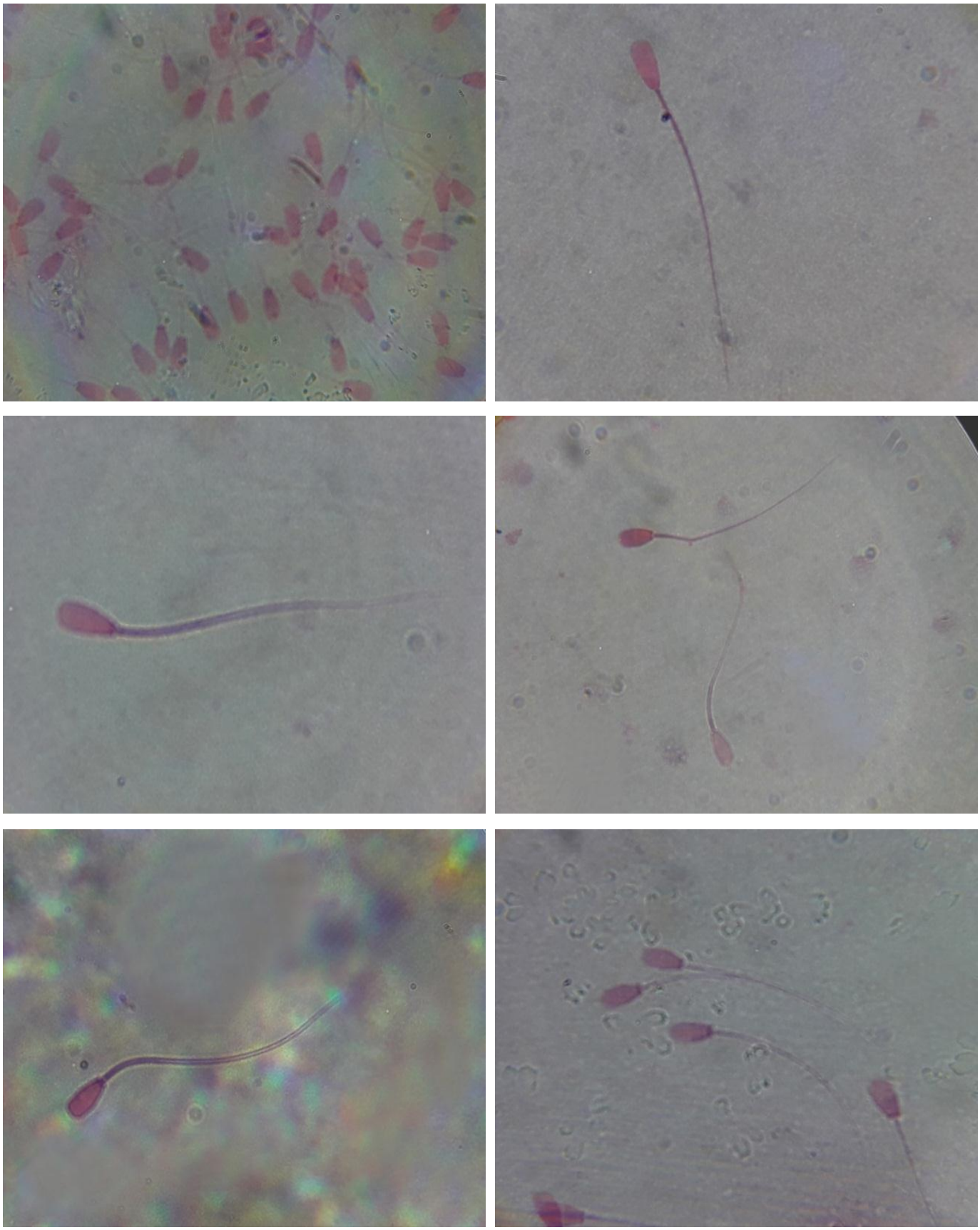


Fig.19. Normal spermatozoa

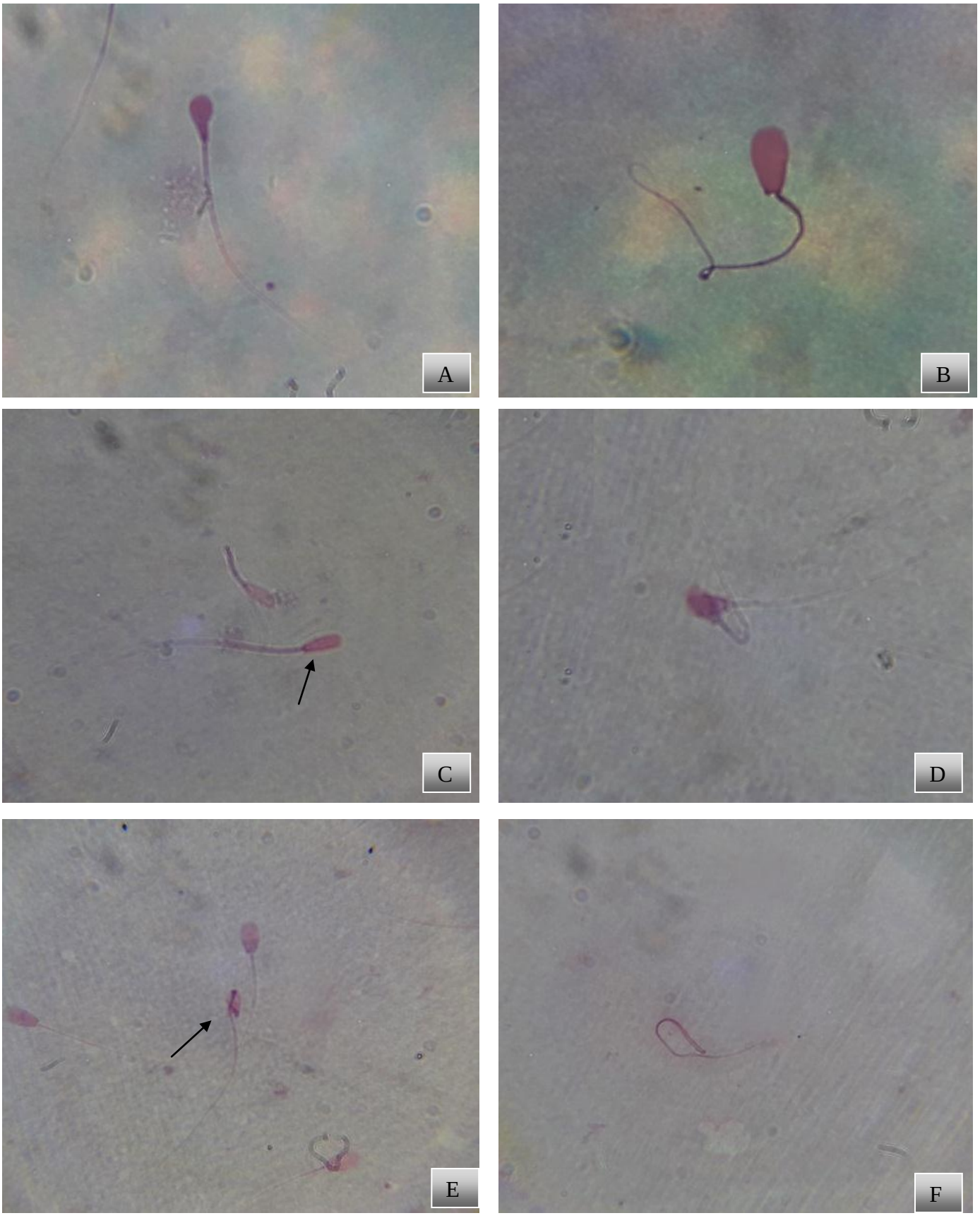


Fig.20. Head abnormalities of spermatozoa. A. Pear shaped B. Abaxial C. Narrow D. Joined head E. Abnormal acrosome F. Head detached

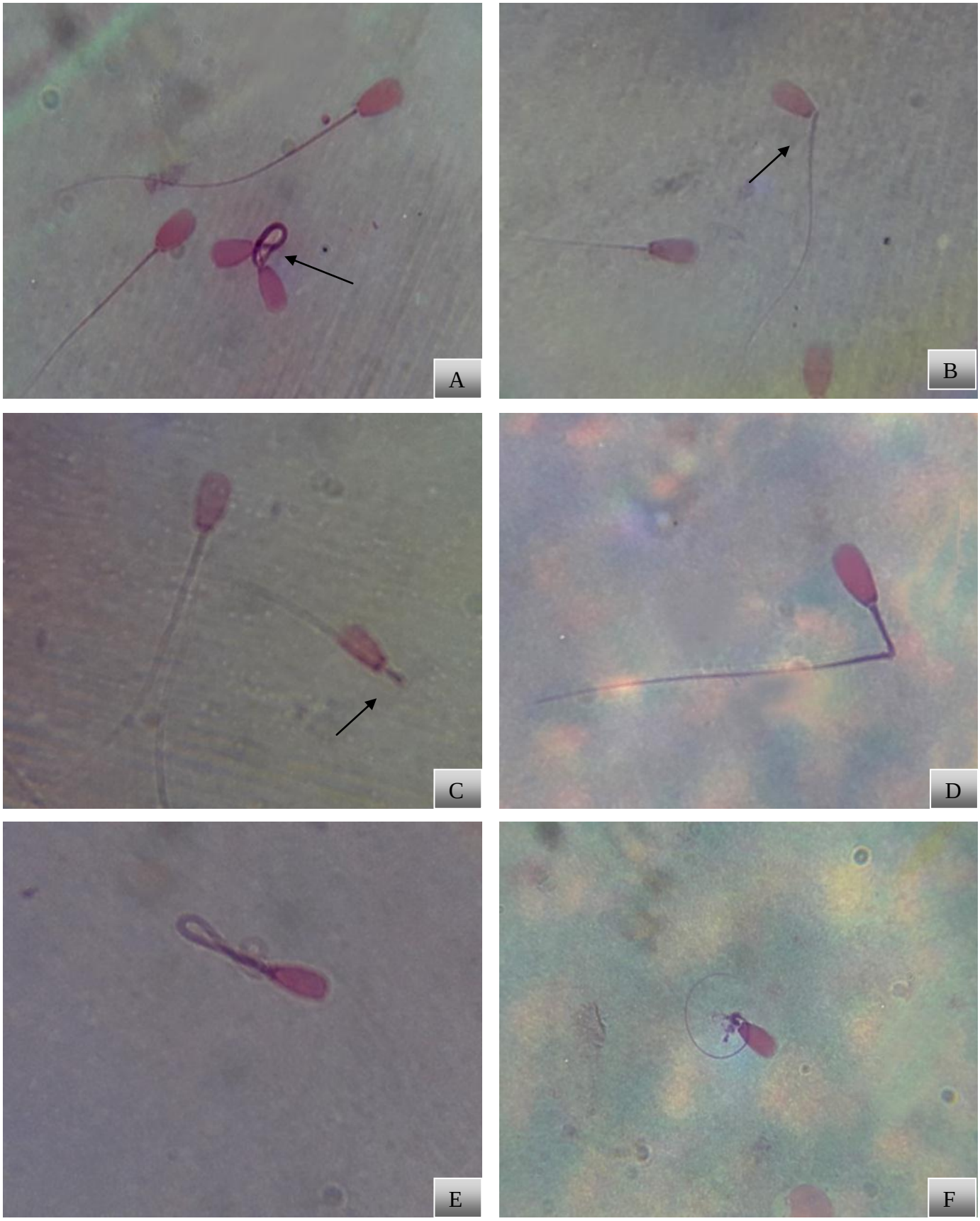


Fig.21. Mid piece abnormalities of spermatozoa. A. Coiled B. Broken at the neck C. Broken at the middle D. Broken at the junction with tail E. Double folded F. Undeveloped

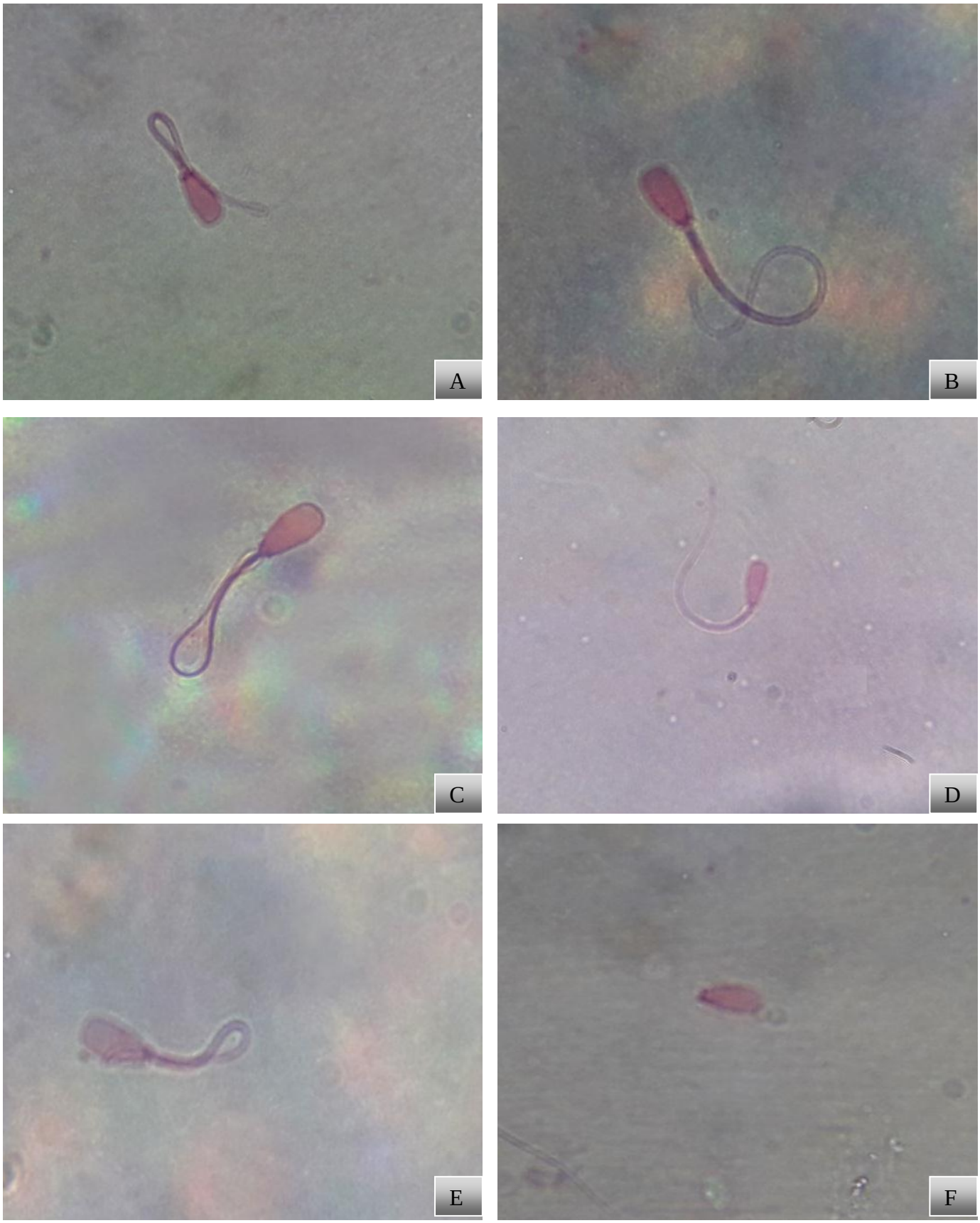


Fig.22. Tail abnormalities of spermatozoa. A. Simple bent B. Coiled at lower part C. Coiled toward mid piece D. Coiled around head E. Swollen tail F. Free loose

Chapter V
Discussion

CHAPTER V
DISCUSSION

Accurate evaluation of freshly drawn bull semen before use is important in artificial insemination. Detection of the quality of semen before use ensures in achieving optimum reproductive efficiency. Therefore all the parameters regarding to quality of semen particularly volume of ejaculate, concentration, pH, motility, mass activity and morphology of sperm have been investigated in this study.

5.1 Characteristics of semen:

5.1.1 Volume:

The study showed that breed affected the volume of semen significantly ($P<0.05$). The mean value of volume of semen was higher in Sahiwal bulls (13.05±4.07 ml) than in Friesian bulls (11.07±3.55 ml). Ahmed *et al.* (1992), Raja and Rao (1982) and Mukherjee and Banerjee (1980), reported a significant ($P<0.01$) breed difference in volume of semen. The results obtained in this study similar to that of Dasinaa and Pagthinathan (2015) who found Higher mean volume (6.0±1.78 ml) in Sahiwal bulls than in (4.1±0.87 ml) Friesian bulls ($P<0.01$). On the other hand, the result of this study differs from that reported by Sane *et al.* (1994), in that significantly ($P<0.05$) highest (7.6 ml) volume of semen was found in Holstein-Friesian×Zebu crosses and lowest (4.0 ml) in Sahiwal×Zebu. Boujenane and Boussaq (2014), Hossain *et al.* (2012) and Gebre Medhin *et al.* (2007) reported no significant differences among the breeds studied but is in contrast with the studies by Lemma and Shemsu (2015), Kunowska-Slosarz *et al.* (2015) and Alemu *et al.* (2014), who found significant volume differences among the bull breeds studied. Atiqur *et al.* (2014) found the highest (7.19±0.19 ml) volume of semen ($P<0.01$) in Holstein-Friesian× Zebu bulls and lowest (4.41±0.21 ml) in Red Chittagong bulls. Hirwa *et al.* (2017) reported that Friesian bulls had superior ($P<0.05$) semen volume (5.76±0.08 ml) to that of Jersey (4.29±0.09 ml) and Inyambo (3.37±0.1 ml). Based on the study, being a tropical breed, Sahiwal bulls are more suitable for the environmental condition of Bangladesh.

During the study, the highest mean volume per ejaculate was found in adult age group (>3.5-4.5 years) and the lowest volume was found in older age group (>4.5 years-above). The mean values of volume of ejaculate differed significantly ($P<0.05$) among different age groups. The study of Ahmad *et al.* (2003) and Lepard *et al.* (1941) closely support the

findings of present study. Ahmad *et al.* (2003) observed that the ejaculatory volume produced by adult age group (3-5 years), were significantly (P<0.05) higher than that of younger (upto 3 years) and older age groups (above 5 years). Lepard *et al.* (1941) found out that mature bulls produced a greater (P<0.05) volume than young bulls. But the present finding is inconsistent with Ruttle *et al.* (1975) and Herman and Swanson (1941). They found no significant age effect on the different age groups of the bulls maintained under range conditions in the tropics. Semen volume has been previously reported to increase with increasing age by Brito *et al.* (2004).

The study showed significant (P<0.05) association of semen volume with the body conditions of the breeding bulls. The mean value of volume of semen was higher (13.33±3.48 ml) in bull group with BCS of 4 to 4.5 than (10.58±3.77 ml) in bull group with BCS of >4.5 to 5. Sitali *et al.* (2017) found that bulls of BCS 3.5 had the highest semen volume while those of BCS 2.0 had the lowest (P<0.05).

The study showed no significant (P>0.05) seasonal effect on the volume of semen. However, the highest mean volume per ejaculate was found in winter (12.23±3.59 ml) and the lowest volume was found in summer (11.48±4.09 ml). Ahmad *et al.* (2003) recorded high ejaculatory volume during spring and low during summer (P<0.05). Djimde and Weniger (1986) reported that, there was a seasonality in semen volume and largest (P<0.05) amount of semen was collected in the month of March-April. Fiaz *et al.* (2010) reported that Friesian bulls produced lower (P<0.05) ejaculatory volume during dry summer season (April-June).

5.1.2 Concentration:

The study showed no significant (P>0.05) effect of breed on the concentration of semen. However, higher mean value of sperm concentration (587.63±33.06 million/ml) was observed in Sahiwal bulls as compared to Friesian bulls (558.37±23.45 million/ml). Kim *et al.* (1983) closely support the findings of the present study. On the other hand, the result of this study differs from those reported by Dasinaa and Pagthinathan (2015) and Everett and Bean (1982). Dasinaa and Pagthinathan (2015) reported that Friesian stud showed the higher concentration (1785.3 million/ml) compared to Sahiwal (1411.5 million/ml) stud bulls (P<0.01). Atiqur *et al.* (2014) found the highest (1257.34±30.46 million/mm^3) value of concentration of semen (P<0.01) in Holstein-Friesian×Zebu breeds of bulls and the lowest (1115.20±30.43 million/mm^3) in Red Chittagong breed of bulls.

The study showed no significant (P>0.05) age effect on the sperm concentration. However, the highest average sperm concentration (592.22±32.45 million/ml) was found in adult age group (>3.5-4.5 years) and the lowest value (549.32±27.51 million/ml) was found in older age group (>4.5 years-above). Ahmad *et al.* (2003) closely support the findings of present study. They found no significant difference in sperm concentration of bulls in different age groups, the finding was highest (1.00±0.01) in adult Sahiwal bulls (3-5 years) and lowest (0.96±0.01) in the young bulls (≤3 years) of the same breed. On the other hand, the results of this study differ from that reported by (Kim *et al.,* 1983). They found that buffalo bull sperm concentration increased significantly (P<0.01) with the age of the animals.

The study showed significant (P<0.05) seasonal effect on sperm concentration. The average values of sperm concentration were 538.60±30.80 million/ml, 582.02±27.71 million/ml and 613.36±29.69 million/ml in summer, rainy season and winter respectively. The mean value was highest in winter and lowest in summer. The study of Salah *et al.* (1992) and Everett and Bean (1982) closely support the findings of present study. Salah *et al.* (1992) reported that ejaculates collected from Friesian bulls during hot summer season had significantly (P<0.01) lower sperm concentration than those collected during winter time. Everett and Bean (1982) reported higher (P<0.05) sperm concentration in December. But the present finding is inconsistent with Lasley and Bogart (1943) and Erb, *et al.* (1942), in that the average concentration of dairy bull semen and total sperm per ejaculate was maximum during April, May, and June. Ahmad *et al.* (2003), Kim *et al.* (1983) and Swanson and Herman (1944) did not find any statistically significant seasonal variation on sperm concentration.

5.1.3 Mass activity:

The study showed significant (P<0.05) breed effect on the mass activity of sperm. Higher value of mass activity was found in Sahiwal bulls (3.39±0.14) than in Friesian bulls (2.77±0.17). Atiqur *et al.* (2014) observed highest (P<0.01) mass activity in Holstein cross and lowest in Red Chittagong breed of bulls. Pal *et al.* (2006) found higher (P<0.05) semen mass activity (2.10±0.06) in Murrah bulls as compared to crossbred bulls (1.99±0.07). In this study there was a significant (P<0.05) effect of age on the sperm mass activity. The highest value (3.26±0.29) was observed in adult bulls (>3.5-4.5 years) and the lowest value (3.07±0.35) was observed in older bulls (>4.5 years-above). Ahmad *et al.* (2003) found highest mass activity value (2.91±0.04) in adult bulls (3-5 years) and lowest

(2.35±0.01) in older bulls (above 5 years) but the effect was non-significant. The study showed significant (P<0.05) seasonal effect on the mass activity of sperm. The highest value (3.31±0.31) was recorded in winter and the lowest (3.00±0.33) in summer. The result agrees with that reported by Ahmad *et al.* (2003) where high mass activity (3.38±0.03) was recorded during spring and low (2.05±0.02) during summer but the effect was non-significant. The study showed that nutrition affected the mass activity of sperm significantly (P<0.05). Higher (3.18±0.33) value was found with that type enriched with Vit. ADE supplementation than (3.10±0.34) with that type enriched with extra protein supplementation (P<0.05). The results obtained in this study similar to that of Susan (2010) who found that the mass activity produced by bulls supplemented with 0.3 ppm organic Se and tocopherol significantly (P<0.05) higher than that of bulls supplemented with basal diet .

5.1.4 Motility:

The study showed that breed affected the motility of sperm significantly (P<0.05). Higher (77.43±2.60%) value of motility was found in Sahiwal bulls than in Friesian bulls (73.08±2.25%). Atiqur *et al.* (2014) found the highest (69.40±0.67%) value of motility of semen (P<0.01) in Sahiwal×Zebu breeds of bulls and lowest (62.12±0.97%) in Red Chittagong breed of bulls. Hoque *et al.* (1997), Sane *et al.* (1994) and Saxena and Tripathi (1981) reported that the percentage of motile sperm was 66.2%-76.0% in the Holstein-Friesian×Zebu bulls and 56.6%-60.1% in Jersey×Zebu bulls. Dasinaa and Pagthinathan (2015) reported higher mass motility (83.2±2.5%) in Friesian bulls than in Sahiwal (80.5±1.46%) bulls (P<0.05) which differs with this study.

Significant (P<0.05) effects of age on sperm motility percentage were observed during this study. The highest value (76.51±3.12%) was observed in adult bulls (>3.5-4.5 years) and the lowest value (75.17±3.32%) was observed in older bulls (>4.5 years-above). The result is also in line with those reported by Ulfina Galmessa *et al.* (2014) and Ahmad *et al.* (2003). Ulfina Galmessa *et al.* (2014) found significantly (P<0.05) higher mean motility (76.40±3.07%) for the younger (<50 months) than (65.00±3.50%) the older bull (>50 months). Ahmad *et al.* (2003) observed that the motility percentage produced by adult bull (upto 3 years) were significantly (P<0.05) higher than that of younger (3-5 years) and older bulls (above 5 years). But the present finding is inconsistent with David *et al.* (2007). They reported that motility, concentration and number of spermatozoa were higher

(P<0.05) up to the age between 2-3 years then declined chronologically. In this study there was a significant (P<0.05) effect of scrotal circumference on the sperm motility percentage. The highest value (77.07±2.82%) was observed in bulls with scrotal circumference of 31.1-33 cm and the lowest (74.81±3.23%) value was observed in bulls with scrotal circumference of 33.1-above. The results obtained in this study similar to that of Ulfina Galmessa *et al.* (2014) who observed higher mean motility for bulls having scrotal circumference <33cm than the bulls having scrotal circumference >33cm (P<0.05).

The study showed significant (P<0.05) seasonal effect on the sperm motility percentage. The highest value (78.45±2.63%) was observed in winter and the lowest value (72.98±2.20%) was observed in summer. The result agrees with those reported by Mostari *et al.* (2005), Li-junjie *et al.* (2001), Fawzy and Rabie (1996), Usmani *et al.* (1993) and Salah *et al.* (1992). Salah *et al.* (1992) reported that ejaculates collected from Friesian bulls during hot summer season had significantly (P<0.01) lower sperm motility than those collected during winter time. Li-junjie *et al.* (2001) and Usmani *et al.* (1993) reported that motility was reduced significantly in hot and humid season. Similarly, Mostari *et al.* (2005) and Fawzy and Rabie (1996) reported that sperm motility was deteriorated during summer in Friesian bulls. Ahmad *et al.* (2003) observed high motility percentage during spring and low during summer (P<0.05). Fiaz *et al.* (2010) reported that the mass motility of semen in Friesian breed was significantly (P<0.05) lower during wet summer (July-September). On the other hand, the result of this study differs from that reported by Kolev and Dimov (1998) where they found significant (P<0.05) increase in mass motility and individual motility during summer for buffalo bulls in Bulgaria. However, Brito *et al.* (2002) and Fonseca (1995) could not find any significant effect of season on mass motility and individual motility during summer. These differences might be due to different breeds and geography.

The study showed that nutrition affected the motility of sperm significantly (P<0.05). Higher (76.79±2.56%) value of motility was found with that type enriched with Vit. ADE supplementation than (74.60±3.50%) with that type enriched with extra protein supplementation (P<0.05). The results obtained in this study similar to that of Susan (2010) who found that the motility percentage produced by bulls supplemented with 0.3 ppm organic Se and tocopherol significantly (P<0.05) higher than that of bulls supplemented with basal diet.

5.1.5 Morphology:

The results of the study indicated that breed had significant (P<0.05) effect on the percentage of normal sperm which is similar to that reported by Coe (1999). Higher mean value (85.07±2.15%) was found in Sahiwal bulls than in (82.41±1.84%) Friesian bulls. The result obtained in this study differs from those in a study on the effect of breed on semen quality of Hereford and Simmental bulls by Buhr *et al.* (1993), where no significant effect of breed was recorded in semen characteristics. Vilakazi (2003) found higher percentage of normal sperm and lower incidence of abnormal sperm that was observed in Friesland bulls compared to Jersey bulls (P<0.05). Roberts *et al.* (1986) observed that, the average live sperm percentage for Holstein bull was 83.5% and with a range from 70%-90%. The difference between breeds/crosses in live sperm percentage showed that the performance of Red Chittagong bull was consistently lower than any other bull. Sitali *et al.* (2017) reported that the *Sussex* breed had the highest head abnormalities (2.05±1.05%) (P<0.05) while the *Santagetrud* breed had the lowest head abnormalities of (0.50±0.18%) (P<0.05) compared with other breeds.

The study showed that age had significant (P<0.05) effect on the percentage of normal sperm. Even though percentages of normal sperm for all age groups were above 70% which is acceptable according to Coe (1999), it also differed significantly (P<0.05) with age. The study indicated that the percentage of normal sperm was higher (84.42±2.40%) in bulls aged >3.5-4.5 years and lower (84.14±2.33%) in bulls younger (2.5-3.5 years) and (83.71±2.44%) older than 4.5 years of age (P<0.05). These results agree with those reported by Dowsett and Knott (1996), Soderquist *et al.* (1996) and Rao (1971), where a variation in total abnormalities and the increased frequency of abnormal sperm cells were observed with advancing age. The results obtained in this study also similar to that of Vilakazi (2003) who reported that higher percentage of normal sperm (P<0.05) and less incidence of sperm defects were observed in bulls aged 36-48 months of age, compared to the bulls younger than 36 months and older than 72 months of age. Younger bulls (less than 36 months) had better semen morphology than their older counterparts (older than 72 months). Overall results on the effects of age on semen morphological traits showed that sperm morphology is better in bulls aged >3.5-4.5 years than bulls aged >4.5 years-above and bulls younger than 3.5 years. David *et al.* (2007) and Collins *et al.* (1962) stated that percentages of live and normal spermatozoa decline as the ram gets older than 3 years which differed with the study.

The study showed significant (P<0.05) association of sperm morphology with the body conditions of the breeding bulls. The average percentage of normal sperm was higher 84.26±2.36% in bulls with BCS of 4-4.5 than 83.71±2.44% in bulls with BCS of >4.5-5. The result agrees with that reported by Sarder (2008) where the highest head abnormalities were recorded in bulls with excellent body condition, followed by average, very good and good body conditions (P<0.05) . Sitali *et al.* (2017) found that the breeding bulls with a BCS of 2.0 had the highest percentage of major defects (10.17±1.43%) while those of BCS 3.5 had the lowest percentage of major defects (4.27±0.78%), (P<0.05). The findings of this study are in contrast with those of Chacon *et al.* (1999), who found no correlation between BCS and sperm morphology.

The results of the study indicated that scrotal circumference had significant (P<0.05) effect on the percentage of normal sperm. Higher average percentage of normal sperm (84.65±2.34%) was recorded in bulls with scrotal circumference of 31.1-33 cm as compared to bulls with 27-29 cm (84.72±2.31%), 29.1-31 cm (84.74±2.17%) and 33.1-above (83.50±2.38%). Sarder (2008) reported that the highest percentage of total sperm abnormalities (17.00%) was recorded in >38 cm groups followed by <34 cm (16.76%), 34-<36 cm (16.39%) and 36-<38 cm (15.73%) groups but was non-significant. Menon *et al.* (2011) found no significant effect of age and SC on all the sperm abnormalities in the beef bulls studied but is in contrast with the findings of Sarder (2008) who reported significant (P<0.05) effects of age and SC on the sperm abnormalities of the breeding bulls from which AI semen was obtained. Chacon (2001) and Chacon *et al.* (1999) reported that bulls with a SC of less than 30cm had more sperm abnormalities (P<0.01).

The results of the study indicated that season significantly (P<0.05) affected the percentage of normal sperm which is considered to be acceptable by Coe (1999) who found significant (P<0.01) variations in the percentage of normal sperm among summer, autumn, winter and spring. In this study, for summer, rainy season and winter average values of percentage of normal sperm were 81.77±1.35%, 84.28±1.67% and 86.18±1.66% respectively. The results of this study indicated that a higher percentage of normal sperm occurred during winter. These results agree with those reported by Mathevon *et al.* (1998) where spring and winter were also associated with a higher (P<0.05) percentage of normal sperm, regardless of the age and breed of the bull. During this study the lowest value of percentage of normal sperm was observed during summer. A lower percentage of normal

sperm during summer may be due to the higher temperature-humidity index (THI) which occurs at maximal level during summer (Curtis, 1993; McDowell, 1972). This is also in line with Mamabolo (1999) and Everret and Bean (1982) where periods of high temperature and humidity were associated with testicular degeneration and a reduction in the percentage of normal sperm. Vilakazi (2003) reported that percentage of normal sperm ($P<0.05$) was significantly higher during spring and winter compared to summer and autumn.

The study showed that nutrition affected the percentage of normal sperm significantly ($P<0.05$). Higher (84.95±1.84%) value was found with that type enriched with Vit. ADE supplementation than (83.07±2.54%) with that type enriched with extra protein supplementation ($P<0.05$). The findings of present study collaborate with the results of Susan (2010) who observed that the percentage of abnormal sperm produced by bulls supplemented with 0.3 ppm organic Se and tocopherol significantly lower ($P<0.05$) than that of bulls supplemented with basal diet.

5.1.6 Color:

During the study, the color was recorded with necked eye as thick-watery, creamy-milky white. Color of semen did not vary between the two breeds. The study of Sitali *et al.* (2017) closely supports the findings of present study where no association was found between breed and semen color ($P<0.05$).

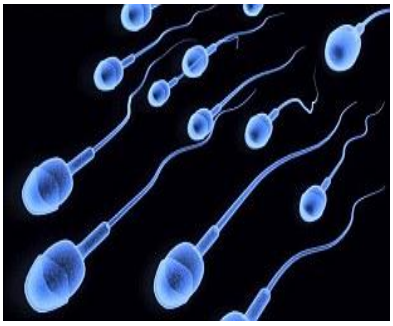
The study showed that adult bulls (>3.5-4.5 years) were more likely to have creamy color semen relative to a watery color compared to younger bulls (2.5-3.5 years). The variation in color may be due to the higher sperm concentration in adult bulls (Perry *et al.,* 1947). The result of this study differs from those reported by Sitali *et al.* (2017) where no effect was found due SC and age on milky and thin milky semen color relative to creamy semen color ($P<0.05$). However, bulls with a BCS of 2.0 were more likely to have watery semen color relative to a creamy color compared to bulls with a BCS of 3.5 ($P<0.05$).

5.1.7 Semen pH:

During the study, pH of fresh semen was found within the range of 6.4-6.7. Shaha *et al.* (2008), Sane *et al.* (1994), Saxena and Tripathi (1981) and Mukherjee and Banerjee (1980) found that the average pH of bull semen was about 6.1-6.5. It was reported by Anderson (1942) that the mean pH of normal bull semen was 6.73±0.02. The pH of seminal plasma ranges from 6.7 to 7.4, which is common in the domestic species (Roberts *et al.,* 1986) and

has the potential to neutralize vaginal acid. Lowering of pH with lactic acid was demonstrated to immobilize bull sperm (Carro *et al.*, 1985; Acott and Carr, 1984). In this study, pH of fresh semen differed between the two breeds ranging from 6.4-6.5 in Friesian bulls and 6.5-6.7 in Sahiwal bulls. Atiqur *et al.* (2014) reported that significantly ($P<0.05$) the highest pH was found in Holstein-Friesian×Zebu and lowest in Red Chittagong breed of bulls.

The study showed that pH did not alter with age of bulls and seasons of the year. The result was found in accordance with Ahmad *et al.* (2003) and Terezenha *et al.* (1991). Terezenha *et al.* (1991) recorded non-significant variations in pH of semen from bulls of young, adult and old age groups and within each age group. Ahmad *et al.* (2003) reported that effect of age and season on pH of semen was non-significant.

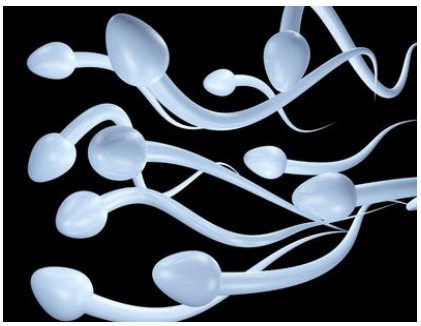


Chapter VI
Conclusions

CHAPTER VI
CONCLUSIONS

The results of the present study lead to the following conclusions:

- Good quality of semen production makes successful breeding program. Physical examination of bulls helps in evaluating the breeding soundness of bulls in a breeding bull station.
- It was evident that the studied factors were important source of variation and had significant effects on the quality of fresh semen of breeding bulls.
- Sahiwal bulls had higher semen quality than Friesland bulls. As a tropical breed Sahiwal bulls ranked top and superior between the two breeds due to its better survived ability under the subtropical environment of Bangladesh.
- The age group >3.5 to 4.5 years produced better semen quality. The production was higher in bulls with BCS ranging from 4 to 4.5 and having scrotal circumference between 31.1 to 33 cm.
- The stressful summer season deteriorated the quality of semen obtained from the breeding bulls while winter was an ideal for the best quality of semen. Vit. ADE supplementation improved the semen quality.
- Further research, considerable attention and prolonged studies should be directed in determining the effects of breed variation, scrotal circumference, BCS, age, season and nutrition on the quality of semen and their interactions.



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