EFFECTS OF BULL CHARACTERISTICS AND TESTICULAR ATTRIBUTES ON SEMEN QUALITY IN KENYAN AI STATION BULLS

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Declaration

This thesis is my original work and has not been presented for a degree in any other university.

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Dedication

This project is dedicated to my loving parents, Gabriel Munywoki and Peninah Katumo, and my brother Jonathan Katumo for their unfading support.

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Abstract

More than 24 million people in East Africa depend on the dairy industry, directly and indirectly through the dairy value chain. Within the sub-Saharan region, Kenya has the best dairy industry, which supports approximately 1.5 million smallholder farmers, with additional stakeholders in the dairy value chain. The dairy subsector contributes 4% to the gross domestic product (GDP) and 12% of the total agricultural GDP.

The success in the dairy sector is attributed to the adoption of assisted reproduction technologies, especially artificial insemination, to improve the genetic makeup and subsequently increase milk production in dairy animals. Artificial insemination (AI) has dramatically enhanced milk production by disseminating improved genetic. However, despite the success in the dairy industry, several knowledge gaps on the selection of bulls for AI exist, necessitating the current study.

The current study aimed at documenting the effects of bull signalment and testicular attributes on semen quality in bulls used for semen production in Kenya. The study was conducted at the Kenya animal genetic resources center. All healthy bulls aged above 16 months and in active collection were recruited. Prospective and retrospective data were collected.

The prospective study was conducted on 96 bulls, and the collected data included the age, body weight, body condition score, breed, scrotal circumference (SC), semen volume, progressive motility, post-thaw motility, and concentration, which were determined and documented. On the other hand, the retrospective study included data on semen quality in 38 bulls aged above 84 months.

Age and weight significantly affected the SC (p-value <0.0001). There was a significant variation in SC across the breeds (p-value <0.0001), where the *Bos indicus* had a greater SC than the *Bos taurus*. Furthermore, a logistic regression model revealed that testicular presence of hyperechoic lesions increased with age (p-value <0.001).

The semen volume significantly increased with age (p-value <0.05), body weight (p-value <0.001), and SC (p-value <0.05). The body condition score negatively and significantly affected the post-thaw semen motility and sperm concentration (p-value<0.05). The breed significantly affected the progressive motility (p-value <0.01), post-thaw motility (p-value <0.05), and sperm concentration (p-value <0.001). The presence of hyperechoic lesions did not significantly affect semen quality in both studies (p-value >0.05), although semen volume was significantly higher in bulls with hyperechoic lesions in the prospective study (p-value <0.05).

From the findings of this study, it is prudent to cull bulls based on semen quality rather than age, as there are old bulls consistently producing good-quality semen. Furthermore, over-conditioning bulls significantly affect sperm concentration and post-thaw motility. Finally, there is a need to substitute the use of an electro-ejaculator in the *Bos indicus* as their semen quality significantly varies from the *Bos taurus*, where an artificial vagina is used.

Keywords: *Age, breed, weight, testicular ultrasound, scrotal circumference, semen quality,*

CHAPTER ONE

1. Introduction

Africa is the fastest-growing continent, and more than half of the world's population growth is expected to be in Africa between now and 2050. Furthermore, the sub-Saharan African human population is projected to double by 2050 (UN, 2022). The growing population in Africa translates to an increased demand for animal protein. To meet the increasing demand for high-quality and quantity of food, Africa must increase the production of livestock products while reducing the production of greenhouse gases. However, Africa does not produce sufficient animal products to cater to its population (Rosegrant et al., 2009).

In sub-Saharan Africa, dairy animals are a significant income generator (Mubiru et al., 2007). More than 24 million people in East Africa depend on the dairy industry (Herrero et al., 2013). Within the sub-Saharan region, Kenya has the best dairy industry (World Bank, 2013), which supports approximately 1.5 million smallholder farmers, with additional stakeholders in the dairy value chain (KDB, 2016).

The dairy subsector contributes 4% to the Gross Domestic Product (GDP) and 12% of the total agricultural GDP (KNBS, 2019). The success in the dairy sector is attributed to the adoption of assisted reproduction technologies, especially artificial insemination (AI), to improve the genetic makeup and subsequently increase milk production in dairy animals. AI has dramatically enhanced milk production by disseminating improved genetics (Hafez & Hafez, 2013).

The primary reason for adopting AI in the dairy industry was to increase productivity through improvements made while selecting sires (Ombelet & Van Robays, 2015). The selected sires are taken through a breeding soundness evaluation (BSE). Breeding soundness evaluation (BSE) is an invaluable tool for selecting bulls for producing semen used for AI, as it is a lowcost procedure that eliminates bulls with low fertility. For efficient and consistent production and distribution of high-quality semen used in AI, desirable traits in bulls are necessary. Economic losses can be incurred following unpredictable fluctuations in semen production (Hering et al., 2014).

The AI industry reports a significant variation in semen quality that leads to an increased risk of producing a low number of straws, especially when there is increased demand in the market (Hering et al., 2014). Breeding soundness evaluation (BSE) depends on physical examination, serving capacity, and satisfactory semen quality, with bulls being termed sterile, sub-fertile, infertile, fertile, or highly fertile (Barth, 2018). Previous studies have identified abnormal scrotal thermoregulation, body condition, stress, age, season, and SC as leading factors affecting semen quality.

Semen analysis is used to evaluate semen quality, and though termed an imperfect tool, it is the cornerstone of male fertility investigation (Barratt, 2007). Consistent high-standard evaluation provides descriptive parameters of the ejaculate. Routine semen evaluation is invaluable for information on sperm production, motility, viability, male reproductive tract patency, accessory sex gland secretions, ejaculation, and emission (Vasan, 2011).

While many studies have described semen quality and bull characteristics, there is still a knowledge gap on the extent to which semen quality correlates with bull characteristics. The same has not been described in bulls used for AI in Kenya, given that the farm management practices, ecological zones, and breed adaptation vary. The current study aims to bridge knowledge gaps on the relationship among bull characteristics, testicular attributes, and semen quality in Kenyan AI station bulls.

1.1 General Objective

To determine the association between age, breed, body weight, testicular characteristics, and semen quality in bulls used to produce semen for artificial insemination (AI) in Kenya.

1.2 Specific Objectives

1. To investigate the effect of bull characteristics (age, body weight, and breed) on testicular attributes (SC and testicular echotexture) in bulls used for semen collection at KAGRC
2. Determine the effect of bull characteristics and testicular attributes on semen quality in bulls used for semen collection at KAGRC
3. To determine the effect of age, breed, and testicular echotexture on semen quality using retrospective data

1.3 Justification

AI has revolutionized the dairy industry in Kenya with a sharp increase in milk production over the years and availing pure breed dairy cattle exported to neighboring countries. In Kenya, KAGRC is mandated to collect semen and distribute it locally and internationally.

Bulls with appropriate characteristics such as weight, body condition, age, and SC are essential to ensure high-quality semen is continuously produced. A correlation between these characteristics and the extent to which they influence semen quality, such as the number of motile sperms, the concentration of spermatozoa, and the percentage of live spermatozoa, have not been fully established, necessitating this study.

CHAPTER TWO

2 Literature Review

2.1 Artificial Insemination

Leeuwenhoek (1678) and Hamm identified the sperm, and described it as *animalcules,* using ground lenses with 270 magnifications. After a century, Spallanzani (1784) successfully inseminated a dog that whelped three puppies after 62 days. One hundred years later, Heape and other researchers in different countries reported the application of AI in horses, rabbits, and dogs. AI became a focal point in research when attempts to pioneer AI began in Russia in 1899 (Foote, 2010).

Ivanoff (1922) introduced semen extenders while training his technicians on selecting superior qualities in stallions to multiply progenies. Milovanov (1964) designed the artificial vagina for semen collection, an alternative to sponges placed on animals' vaginas. Ivanoff's work triggered studies outside Russia as Dr. Ishikawa developed a similar program in Japan's cattle, goats, sheep, poultry, and swine. In 1936, Denmark established an AI program, recruiting 1070 cows. The conception rate following AI service was 59%, slightly higher than the use of natural service, stimulating the adoption of AI service in the dairy sector in the US and other countries in the West (Foote, 2010).

Denmark further established rectovaginal fixation of the cervix, a method that facilitated the depositing of semen at the posterior aspect of the cervix or the level of the uterine body. This invention allowed a small number of spermatozoa to cause conception (Foote, 2010). Denmark also invented using a straw to package semen (Sørensen, 1940), later commercially produced by Cassou (1964) and distributed worldwide.

Progressive growth in AI was observed around the 1940s in the US, where many procedures and methodologies developed and gained worldwide acceptance and subsequent consumption. In 1936, Brownell served cows using AI in the Cornell herd. Artificial insemination (AI) was also used in serving cattle in Minnesota and Wisconsin.

The use of AI grew with the establishment of cooperatives in New Jersey and New York, facilitating collaboration between cooperatives and research institutions. The interaction culminated in the insemination of numerous cattle experimentally and more than 100 publications on BSE, both macroscopic and microscopic, and bull fertility testing (Foote, 1998; Foote, 1999).

2.2 Bull Fertility

Fertility is the ability of animals to produce viable offspring. There are numerous fertility endpoints between insemination and birth, including fertilization rate, conception rate, and non-return to estrus rate (NRR) (Utt, 2016). Fertilization rate, as suggested by the name, refers to the sperm's ability to fertilize an ovum and can be evaluated 48 hours following insemination using evaluation endpoints such as second polar body extrusion, identification of pronuclei, and first cell division. (Utt, 2016).

Sterility, on the other hand, means the permanent and complete inability to reproduce, subfertility refers to the depressed ability to reproduce, and infertility is the temporary inability to reproduce. The term highly fertile is often used in bull evaluation systems to denote the full reproductive potential for fertility that can be reached without any reduced fertility caused by the bull (Barth, 2018).

For optimum interaction with the ovum and provision of the necessary chromosomal complement, spermatozoa must exist in sufficient quantities, exhibit good motility and morphology, and pass via the cervix, uterus, and ampullae. Furthermore, the spermatozoa must undergo requisite processes such as capacitation, acrosome reaction, binding to the zona pellucida, and nuclear decondensation. Any spermatozoal defects will lead to male infertility (Eliasson, 2010; Lamb, 2010).

2.3 Breeding Soundness Evaluation (BSE)

BSE is a uniform methodology used to assess the probability of establishing pregnancy in a given number of open, healthy, and cycling heifers or cows in a breeding season (Strickland, 2000). Standardized procedures for BSE were first reported by the American Society of Theriogenology (Chenoweth et al., 1993). Bull breeding soundness evaluation (BSE) can be determined by breeding numerous heifers with normal fertility while assessing the pregnancy rates or subjecting bulls to an evaluation of breeding sound (Kastelic & Thundathil, 2008).

Bull breeding and soundness evaluation vary from country to country, but three cardinal principles remain consistent in all the systems. Bulls selected for breeding must exhibit physical soundness, desire to mate and deliver good-quality semen to females. A tentative fourth cardinal principle involves freedom from transmissible diseases. Depending on the epidemiology, trichomoniasis and campylobacteriosis testing may be indicated as part of BSE (Barth, 2018).

Following a BSE, bulls are classified into an unsatisfactory potential breeder, deferred, and satisfactory potential breeder. A satisfactory potential breeder is a fertile bull that has passed the physical evaluation, including meeting the minimum requirements for SC, having sperm motility of 30% or higher, and producing at least 70% of normal spermatozoa (Chenoweth et al., 2010)

Unsatisfactory potential breeders are considered sub-fertile or sterile as the bull failed at least one of the BSE components. The bull can impregnate cows but cannot be recommended to breed due to inefficiency. The deferred breeder bull means that it failed one of the BSE components due to a condition expected to resolve with time or therapy and is subject to review later (Chenoweth et al., 2010).

2.4 Scrotal Circumference (SC)

The SC is an essential macroscopic parameter usually determined during a BSE in bulls. The SC correlates with semen volume and sperm concentration (Latif et al., 2009). The SC measurements at <15 months are expected to be 30cm, 31cm at >15- 18 months, 32cm at >18-21 months, 33cm at >21 – 24 months, and 34 at > 24 months (Chenoweth et al., 1993)

Scrotal circumference (SC) is highly correlated with paired testicular weight, affecting daily semen production and, consequently, semen quality (Barth, 2007). Scrotal circumference (SC) also correlates with progenies, as bulls having a large SC produce half-sib heifers with early puberty and excellent fertility. SC heritability in young bulls between 12-24 months is ~0.5; therefore, it responds favorably to selection (Barth, 2007).

A study done by Coe and Gibson (1993) in beef bulls of 13 different breeds aged 200 days revealed that calves with an SC measuring >23cm had a 95% chance of attaining an SC of >34cm at one year, and calves with SC<23 were 54% likely to attain SC of >34 at one year. SC measurements are taken by squeezing the testicles ventrally and using scrotal tape at the largest circumference to apply tension. Primary prerequisites are undertaken, such as ensuring that the scrotum has a distinct neck and testicle should move freely, is firm, resilient, and similar in size (Barth, 2007).

Bulls that have large SC are preferred while selecting bulls at early puberty. A study by Barth and Waldner (2002) on beef bulls revealed that many post-pubertal bulls with average or above-average SC have desirable semen quality compared to bulls below the recommended SC. Furthermore, bulls with an SC measuring <30cm have poor sperm morphology (Barth & Waldner, 2002).

Scrotal circumference (SC) does not significantly affect the percentage of morphologically normal spermatozoa. Moreover, bulls with SC measuring below the recommended parameters have low percentages of spermatozoa exhibiting forward progressive motility compared to bulls that meet the minimum requirement. Regarding sperm defects, bulls with SC measuring below the minimum requirement have high numbers of sperm with head and midpiece defects (Barth & Waldner, 2002).

Gopinathan et al. (2018) reported that Holstein Friesian bulls had a positive yet contradicting association between SC and sperm parameters. Bulls with SC measuring 36-38 cm produced the highest semen volume, sperm concentration, and ejaculate doses. However, the highest initial and post-thaw sperm motility was recorded with SC <34 cm, and values were reduced with increasing SC (Gopinathan et al., 2018).

2.5 Semen Collection

Semen collection is an integral component of BSE as it precedes semen analysis. Different options are available for semen collection, and these include; artificial vagina (AV), electro-ejaculation (EEJ), transrectal massage (TM), and aspiration of semen deposited in a cow's vagina following natural service (Palmer, 2021).

Electro-ejaculation (EEJ) is a relatively fast and efficient method that eliminates the need to train animals, mount animals, and specialized animal handling (Palmer, 2021). However, using EEJ is not welfare-friendly as it is perceived to inflict pain on bulls and, therefore, illegal in different countries (Mosure et al., 1998). Furthermore, EEJ causes an increase in the temperature of the scrotal skin covering the cauda epididymides (Kastelic et al., 1996)

The use of EEJ was reported in 1936 (Gunn, 1936). The current electro-ejaculators utilize a sine-wave pulse (frequency 20-30 cycles) (Furman et al., 1975). Increased Electrical stimulation leads to unnecessary muscle contractions. The maximum voltage the Lane Pulsator IV achieves is 16 V, with the maximum current being <900 mA (Marden, 1954).

During EEJ collection, the bull is adequately restrained, and a transrectal examination of the seminal vesicles, ampullae, prostate, inguinal rings, and urethra is completed. Following examination, the aforementioned organs are massaged for 10-60 seconds to stimulate the bull and relax the anal sphincter before probe insertion. Once the probe is inserted, electrical stimulation follows while observing the bull’s response. Following a slight contraction of the hind limb musculature, the bull's response is noted, and electrical stimulation can be removed.

The use of TM involves massaging the ampullae and the urethralis muscle (McGowan, 1995). However, the method is unreliable due to low semen volumes, as Persson et al. (2007) observed, where out of three bulls, semen collected was only enough for sperm morphology evaluation but not for volume and concentration analysis. In 4 bulls, no semen was obtained.

False mounts and AV is the most common technique for semen collection in most Artificial breeding settings. It allows for evaluating other variables, such as sex drive and mating ability (Barth et al., 2004). The AV comprises a glass container with a calibrated volume scale, a water jacket, a flexible latex sleeve, and a latex cone that joins to the collection tube. The AV further consists of a cylindrical casing, a valve that facilitates the pouring of water and blowing of air, a thermal protector, and a mechanical container. The AV water temperature should be set at 40-42 ˚C, while the container temperature should fall between 35-37 ˚C (Barszcz et al., 2012).

2.6 Semen Evaluation

Semen is composed of sperm and seminal fluids that form the liquid part. Sperm production occurs in the seminiferous tubules and the seminal plasma from the excurrent duct and the accessory sex glands (Patel et al., 2018). Characteristics of semen include volume, color, texture, smell, spermatozoa concentration, and pH.

Normal bull ejaculate volume is 2-8 ml; the color should be white and a characteristic cream hue. A pink or red color suggests blood presence and may be due to penile abrasion, cavernous bodies' fistulas, or urinary stones. A green color suggests pus presence; yellow color suggests urine presence and watery white color suggests low sperm concentration or water addition from the artificial vagina during semen collection (Barszcz et al., 2012)

The semen texture should be milky, creamy, or milk with cream. The mucus from the urogenital system inflammation may change the texture to be viscous. The smell should be similar to cow's milk. The normal sperm concentration should be 0.6 to 1.5 x 10^{6} /mm^{3,} and the pH should be 6.2-6.8. Semen impurities such as fur and soil are unacceptable. Furthermore, semen should be free of deposits that may appear after semen collection, suggesting the presence of inflammatory cells and dead spermatozoa (Barszcz et al., 2012)

2.6.1 Sperm Concentration

There are different methodologies used to determine sperm concentration. These include using photometry or densitometry and direct methods involving counting individual sperm cells in a determined volume using the haematocytometric method (Vianna et al., 2007). The spectrophotometric analysis does not include the direct enumeration of spermatozoa; it is precise and accurate when adequately calibrated. The method requires a small sample volume, is relatively inexpensive, and is easy to train technicians, making it the most common technique in determining sperm concentration (Brito et al., 2016).

A sample of 0.02 ml of semen is taken and diluted at 1:200 using Natrium chloratum 0.9% as a physiological solution and poured into a macro-cuvette. The analysis produces a print-out showing; sperm concentration, the ejaculate volume, sperm concentration in a straw, the extender quantity to be used, and the number of straws. Other techniques include flow cytometry, nucleocounter, and computer-assisted semen analysis (Brito et al., 2016).

2.6.2 Sperm Morphology

Sperm morphometric characteristics are among the most critical bull fertility indicators. Normal sperm morphology affects fertility significantly, either *in vivo* or *in vitro;* hence, morphometric characteristics form a critical part of semen analysis (Eggert-Kruse et al., 1995). Abnormal types are differentiated and classified based on the sperm cellular morphology and include a defective spacer, head, neck, and default queue (Gago et al., 1999).

Different smear preparation and fixation techniques have been adopted to assess sperm morphology, but no particular technique has been standardized as optimal for the different species. Variability between different settings and institutions regarding the accurate technique for sperm morphology exists.

Computerized sperm morphology analysis is called automated sperm morphometric analysis. This method allows for the morphometric assessment of live spermatozoa (Rijsselaere et al., 2004). The system efficiently differentiates between normal and abnormal spermatozoa by bypassing technical variations. Furthermore, it precisely estimates sperm morphology. However, sperm morphometric analysis can be improved through staining (Tanga et al., 2021).

2.6.3 Sperm Motility

Sperm motility assessment involves using light and phase-contrast microscopes under 20 and 40X objective lenses, yielding substantially desirable results (Cuche et al., 2000). The microscope has a stage warmer that allows temperature adjustment to 37 °C and magnification to achieve clear sperm visualization (Love, 2016). A light microscope is, however, avoided due to clear visualization concerns such as difficulty in identifying immotile sperm, especially under low magnification, which may lead to false high-motility values (Love, 2016). On the other hand, phase contrast microscopy is affected by working conditions (ambient temperature, heated stage), magnification, environmental conditions, and personal experience (Brito, 2007).

In phase contrast microscopy, mass and progressive sperm motility are described (Eskenazi et al., 2003). Based on the observed motility patterns, sperm can be classified as progressive motile (PR) or non-progressive motility (NP) (Iranpour et al., 2000). While recording motility, it is paramount to consider total motility, which is given by PR+NP or progressive motility. Only the percentage of PR sperm correlates with pregnancy rates. Therefore, it is prudent to differentiate between NP and PR (Simon & Lewis, 2011).

Progressive motility is reported as a percentage, which suggests sperm fertility and indicates desirable spermatogenesis and sperm maturation during epididymal transit (Cabrillana et al., 2017). In animals with external fertilization, semen exposure to the fertilization medium leads to hypotonic shock that triggers sperm motile function (Morisawa et al., 2004). In mammals, progressive sperm motility is associated with adenylyl cyclase and cAMP signaling pathways (Okamura et al., 1985).

Specific sperm motility can also be described as swirling oscillation, observed under light microscopy with a warm stage (David et al., 2015). Motility can also be assessed using Computer-assisted semen analysis (CASA). It is the first primary system to provide sperm motion and concentration analysis.

The CASA software offers instant quantification of spermatozoal concentration and progressive motility. This software produces better results than phase-contrast microscopy due to generating highly reproducible and accurate data bearing different kinematic sperm parameters (Günzel-Apel et al., 1993). Furthermore, through microscopy, CASA overcomes the variability of daily semen evaluation (Verstegen et al., 2002).

The CASA system, however, has some drawbacks as it is affected by high sperm concentration, interfering with progressive motility (Rijsselaere et al., 2002). The CASA software can also face limitations from low-contrast images and dirt artifacts that negatively affect accuracy. In such instances, sperm concentration becomes overestimated and sperm motility underestimated. These shortcomings have, however, been partly addressed in the last two decades. More CASA limitations are based on video frames associated with systems with frame rate restrictions and poor-resolution cameras. Reliable parameters are obtained when the concentration is between 20-50x10^6 sperm/ ml (Rijsselaere et al., 2003).

2.7 Age and Semen Characteristics

Semen volume increases with age, and peak volumes can be obtained between 84 and 108 months (Argiris et al., 2018). A study conducted on Australian Simmental bulls by Fuerst-Waltl et al. (2006) revealed that ejaculate volume increases with age, with the highest volumes obtained in bulls aged 72 months or older.

Concentration decreases with an increase in age, with the highest concentrations found in bulls aged between 24-36 months and the lowest concentrations obtained in bulls aged 20-22 months. Rehman et al. (2016) recorded that volume increased with age, highest at 36-60 months and lowest in bulls aged 12-36 months. Conversely, motility does not have a consistent trend except for a slight tendency to decrease with an increase in age (Fuerst-Waltl et al., 2006).

According to Brito et al. (2002), semen volume, total spermatozoa number, and viable spermatozoa increase with age. There is, however, no significant relationship between age and sperm concentration, sperm defects, and motility. Sperm concentration, motility, and percentage of dead spermatozoa do not show significant differences among the different ages.

2.8 Testicular Ultrasonography

Diagnostic ultrasound is a popular tool applied in soft tissue imaging. While ultrasound remains widely used in the female reproductive system, it has limited use in the male reproductive system. It is not indicated for routine BSE by the Society for Theriogenology (Chenoweth et al., 1993).

However, ultrasonography is vital as it localized and objectively assesses tissue morphological changes. Ultrasonography in male domestic animals is routinely used for palpable reproductive tract abnormalities. Furthermore, the use of ultrasonography in the male domestic species is indicated when males have hemospermia, pyospermia, oligospermia, azoospermia or when bulls are producing immotile and morphologically abnormal sperms (Momont & Checura, 2014).

The ultrasound used for testicular ultrasonography is similar to that used for the female reproductive tract, a linear-array probe (Gnemmi & Lefebvre, 2009). The transducer generates sound waves at a high frequency (5-7.5 MHz), which are reflected and processed to form an image in real-time (Kastelic & Brito, 2012). Detailed ultrasonograms of the penile and epididymis tissues are obtained with probes of higher frequencies and smaller footprints (Momont & Checura, 2014).

Restraint during examination varies, but operator safety is a priority. Most bulls will tolerate perineal, rectal, and scrotal examination with little restraint. During a scrotal examination, a kick bar is indicated with care taken to avoid placing arms in dangerous spots. Sedation using xylazine at a dosage rate of 0.01-0.02/kg administered intravenously is indicated when dealing with fractious bulls. When imaging the testes, shaving is unnecessary as the growing hair is usually scanty. A transcutaneous scan is used to image the scrotum, and quality images are obtained by maintaining a firm grip on the scrotal neck dorsally while squeezing the testicles ventrally (Momont & Checura, 2014).

A coupling gel obliterates air or gas between the scrotum and the probe. Isopropyl alcohol (70%) is also an alternative, but it evaporates and may need re-application and is not safe to come into contact with probe surfaces. (Momont & Checura, 2014). Diagnostic ultrasonography is safe for use in bulls as it does not cause discernible effects on semen quality (sperm concentration, motility, and morphology) as shown in yearling beef bulls during a 3-minute examination per testis using a frequency of 5 MHz (Coulter & Bailey, 1988).

B-mode ultrasonography was first described by Pechman and Eilts (1987) to determine bull testicular echographic anatomy. Sagittal and transverse planes are obtained using the lateral, cranial, and caudal testicular approach. The examination includes the entire testicular parenchyma, the spermatic cord, the scrotal wall, and the epididymis. The ductus deferens and the body of the epididymis are challenging to visualize unless grossly pathological (Momont & Checura, 2014).

Ultrasonically, the testicular parenchyma appears homogeneously with stippled medium echogenicity. The parenchyma surrounds the hyperechoic mediastinum testis, which measures <5mm in width. The parenchyma comprises seminiferous tubules with sertoli and germinal cells, interstitial Leydig cells, and associated neural, vascular, and stromal tissues (Momont & Checura, 2014). Echographic changes may indicate physiological or pathological changes. Increased testicular echogenicity, as depicted by pixel intensity during sexual development, indicates tissue intensity. Dramatic echogenicity increase has been noticed in bulls at 20-46 weeks of age (Kastelic & Brito, 2012).

These lesions appear as hyperechoic foci scattered within the testicular parenchyma or may be localized. Large hyperechoic lesions radiate from the rete testis to the testicular periphery, suggesting the complete involvement of one or more seminiferous tubules (Momont & Checura, 2014). Histologically, an increase in echogenicity usually suggests fibrosis (Barth et al., 2008), but increased echogenicity is not confirmatory of etiology.

Tumors have a general hyperechoic appearance compared to the normal testicular parenchyma, but hypoechoic or mixed echogenicity may occur (Nyland et al., 1995). Mineralized lesions exhibit a hyperechoic appearance and are usually accompanied by an absolute shadowing that causes a loss of image caudal to the lesion (Momont & Checura, 2014).

Fibrotic lesions in young bulls aged 3-20 months do not translate to poor semen quality. However, a dramatic increase in fibrosis suggests an increased likelihood of poor semen quality (Barth et al., 2008). The etiology of fibrotic testicular lesions is unknown. However, possible causes may include inflammatory or infectious conditions, developmental anomalies of seminiferous tubules and connecting ducts, autoimmune disorders, and degenerative conditions or aging (Momont & Checura, 2014).

Experimental inoculation of ram testes with *Trueperella pyogenes* leads to the development of hyperechoic lesions due to the fibrotic tissue (Gouletsou et al., 2004). Administration of calcium chloride also causes fibrotic lesions and an abnormal mediastinum testis (Pereira et al., 2018). Bovine respiratory syncytial virus outbreak in bulls has been associated with the prevalence of fibrotic testicular lesions, although the mechanism is ill-defined (Barth et al., 2008).

Scrotal insulation, which dramatically reduces semen quality, has not revealed any ultrasonically detectable testicular changes within 4-6 months (Sidibe et al., 1992; Arteaga et al., 2005). Aging in the bull is associated with marked progressive fibrosis at the ventral aspect of the testis (Humphrey & Ladds, 1975).

3. CHAPTER THREE

3.1 Materials and Methods

3.1.1 Study area

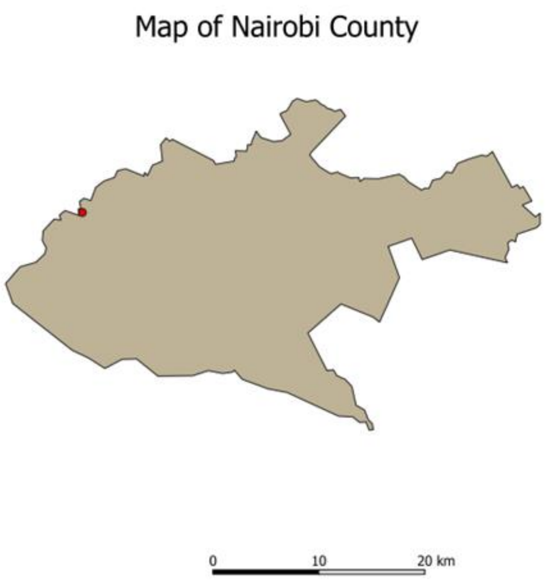

Figure 3.1: The study Area

The study was conducted at KAGRC at -1.24238767621, 36.7338180542 Lower Kabete, Nairobi, Kenya. The center is 15 km from Kenya's capital, Nairobi, with an elevation of 1777.36 meters above sea level. KAGRC is mandated to produce dairy and beef semen for the local and international markets.

3.1.2 Study Groups

KAGRC has 111 bulls of different breeds and ages. The breeds include Friesian, Ayrshire, Guernsey, Jersey, Composite/Magic 50 (crossbreed of Friesian, Sahiwal, and Gir breeds). Out of the 111 bulls, 107 had attained the minimum semen collection age (16 months) during the study period, and their distribution is shown in Table 3.1. The inclusion criteria required healthy bulls in active semen collection and aged >16 months.

Table 3.1: Breed distribution across the bull population

Breed	Number of bulls (*n*)
Ayrshire	42
Boran	5
Friesian	42
Guernsey	5
Jersey	7
Sahiwal	6

3.1.3: Study Design: Objective One

To investigate the relationship between testicular characteristics and age, body weight, body condition score, and breed of bulls used for semen collection at KAGRC

Ninety-six bulls met the inclusion criteria for recruitment to the study and the breed distribution is as follows; Ayrshire (*n*=40), Boran (*n*= 3), Friesian (*n*= 36), Guernsey (*n*= 5), Jersey (*n*= 7), and Sahiwal (*n*= 5). Their weights were taken using livestock weighing scale (AEA LTD, Nairobi, Kenya).

The bulls were then restrained in a crash for testicular examination. The SC was measured using a scrotal tape (Lane Manufacturing Co., Denver, CO, USA) using the procedure described by Foote (1969) by squeezing the testicles ventrally and taking measurements from the widest scrotal diameter, as shown in Figure 3.2 below.

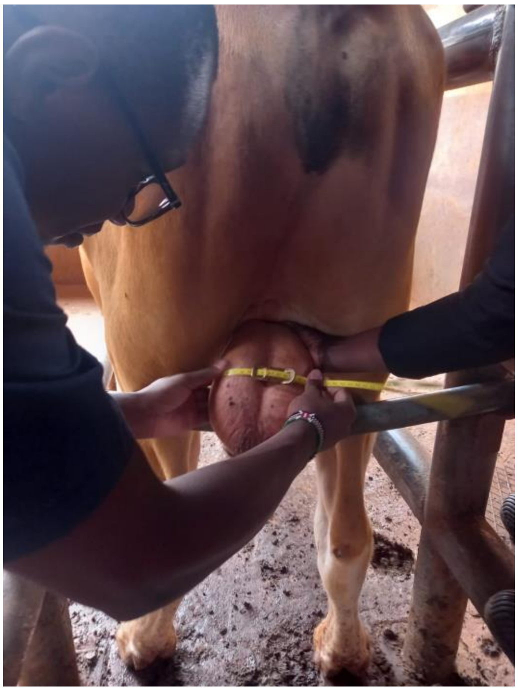

Figure 3.2: Scrotal circumference measurement

The testicles were also examined using a portable ultrasound machine (Minitüb GmbH, Hauptstraße 41, 84184 Tiefenbach, Germany). The testicles were not shaved, as the scanty scrotal hair did not interfere with the ultrasound image. The testicles under examination were squeezed ventrally and secured with one hand at the neck of the scrotum. B-mode ultrasonography was used to image the bull testicular tissue using a linear probe at a frequency of 6.5 MHz.

An ultrasound coupling gel applied uniformly on the footprint of the probe provided eliminated air between the probe and the scrotal surface. Both testicles were imaged using caudal and cranial views. Both transverse and sagittal planes were used, but the sagittal view revealed an excellent exposure of the individual testicle and the mediastinum testes, with both the dorsal and ventral borders. Data was recorded in Microsoft Excel 2016 (Microsoft.
Corporation; Redmond, WA).

The body condition score was determined by observing the fat cover over the spine, short ribs, hip bones, and tail head. Age was determined by retrieving bull records and was recorded in months

Figure 3.3: Imaging bull testicles with a portable Ultrasound Machine

3.1.3: Study Design: Objective Two

To determine the correlation between age, breed, and testicular characteristics and semen quality for bulls used for semen collection at KAGRC

The individual bull parameters were obtained under objective one, and their respective biodata (age, breed, and name) were recorded. Semen collection was done using false mounts and an AV (IMV Technologies, Normandy, France) in the Friesian, Ayrshire, Jersey, and Guernsey. Before the AV collection, bulls were allowed to have a first false mount, as shown in Figure 3.4. The second mount was collected, as shown in Figure 3.5. In the Boran and Sahiwal breeds, an Electro-ejaculator (Electronic Research Group, Midrand, South Africa) was used to collect semen.

Collected semen was observed for macroscopic characteristics (color and volume) and maintained at body temperature in a dry bath, awaiting processing. A warm-stage light microscope (Ernt Leitz Wetzlar Gmbh, Germany) was used to observe mass motility at a magnification factor of X10 and individual motility at a magnification factor of X25. The sperm concentration was estimated using a photometer (IMV Technologies, Normandy, France). Forty μL of the collected semen was mixed with 3960 Ml of Natrium chloratum to kill the spermatozoa and poured into a cuvette to quantify spermatozoa concentration per ml.

Processed semen was diluted with OptiXcell 2 (IMV Technologies, Normandy, France), which contains mineral salts, carbohydrates, buffer, glycerol, antioxidants, ultra-pure water, phospholipids, and antibiotics (Gentamycin, Lincomycin, Tylosin, Spectinomycin). Following dilution, the semen was filled in straws, chilled for 15 minutes, frozen for 4 hours, and stored at -196°C.

One dose per ejaculate was thawed, and the post-thaw motility was observed under a warm stage light microscope at X25 objective lens. All the information was recorded and compiled in Microsoft Excel 2016 (Microsoft Corporation; Redmond, WA) and converted into Comma Separated Values for exportation into the statistical analysis software.

Figure 3.4: False first mount

Figure 3.5: AV collection during the second mount

3.1.4. Study Design: Objective Three

To determine the relationship among age, breed, testicular echotexture, and semen quality using retrospective data

Thirty-eight bulls met the inclusion criteria for the retrospective analysis of semen volume, progressive sperm motility, and sperm concentration. The inclusion criteria included bulls above KAGRC's recommended culling age of seven years and are still under active semen collection.

The breed distribution was as follows; Ayrshire 47.37% (*n*=18), Friesian 39.47% (*n*=15), Sahiwal 7.89% (*n*=3), Guernsey 2.63% (*n*=1), and Boran (*n*=1) 2.63%. Retrospective data on semen volume, progressive motility, and sperm concentration was retrieved and filtered using Microsoft Excel 2016 (Microsoft Corporation; Redmond, WA) and exported to R Studio 2020 (R Studio, PBC, Boston, MA) for analysis and visualization.

CHAPTER FOUR

4.1 Results: Objective one

4.1.1 The Relationship Between Age and Scrotal Circumference

Table 4.1: Summary of the mean SC across the different bull age groups

Age group (Years)	Number of bulls (*n*)	SC Mean
≤3	18	34.41±1.994961
>3-5	20	37.39±2.541503
>5-7	18	37.29±2.266501
>7-9	21	40.33±2.450578
>9	19	41.53±3.061781

Table 4.1 above shows the SC means of the different age groups. Age significantly affects the SC (p-value <0.0001). Animals under 36 months had the lowest SC mean (34.41 cm), and those above 108 months had the highest SC mean (41.11 cm).

A linear regression analysis revealed a statistically significant relationship between the age of the bull and the SC (p-value: 1.113e-15) with an intercept of -192.018 and a coefficient of 6.921 and 95 degrees of freedom. Below is a summary of the linear model.

Table 4.2 A summary of the linear model output

Coefficients:

	Estimate Std. Error	t value
(Intercept)	-192.018 27.685	-6.936
Scrotal Circumference	6.921 0.720	9.612
	Pr(>|t|)	
(Intercept)	4.88e-10 ***	
Scrotal Circumference	1.11e-15 ***	
Signif.codes: 0 ‘***’ 0.001	‘**’ 0.01 ‘*’ 0.05	‘.’ 0.1 ‘ ’ 1
Residual standard error:	24.54 on 95 degrees	of freedom
Multiple R-squared: 0.4931,	Adjusted R-squared:	0.4877
F-statistic: 92.4 1 and	95 D.F., p-value:	1.113e-15

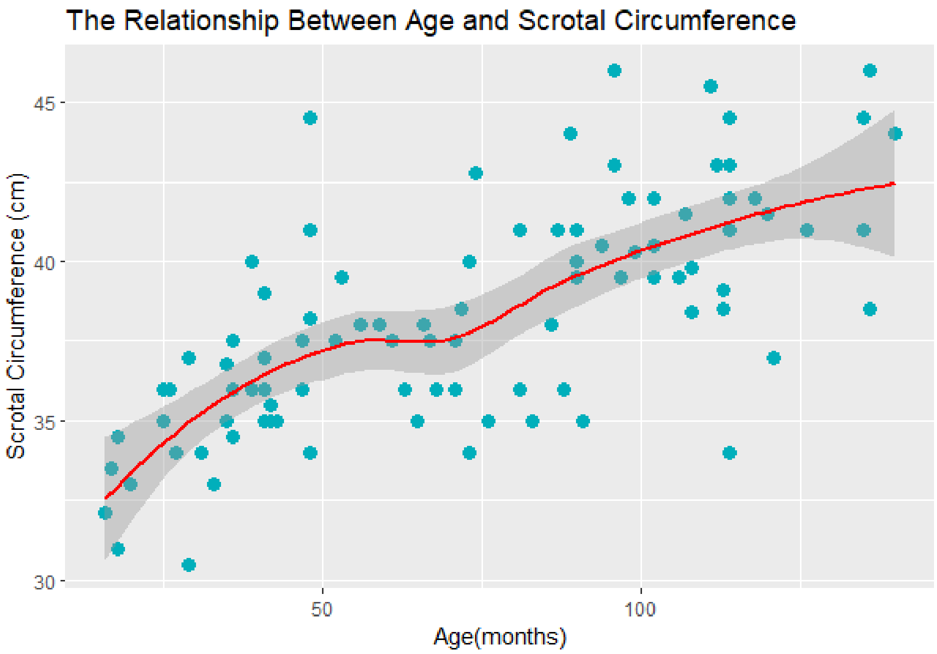

Figure 4.1: A scatter plot showing the relationship between age and SC

The scatter plot in Figure 4.1 above shows a positive relationship between age and SC. The SC increases exponentially until 50 months. Thereafter, SC still increases but at a relatively slower rate.

4.1.2 The Relationship Between Body Weight and SC

A statistically significant relationship was found between body weight and SC (p-value <0.0001). The y-intercept was at -312.132 and had a coefficient of 24.129. A scatter plot further demonstrates the relationship in Figure 4.2. The summary of the linear model from the r output is shown in Table 4.3 below.

Table 4.3: Weight distribution across the age groups

Age group (Years)	Mean Weight (Kg)
≤3	409.56±62.89227
>3-5	546.20±79.52861
>5-7	651.67±77.29927
>7-9	703.67±62.44624
>9	734.11±96.12544

Table 4.4 A summary of the linear model output

Coefficients:

	Estimate Std. Error	t value
(Intercept)	-312.132 125.154	-2.494
Scrotal Circumference	24.129 3.255	7.413
	Pr(>|t|)	
(Intercept)	0.0144*	
Scrotal Circumference	5.09e-11 ***	
Signif.codes: 0 ‘***’ 0.001	‘**’ 0.01 ‘*’ 0.05	‘.’ 0.1 ‘ ’ 1
Residual standard error:	110.9 on 95 degrees	of freedom
Multiple R-squared: 0.3665,	Adjusted R-squared:	0.3598
F-statistic: 54.95 on 1 and	95 D.F., p-value:	5.093e-11

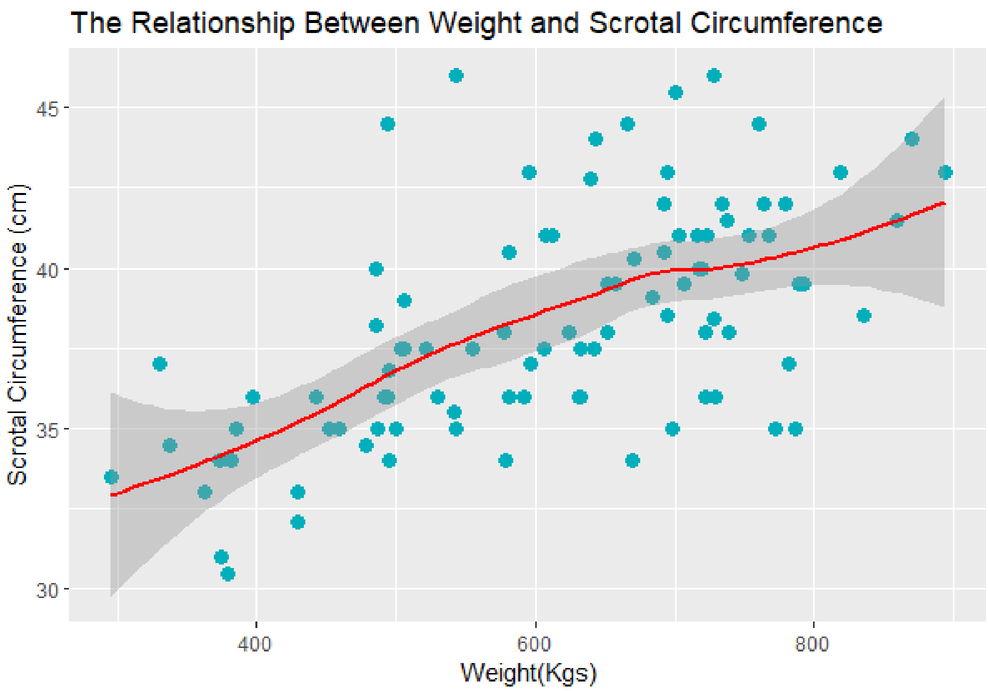

Figure 4.2: A scatter plot showing the relationship between weight and SC

There is a significant relationship between weight and the SC. The SC increases steadily and averages 40 cm at 700 kg. Past 700 kg, the SC increase is relatively slower.

4.1.3 The Relationship Between Breed and Scrotal Circumference

Before an Analysis of Variance (ANOVA) test was done, a box plot was plotted to visualize the data and show the locality and skewness of data groups through their quartiles. The different breeds' mean and median can be observed. The box plot reveals variance in the mean and median between one breed to the next.

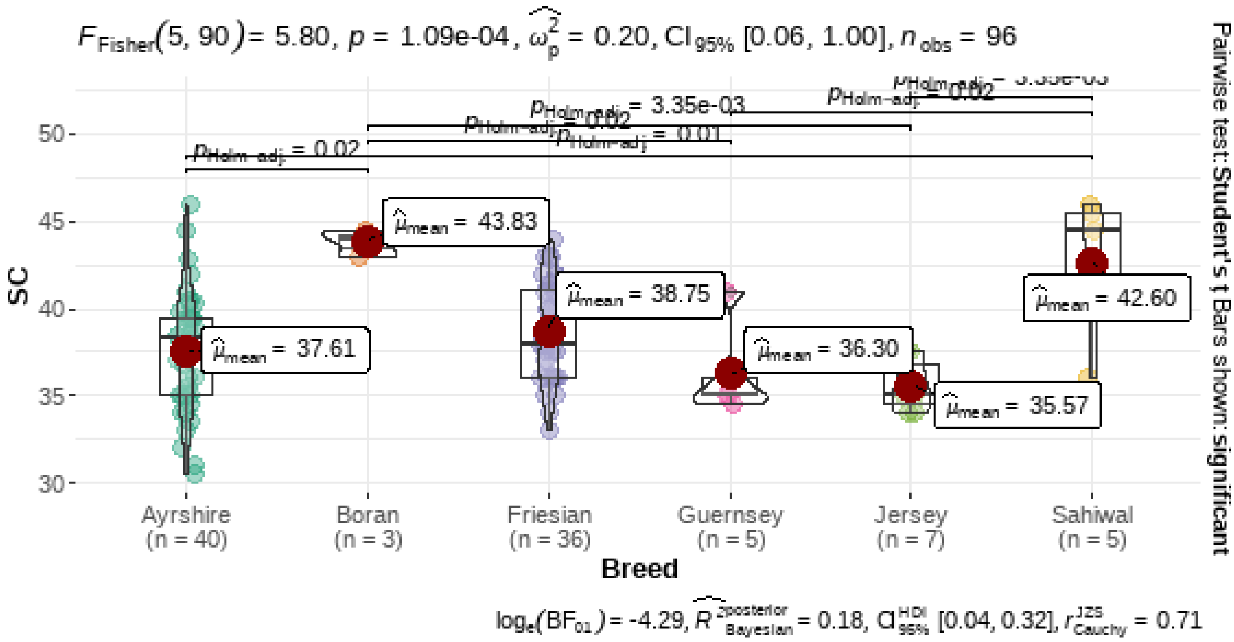

Figure 4.3: A violin box plot showing the means, medians, and distribution of the data on SC of the different breeds

After conducting a Shapiro-Wilk test (p-value >0.05), the data followed a normal distribution. Furthermore, Levene's test was conducted to determine the homogeneity of variance. A Fisher's One-way ANOVA was conducted to determine the variation between the overall SC means of the different breeds. The results indicated a high F (5.80) Statistic, an ES value of 0.20 (>0.14) at 95% CI [0.06, 1.00]. The p-value (p-value <0.001) and BF_{ij} indicated a very strong effect of breed on SC ($BF_{ij} = -4.29$). The R^2 value was 0.18 at 95% CI [0.04, 0.32], indicating that the model used was moderately fitting for the data.

Table 4.5: Breed vs. mean SC

Breed	SC (cm)
Ayrshire *(n=40)*	37.6±3.46
Boran *(n=3)*	43.8±0.764
Friesian *(n=36)*	38.8±2.91
Guernsey *(n=5)*	36.3±2.68
Jersey *(n=7)*	35.6±1.48
Sahiwal *(n=5)*	42.6±4.17

The SC varied from one group to the next (p-value <0.0001). The *Bos indicus* had the highest SC means. The Boran had an SC mean of 43.8cm, followed by the Sahiwal with 42.6cm, as shown in Table 4.4 above. Among the *Bos taurus,* there were individual variations. The Friesian had the highest mean SC of 38.8cm, followed by the Ayrshire with 37.6cm. Guernsey had the second-lowest SC mean of 36.3cm, and Jersey had the lowest SC mean of 35.6cm.

4.1.4 Testicular Ultrasonography Findings

Testicular findings were recorded as either normal or with hyperechoic testicular lesions. Figure 4.4 (a) shows what was considered normal, and 4.4 (b) shows what was considered to have hyperechoic lesions.

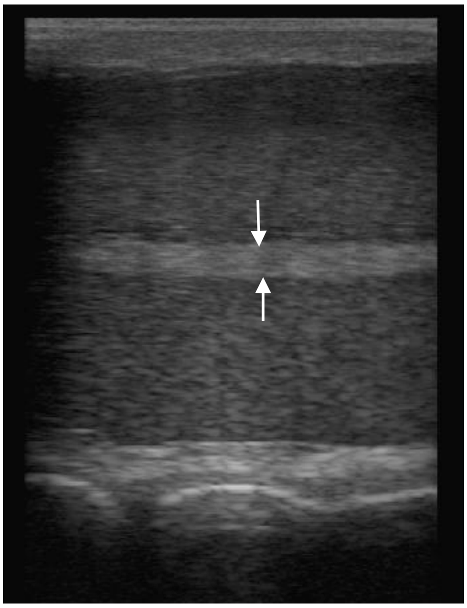

Figure 4.4 (a): Normal testicular ultrasound findings The arrow points towards the hyperechoic mediastinum testis. The rest of the testicular parenchyma shows stippled medium echogenicity

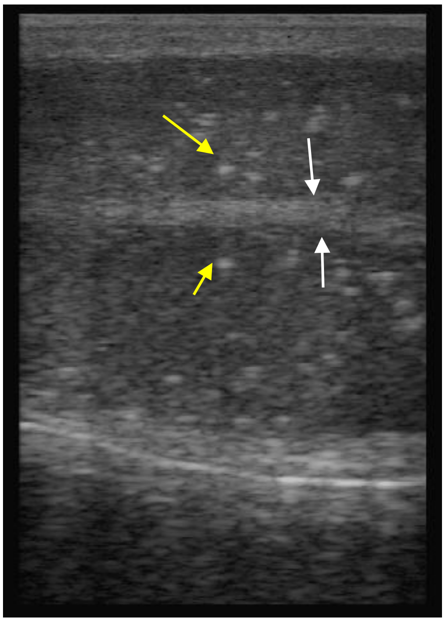

Figure 4.4 (b): Hyperechoic testicular lesions. The yellow arrows point towards the hyperechoic foci

Figures 4.4 (a) and 4.4 (b) show caudal ultrasonographic views along the sagittal plane contrasting findings from two different bulls. The white arrows in both figures show the mediastinum testis. The scrotal wall appears dorsally and ventrally. The yellow arrows in Figure 4.4 (b) show hyperechoic foci, that may be suggestive but not confirmatory of testicular fibrosis. Figure 4.5 below shows the representation of the ultrasound findings across the imaged bulls.

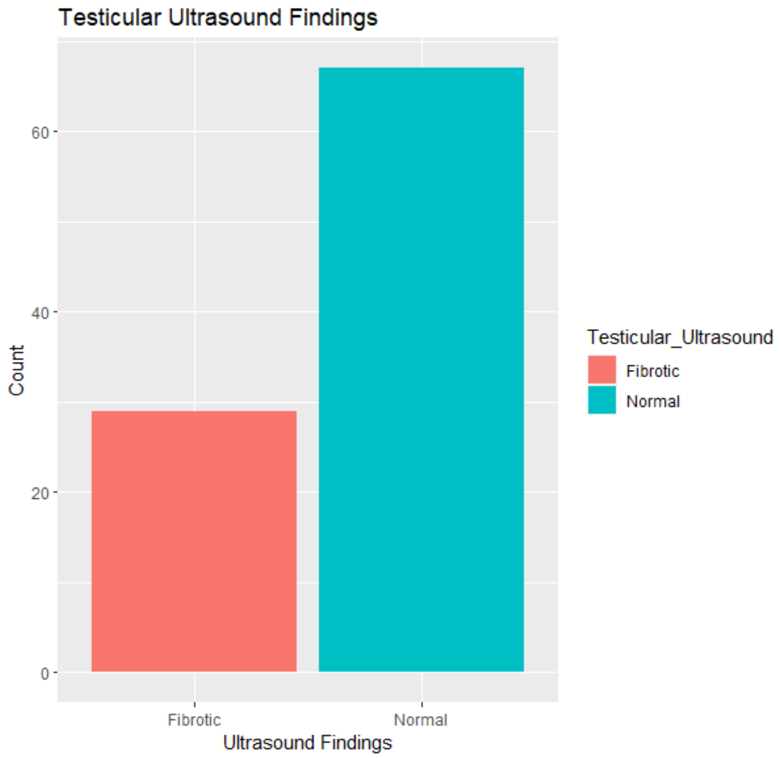

Figure 4.5: Visual representation of the testicular ultrasound findings

Twenty-nine (30.21%) bulls had hyperechoic testicular lesions, while 67 (69.79%) had a normal appearance.

Table 4.6: Comparison between age and Presence of Hyperechoic Testicular Lesions

Age group (Years)	Number Pathologic	Percentage (%)
≤3	0	0
>3-5	3	10.34
>5-7	8	27.59
>7-9	9	31.03
>9	9	31.03

A logistic regression model reveals a significant relationship between age and the presence of hyperechoic testicular lesions (p-value <0.001). Hyperechoic testicular lesions have been associated with aging in bulls and have been observed to set in at 48 months, with 10.34% of the cases recorded starting from this age bracket. The prevalence of hyperechoic testicular lesions is relatively high beyond 60 months. 27.59% of the cases were in bulls aged 60-84 months. The prevalence of hyperechoic testicular lesions was highest in bulls aged beyond 84 months (62.06%).

Table 4.7: Prevalence of Hyperechoic Testicular Lesions in the Different Breeds

Breed	Number with hyperechoic testicular lesions
Ayrshire *(n=40)*	12
Boran *(n=3)*	0
Friesian *(n=36)*	14
Guernsey *(n=5)*	2
Jersey *(n=7)*	1
Sahiwal *(n=5)*	0

Table 4.7 above shows the prevalence of hyperechoic testicular lesions in the different breeds. hyperechoic testicular lesions were only observed in the *Bos taurus* as no case was recorded in the *Bos indicus*, even in the older bulls. However, the sample size of the *Bos indicus (n=7)* was relatively smaller than that of the *Bos taurus (n=89).*

4.2. Results: Objective Two

The inclusion criterion for objective two was all healthy bulls in active collection. Animals meeting the inclusion criteria (*n*=90*)* were distributed among the various breeds, namely Ayrshire (*n*=39), Friesian (*n*=34), Jersey (*n*=7), Boran (*n*=5), and Sahiwal (*n*=5). A total of 487 ejaculates were examined.

4.2.1 Relationship Between Age and Semen quality

Multivariate regression revealed that age significantly affects semen quality (p-value <0.05). However, Individual linear regression shows that age significantly affects only volume (b_1=6.85, t_1=3.33, p-value <0.05). The effect of age on motility (b_1= -0.50, t_1= -1.70, p-value >0.05), post-thaw motility (b_1= -0.38, t_1= -1.17), p-value >0.05), and concentration (b_1= -0.02, t_1= -1.07, p-value >0.05) were not statistically significant. There is, however, a negative but statistically non-significant relationship between age and motility, post-thaw motility, and concentration. Figures 4.6(a), 4.6(b), 4.6(c), and 4.6(d) show the effect of age on the respective semen quality.

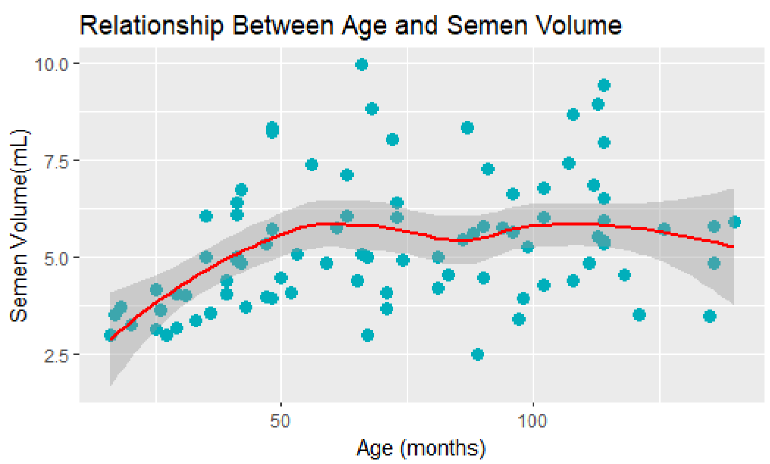

Figure 4.6 (a): Relationship Between Age and Semen Volume

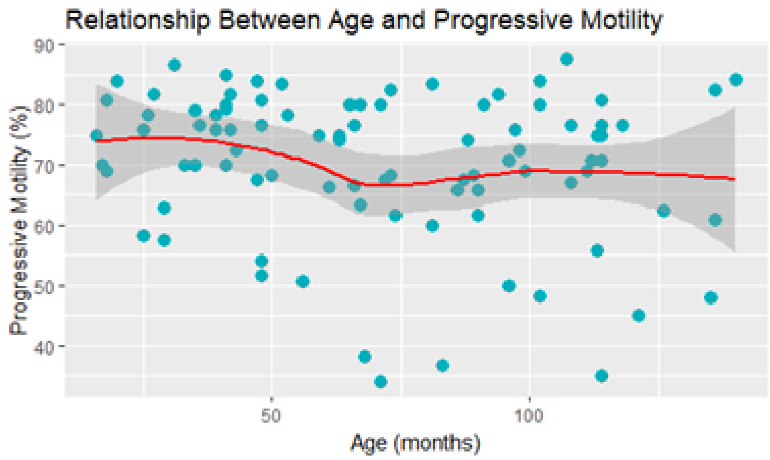

Figure 4.6 (b): Relationship between age and progressive motility

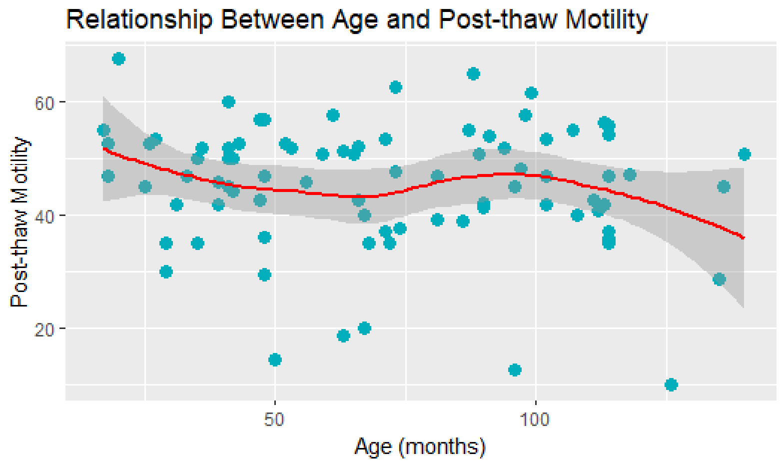

Figure 4.6 (c): Relationship between age and post-thaw motility

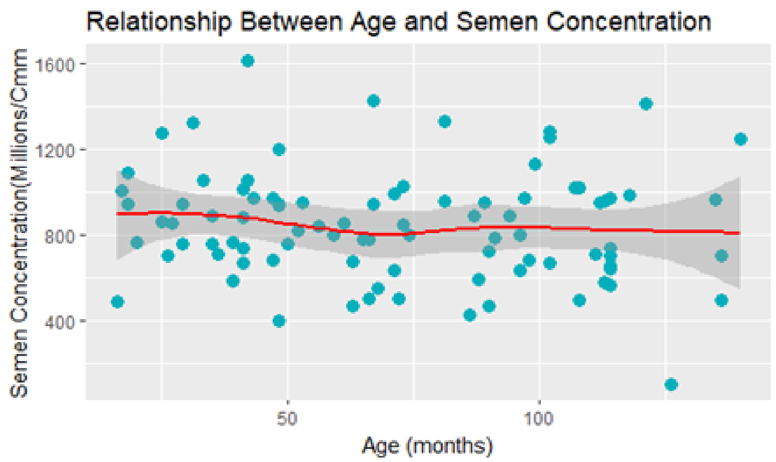

Figure 4.6 (d): Relationship between age and sperm concentration

Table 4.8: A summary of the semen quality against the different age groups

Age group (Years)	*(n)*	Mean volume (ml)	Mean Progressive Motility (%)	Mean Post-thaw motility (%)	Mean sperm concentration (millions/ml)
≤3	16	3.77±0.80	73.49±8.68	47.17±9.37	901.37±215.39
>3-5	20	5.37±1.40	73.45±10.37	46.21±10.34	878.97±248.07
>5-7	18	5.66±1.83	66.37±15.58	42.73±11.96	827.80±272.17
>7-9	19	5.66±1.62	70.88±10.33	47.80±11.59	824.67±252.53
>9	17	5.91±1.65	67.28±14.11	41.84±11.92	787.21±301.95

Table 4.8 summarizes the data on semen quality into five age groups. Semen volume is lowest in bulls aged below 36 months (3.77 ml) and highest in bulls aged above 108 months (5.91 ml). The variance in mean volume among the groups is statistically significant (H=22.27 (4), p-value <0.001). The progressive motility is highest in the first 60 months, after which there is a decrease. The difference is, however, not statistically significant (H=4.29 (4), p-value >0.05).

The Post-thaw motility is highest in bulls aged 84-108 months (47.8%) and lowest in bulls aged above 108 months (41.84%), although the variance is not statistically significant (H=4.021 (4), p-value >0.05). The concentration decreases with age, highest in bulls below 36 months (901.37 million/ml) and lowest in bulls above 108 months (787.21 million/ml). The group differences are not statistically significant (p-value >0.05).

4.2.2 Relationship Between Scrotal Circumference and Semen quality

A linear regression model revealed that the SC significantly affects volume (b_1= 0.58, t_1= 2.60, p-value <0.05). The SC does not significantly affect the progressive motility (b_1= -0.01, t_1= -0.46, p-value >0.05), post-thaw motility (b_1= -0.05, t_1= -1.52, p-value >0.05), and sperm concentration (b_1= -0.003, t_1= -1.92, p-value >0.05). Age is however a potential confounder, explaining this outcome as concentration is expected to increase with SC.

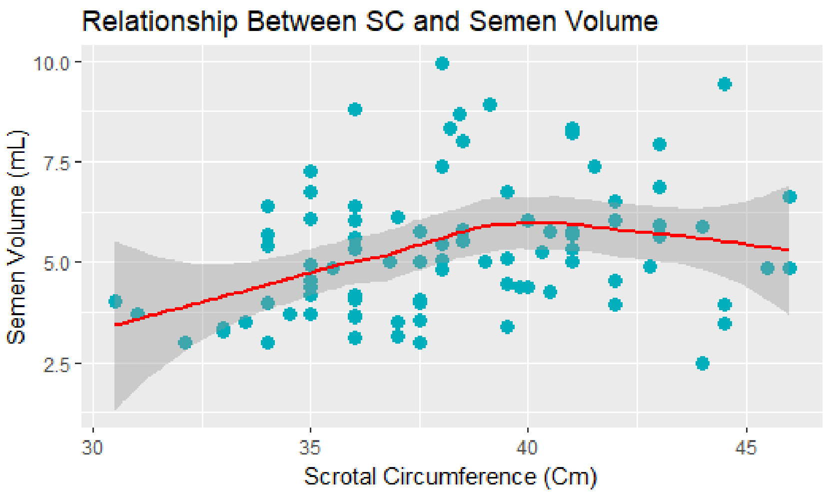

Figure 4.7 (a): Relationship between SC and volume

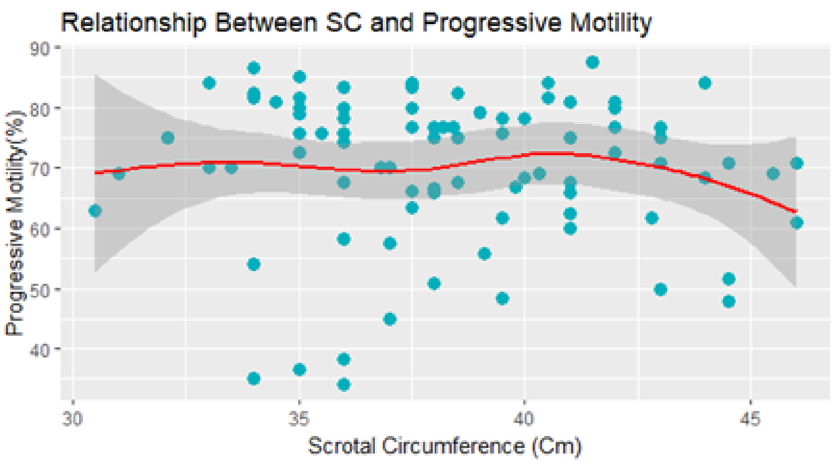

Figure 4.7 (b): Relationship between SC and progressive motility

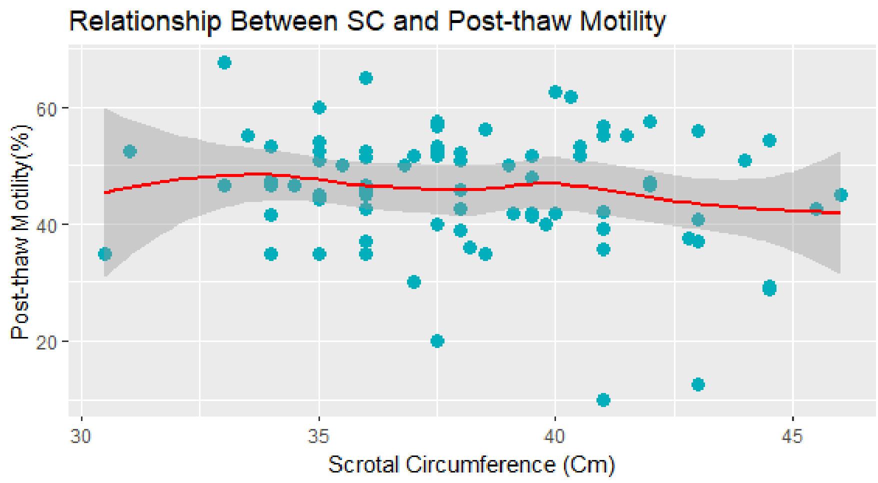

Figure 4.7 (c): Relationship between SC and post-thaw motility

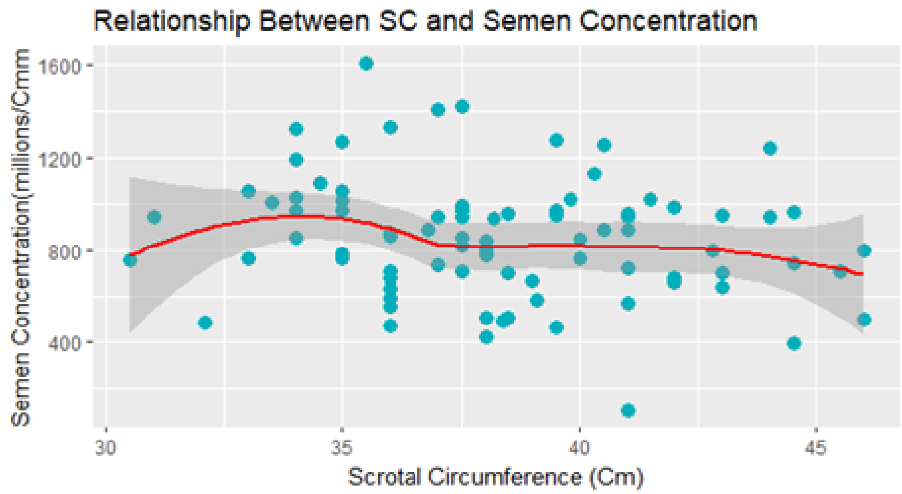

Figure 4.7 (d): Relationship between SC and sperm concentration

4.2.3 Relationship Between Body Weight and Semen quality

A linear regression model reveals that weight significantly affects semen volume (b_1= 39.94, t_1= 5.16, p-value <0.001). However, the weight does not significantly affect the progressive motility (b_1= -1.18, t_1= -0.997, p-value >0.05), the post-thaw motility (b_1= 0.64, t_1= 0.44, p-value >0.05), and the concentration (b_1= -0.08, t_1= -1.39, p-value >0.05).

4.2.4 Relationship Between Body Condition Score and Semen quality

The average body condition score was 3.36. A linear regression reveals that there is a negative relationship between the body condition score and the post-thaw motility (b_1= -0.01, t_1= -2.12, p-value <0.05) and the concentration (b_1= -0.0004, t_1= -2.02, p-value <0.05). There was no statistically significant relationship between the body condition score and semen volume (b_1= 0.02, t_1= 0.76, p-value >0.05) and progressive motility (b_1= -0.004, t_1= -0.96, p-value >0.05).

4.2.5 Relationship Between Breed and Semen quality

Table 4.9: Relationship Between Breed and Semen Volume, Progressive Motility, Post-thaw motility, and Sperm concentration

Breed	Number of Bulls (*n*)	volume (ml)	Progressive Motility (%)	Post-Thaw Motility (%)	Concentration (millions/ml)
Ayrshire	39	5.61±1.86	71.10±10.35	48.07±8.90	870.23±254.65
Boran	5	4.64±1.81	61.93±12.14	25.01±15.73	796.55±152.86
Friesian	34	5.29±1.51	69.82±14.15	47.37±7.20	815.72±209.54
Jersey	7	4.41±1.30	80.24±7.79	43.69±12.56	1135.62±220.04
Sahiwal	5	4.93±0.66	62.33±6.83	33.88±14.69	477.94±244.08

The volume was highest in Ayrshire (5.61 ml) and lowest in Jersey (4.41 ml). The breed does not significantly affect semen volume (H=3.89 (4), p-value >0.05). The progressive motility was highest in Jersey (80.24%) and lowest in Boran (61.93%). The variation among the breeds is statistically significant (H=14.79 (4), p-value <0.01).

The post-thaw motility significantly varied from one breed to the other (H=12.943 (4), p-value <0.05), and it was highest in Ayrshire (48.07%) and lowest in Boran (25.01%). One-way ANOVA shows that the breed also significantly affected the concentration (p-value <0.001). The highest concentration was recorded in Jersey (1135.62 million/ml) and the lowest in Sahiwal (477.94 million/ml).

4.2.6 Effects of Testicular Hyperechoic Testicular Lesions on Semen Quality

Table 4.10: Effects of Hyperechoic Testicular Lesions on Semen Quality

Testicular Ultrasound Findings	Number of bulls (*n*)	Semen volume (ml)	Progressive Motility (%)	Post-thaw Motility (%)	Sperm concentration (millions/ml)
Normal	26	5.05±1.67	69.92±12.91	44.55±12.91	832.97±261.59
Hyperechoic lesions	62	5.88±1.51	71.21±10.93	49.37±10.93	865.93±256.50

A Shapiro-Wilk test revealed that only the sperm concentration followed a normal distribution (p-value >0.05), and therefore, a two-way Fisher's ANOVA was conducted. The semen volume, progressive motility, and post-thaw motility did not follow a normal distribution (p-value <0.05), and therefore, the Kruskal-Wallis test was used to determine the variance.

The volume varied significantly between the two groups (H=6.0988 (1), p-value <0.05). Bulls with hyperechoic testicular lesions had the highest semen volume (5.88 ml) compared to bulls with normal testicular echotexture (5.05 ml). The presence of hyperechoic testicular lesions does not, however, affect sperm motility (H=0.06 (1), p-value >0.05), post-thaw-motility (H= 2.94 (1), p-value >0.05), and concentration (p-value >0.05).

4.3 Results: Objective Three

Retrospective data on 11019 ejaculates was retrieved and uploaded to R studio for data analysis. The data included the age, breed, semen volume, progressive sperm motility, and sperm concentration. Only data from the Ayrshire and Friesian was adequate to obtain averages distributed throughout the years.

However, data from three bulls (1 Ayrshire and 2 Friesian) were not included in the linear model and line graphs due to their small sample size and lack of averages, as shown in Table 4.11 below.

4.3.1 The Relationship Between Age and Breed and Semen Volume

Table 4.11: The relationship between age and breed and semen volume

Age (years)	Ayrshire volume (ml)	Number of bulls *(n)*	Number of ejaculates	Friesian volume (ml)	Number of bulls *(n)*	Number of ejaculates
1	3.54±0.88	16	389	3.14±0.84	15	438
2	4.60±0.96	18	710	3.97±0.74	15	991
3	5.35±1.21	18	687	4.80±0.76	15	750
4	5.86±1.69	14	463	5.35±0.51	12	471
5	6.44±1.89	12	231	5.81±0.74	13	639
6	6.09±1.26	14	519	5.54±0.57	10	426
7	6.35±1.64	18	836	5.80±0.94	13	605
8	6.15±1.94	11	540	5.59±0.60	12	747
9	6.64±2.61	4	95	5.35±0.79	9	557
10	4.14	1	66	6.34	2	157
11	3.82	1	13	6.18	1	73

Table 4.11 shows the relationship between age and the average semen volume for the Ayrshire and the Friesian from 12-108 months. The averages from 120-132 months reflect the yearly averages for one bull per breed whose age exceeds 108 months. The volume in Ayrshire was related to age (p-value <0.01). The volume increases with age; the highest volume is recorded at 108 months (6.64 ml).

Only one Ayrshire bull was older than 108 months; its volume at 120 months was 4.14 ml, and at 132 months, 3.82 ml. The Friesian semen volume also relates to age (p-value <0.05).

The volume increases with age, peaks at 60 months (5.81 ml), and decreases from 96 months. However, only two Friesian bulls were aged 120 months, and one was aged 132 months, and the mean volume at 120 months was 6.34 ml and 6.18 ml at 132 months, and these values were higher than the highest mean volume recorded in the Friesian across the other years.

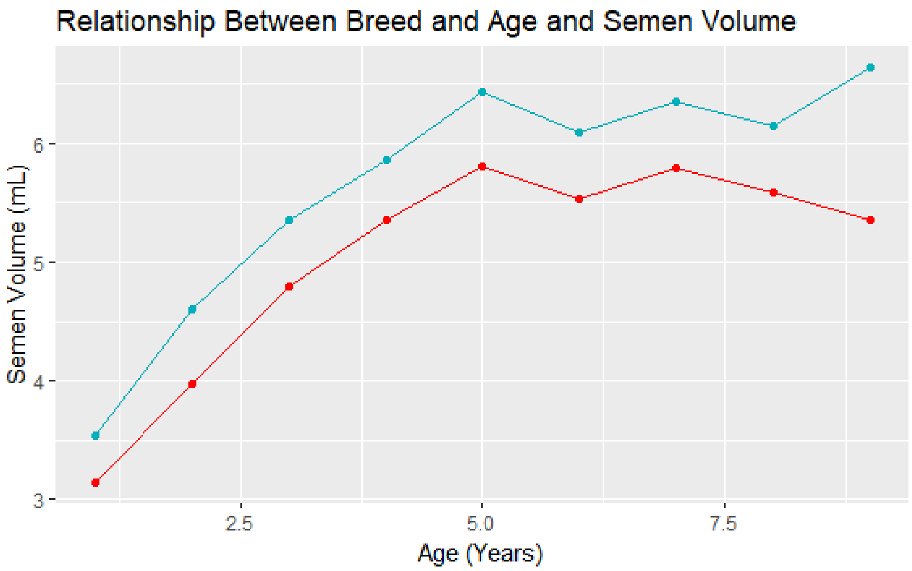

Figure 4.8: A linear graph showing the relationship among age (12-108 months), breed, and mean semen volume. The two breeds are Ayrshire (blue), and Friesian (Red)

4.3.2 The Relationship Between Age and Breed and Progressive Motility

Table 4.12: The Relationship Between Age and Breed and Progressive Motility

Age (years)	Ayrshire progressive motility (%)	Number of bulls *(n)*	Number of ejaculates	Friesian progressive motility (%)	Number of bulls *(n)*	Number of ejaculates
1	73.27	16	389	72.88	15	438
2	75.88	18	710	79.05	15	991
3	80.61	18	687	81.30	15	750
4	82.21	14	463	79.30	12	471
5	84.40	12	231	83.82	13	639
6	82.16	14	519	83.03	10	426
7	78.15	18	836	82.32	13	605
8	76.84	11	540	80.06	12	747
9	77.35	4	95	76.75	9	557
10	78.05	1	66	84.45	2	157
11	82.31	1	13	78.90	1	73

Table 4.12 shows the relationship between age and breed and progressive sperm motility. The progressive motility does not increase with age (p-value > 0.05). However, there is an increasing trend, though minimal, in the Ayrshire (*rho*=0.2) and the Friesian (*rho*=0.27), as shown in figure 4.3.2, where motility increases up to 60 months in both the Ayrshire (84.40%) and Friesian (83.32%) and starts dropping from 72 months. The highest motility in the Ayrshire (84.40%) and Friesian (83.03%) are recorded at 60 months. However, the three bulls above 108 months maintained high progressive motilities even at 120 and 132 months.

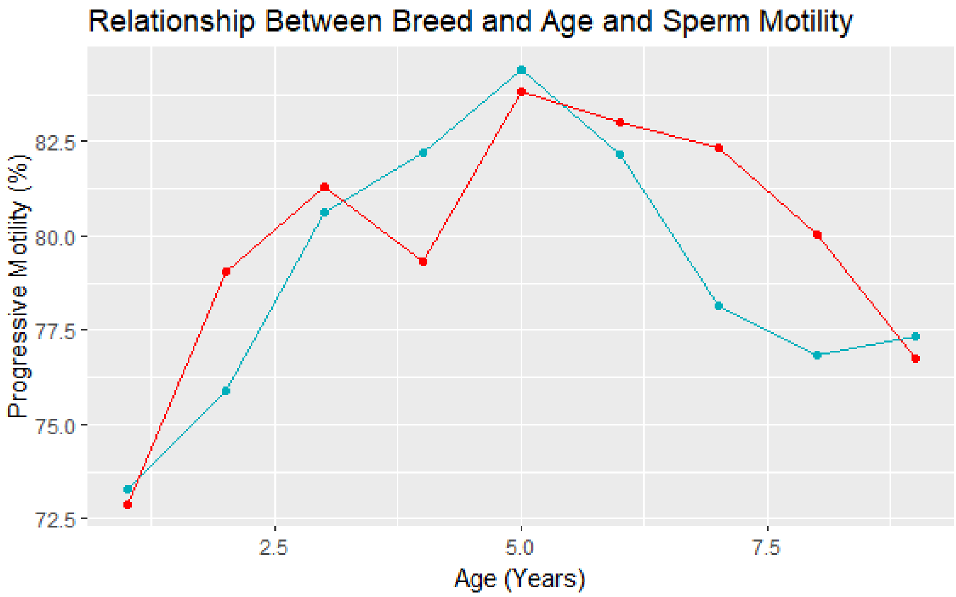

Figure 4.9: A linear graph showing the yearly averages of the progressive motility for Ayrshire (Blue) and Friesian (Red) from 12-108 months.

4.3.3 The Relationship Between Age and Breed and Sperm concentration

Table 4.13: The Relationship Between Age and Breed and Sperm concentration

Age (years)	Ayrshire concentration (millions/ml)	Number of bulls *(n)*	Number of ejaculates	Friesian concentration (millions/ml)	Number of bulls *(n)*	Number of ejaculates
1	824.82±344.99	16	389	868.57±191.98	15	438
2	1059.83±231.62	18	710	1081.38±234.64	15	991
3	1118.98±283.48	18	687	1186.15±164.63	15	750
4	1092.86±298.31	14	463	1187.81±235.10	12	471
5	1100.90±220.33	12	231	1261.31±195.50	13	639
6	1063.26±383.93	14	519	1121.87±310.53	10	426
7	835.19±320.95	18	836	1116.44±295.03	13	605
8	716.93±151.56	11	540	1025.12±273.87	12	747
9	798.97±458.54	4	95	875.67±169.36	9	557
10	1096.67	1	66	1199.14	2	157
11	824.69	1	13	1190.6	1	73

Table 4.13 shows the average sperm concentrations at different ages in the Ayrshire and the Friesian breeds from 12-96 months. Additionally, there are two bulls (1 Ayrshire and 1 Friesian) aged 132 months, whose parameters are also displayed. In Ayrshire, age does not significantly affect concentration (p-value>0.05, *rho*= -0.45)) but as seen in Figure 4.10

below, the highest mean sperm concentration is obtained at 36 months (1118.98 million/ml) in Ayrshire. Sperm concentration drops from 72 months. The Friesian sperm concentration is not significantly affected by age (p-value >0.05, *rho*= -0.08)).

However, the sperm concentration peaks at 60 months (1261.31 million/ml) in both Ayrshire and Friesian and starts decreasing from 72 months. The other three bulls' concentrations remained high despite old age, as shown in Table 4.3.3 above. At 120 months, the Ayrshire bull had a high concentration (1096.67 million/ml) which lies within the average peak values, and at 132 months, the volume drops to 824.69 million/ml. The Friesian average sperm concentration for two bulls at 120 months (1199.14 million/ml) and one bull at 132 months (1190.6 million/ml) remains high. Therefore, the average sperm concentration at 120 and 132 months is closer to the sperm concentration obtained at the peak.

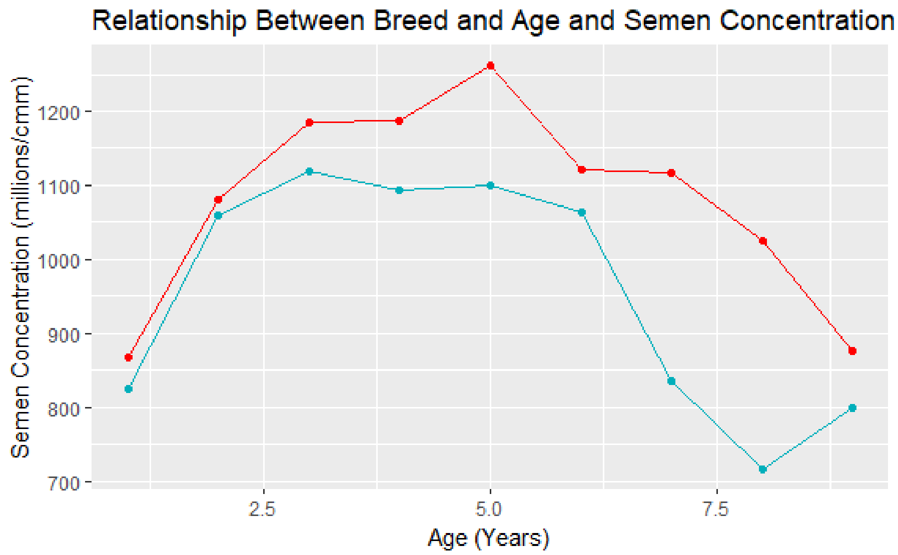

Figure 4.10: A line graph showing the yearly sperm concentration averages for Ayrshire (Blue) and Friesian (Red).

4.3.4 Relationship Between Age and Semen Volume

Table 4.14: Relationship between Age and Semen Volume

Age (Years)	Mean volume	Number of bulls *(n)*	Number of ejaculates
1	3.42±0.95	32	841
2	4.34±0.93	35	1775
3	5.16±1.04	36	1493
4	5.79±1.43	29	956
5	5.99±1.37	29	900
6	5.73±1.13	27	988
7	6.09±1.68	35	1474
8	5.78±1.46	26	1384
9	5.52±1.62	17	812
10	4.81±1.16	5	303
11	4.40±1.57	3	93

Table 4.14 shows the yearly averages of bulls from across all breeds. Spearman's correlation shows that age does not significantly affect semen volume (p-value >0.05), but there is a minimal positive correlation (*rho*= 0.18). However, the volume increases from the first year in collection and peaks between 60-84 months. The highest semen volume is recorded at 84 months (6.09 ml). From 84 months, the volume starts to decrease and is at its lowest at 132 months. As shown in Figure 4.11 below

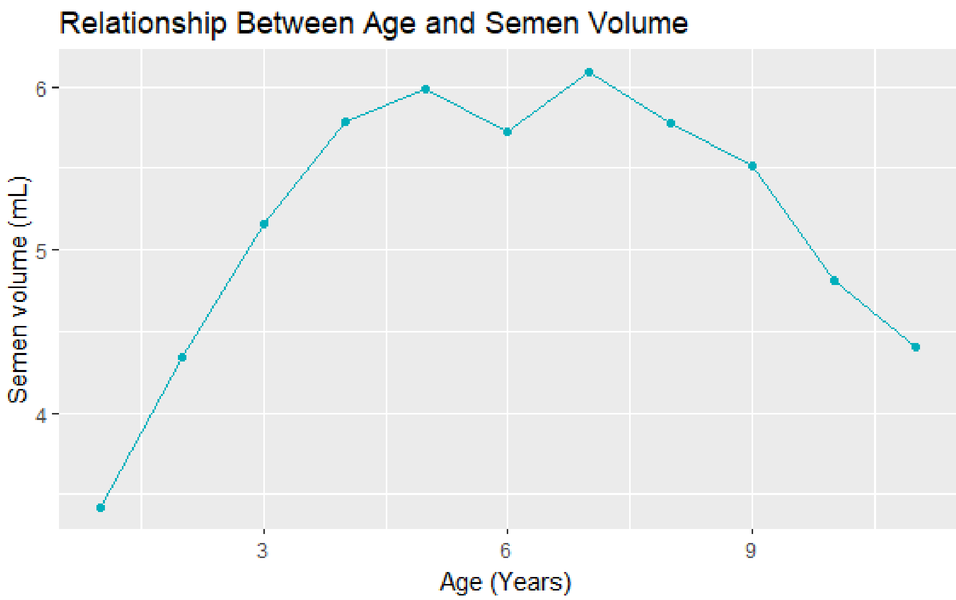

Figure 4.11: A line graph showing the relationship between age and semen volume

4.3.5 Relationship Between Age and Progressive Sperm Motility

Table 4.15: Relationship Between Age and Progressive Motility

Age (Years)	Mean Progressive motility (%)	Number of bulls *(n)*	Number of ejaculates
1	73.33± 9.31	32	841
2	77.04±5.77	35	1775
3	79.76±6.13	36	1493
4	80.52±5.54	29	956
5	83.27±3.83	29	900
6	81.37±5.89	27	988
7	78.84±8.18	35	1474
8	78.43±5.02	26	1384
9	76.53±5.42	17	812
10	79.34±4.11	5	303
11	75.68±8.70	3	93

Table 4.15 shows the relationship between age and progressive sperm motility. Spearman's correlation was used to compare the non-linear data, revealing that age does not significantly affect sperm motility (p-value >0.05), with minimal negative correlation (*rho*= -0.1). Progressive sperm motility is lowest during the first year (73.33%) of collection but increases ith age and peaks at 60 months (83.27%). After 60 months, the motility decreases with time.

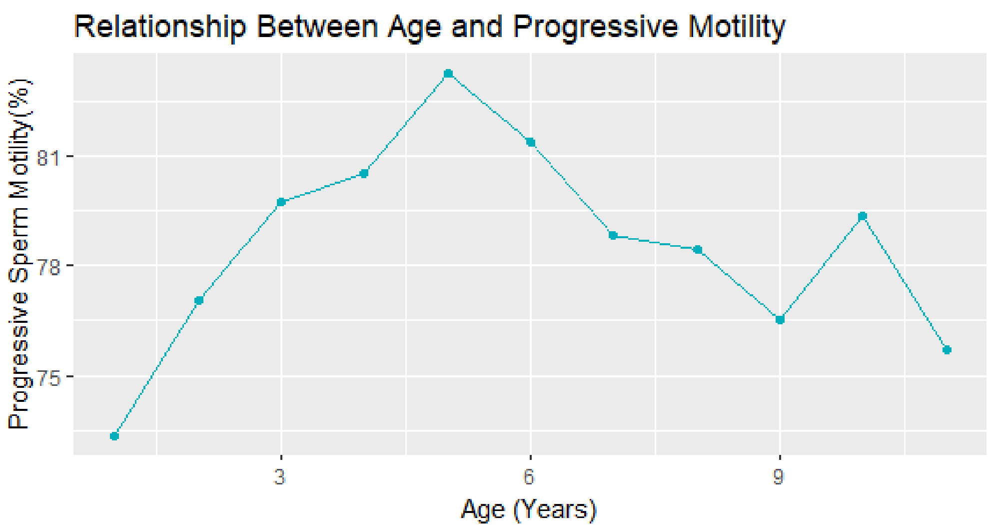

Figure 4.12: A line graph showing the relationship between age and progressive semen motility

4.3.6 Relationship Age and Sperm concentration

Table 4.16: Relationship between Age and Sperm concentration

Age (Years)	Mean concentration (million/ml)	Number of bulls *(n)*	Number of ejaculates
1	851.52±277.81	32	841
2	1061.61±225.70	35	1775
3	1111.18±298.56	36	1493
4	1101.18±303.22	29	956
5	1112.16±300.36	29	900
6	1000.13±410.14	27	988
7	913.65±346.56	35	1474
8	878.63±276.42	26	1384
9	798.56±270.33	17	812
10	888.02±359.23	5	303
11	846.60±333.5	3	93

Table 4.16 shows the relationship between age and sperm concentration. Spearman's test for non-linear data reveals no significant relationship between age and sperm concentration (p-value >0.05). Sperm concentration decreases with age (*rho*=0.54). The concentration increases from the first year of collection and peaks at 36-60 months, as shown in Figure 4.13 below. The highest concentration is observed at 60 months (1112.16 million/ml). The concentration then starts decreasing after 60 months, with the lowest concentration at 108

months (798.56 million/ml)

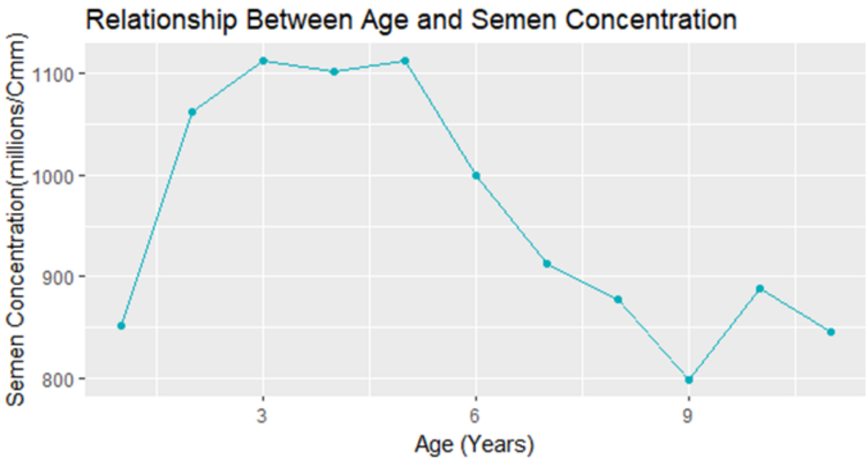

Figure 4.13: A line graph showing the relationship between age and sperm concentration

4.3.7 Relationship Between Breed and Semen quality

Table 4.17: Relationship Between Breed and Semen Volume, Progressive Motility, and Concentration

Breed	Number of Bulls *(n)*	Number of ejaculates	Mean Volume (ml)	Mean Progressive Motility (%)	Mean Concentration (million/ml)
Ayrshire	18	4549	5.49±1.20	78.74±3.68	973.10±192.59
Boran	1	32	3.98	55.03	310.39
Friesian	15	5854	4.97±0.52	79.74±2.02	1081.60±147.30
Guernsey	1	263	7.29	80.92	1128.88
Sahiwal	3	321	4.62±0.56	73.20±3.54	523.78±47.60

Table 4.17 shows the relationship between the breed and semen volume, progressive motility, and concentration. The breed comparison did not include Boran and Guernsey due to the small sample size (*n*=1*)*. A Shapiro-Wilk test revealed that the volume did not follow a normal distribution (p-value <0.05). Therefore, a non-parametric (Kruskal-Wallis) test was used to determine variance among the breeds. The breed does not significantly affect semen volume (H=3.2301 (2), p-value >0.05). Nonetheless, the Ayrshire had the highest semen volume (5.49 ml), and the Sahiwal had the lowest (4.62 ml).

The motility and concentration followed a normal distribution (p-value >0.05). A one-way ANOVA was therefore indicated. The breed significantly affects the progressive motility (p-value <0.01). Semen motility is highest in the Friesian (79.74%) and lowest in the Sahiwal (73.20%). Sperm concentration varies significantly from breed to breed (p-value <0.001). Concentration is highest in the Friesian (1081.6 million/ml) and lowest in the Sahiwal (523.78 million/ml). It is, however, worth noting that the method of semen collection differs. In the *Bos taurus*, semen collection was done using the AV instead of the EEJ in the *Bos indicus.*

4.3.8 Effects of Hyperechoic Testicular Lesions on Semen Quality Using Retrospective Data

Table 4.18: Relationship between Testicular Ultrasound Findings and Semen Volume, Progressive Motility, and Sperm concentration

Testicular ultrasound Findings	Number of Bulls (*n*)	Mean volume (ml)	Mean Progressive motility (%)	Mean concentration (million/ml)
Normal	20	5.11±1.12	76.78±6.34	909.28±282.88
Hyperechoic lesions	18	5.35±0.90	79.62±2.79	1031.37±175.15

Table 4.18 shows the average volume, progressive motility, and sperm concentration. Of the 38 selected bulls, 18 had hyperechoic testicular lesions. A Shapiro-Wilk normality test was done to determine the normality of the data. The volume and progressive motility did not follow a normal distribution (p-value <0.05), necessitating a non-parametric test.

Kruskal-Wallis test revealed that the presence of hyperechoic testicular lesions does not significantly affect semen volume and progressive motility (H=3.5012 (1), p-value >0.05). Sperm concentration followed a normal distribution (p-value >0.05), so a parametric test was used. One-way ANOVA revealed no significant variance between the bulls with normal and fibrotic testicular findings (p-value >0.05).

CHAPTER FIVE

5.1 Discussion

The current study evaluated the effects of bull characteristics and testicular attributes on sperm parameters in bulls kept at a government-owned animal genetic resources center. The study has demonstrated that semen quality, to an extent, is affected by age, breed, body weight, SC, and BCS. The generated information will help guide decision-making or bull management in AI stations by providing vital information on culling criteria and the selection of bulls for AI based on bull parameters and attributes. Ninety-six bulls were initially recruited into the study and retained for objective one.

However, only 88 bulls from objective one were retained for objective two because of morbidities and mortalities encountered during the study period. Two Boran breed bulls whose body weight and SC had not been determined (due to difficulties in restraint) were introduced, bringing the total tally to 90. Additionally, 10-year retrospective data on 38 bulls aged above 84 months was mined to compare the effect of age, breed, and testicular echotexture on semen quality.

Scrotal circumference (SC) is a parameter used to select bulls for breeding, with those with a high SC being more desirable since semen volume increases with increasing SC (Latif et al., 2009). According to Chenoweth et al. (1993), a bull should have a mean SC of 31cm at one year of age; in the Current study, the youngest bull, aged 16 months, had a SC of 32.1cm, while the oldest bull aged 140 months had a SC of 44 cm.

It is further noted that the SC increases with age. Young bulls between 16 and 36 months recorded the lowest mean SC (34.41cm), while bulls above 108 months had the highest mean SC (41.53cm). The study agrees with previous reports that age affects the SC (Brito et al., 2002; Devkota et al., 2008; Perumal, 2014) in *Bos indicus* and *Bos taurus*. Further, the current study depicts SC to increase exponentially up to 60 months; thereafter, the increase is relatively slow, even in bulls over 100 months. Contrary to the finding by Ahmad et al. (2011) that SC increases with age from birth up to 100 months and plateaus thereafter.

The body weight significantly affected the SC (p-value <0.0001), with heavier bulls exhibiting a larger SC. These findings are consistent with previous studies that found a high correlation between body weight and SC (Devkota et al., 2008; Fordyce et al., 2014; Perumal, 2014)

The SC exhibits a significant variation from one breed to the next (p-value <0.0001, BF_{ij} = -4.29) across the ages; the *Bos indicus* had a larger SC than the *Bos taurus*. The Boran had the highest mean SC (43.83cm), and the Sahiwal had the second-highest mean SC (42.60 cm). Among the *Bos taurus*, the Friesian had the highest mean SC (38.75cm), followed by the Ayrshire (37.61cm).

Guernsey had a mean SC of 36.30 cm, and Jersey had the lowest mean SC (35.57 cm). However, Brito et al. (2002) found no significant differences in the SC between the *Bos taurus*, cross-breeds, and *Bos indicus*. Further, the Sahiwal had a greater SC in the current study (42.60 cm) contrary to Ahmad and Asmat (2005), who recorded a mean SC of 32.38cm in Sahiwal aged 60 months and above.

Semen volume comprises sperm cells and seminal plasma from the accessory sex glands, and it has been demonstrated that a high semen volume doesn’t necessarily correlate to quality semen (Argiris et al., 2018). Semen volume is affected by a myriad of factors; among them are: the age of the bull, collection frequency, bull handling, temperature during collection, and semen collector (Fuerst-Waltl et al., 2006). The current study reveals that semen volume is significantly affected by age (p-value <0.01). Semen volume was lowest in bulls below 36 months (3.77 ml) and highest in bulls aged above 108 months (5.91 ml).

A retrospective analysis of data on semen volume revealed that the average semen volume was lowest in bulls aged below 24 months (3.42 ml) and highest in bulls aged 84 months (6.09 ml), with a decreasing trend observed thereafter. The study concurs with the findings by Fuerst-Waltl et al. (2006), who reported that volume increases with age, and the highest volume was reported in bulls above 72 months.

Similarly, Argiris et al. (2018) reported that volume increased with age; the highest volumes in two AI stations were obtained at 84 months and above 108 months, respectively. Semen volume increases with age due to physiological changes such as increased body mass, hypothalamic-pituitary-testicular axis activity, simultaneous accessory glands, and testicular development (Almquist, 1978; Balić et al., 2012).

Forward progressive motility is an essential parameter in sperm quality evaluation. The sperm can exhibit a forward motion in a defined direction, which is vital for movement inside the female reproductive system (Hafez & Hafez, 2013). High forward progressive motility; >81% has been associated with high fertility; as such, ejaculates have more embryos reaching the 2-cell stage, 4-cell stage, and blastocyst stages in in-vitro fertilization trials (Li et al., 2016).

The forward progressive motility in this study was highest in bulls aged below 60 months in the prospective (73.5%) and retrospective studies (83.3%). After 72 months in both groups, there was a decrease in the mean forward progressive motility, although the difference is not statistically significant (p-value >0.05). Brito et al. (2002) and Rehman et al. (2016) also found no significant effect of age on forward progressive motility. The recorded decline in forward progressive motility could be due to age-related physiological and hormonal changes and oxidative damage due to reactive oxygen species (Sloter et al., 2006).

The sperm concentration is the number of sperm cells in an ejaculate, and it determines the number of doses one can obtain from an ejaculate. As reported with volume, the concentration can be influenced by factors such as the frequency of semen collection (Fuerst-Waltl et al., 2006). In concurrence with other reports (Brito et al., 2002; Rehman et al., 2016; Argiris et al., 2018), there was an insignificant effect of bull age on sperm concentration (p-value >0.05).

The current study noted that sperm concentration was highest in bulls below 36 months (901.37 million/ml). A decreasing trend after 36 months was recorded, with the lowest concentration recorded in bulls aged above 108 months (787.21 million/ml). In the retrospective group, sperm concentration was highest from 24-60 months and started decreasing from 72 months. The decrease in sperm concentration is primarily due to testicular degeneration that sets in with old age (Barth et al., 2008). However, the decrease in concentration with age was not perceived to affect fertility significantly.

Ahmad et al. (2003) and Murphy et al. (2018) have had conflicting reports on the effect of age on post-thaw motility. In agreement with Ahmad et al. (2003), this study found no significant effect of age on this parameter (p-value >0.05), although it was highest in bulls aged 84-108 months (47.80%).

The current study found a significant relationship between SC and semen volume (p-value <0.05). Latif et al. (2009) and Rashid et al. (2015) also reported similar findings, where semen volume increases with SC due to a large seminiferous tubule surface area hence, high semen production. A large SC does not necessarily negatively affect these parameters as they are mostly age-related effects and it has already been shown in this study that the SC increases with age. Age, is therefore, a potential confounder in this finding.

Body weight is a significant parameter in bulls, as an increase in bull weight has a corresponding effect on semen volume (Siddiqui et al., 2008). The current study revealed similar findings, as body weight had a significant positive effect on semen volume. Compared to the age and the SC, body weight had the highest significance (p-value <0.001).

However, the body weight did not significantly affect the other parameters (p-value >0.05). A similar study by Gopinathan et al. (2018) reported a significant effect of body weight on semen volume and no significant effect on post-thaw motility. However, contrary to the current study, there was a significant effect on sperm concentration and forward progressive motility.

The breed significantly affected sperm concentration (p-value <0.001), forward progressive motility (p-value <0.01), and post-thaw motility (p-value <0.05). The volume did not significantly differ among the breeds in both study groups (p-value >0.05). These findings in semen volume differ from Nasrin et al. (2008) and Lemma and Shemsu (2015), but this could be due to the different breeds studied.

Jersey had the highest forward progressive motility (80.24%) (p-value <0.01), and this is in agreement with Lemma and Shemsu (2015). Sperm concentration was also highest in Jersey (1135.62 million/ml) (p-value <0.001). Boran had the lowest forward progressive motility (61.93%) (p-value <0.01), while the Sahiwal had the lowest sperm concentration (477.94 million/ml) (p-value <0.001). However, Lemma and Shemsu (2015) reported the highest concentration in the Boran (1.35 billion/ml). In the current study, the post-thaw motility was highest in the Ayrshire (48.07%) and lowest in the Boran (25.01%). There is, however, a scarcity of literature comparing breeds and post-thaw motility.

The current study can’t however, conclude that *Bos indicus* have poor semen parameters as seen in the results as the methods used for semen collection are different. The *Bos indicus* (Sahiwal and Boran) had consistently poor parameters in the current study. This difference can be attributed to the use of EEJ in semen collection, which interferes with semen quality by raising the temperature on the scrotal skin covering the cauda epididymides (Kastelic et al., 1996) and it is an involuntary process that has been associated with pain during ejaculation (Mosure et al., 1998).

The BCS had a significant negative relationship with sperm concentration and post-thaw motility (p-value <0.05). Over-conditioned bulls had low sperm concentration because obesity is associated with poor thermoregulation due to scrotal insulation (Setchell, 1978) and no significant effect on the semen volume and forward progressive motility (p-value >0.05). Beran et al. (2011) also found no significant effect of BCS on semen volume. However, the same study reported a significant positive relationship between BCS and sperm concentration. There is however need for further research to investigate the link between high BCS and post-thaw motility.

A testicular ultrasound to determine the echotexture revealed the presence of hyperechoic testicular lesions in 29 of 96 bulls (30.21%). Both sagittal and transverse views were used, but the sagittal view revealed an excellent exposure of the individual testicle and the mediastinum testes, with both the dorsal and ventral borders. The presence of hyperechoic testicular lesions increased with age, according to a logistic regression model that implied a strong statistically significant relationship (p-value <0.001). The presence of hyperechoic testicular lesions is suggestive but not confirmatory of testicular fibrosis.

A study conducted by Barth et al. (2008) in bulls revealed that fibrotic lesions were present as early as 5-6 months persisted until at least 12-14 months and disappeared thereafter. While the cause of the fibrotic lesions in the young bulls remained unclear, the current study used bulls aged above 16 months, and no hyperechoic testicular lesions were noticed until 48 months of age. However, the prevalence was highest in bulls above 84 months (62.06%), implicating age as a potential risk factor. Furthermore, aging in the bull is associated with marked progressive fibrosis at the ventral aspect of the testis according to Humphrey and Ladds (1975).

The presence of hyperechoic testicular lesions did not significantly affect semen quality (p-value >0.05) in prospective and retrospective studies. However, in the prospective study, the group with hyperechoic testicular lesions had the highest semen volume (p-value <0.05). This finding is primarily because the current study established a very strong relationship between age and the presence of hyperechoic testicular lesions (p-value <0.001) and a relationship between age and semen volume (p-value <0.01). Furthermore, the older bulls have fully developed accessory sex glands, hence high seminal plasma.

The current study's findings agree with Barth et al. (2008) and Tomlinson et al. (2017). Barth et al. (2008) recorded normal semen morphologies of up to 94% even in bulls with severe testicular fibrosis, suggesting that the remaining normal testicular parenchyma continues to produce normal sperms.

The study provides critical information for use during BSE in Kenya and other countries within the tropics. The current findings have bridged knowledge gaps on the culling criteria, methods of semen collection, and appropriate BCS for AI bulls. The study has also provided detailed information on the relationship between bull signalment and testicular parameters, including the determination of testicular echotexture, the predisposition, and its effects on semen quality. However, more studies are required to clarify age-related hyperechoic testicular lesions pathophysiology and why it doesn’t significantly affect semen quality.

5.2 Conclusions

a) Age and body weight have a significant positive effect on semen volume, the SC was also noted to significantly increase with age and body weight

b) Prevalence of hyperechoic testicular lesions prevalence increases with age; however, it did not have a significant effect on semen quality

c) Peak semen volumes are obtained between 60-84 months of age, while sperm concentration peaks between 24-60 months. Additionally, forward progressive motility, post-thaw motility, and sperm concentration significantly vary from breed to breed. Jersey has the highest sperm concentration and progressive motility, while Ayrshire has the highest post-thaw motility.

d) Over-conditioned bulls have low sperm concentration and post-thaw motility

5.3 Recommendations

i. Bull semen quality rather than age should dictate the culling of bulls, as the bulls beyond the recommended culling age of 84 months exhibited desirable semen quality in the current study.

ii. Use AV or alternative semen collection technique to replace EEJ for *Bos indicus* cattle as the latter adversely affects semen motility, sperm concentration, and post-thaw motility. The findings documented herein differ significantly from other studies that have used other methods for semen collection.

iii. Further studies are required to understand age-related hyperechoic testicular lesions pathophysiology and why it doesn't affect semen quality.

iv. There is also need for further research to investigate the relationship between obesity and poor post thaw motility, as literature on the same is scarce.

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