**MEKELLE UNIVERSITY**

**COLLEGE OF VETERINARY SCIENCE**

**PROGRAM: MSC IN VETERINARY REPRODUCTION AND OBSTETRICS**

# ASSESSING BULL SEMEN QUALITY ACROSS BREEDS AND AGE GROUPS: INSIGHTS FROM MEKELLE ARTIFICIAL INSEMINATION CENTER, TIGRAY, ETHIOPIA

**By:**

**Tesfay Gebremariam Naizgi**

**A thesis proposal Submitted to the College of Veterinary Science, Mekelles University**

**In Partial Fulfillment of the Requirement for the Degree of Master of Veterinary Reproduction and Obstetrics.**

**January, 2025**

**Mekelle, Ethiopia**

## Declaration

This certification approve that the thesis titled ″Comparative study on the evaluation of bull semen quality during pre and post war in mekelle artificial insemination center″ is submit by Mr. Tesfay Gebremariam as a part of the requirements for the degree of Master **in** veterinary reproduction and obstetrics. It additional that the research has not been submitted previously for any other publication.

Name Tesfay Gebremariam

Signature _____

Date of Submission _____

This thesis has been submitted for examination with our approval as university advisors.

Main Advisor: Dr. Berihu Gebrekidan (DVM, MSc, associate professor) Signature_____

Co-Adviser: Dr.Gebregiorgis Ashebir (Associate. Professor, CVs, MU) Signature_____

**ASSESSING BULL SEMEN QUALITY ACROSS BREEDS AND AGE GROUPS: INSIGHTS FROM MEKELLE ARTIFICIAL INSEMINATION CENTER, TIGRAY, ETHIOPIA**

**By:**

**Tesfay Gebremariam Naizgi**

**BOARD OF EXTERNAL EXAMINERS**

**1.**_____

**2.**_____

Signature

Dr. Berihu Gebrekidan (DVM, MSc, associate professor) _____

Dr.Gebregiorgis Ashebir (Associate. Professor, CVs, MU) _____

# Contents

**ACKNOWLEDGMENTS**

My special thanks to the Tigray Bureau of Agricultural and Rural Development for sponsoring my study and giving me study leave permission.

I would like to thank my major Dr.Berihu Gebrekidan and co-advisors, Dr. Gebregiorgis Ashebir advisor, scholarly advice, guidance, constructive comments, reference provision, careful correction and finalization of the thesis. I would also want to thank my friends Tadesse Gugssa and Tewelde Mehari for their constructive ideas and facilitation of the Mekelle AI center.

I also appreciate the contribution of the MAIC staff for allowing me to work on the semen of their animals and in their laboratory.

I would like to extend my gratitude to my family for their substantial moral support and for sharing their ideas for my proposal. Additionally, I want to thank everyone who assisted me, both directly and indirectly, throughout the entire program of my work.

**LIST OF FIGURES**

**LIST OF TABLES**

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AI | Artificial Insemination |
| HF | Holstein Fresian |
| AGP | Agricultural Growth Program |
| ASMA | Artificial Sperm Morphology Assessment |
| BOARD | Bureau of Agriculture and Rural development |
| BSE | Breeding Soundness Evaluation |
| DMR | Distal Midpiece Reflex |
| EMA | Ethiopian Mapping Agency |
| GDP | Gross Domestic Product |
| LN | Liquid Nitrogen |
| MSPL | Mekele semen processing Laboratory |
| NAIC | National Artificial Insemination Center |
| Sida | Swedish International Development Agency |
| SPSS | Statistical Package for Social Sciences |
| TAIC | Tigray Artificial Insemination Center |

**ABSTRACT**

The low productivity of Ethiopian indigenous cattle can be improved through selection and crossbreeding with high-producing exotic breeds. This study aimed to evaluate the effects of age and breed on bull semen quality at the Regional Artificial Insemination Center (RAIC). A total of five Holstein Friesian (HF) and Begait bulls aged 3-9 years were used. Semen was collected using an artificial vagina, and evaluations were conducted using a photometer, stage heated microscope and eosin-nigrosin staining for sperm viability and abnormality assessments. Independent sample t-tests were employed to compare semen quality parameters between groups. The results indicated no significant differences ($p > 0.05$) between HF and Begait bulls in motility, concentration, and viability, except for semen volume, which was significantly higher in HF bulls ($p = 0.001$). The highest initial motility (78.75 ± 1.35%), concentration (1.75 ± 0.15), and live sperm count (81.00 ± 0.85%) were observed in bulls aged 4-6 years. Lower values were recorded in younger (< 4 years) and older (> 6 years) bulls. Progressive forward motility was negatively influenced by defective sperm cells (13.1 ± 1.4%), with tail abnormalities significantly affecting semen quality ($p < 0.05$). Minor sperm defects (10.6 ± 1.2%) were more prevalent than major defects (2.5 ± 0.2%), with bent-tail defects being predominant. Post-war conditions led to a significant increase in defective sperm cells, potentially due to aging and seasonal variations. The study concludes that age significantly affects semen quality, while breed does not. Introducing younger, proven bulls into breeding programs is recommended to enhance fertility rates and semen quality.

**Keywords:** age, breed, bull, semen quality

## 1. INTRODUCTION

Livestock is an integral part of the agricultural sector, Ethiopia has the largest livestock population in Africa, with 65 million cattle, 40 million sheep, 51 million goats, 8 million camels and 49 million chickens in 2020 (Central Statistics Agency, CSA, 2020a). Between 2000 and 2016, the average stock of livestock, measured in tropical livestock units (TLU) per 100 people, stood at 51 TLU, which is more than double the continental median of 23 TLU. The gross production value average growth rate during the same period was 4.5% — also twice the continental median of 2.2% (FAO, 2019). The national herd supports, at least in part, the livelihoods of more than 11.3 million rural households, including 27– 35% of the highland livestock keepers, and a large proportion of the lowland herders, who live below the Government of Ethiopia established poverty line (Shapiro et al., 2017).

Although the country holds the largest livestock population in Africa, production is too low mainly due to poor genetic performance, nutrition, management, infertility, reproductive disorders and diseases accompanied with lack of veterinary and AI professionals hindering the growth of the dairy industry in the country (Mureda and Mekuriaw 2007; Mekonnen*et al.,* 2010; Bitew and Prasad 2011; Haileselassie*et al*., 2011).

In livestock breeding AI is a relevant technology in the sector. Artificial insemination (AI) is a process by which sperm is collected from the male, processed, stored, and artificially introduced into the female reproductive tract for the purpose of conception (Webb, 2003).Artificial insemination is widely used for livestock breeding around the world, and a necessary tool in sustainable farm animal breeding (Gamborg and Sandoe, 2005). This technology has been used in cattle for over 65 years (Betteridge, 2003).

The first successful AI was performed in Italy in 1780 and over 100 years later, in 1890, it was used for horse breeding. In Ethiopia, AI was introduced in 1938 in Asmara, then part of Ethiopia, which was interrupted due to the Second World War and restarted in 1952. ( Yemane *et al.,* 1993). It was again discontinued due to unaffordable expenses of importing semen, liquid nitrogen and other related inputs requirement. In 1967, an independent service was started in the then Arsi Region, Chilalo Awraja under the Swedish International Development Agency (Sida).

The technology of AI for cattle has been introduced at the farm level in the country over 35 years ago as a tool for genetic improvement (Zewdie et al., 2006). The present National Artificial Insemination Center (NAIC) was established in 1984 to coordinate the overall AI operation at national level (GebreMedhin, 2005). The efficiency of the service in the country, however, has remained at a very low level due to infrastructure, managerial, and financial constraints, as well as poor heat detection, improper timing of insemination and embryonic death (Shiferaw *et al*., 2003).

AI has many advantages including prevention of reproductive diseases, control of inbreeding, minimizing the cost of keeping bulls for natural service and others, however, it has disadvantages such as poor conception rates due to poor heat detection and inefficiency of AI technicians, dissemination of reproductive diseases and poor quality of semen causes failure conception rate. A successful AI program must include efficient and accurate heat detection and timely AI relative to ovulation. The failure to detect heat is the most common and costly problem of AI programs and the major limiting factor of reproductive performance on many dairy farms (Nebel and Jobst, 1998). The efficiency of cow insemination depends, among other factors, on the ability of the inseminator to deliver the semen to the appropriate site in the reproductive tract at the appropriate stage of estrus. Extensive training of inseminators has been one of the most significant contributions to the successful commercial application of AI in dairy cattle breeding (Foote, 1996).

Before semen is used for artificial insemination, it undergoes the quality parameters include Volume, density ,initial motility, sperm concentration, motility before filling, and motility after thawing (Sankhi *et al*.,2019). Vitality is the proportion of live spermatozoa in a sample, as determined by the evaluation of membrane integrity (Moskovtsev *et.al*,2013). The routine semen analysis relies on assessing a number of parameters such as color, volume sperm concentration, motility, viability and morphology for the prediction of fertility in the male (Selvaraju et al., 2008b) Semen evaluation helps determine the fertility potential of the spermatozoa and ensures that only high-quality semen is used for breeding. One important factor which limits the reproductive performance and fertility of bulls have been reported to be the quality of semen they produce, which can be affected by numerous exogenous and endogenous factors (Hafez, 1993; Blezinger, 1999).

In Tigray, Artificial insemination service was initially started before 30 years (Ashebir *et al.2016),* with limited resources in restricted areas, specifically around Mekelle and the eastern zone of Tigray (Adigrat). The semen was provided from the Kality Artificial Insemination Center (NAIC) until 2008 E.C. Later, in 2009 E.C. The Tigray Artificial Insemination Center was established in 2009 E.C through collaboration between the BOARD and AGP, features 12 improved breeds, This includes 5 Holstein Friesian, 3 Jersey, and 3 Begaiet indigenous bulls. And , there are four LN2 plants located in Mekelle, Maichew, and Shire Endaselassie, which have the capacity to produce 150,000 doses (semen straws) per year. The AI center was operational prior to the onset of the war.

The aim of this thesis was to conduct a laboratory investigation of bull semen quality, focusing on the Holstein Frisian and Begait breeds. This study assessing bull semen quality, across breeds and age groups. as well as the risk factors that affect semen quality at the Mekele Artificial Insemination Center.

### 1.1.Statement of the Problem

Because of the war, the animal breeding system in the Tigray region has significantly broken down. The Mekele Artificial Insemination Center has faced various challenges, including issues with bull's management, semen collection and liquid nitrogen production, and the conflict has severely affected the functioning and effectiveness of the animal breeding system in the region, hindering efforts to improve livestock production and genetic potential.

One of the methods used to predict the potential fertility of a bull is breeding soundness evaluation (BSE) (Hopkins and Spitzer, 1997) or bull breeding soundness evaluation (BBSE) (Alexander, 2008). Worldwide, BSE has been used to assess the breeding potential of bulls (Godfrey and Dodson, 2005; Makhzoomi *et al*., 2007 and Hoflack et al., 2006),

The issue of bull semen quality at the Mekele Artificial Insemination Center (MAIC) has arisen as a consequence of the conflict in the region. To revitalize the animal breeding sector in the region, it will be crucial to restore and rebuild the breeding units, artificial insemination (AI) services, and infrastructure of the AI center.

This research aims to assess semen quality and the risk factors that can affect it at the AI center, since Semen quality is crucial for achieving high fertility rates, genetic improvement, disease control, and economic success in cattle breeding.

## 1.2. Objective

### 1.2.1. General Objective:

The overall objective of this research is to assess bull semen quality across breeds and age groups at the Mekelle Artificial Insemination Center, addressing critical factors affecting bull fertility and livestock productivity in the region.

### 1.2.2. Specific Objectives:

- To evaluate the quality of Bull semen with the parameters, including volume, color, concentration, morphology, and viability, before and after the war.
- To Identify risk factors such as breed, age, and handling practices that affect semen quality to provide actionable insights for improving AI outcomes

# 2. LITERATURE REVIEW

## 2.1. Livestock Production in Ethiopia

The Ethiopian livestock population is almost entirely composed of indigenous animals. Recent estimates showed that 97.8%, 1.9%, and 0.3% of cattle are indigenous, hybrid, and exotic breeds, respectively (CSA, 2020a).It has been known that the indigenous cattle breeds of Ethiopia have low productivity despite their special merit of overcoming harsh environmental conditions of the country; on the other hand the high performing exotic cattle cannot cope with the harsh environment of the country (MOA, 1996). So improvement of cattle productivity without losing traits that are essential for survival has been proposed. In Ethiopia few cattle breeds have been categorically identified and those identified are not adequately assessed and conserved (MOA, 1996).

The practical solution to improve the low productivity of Ethiopian indigenous cattle was by selection and crossbreeding of the indigenous cattle breeds with high-producing exotic cattle (Tadesse, 2002). Ethiopian cattle crossbreeding work was initiated in the early 1950s, and was not began based on clearly defined breeding policy with regard to the level of exotic blood inheritance and the breed types to be used. The unplanned crossbreeding had also threatened the genetic resources base of Ethiopia (Aynalem et al., 2011). Crossbred dairy cattle in Ethiopia are mainly crosses of Zebu and Holstein Friesian (Nuraddis and Ahmed, 2017). In the Tigray region, crosses of the Begait breed and Holstein Friesian have become common. Ethiopian cattle breeding are mostly uncontrolled, and genetic improvement is difficult (Tegegn and Zelalem, 2017). There are quite insignificant total numbers of crossbred female cattle produced through the crossbreeding programmed for many decades in Ethiopia indicating unsuccessful crossbreeding through AI (Sinishaw, 2004; Desalegn, 2008; CSA, 2011). The efficiency of the service in the country, however, has remained at a very low level due to infrastructure, managerial, and financial constraints, as well as poor heat detection, improper timing of insemination and embryonic death (Shiferaw *et al*., 2003).

### 2.1.1. Holstein Friesian Breeds

Holstein Friesian breeds are firstly originated in Holland and worldwide distributed then after. It is being raised in Scandinavian countries, Britain, North America, Africa, Asia, particularly Middle East due to its adaptability to hot weather and high production. The average production of milk is 5000 kg within 305 days of production, while the butter fat percentage ranges between 3.5-3.8% which is comparatively low due to large quantity of milk production. Milk has white color due to the efficiency of cow to convert carotene yellow pigment to Vitamin A is a colorless substance. Holstein Friesian bulls reach sexual maturity at about 6 to 12 months, but optimal semen production generally begins around 12 to 15 months.

Currently, the value of the Holstein breed is very large, as it is characterized by the highest milk productivity and is used to improve the dairy breeds all over the world. It is distinguished by good adaptability to various climatic and economic conditions (Dmitriev et al., 1989).

Several other indicators of productivity are characteristic to the Holstein breed of Dutch breeding. The average milk productivity of cows in the Netherlands in 2009-2010, 305 days of lactation amounted to 8832 kg of milk, the content of milk fat – 4.25%, milk protein – 3.45% (Tekeev, 2015).

Over the past 40 years, milk yields in many European countries have more than doubled as a result of achievements in breeding, feeding and management of the herd. At present, the average increase in milk productivity is 1.5% per year; the main role is given to the effective use of artifi cial insemination, which determines the high genetic potential of the herd (Lin and Togashi, 2005).

### 2.1.2. Begait Breeds

According to a report by the Central Statistical Agency (CSA) in 2015, 98.7% of the total cattle reared in Ethiopia are indigenous. These cattle contribute significantly to the draught power of smallholder farmers, generate critical cash income, provide collateral for local informal credit, and serve various socio-cultural functions in the country (Ulfina *et al.,* 2005; Melaku, 2011).

The Begait breeds are one of the major and comparatively productive indigenous cattle breeds found in the northern part of Ethiopia, specifically in the Tigray region, including the Tahtay

Adyabo, Kafta Humera, Welkayit, and Asgeda-Tsembla districts. These cattles are characterized by their larger body size, well-developed udders, and long teats (Zerabruk: Gebretnsae *et al*., 2017)., And they typically have a white coat with black spots or splashes (Mason, 1996) The presence of different coat color patterns in Begait cattle are similar those reported by Mekuriaw and Kebede (2015) and Mulugeta (2015). The body weight of Begait bulls and cows are similar to some local breeds such as Kereyu, Nkasi and Boran cattle reported by Sheferaw (2006), Mwambene *et al* (2012) and Banerjee *et al* (2014). The weight of the Begait cattle is higher than those of other Ethiopian breeds of cattle, Horro and Arsi Bale which is ascribed to the genetic makeup of the cattle. and are primarily used for milk and meat production. They generate an average of 6.2 litters/day for 195 days and 2.7litters /day for 140 days of milk per day. Ndambi *et al.,* (2012). Begait cattle determine relatively high productivity potential due to their superior adaptation for survival and reproduction in the harsh environments of the region (Okomo-Adhiambo, 2002). They are notably tolerant of many common diseases affecting cattle in the area and can graze on sparse vegetation while maintaining their body condition (Rezene, 2007).

### 2.2. Semen Collection and Processing

Before semen is collected, bulls should undergo false mounting (Almquist and Hale, 1956; Almquist, 1973), as it is the most effective method for sexual preparation and serves as a clear indicator of sexual arousal. This process allows a bull to mount a teaser without triggering the ejaculation response. To maximize sperm harvest per ejaculate, a total of three false mounts should be performed, followed by a fourth mount for semen collection. The first two false mounts can occur in quick succession, followed by 2 minutes of active restraint (Almquist, 1973).

During the collection of semen, appropriate and specialized facilities, equipment, and procedures have been implemented to prevent injury to both the bulls and their handlers. These measures aim to maximize the physiological responsiveness of the bulls in producing semen and to enhance both the quantity and quality of the collected semen (Garner, 1991). The semen collection area was cleaned, quiet, and free of disruptions and other stressful procedures. Reports indicate that proper sexual stimulation of the bull can increase spermatozoa motility by 50% (Salisbury *et al.,* 1978).

Semen was collected during morning hours with the help of artificial vagina as per the method suggested by Arthur *et al.* (1982). Prior to collection of semen, all parts of artificial vagina set were cleaned, sterilized and assembled. The inner liner was put into the cylinder and both ends of inner liner were reflected over the cylinder forming water like space between them. The cone along with vial was slipped over one of the ends of the cylinder and then tightened with rubber band. Two third of the outer jacket of vagina was filled with warm water. The temperature inside the artificial vagina was $110^0$ -$115^0$ F. An air screw was used for blowing air between two layers to create desired pressure. Required amount of lubricant was applied inside the artificial vagina with a glass rod. When the bull was sufficiently excited to jump over the teaser bull, the penis of the bull was directed into the artificial vagina by holding the sheath to collect the semen in a vial.

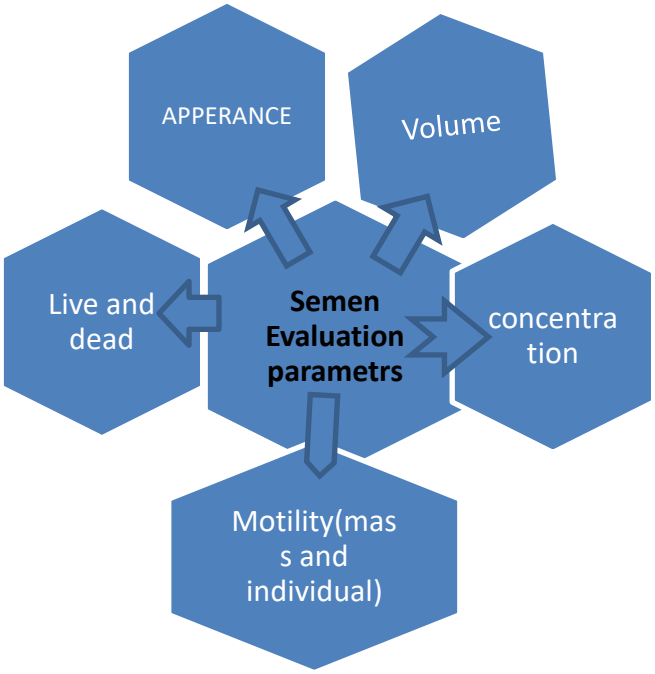
After collection, the tube containing the freshly collected semen was capped with aluminum foil as soon as it was placed in the dynamic pass box for transfer to the laboratory. The collected tube remained capped until processing, at which point it was placed in a water bath at 37°C. The evaluation included assessments of volume, color, morphology, motility (both individual and mass), and concentration.

### 2.3. Semen Quality Assessment

Semen assessment is essential for determining the fertility potential of spermatozoa, as semen quality significantly influences the success of cattle breeding programs. Continuous evaluation of semen quality is crucial, serving as the foundation for deciding whether semen can be processed into liquid or frozen forms. Research has shown that fresh semen is suitable for freezing when its motility reaches 70% (Sarastina *et al.,* 2006). The primary parameters to assess bull fertility are the motility and morphology of spermatozoa (Parker *et al*., 1999).

Semen volume, concentration, motility, and abnormalities of spermatozoa that meet normal standards may be influenced by several factors, including feed provided, environmental adaptation, frequency of ejaculation, collection methods, and pre-stimulation (Garner & Hafez, 2010; Feradis, 2010; Ismaya, 2014).

The quantity and quality of bull's semen (including the ejaculate Appearance, volume, sperm concentration, sperm motility, and viability) are affected by genetic as well as non-genetic factors , including diet, age, and diseases (Pan *et al*., 2013).




Semen evaluation parameters

### 2.3.1. Techniques to evaluate semen quality

Semen evaluation helps determine the fertility potential of the spermatozoa. the main goal of semen quality tests is to accurately predict semen fertility using rapid, inexpensive techniques (Gomes, 1977). Some tests like individual motility and morphologically normal percentage are known to have a significant positive correlation with fertility (Das *et al*., 1999). The heritability estimates of semen quality and quantity traits are known to be within the range of 0.02(2%) to 0.28(28%) with heritability of motility being about 0.2 (20%) (Stalhmmar, 1995). Similarly, other researchers reported the heritability of some seminal attributes like semen volume, and spermatozoa concentration and total count to be 0.3 to 0.5 (Graffer *et al*., 1998).

The ideal method of evaluating the fertility of breeding male, other than its ability to produce pregnancy, is by the examination of its semen (Hafez, 1993). Reliable estimations of the semen

quality require careful examinations of a minimum of three ejaculates collected at intervals of a few days (Hudison, 1972; Garner, 1991).

Semen examination has great diagnostic value in determining the cause, severity, and degree of testicular or accessory gland pathology or infertility, being of value in estimating the fertility of the male, as a definite correlation between testicular pathology, disease of the reproductive tract and accessory gland, and the semen characteristics and fertility has been found (Roberts, 1971; Faulkner and Pineda, 1980).

#### *2.3.1.1.Appearance (color)*

It has been known that the bull semen which is concentrated has creamy, milky or creamy white and opaque color (Roberts, 1971; Hafez, 1993; Bearden and Fuquay, 2000). On the other hand, semen with few spermatozoa and that from animal with reproductive system infections has been reported respectively to be translucent and curdy in appearance (Hafez, 1993). Some bulls have been known to consistently produce yellow semen which is due to a harmless pigment known as riboflavin. However, the semen should be free from hair, dirt, urine, blood, faeces and other contaminants (Garner, 1991; Hafez, 1993; Bearden and Fuquay, 2000).

The semen with the spermatozoa concentration of one million to 1.2 million per cubic millimeter or higher has been classified as creamy consistency while the spermatozoa with concentration of $5 \times 10^5$ to $6 \times 10^5$ per cubic millimeter has been classified as a thin milky consistency, and those with the spermatozoa concentration of less than $3 \times 10^5$ spermatozoa per cubic millimeter has been classified as watery, translucent or clear (Roberts, 1971).

#### *2.3.1.2.Motility of sperm cells*

Sperm motility is a critical parameter in assessing semen quality and fertility potential. Motility of spermatozoa at time of collection has been used commonly as a measure of the fertilizing ability of the sperm (Roberts, 1971; Bhosrekar, 1990). Spermatozoa with morphologic abnormalities have been known to show effect no progressive motility (Bearden and Fuquay, 2000). Several endogenous and exogenous factors (Roberts, 1971; Bhosrekar, 1990; Hafez, 1993) have been recognized to affect sperm motility. Excessive heat, and chemical or foreign agents, and sudden reduction in temperature during collection depress sperm motility. Normally 40-75 % (Sorensen, 1979; Hafez, 1993) and 50% to 80 % (Bearden and Fuquay, 2000) of bull

sperm has been found motile, however, spermatozoa motility below 50 % (Roberts, 1971), 40 % (Bearden and Fuquay, 2000) has been known to be associated with low conception rate or poor fertility.

Small "standing" drop is placed on a pre-warmed slide and evaluated under low power microscopy (40X-200X) for gross motility. Thick, dark, rapidly oscillating swirls are indicative of excellent motility (defined as a high velocity or high speed motility), a high percentage of sperm that are progressively motile, and a sample of high concentration. Sperm motility was subjectively estimated according to the standard method (Ax *et al.,* 2000).

- **The mass motility:** The mass motility is the collective movement of spermatozoa. It is the most influenced parameter in semen analysis and examined as soon as possible. Semen was evaluated subjectively using a phase contrast microscope with 100x low magnification power without cover slip score (0-4) mass activity was recorded according to the criteria of Nath (1988); it can also assessed by placing a drop of semen onto a warmed slide under x100 magnification (x10 eyepiece and x10 objective). The swirl is graded according to the Society for Theriogenology Handbook as follows:

  | | |
  |---|---|
  | Rapid Swirling | Very Good |
  | Slower Swirling | Good |
  | Generalized Oscillation | Fair |
  | Sporadic Oscillation | Poor |

  The minimum threshold recommended is fair.

- **Individual motility:** The progressive movement of sperm cells is indicated to as individual motility. To assess this, a drop of undiluted semen is placed on a pre-warmed glass slide (37°C), and a cover slip is added. After collecting semen samples, the percentage motility of spermatozoa is evaluated under a phase contrast microscope at 40× magnifications, with the heated stage adjusted to 37°C. A progressive motility above 50% and abnormalities in spermatozoa below 20% indicate that the semen quality meets the standards for bull semen (Ax *et al*., 2008).

Several endogenous and exogenous factors (Roberts, 1971; Bhosrekar, 1990; Hafez, 1993) have been recognized to affect sperm motility. Excessive heat, and chemical or foreign agents, and sudden reduction in temperature during collection depress sperm motility. Normally 40-75 % (Sorensen, 1979; Hafez, 1993) and 50 to 80 % (Bearden and Fuquay, 2000) of bull sperm has been found motile, however, spermatozoa motility below 50 % (Roberts, 1971), 40 % (Bearden and Fuquay, 2000) has been known to be associated with low conception rate or poor fertility

#### *2.3.1.3.Concentration*

Spermatozoa concentration refers to the number of spermatozoa per milliliter of semen (Hafez, 1993) and the sperm concentration of the bull has been known to range from 800 to 1200 (Sorensen, 1979), 300 to 2000 (Setchell, 1991), 800 to 2000 (Hafez, 1993), 1000 to 3000 (Bearden and Fuquay, 2000) million per milliliter of semen. According to Roberts, 1971, the concentration of the spermatozoa in the ejaculate of the fertile bull varies from $3 \times 10^5$ to $2 \times 10^6$ per cubic millimeter, with an average of $8 \times 10^5$. The sperm concentration range from $3 \times 10^5$ to $3 \times 10^6$ /$mm^3$, and low sperm concentration has been known to be the feature of testicular hypoplasia and degeneration (Arthur *et al.,* 1989).

Higher concentrations of sperm can lead to better pregnancy rates by increasing the possibility of successful fertilization. Concentrated semen, often sourced from genetically superior bulls, enhances the genetic quality of the offspring. It also helps maintain sperm vitality during storage and transport, ensuring that more viable sperm are available at the time of insemination. The concentration (million/mL) was evaluated using photometric analysis (IMV photometer). Sperm concentration among breeds already meets the standard for bulls, which is 200-1800 million/mL (Ax *et al*., 2008). The concentration of spermatozoa has been reported to vary with sexual development and maturity of the bull, with feeding regimen, and with reproductive health of the testes, season of the year and geographical localities (Salisbury *et al.,* 1978).

#### *2.3.1.4. Morphology of sperm cells*

Any morphological deviation from the normal structure of spermatozoon has been considered abnormal (Sullivan, 1978). Ejaculated semen has been reported to contain some morphologically abnormal spermatozoa (Faulkner and Pineda, 1980; Hafez, 1993) which has not been associated with lowered fertility until the proportion of abnormal spermatozoa exceeds 20 %.

Sperm morphology represents one of the more important aspects of semen assessment (Garner 1997). Difficulties arise in determining which abnormalities are most problematic, and what are their acceptable levels of tolerance. Sperm morphology classification systems have attempted to categorize abnormalities in terms of their assumed origin (primary/secondary), significance (major/minor) and presumed functional contribution to infertility (compensable/un compensable). Another approach is to assess the number of viable sperm which are free of abnormalities in relation to a estimated fertility Athreshold @ - the route followed by the Society for Theriogenology with its breeding soundness guidelines for bulls (Chenoweth et al 1992).

The morphological abnormalities of sperm was assessed using stained slides with Eosin Nigrosin stain or dye product. The abnormality of the head, mid and tail had been evaluated using phase contrast microscope at the magnification of 100X. Using pipette and putting a drop of sperm was placed on a pre-warmed microscope slide ($35^0$c) and then stained with Eosin-Nigrosin staining methods and products (Rege *et al.,* 2000).

Smeared samples were stained with carbolfuchsin-eosin according to the method described by Williams in 1920 and modified by Lagerlof in 1934 (Kavak *et al*., 2004). Steps of Williams staining protocol were as follow. The air-dried, fresh semen thin smeared samples from AI centers/stations were fixed in flame, z\washed with absolute alcohol for 4 minutes, and air dried.

The proportion of morphologically abnormal sperm cells was examined on wet mount slide using two or three drops of semen diluted in Hancock's solution (Hancock, 1952). A drop of this mixture was placed on a slide and covered with a cover slip. The percentage of total spermatozoa abnormality (acrosomal abnormality, detached heads, abnormal mid-pieces and tail defects) was determined by counting a total of 400× spermatozoa under the phase contrast microscope (magnification 1000×, oil immersion).

Optimal fertility of the spermatozoa is known to depend on the structural and biochemical intactness of the acrosome (Sullivan, 1978). The base of the head of the spermatozoa is recognized to be concave, the implantation groove to which the tail is fastened (Bhosrekar, 1990).

Furthermore, parameters of head shape can be evaluated such as ellipticity, circularity, elongation, and regularity (Álvarez *et al*., 2008). Nevertheless, the accuracy of sperm

morphology assessment depends on the careful preparation, fixation and staining of spermatozoa. Rubio-Guillen *et al*. (2007) showed that by applying ASMA techniques and multivariate cluster analysis, it is possible to determine three indirect subpopulations of spermatozoa with different morphometric characteristics coexisting in bull ejaculates. The proportion of spermatozoa in each sperm subpopulation showed considerable differences among males and varied significantly throughout the cryopreservation procedure. The cryopreservation of spermatozoa has been found to affect chromatin structure and morphometric of the sperm head (Arruda *et al*., 2002; Esteso *et al*., 2003; Gravance *et al*., 1998; Hidalgo *et al*., 2006; Rijsselaere *et al*., 2004). Therefore, it is presumed that the adverse effects of cryopreservation on sperm chromatin and head morphology may be responsible for the lowered fertility of spermatozoa observed after cryopreservation. The number of sperm with distal droplets within the ejaculate varies widely between sequential ejaculates (McAuliffe *et al*., 2010). Case studies indicate that bulls with high numbers of distal droplets in natural service achieve normal pregnancy rates (Barth A., 2013). Therefore, distal droplets are not generally considered a defect by the author or other researchers (Johnson WH, 1997; Barth A., 2013) and are categorized as normal.

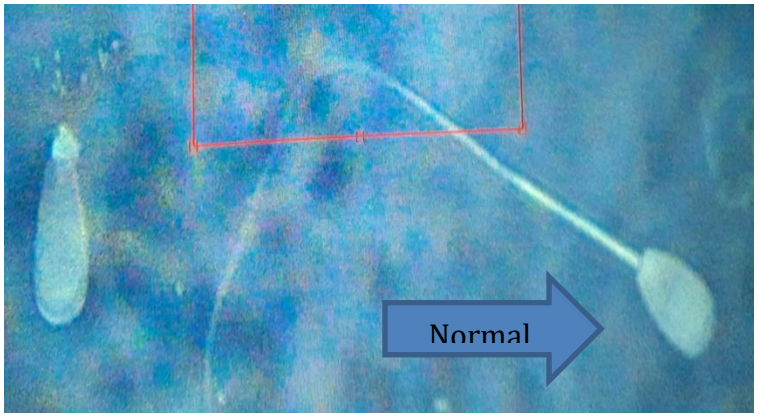



**Figure 1** Results of sperm cell examination; (A) head detached bull sperm cell (B) Normal spermatozoa

### *2.3.1.5. PH*

The PH level of bull semen can have a significant impact on sperm motility, viability, and mitochondrial activity. The pH of semen has been reported to be measured by pH paper, bromothymole blue or a pH meter (Roberts, 1971). The pH of bull semen is slightly acidic, about

6.7, and has been reported to rise (≥ 7) when there is incomplete ejaculation, excessive use of the bull or in yearling bulls and in pathologic situation of the testis, epididymis, ampulla or seminal vesicle (Roberts, 1971; Arthur *et al*., 1989). Dense semen samples which possess excellent motility have been known to show lower values of pH (Arthur *et al*., 1989).

### *2.3.1.6. Live and dead spermatozoa's*

Live and dead cell counts provide vital information about the quality and fertility potential of bull semen used in breeding programs. To determine the proportion of live and dead spermatozoa, various staining techniques are employed. One common method is the eosin-nigrosin staining technique, where live sperm remain unstained white, while dead sperm take up the stain red (Hafez, 1993).

Differential staining to determine the proportion of live to dead cells is undertaken by supra vital staining with stain mixture such as Nigrosin-Eosin (Salisbury *et al*., 1978; Hafez, 1993). Furthermore the results of these techniques correlate with visual estimates of progressively motile cells (Hafez, 1993). Commonly accepted standards for percentage of live spermatozoa in bull semen collected by artificial vagina and to be used for AI was given by Lindasay *et al*. (1982) to be 70 %, and 75 % by Sorensen (1979).

The determination of sperm viability is one of the basic parts of semen examination. The eosin nigrosin (EN) and propidium Iodide (PI) staining procedures are the most common techniques that are used to detect sperm viability in different species. The EN staining method is well known, widely used and a simple test (Björndahl et al., 2003). In eosin nigrosin staining technique, when eosin stains membrane

damaged dead sperm cells, the nigrosine stains background and subsequently; the dead red stained and live non-stained sperm cells could be easily distinguished under bright field microscope (Agarwal *et al*., 2016: Björndahl *et al*., 2004). Similarly, PI stains only membrane damaged (dead) sperm cells. The red stained (dead) sperm cells are detected under fluorescents microscope. Additionally, the combination of PI and SYBR-14 is also used to detect sperm viability with flow cytometer (Garner and Johnson, 1995). Thus, the present study was designed to investigate the comparative sensitivity of EN and PI staining procedures to detect viability of frozen thawed rabbit sperm.

The vital staining method was adopted from the procedure described by Dott and Foster (1972). Briefly, one drop of thawed sperm was mixed with one drop of eosin-nigrosin on a clean, preheated slide and incubated for 30 seconds. A smear was then prepared using 10 µl of this mixture on a glass slide. To determine the percentage of live sperm cells, 200 sperm cells were counted across the slide. If any part of the sperm, such as the nucleus, was stained, it was considered a dead cell, as indicated by the formula below (Salisbury et al., 1978).

No of live sperm counted × 100=_____%

No of total sperm counted

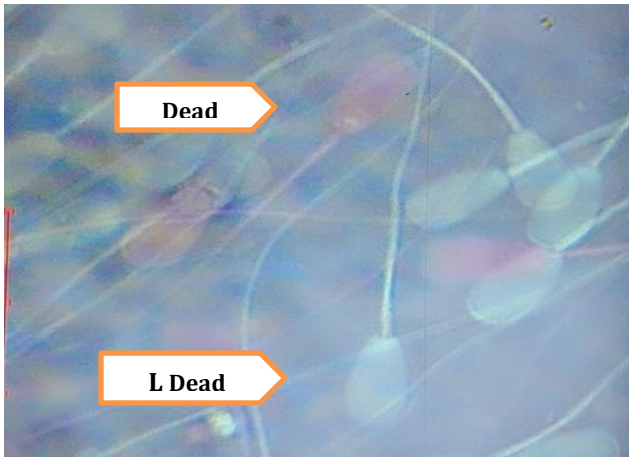



Figure 2**:** Results of sperm abnormalities examination; (A) dead spermatozoa Red head, (B) Normal spermatozoa White head

### 2.4. Factors Affecting Bovine Semen Quality

An abnormal spermiogram with supporting evidence from the history and physical examination can give insights into reasons for abnormal testicular function, and consequently allow formation of a prognosis for recovery or potential treatment. When an abnormal spermiogram is found, the types and number of abnormalities combined with history regarding environment, nutrition and health status can be used to compile a reason for spermiogram disturbances noted. The veterinarian can then use that information to make a diagnosis and prognosis for recovery. The most common causes of abnormal spermatogenesis in males include: abnormal testicular thermoregulation; hormonal imbalances, particularly those associated with stress; and effect(s) of toxins or expression of deleterious genes (Barth AD, 1994).

Stress, for any reason (environment, social or illness), causes a rise in cortisol which has a negative feedback to the hypothalamus and pituitary causing a decrease in FSH, LH and testosterone. This leads to sperm changes not only in the testes but epididymis as well. The most notable defect associated with stress is the distal midpiece reflex (DMR). DMR defects are due to an abnormal environment in the caudaepididymis, specifically, the distal third of the cauda epididymis. (Barth AD, *et al*., 1989). High concentrations of testosterone are necessary for normal epididymal function. ( Goyal H. 1983).

### 2.4.1. Age

It is commonly known that the age of a bull at collection affects semen characteristics (Mathevon *et al*., 1998; Brito *et al*., 2002a), with older mature bulls having greater semen volume and quality than younger bulls (Brito *et al*., 2002a; Fuerst-Waltl *et al.,* 2006). This increase is primarily believed to be due to physiological changes such as an increase in body mass (Balić *et al*., 2012) and the simultaneous development of the testis and accessory glands post puberty and during sexual maturation which consequently leads to an increase in semen production (Almquist, 1978). However, peak ejaculate volumes and total sperm number are achieved at different ages in different breeds (Snoj *et al*., 2013).

Investigating genetic parameters of semen quality traits assumed constant genetic variance across bull's age (Atagi *et al*., 2017; Berry *et al*., 2019; Olsen *et al*., 2020; Gebreyesus *et al*., 2021). Though, semen quality traits have been shown to have high phenotypic variability pre-puberty and around puberty and tend to stabilize as bulls mature (≥15–17 months of age; Karoui *et al*., 2011; Gebreyesus *et al*., 2021).

### 2.4.2. Nutrition

Bull fertility is significantly impacted by nutrition, particularly during the calf hood period when the testes seminiferous epithelium is developing. Factors influencing bull fertility include not only genetic make-up but also management practices and environmental conditions such as climate, stress, and pollution. Similarly, behavior, including experience and temperament, plays a crucial role (Rekwot PI et al., 1988; Hernandez E et al., 1991; Mukhopadhyay CS et al., 2011).

Apart from these, reproductive performance (semen quality, libido etc.) of bull is directly dependent on body vigor, physical soundness, structural conformation, masculinity and general health. The physical soundness of a bull is depends on feeding management and attainment of appropriate weight in different breeds of the bull. Thus, nutritional status of a bull is one of the main attribute in determining semen quantity and quality (Martin. GB. *et al*., 2011).

### 2.4.3. Disease of Testis, Epididymis and Accessory Glands

Pathologic condition of testis, epididymis, and seminal vesicle has been recognized to interfere with fertility by disturbing spermatogenesis or sperm maturation, leading to abnormal semen characteristics or preventing the passage of spermatozoa from testes to urethra (Blezinger, 1999). Some of such disease conditions include: testicular degeneration, orchitis, epididymitis and seminal vesiculitis (Hafez, 1993).

Disease prevention in bulls has been considered as essential as in breeding females and new bulls need to be screened by a qualified veterinarian for infectious agents prior to entering a new herd. Bulls have been recommended to be purchased only from reputable seed stock producers with adequate herd health plans; including vaccination against infectious diseases, e.g. leptospirosis and campylobacteriosis. Bulls are also recommended to be tested annually for brucellosis, but not be vaccinated for brucellosis. In some instances, bulls need to be vaccinated for bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and trichomoniasis (Hansen GR (2006). Unless it is made possible to make full control of the health of bulls selected for semen production, the disadvantages of artificial insemination in disseminating diseases will be much higher ( Zewdie E 2007).

According to the international animal health code (2001) of the Office International des Epizooties (OIE)), donor and teaser animals should be tested for the following specific diseases: Bovine Brucellosis, Bovine Tuberculosis, Bovine Viral Diarrhea, Infectious Bovine Rhinotracheitis, Campylobacter fetus/subspecies veneralis, Trichomonas fetus. Nevertheless, semen-producing bulls at NAIC are tested only for brucellosis and tuberculosis and yet not on regular basis due to many associated constraints (Agegnehu B 2007).

### 2.4.4. Temperature and Season

Temperature has significant impact on bull semen quality, and heat stress can negative impact a bulls fertility .Testicular temperature of bulls must be 2 to 6 $^0$c cooler than body temperature to allow normal spermatogenesis and maturation of sperm. Exposure of bulls to increase ambient temperature result in reduced semen quality (Casady *et al.,* 1953: skinner and louw, 1966; Meyerhoeffer *et al*., 1985) If semen is exposed to a sudden fall in temperature there is an irreversible decline in the activity of the spermatozoa (Chang & Walton, 1940), and this reduction in activity is associated with an increase in the number of dead, i.e. eosinophil cells (Lasley, Easley & McKenzie, 1942). This phenomenon of 'temperature shock' is encountered in bull semen cooled abruptly by pipetting semen at $30^0$ C. into an aqueous solution of nigrosin and eosin at $20^0$ C. (Hancock, 1951).

### 2.4.5. Scrotal Circumference

Scrotal circumference has been recognized to be highly heritable, and to serve as an indicator of puberty, total sperm production, semen quality, pathologic condition of testes, potential sub fertility or infertility of bulls, and consequently failure to select properly for this trait has the potential of decreasing fertility and production for the generations to come (Ott, 1986). The bulls with larger testes have been known to give

larger volume of semen, larger sperm motility and percentage of normal spermatozoa (Ott, 1986). The scrotal circumference estimates for most yearling bulls has been recognized to be 32 to 36 cm; the average estimate being 26.1 cm (Coulter, 1986), and 27.9 cm (Ott, 1986) at puberty. As the scrotal circumference increases, the probability of having seminal quality which is acceptable increases until a scrotal measurement of 38.0 cm is attained but no bull with the scrotal measurement less than 30 cm has been known to produce semen of acceptable quality (Coulter, 1986).

### 2.4.6. Other Factors

Other factors like frequency of service (ejaculation), poor semen collection technique, and cow to bull ratio, procedure of handling the semen during and after collection, analytical techniques, and variation among technicians, pharmacologic agents and normal physiologic variations have been recognized to influence the semen quality of the bull (Faulkner and Pineda, 1980; Hafez, 1993; Blezinger, 1999).

## 3. MATERIALS AND METHODS

### 3.1. Study Area

The study was conducted at the laboratory of the Mekele Artificial Insemination Center (MAIC) (Figure 1). Mekelle, the capital city of the region, is located 783 km from Addis Ababa, at a longitude of 39°33'E and a latitude of 13°32'N. The altitude of Mekelle ranges between 1965 m and 2220 m above sea level and varies from 2000 m to 2200 m in different areas. The town is surrounded by mountain ranges to the east and north. In terms of climate, the area is classified as "Woina Dega" (temperate), with an effective temperature ranging between 14°C and 20°C according to the Ethiopian Mapping Agency (EMA, 1981). The mean annual rainfall ranges from 11.3 mm to 39.1 mm, and temperatures vary from 12°C in November and December to 27°C in January and March. The moisture index (P/ET) is between 0.25 and 0.5, indicating a moderately dry area. Overall, Mekelle enjoys a humid and hot climate. In 2013 the population of Mekelle was projected to be around 300,000 assuming the 5.4% growth rate.

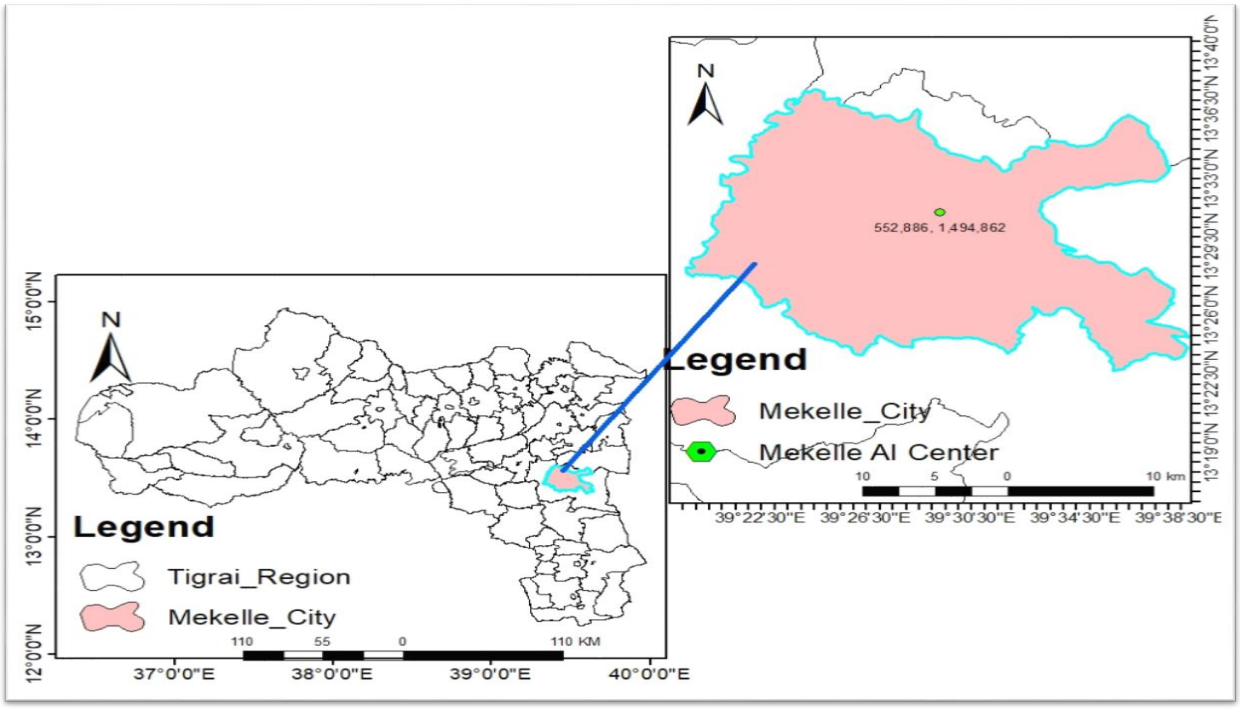



**Figure 3: Map of study area**

### 3.2. Study Animals /Unit/

The study focuses on five bulls: three Holstein bulls and two Begait bulls. The Holstein bulls are identified by the following ID numbers: 100004658, 100004665, and 100004684. The Begait bulls are identified by ID numbers 100005028 and 100005029. All bulls (Figure 1) were kept under the same intensive management conditions at the Mekele Artificial Insemination Center. Semen was collected and analyzed prior to the war, as well as fresh semen collected after war. The bulls, aged approximately 6.5 to 9 years, were all sexually mature, belonging to the Holstein-Friesian and Begait breeds. Throughout the study, they were reared under identical feeding and management conditions, receiving green grass and hay.

### 3.3. Study Design and Data Collection

This study was designed to assess the relationship between semen quality parameters and various factors, including breed, age, and morphology. Data were collected from 5 animals representing breeds, categorized by age groups and morphological traits. Semen samples were obtained using manual collection and quality parameters such as motility, concentration, and morphology were measured using scientific methods.

Variables

- Dependent Variables:
    - Breed (categorical: e.g., Breed A, Breed B, etc.)
    - Age (categorical: e.g., <2 years, 2–4 years, >4 years)
    - Morphology (categorical: normal, abnormal)
- Independent Variables:
    - Semen quality parameters, including:
        - Sperm motility (%)
        - Sperm concentration (million/mL)
        - Sperm viability (%)
        - Morphological defects (%)

### 3.4. Data Analysis

Data analysis was conducted using SPSS version 20. The following statistical tests were applied to address the study objectives. Descriptive statistics, including means, standard deviations, and ranges, were computed for all variables. Frequencies and percentages were used for categorical variables such as breed,

age, and morphology. An independent sample t-test was used to compare semen quality parameters between two groups within categorical variables, such as normal versus abnormal morphology. One-way ANOVA was employed to determine whether there were significant differences in semen quality parameters across multiple groups, such as breeds or age categories. Post-hoc tests (Tukey's HSD) were conducted to identify specific group differences when ANOVA results were significant. Pearson's correlation coefficients were calculated to assess the linear relationships between semen quality parameters (e.g., motility, concentration, viability) and continuous variables, such as age.

A one-way ANOVA was conducted to examine whether semen quality parameters varied significantly across multiple groups, such as different breeds or age categories. The general equation for the ANOVA model is:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where:

- $Y_{ij}$ represents the observed value of the dependent variable.
- $\mu$ is the overall mean.
- $\alpha_i$ is the effect of the $i^{th}$ group (e.g., breed or age category).
- $\epsilon_{ij}$ is the error term associated with the $j^{th}$ observation in the $i^{th}$ group.

### 3.5. Statistical Significance

All statistical tests were conducted as two-tailed tests, with a significance level set at $p<0.05p < 0.05$. Before performing the analyses, assumptions for each statistical test, such as normality and homogeneity of variances, were thoroughly checked. Where assumptions were violated, appropriate data transformations or non-parametric alternatives were employed.

#### 3.5.1. Sampling procedures

Before the conflict in 2020 G.C., five bulls (three Holstein Friesian and two Begait bulls) were used for semen production at the Mekele Artificial Insemination Center (MAIC). Semen was collected weekly, following optimal recommended procedures (Salisbury *et al*., 1978; Bhosrekar, 1990).

Prior to collection, the bulls were washed and groomed, and the semen collection environment was cleaned and rinsed with water. A general examination for any diseases was conducted, and proper treatment of the

bulls was ensured, including minimizing distractions. The teaser bull was also cleaned before and during the semen collection process to stimulate the bulls and facilitate false mounts, ultimately aiding in semen collection. The bulls received adequate sexual stimulation from a familiar handler, with two or more false mounts prior to collection. The artificial vagina was used for all semen collections. However, routine semen collection was interrupted due to absence of laboratory equipment, especially the production of liquid nitrogen.

### 3.5.2. Examination of the collected semen

#### *3.5.2.1.Physical examination of the semen and the spermatozoa*

Immediately following collection, the semen was kept at $34^0$C (Bhosrekar, 1990) in water bath (IMV, L'AIGLE, France) and examined grossly or macroscopically (for appearance, volume, and presence of foreign materials such as dust or pus), microscopically (for mass activity and individual motility, live/dead count and morphology of the spermatozoa) and concentration, sperm total count, viable number (percent motile multiplied by total count) of the spermatozoa following the recommended procedures (Salisbury *et al*., 1978; Roberts, 1971; Morrow, 1986; Bhosrekar, 1990; Garner, 1991; Hafez, 1993).

Gross examination was made as rapidly as possible after collection for the presence of any dust, hair, or any foreign body, and to note the color of the semen for each animal in each breed and was recorded as creamy, white, yellow, watery, or brown. The volume of the ejaculate from each animal in each group was recorded in graduated collection. A drop of undiluted semen was placed on warmed slide ($37^0$C) and examined on a stage warmed thermostatically set (at $37^0$C) phase contrast microscope (Nikon, Japan) at magnification of 100 times (100x) and will be scored for mass activity from 0 to 5 according to the intensity of the wave motion. The individual motility of sperm cells was estimated as a percentage by examining undiluted semen placed on warmed slide covered under warmed cover slip and then those sperm cells which exhibited progressive movements under stage warmed ($37^0$C) microscope at a magnification of 200x and scored 0 to 100 % according to the estimated percentage of spermatozoa which move in a progressive forward direction. Sperm cell concentration was determined by using calibrated spectrophotometery (IMV, Technologies France). The sperm concentration obtained was multiplied by the total volume of the ejaculate to calculate the total count of spermatozoa. This total count was then multiplied by progressively forward-moving percentages (individual motility) to determine the viable number of spermatozoa (Brito *et al*., 2002).

For the morphological examination of the spermatozoa, one milliliter of Hancock solution (buffered for mole saline) was kept at 34°C in a water bath. One drop of fresh semen was added with a warm (37°C) Pasteur pipette and gently mixed to preserve the spermatozoa.

Later, morphological abnormalities specifically head, mid-piece, tail defects, and proximal and distal droplets were examined by placing a small drop of the sample on a clean, grease-free glass slide and covering it with a cover slip under a phase contrast microscope (200x) using Hancock's technique. By this method, 200 spermatozoa were counted, and the abnormalities were visualized and recorded as head, mid-piece (body), and tail abnormalities (Roberts, 1971; Salisbury *et al*., 1978).

The characterization of head abnormalities (including acrosome defects) was conducted on 500 spermatozoa stained using the Williams technique (Williams, 1920; Bane, 1982) from the sample preserved with Hancock solution (Annex 2).

The proportion of live spermatozoa was determined on smears prepared from fresh semen by the Eosin-Nigrosin technique during which 500 spermatozoa were counted under 1000x or oil immersion lens (light microscope). In this technique: two drops of mixed stain and one drop of fresh semen were mixed and smears were made on glass slide (by using spreader glass), allowed to dry in air, and finally examined under Bright field light microscope (100x). Cells which accepted Eosin subsequently stain pink, against dark background of Nigrosin and were taken as dead. Similarly, sperm cells which exhibited such property to at least half of the sperm head were taken as dead. On the other hand, those sperm cells that didn't accept the stain and remained white were taken as alive. Sperm dimension measurements were done by measuring the head, midpiece and tail of the spermatozoa from those stained by the William stain technique.

## 4. RESULT

### 4.1. The Effect of Breed Type on Semen Quality

Table 1 presents the comparison of semen quality parameters between Holstein Friesian (HF) and Begait bulls. The analysis revealed no significant differences ($p > 0.05$) in most parameters, except for semen volume, which was significantly higher in HF bulls ($p = 0.001$).

**Table 1. The Effect of Breed Type on Semen Quality (Mean ± SE)**

| Breed Type | Concentration | Mass Motility | Initial Forward Motility | Live Cell Count | Dead Cell Count | Major Defects | Minor Defects | Total Defects | Semen Volume (ml) | Volume Of Extender (ml) | Number Of Doses |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HF | 1.2 ± 0.1 | 3.7 ± 0.1 | 75.4 ± 1.0 | 78.8 ± 0.3 | 21.2 ± 0.3 | 2.2 ± 0.2 | 7.2 ± 1.2 | 9.3 ± 1.4 | 7.3 ± 0.4 | 53.0 ± 5.5 | 260.8 ± 25.3 |
| begait | 1.2 ± 0.2 | 3.3 ± 0.2 | 72.8 ± 2.1 | 77.5 ± 0.8 | 22.5 ± 0.8 | 2.8 ± 0.1 | 10.5 ± 1.3 | 13.3 ± 1.4 | 5.3 ± 0.3 | 40.9 ± 7.8 | 200.5 ± 32.8 |
| p-value | 0.927 | 0.107 | 0.228 | 0.073 | 0.073 | 0.05 | 0.70 | 0.59 | 0.001 | 0.198 | 0.149 |

## 4.2.The effect of war on semen quality

The impact of pre-war and post-war conditions on semen quality parameters is summarized in **Table 2**. While no significant changes were observed in concentration, pH, or mass motility, there was a significant increase in minor and total defective cells post-war (*$p = 0.002$ and $p = 0.004$*, respectively).

**Table 2. The effect of war on semen quality (Mean ± SE)**

| War Period | Concentration | pH | Mass Motility | Initial Forward Motility | Live Cell Count | Dead Cell Count | Major Defects | Minor Defects | Total Defects | Volume of Extender (ml) | Number of Doses |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pr-war | 1.3 ± 0.1 | 6.0 | 3.7 ± 0.1 | 76.0 ± 1.2 | 79.2 ± 0.5 | 20.8 ± 0.5 | 2.2 ± 0.2 | 5.0 ± 0.7 | 7.2 ± 0.9 | 43.1 ± 5.4 | 210.5 ± 24.5 |
| Post-war | 1.1 ± 0.2 | 6.0 | 3.4 ± 0.2 | 73.4 ± 1.5 | 77.8 ± 0.5 | 22.2 ± 0.5 | 2.5 ± 0.2 | 10.6 ± 1.2 | 13.1 ± 1.4 | 51.1 ± 6.5 | 252.4 ± 29.0 |
| p-value | *0.348* | *N/A* | *0.313* | *0.234* | *0.055* | *0.055* | *0.297* | *0.002* | *0.004* | *0.404* | *0.326* |

## 4.3.The effect of sperm morphology and viability on semen quality

The analysis of sperm morphology defects using one-way ANOVA revealed significant differences among defect types ($p < 0.05$). Table 3 summarizes the mean percentages of major and minor defects across groups.

**Table 3. Descriptive Statistics for Major Defects (Mean ± SE)**

| Defect Type | N | Mean ± SE | p-value |
|---|---|---|---|
| **Pear-shaped** | 10 | 1.20 ± 0.29 | *0.009* |
| **Coiled tail** | 10 | 0.90 ± 0.23 | |
| **Proximal droplet** | 10 | 0.20 ± 0.13 | |
| **Irregular-shaped** | 5 | 0.20 ± 0.20 | |

The Tukey HSD test revealed that the Simple bent tails group *(4.20±0.79)* had significantly higher mean defect counts compared to all other groups ($p<0.05$) (Table 4). The Distal droplet group (0.40±0.27) exhibited the lowest mean defect count, which was significantly different from the Simple bent tail group ($p<0.001$) but not significantly different from the other groups. The Small normal head (0.90±0.41), Detached head (1.90±0.62), and Short tail (1.10±0.31) groups showed intermediate mean defect counts. Among these, the Detached head group was significantly different from the Simple bent tail group ($p=0.024$), while the other comparisons were not statistically significant. These findings underscore the distinctiveness of the Simple bent tail group and the relative similarity among the remaining groups.

**Table 4. Mean comparison of minor defects across defect groups**

| Defect type | N | Mean ± SE | p-value |
|---|---|---|---|
| **Simple bent tail** | 10 | 4.20 ± 0.79 | < 0.001 |
| **Distal droplet** | 10 | 0.40 ± 0.27 | < 0.001 |
| **Small normal head** | 10 | 0.90 ± 0.41 | < 0.001 |
| **Detached head** | 10 | 1.90 ± 0.62 | 0.024 |
| **Short tail** | 10 | 1.10 ± 0.31 | 0.001 |

### 4.4.The effect of breed age on semen quality

Semen quality parameters were analysed across three age groups using one-way ANOVA. Significant effects were observed for concentration, mass motility, and live cell count (**$p < 0.05$**).

**Table 5. The effect of age on semen quality (Mean ± SE)**

| Age Group | Concentration | Mass Motility | Initial Forward Motility | Live Cell Count | Total Defects |
|---|---|---|---|---|---|
| **<4 years** | 1.25 ± 0.13 | 3.75 ± 0.25 | 76.56 ± 1.12 | 78.50 ± 0.75 | 21.50 ± 0.95 |
| **4–6 years** | 1.75 ± 0.15 | 3.88 ± 0.18 | 78.75 ± 1.35 | 81.00 ± 0.85 | 19.00 ± 0.85 |
| **>6 years** | 0.88 ± 0.10 | 3.13 ± 0.20 | 70.00 ± 1.50 | 76.75 ± 0.90 | 23.25 ± 1.05 |
| ***p-value*** | *0.009* | *0.006* | *0.001* | *0.000* | *0.000* |

#### 4.4.1. Correlation between age and other semen quality parameters

The correlation between age and other semen quality parameters are summarized in Table5.

Age is negatively correlated with mass motility ($r = -0.510$, $p < 0.01$), initial forward motility ($r = -0.501$, $p < 0.01$), and live cell count ($r = -0.437$, $p < 0.01$), indicating that as bulls age, sperm motility and viability tend to decline. On the other hand, age positively correlates with dead cell count ($r = 0.437$, $p < 0.01$), major defective cells ($r = 0.455$, $p < 0.01$), minor defective cells ($r = 0.793$, $p < 0.01$), and total defective cells ($r = 0.767$, $p < 0.01$), demonstrating an increase in sperm defects with advancing age.

Semen volume shows positive correlations with mass motility ($r = 0.374$, $p < 0.05$), initial forward motility ($r = 0.316$, $p < 0.05$), and live cell count ($r = 0.543$, $p < 0.01$), suggesting that higher semen volume is associated with better motility and a greater proportion of live sperm. Conversely, negative correlations exist between semen volume and dead cell count ($r = -0.543$, $p < 0.01$), major defective cells ($r = -0.367$, $p < 0.05$), minor defective cells ($r = -0.334$, $p < 0.05$), and total defective cells ($r = -0.348$, $p < 0.05$), implying that increased semen volume is linked to fewer defective sperm cells.

Sperm concentration exhibits strong positive correlations with mass motility ($r = 0.759$, $p < 0.01$), initial forward motility ($r = 0.761$, $p < 0.01$), and live cell count ($r = 0.575$, $p < 0.01$), indicating that higher sperm concentrations are associated with improved motility and viability. Conversely, sperm concentration is negatively correlated with dead cell count ($r = -0.575$, $p < 0.01$), major defective cells ($r = -0.571$, $p < 0.01$),

minor defective cells ($r = -0.718$, $p < 0.01$), and total defective cells ($r = -0.718$, $p < 0.01$), reflecting a reduction in sperm defects as concentration increases.

Mass motility and initial forward motility are highly interrelated, as evidenced by their strong positive correlation ($r = 0.931$, $p < 0.01$). Both parameters positively correlate with live cell count ($r = 0.649$ and $r = 0.666$, $p < 0.01$) and negatively correlate with dead cell count, major defective cells, minor defective cells, and total defective cells ($p < 0.01$ for all). These results underscore the association between higher motility and better sperm quality.

Major defective cells strongly correlate with minor defective cells ($r = 0.773$, $p < 0.01$) and total defective cells ($r = 0.828$, $p < 0.01$), indicating that defects tend to co-occur. Additionally, minor defective cells almost perfectly correlate with total defective cells ($r = 0.996$, $p < 0.01$), suggesting that minor defects dominate the total defect count.

Finally, the volume of extender and number of doses are positively correlated with semen quality parameters, including sperm concentration ($r = 0.728$ and $r = 0.717$, $p < 0.01$), mass motility, and initial forward motility ($p < 0.01$ for both). These findings highlight that better semen quality facilitates the use of more extender and allows for a higher number of doses.

**Table 6. Correlation among semen quality parameters and age group of the breeds**

| | Age | Semen volume (ml) | concentration | Mass motility (1-4) | Initial forward Motility % | Live cell count % | Dead cell count % | Major Defective cell% | Minor defective cell | Total defective cell | Volume of extender | Number of Doses |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 1 | -0.059 | -.359* | -.510** | -.501** | -.437** | .437** | .455** | .793** | .767** | -0.089 | -0.072 |
| Semen volume (ml) | | 1 | 0.253 | .374* | .316* | .543** | -.543** | -.367* | -.334* | -.348* | .459** | .484** |
| concentration | | | 1 | .759** | .761** | .575** | -.575** | -.571** | -.718** | -.718** | .728** | .717** |
| Mass motility (1-4) | | | | 1 | .931** | .649** | -.649** | -.464** | -.757** | -.736** | .589** | .576** |
| Initial forward Motility % | | | | | 1 | .666** | -.666** | -.396* | -.744** | -.715** | .577** | .546** |
| Live cell count % | | | | | | 1 | -1.000** | -.535** | -.710** | -.705** | .398* | .407** |
| Dead cell countout % | | | | | | | 1 | .535** | .710** | .705** | -.398* | -.407** |
| Major Defective cell% | | | | | | | | 1 | .773** | .828** | -.507** | -.533** |
| Minor defective cell | | | | | | | | | 1 | .996** | -.501** | -.490** |
| Total defective cell | | | | | | | | | | 1 | -.516** | -.510** |
| *. Correlation is significant at the 0.05 level (2-tailed). | | | | | | | | | | | | |
| **. Correlation is significant at the 0.01 level (2-tailed). | | | | | | | | | | | | |

## 5. DISCUSSION

In this study it was observed that the comparison of semen quality parameters between Holstein Friesian (HF) and Begait bulls breeds the analysis revealed no significant differences ($p > 0.05$) in most parameters ( motility, concentration and viability) , except for semen volume, which was significantly higher in HF bulls (*$p = 0.001$*). The overall volume (ml ± SE) of semen produced by Holistein bulls was found 7.3 ± 0.4 ml. This was higher finding as reported by (JLTA, 2004). The lowest volume of semen produced by Begait bulls was observed 5.3 ± 0.3 ml in age of 3 and 4 year of bull. As they are becoming older, they give larger volume of semen which is also justified by the reports of (Waltl *et al.,* 2004). in semen production and quality can be explained by the fact that size of the testis is regarded as the main factor determining the total number of sperm production correlates with age of the bulls (Brito *et al.,* 2002)

In this study, initial or individual motility was highest in bulls aged 4-6 years (78.75 ± 1.35%), followed by younger bulls (< 4 years: 76.56 ± 1.12%) and lowest in older bulls (> 6 years: 70.00 ± 1.50%). This finding is consistent with the 77.46 ± 0.55% reported by Seyoum *et al.* (2020), but lower than the 89.62 ± 0.51% recorded in Indonesia by Indriastuti *et al.,* (2020). Ahmed *et al.* (2014) reported higher values, possibly due to differences in breed or environmental conditions. The relatively high motility observed here may result from proper lab protocols and the involvement of well-trained technicians.

In this study, semen concentration was highest in mature HF and Begait bulls aged 4-6 years (1.75 ± 0.15), compared to younger (< 4 years: 1.25 ± 0.13) and older (> 6 years: 0.88 ± 0.10) bulls. Similar findings were reported by Muhammed *et al.* (2000). This superior concentration in middle-aged bulls may result from proper bull handling, better management, and standardized laboratory procedures. In contrast, Ahmed *et al.* (2014) reported lower values for Jersey, Sahiwal, and local bulls, likely due to seasonal factors. While Sankhi *et al.* (2018) recorded a comparable mean value (1.084 ± 0.014) in Nepal, Indriastuti *et al,.* (2020) reported much higher sperm concentration (1164.81 ± 9.10). Differences in sperm concentration could be attributed to genetic variation, nutrition, age, management practices, and possible technical errors during semen collection (Andrable *et al.,* 2002).

The study demonstrated that bulls aged 4-6 years had the highest live sperm count (81.00 ± 0.85%), whereas lower values were recorded in younger (< 4 years: 78.50 ± 0.75%) and older (> 6 years: 76.75 ±

0.90%) bulls. These values were consistent with the recommended guideline of > 70% live sperm, suggesting that middle-aged bulls exhibit superior semen quality. Age was identified as a significant factor influencing sperm viability, corroborating findings by Lemma and Shamsu (2015). Additionally, abnormal sperm percentages were notably higher in older bulls (23.25 ± 1.05%) compared to adult (19.00 ± 0.85%) and young bulls (21.50 ± 0.95%). Ahmad *et al.*, (1986) similarly reported variations in abnormal sperm with age, though the inverse trend in their findings may result from breed and seasonal influences, as noted by Seifi et al. (2020). These results highlight the importance of age management in breeding programs to maintain optimal semen quality.

This study revealed that progressive forward motility of bovine sperm was influenced by defective sperm cells (13.1 ± 1.4%), with tail abnormalities significantly ($p < 0.05$) affecting semen quality. Although not statistically significant, the study found a greater proportion of minor sperm cell defects (10.6 ± 1.2%) than major defects (2.5 ± 0.2%), with bent-tail defects being predominant. This aligns with Adugna (2016), who reported that bent-tail sperm should not exceed 8% of total defective spermatozoa. According to the International Atomic Energy Agency (IAEA, 2005), bent-tail spermatozoa in fertile bulls should range between 8-12% of total defective cells. A significant relationship ($p \leq 0.05$) was observed between forward motility and bent-tail abnormalities. Since bent-tail defects often occur during or immediately after semen collection and handling, it is crucial to ensure proper handling procedures (Pearson, 2008).

This study highlights the effects of pre-war and post-war conditions on semen quality parameters. While no significant changes were noted in concentration, live cell count, or mass motility, a significant increase in minor and total defective cells was observed post-war ($p = 0.002$ and $p = 0.004$, respectively). This increase may be attributed to aging of the bulls and seasonal variations during the collection period. The three-year gap in semen collection due to the war likely contributed to the rise in both minor and major defects, emphasizing the importance of consistent collection and management practices to maintain optimal semen quality.

## 6. CONCLUSION AND RECOMMENDATION

The quantity and quality of fresh semen are affected by various factors, including genetics, age, nutrition, environmental temperature or seasonal changes, ejaculation frequency, libido, physical condition, transportation, scrotal size, and overall health (Ismaya, 2014).This study demonstrated that while breed did not significantly affect semen quality parameters such as motility, concentration, and viability, age had a notable influence on semen abnormalities, with bulls aged 4-6 years producing the highest quality semen. This superior performance in the 4-6 years age group may be attributed to better testicular development and favorable management practices. Defective sperm cells, particularly bent-tail abnormalities, were identified as key factors reducing semen quality, underscoring the need for thorough morphological examination during semen evaluation. Despite this results, further validation is necessary due to the limited number of semen-producing bulls and operational challenges at the Mekelle AI center caused by conflict. Currently, both Holstein Friesian and Begait bulls are being maintained without active semen collection, highlighting the need for improved management and operational continuity. Addressing these issues could enhance the center's ability to produce high-quality semen in the future.

Based on the above findings, the following recommendations are required to enhance semen quality and overall breeding performance:

- Establish a targeted acquisition and breeding program focusing on bulls aged 4-6 years with demonstrated high fertility and genetic potential
- Since bent-tail sperm defects are common during and after semen collection, meticulous care is required in handling fresh semen to minimize defects.
- Personnel involved in semen collection, sterilization, laboratory processing, and quality control should prioritize proper handling, sterilization, and maintenance of equipment to ensure optimal semen quality.
- Bulls older than six years should undergo regular performance evaluations, as their semen quality declines with age, requiring careful selection or replacement.
- Younger, proven bulls should be introduced into the breeding program to improve fertility rates and ensure consistent high-quality semen production.

- Maintaining optimal nutrition tailored to bull age and breed, along with regular health monitoring, is crucial for sustainable semen production.
- Enhancing the operational capacity of AI centers through proper training, resource allocation, and continuous quality control is vital for improving breeding outcomes in Holstein Friesian and Begait bulls.
- Post-war recovery efforts, **w**ork with local and international organizations to rebuild critical AI infrastructure, restore operational supply chains, and train personnel in conflict-resilient management practices

## 7. REFFERENCE


Agegnehu B (2007) Performance evaluation of semen production and distribution team for fiscal year. NAIC, Addis Ababa

Ahmed, M.M., Bezabih Emana, M.A., Tangka, J.F. and Ehui, S. (2003) Economic and Nutritional Impacts of Market-Oriented Dairy Production in the Ethiopian Highlands. Socio-Economics and Policy Research Working Paper 51. ILRI (International Livestock Institute), Nairobi, 27.

Almquist and Hale, (1956) Almquist JO and Hale EB 1956. An approach to the measurement of sexual behavior and semen production of dairy bulls. Proceedings, 3rd International Congress on Animal Reproduction, Plenary Papers, Cambridge, England, pp. 50–59.

Almquist, et al. (1973) J O Almquist .Effects of sexual preparation on sperm output, sperm

Almquist, J. 1978. Bull semen collection procedures to maximize output of sperm Technical Conference on Artif. Insem. Reprod. 33–36.

Amare, T.S., Reda, A.A., 2012. Quality evaluation of cryopreserved semen used in artificial insemination of cattle in selected districts of western gojjam zone of Amhara region. Ethiop. J. Reprod. Infertil. 3 (1), 1–7, 2012.

Arthur, G. H., Noakes, D. E. and Pearson, H. (1989): Veterinary Reproduction and Obstetrics. 6 th ed. London: Bailliere Tindal, pp. 509-584.

Arthur, G.H, Noakes, D.E. and Pearson, H. 1982. Veterinary Reproduction and Obstetrics. 6th Edn., The English Language Book Society and BalliereTindall, Londo

Ashebir,G.,Birhanu ,A. and Gugsa, T.,(2016): Status of artificial insemination in Tigray Regionaln State, ″ Constraints and acceptability under field condition ". J Dairy Vet Animal Res,3(3), p.00078.

Ax, R.L., Dally, M., Didion, B.A., Lenz, R.W., Love, C.C., Varner, D.D., Hafez, B., Bellin, M.E., 2000. Semen evaluation. In: Hafez, B., Hafez, E.S.E. (Eds.), Reproduction in Farm Animals, 7th ed. Williams & Wilkins, Lippincott, pp. 365–375

Ax, R.L., Dally, M.R., Didion, B.A., Lenz, R.W. and Love, C.C., Varner, D.D., Hafez, B. and Bellin, M.E. 2008. Semen evaluation. Reproductive in Farm Animals. 8th Edition. Edited by Hafez and Hafez. Lea and Febiger. PP : 365-375.

Aynalem, H., Workneh, A., Noah, K., Tadelle, D. & Azage, T. (2011). Breeding strategy to improve

Ethiopian Boran cattle for meat and milk production. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 26. Nairobi, Kenya, ILRI.

Balić, I. M., S. Milinković -Tur, M. Samardžija, and S. Vince. 2012. Effect of age and environmental factors on semen quality, glutathione peroxidase activity and oxidative parameters in simmentalbulls.Theriogenology.78:423– 431. doi:10.1016/j.theriogenology.2012.02.022

Barth AD, Bowman PA. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. The Can Vet J 1994;35:93-102

Barth AD, Oko, R.J. Abnormal Morphology of Bovine Spermatozoa. Malden, USA: Blackwell

Bearden, H. J. and Fuquay, J. W. (2000): Applied Animal Reproduction. 5th ed. Upper Saddle, New Jersey: Prentice Hall Inc., pp. 138-147.

Bhosrekar, M. R. (1990): Semen Production and Artificial Insemination. 1st ed. India: BAIF Development Foundation. pp. 4-205.

Bjorndahl, L.; Soderlund, I.; Kvist, U.(2003), Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Hum. Reprod. 18, 813–816.

Blezinger,S.(1999):Cattle Today: Many Factors Affect Bull Performance. http://www.cattletoday.com

BoARD (Bureau of Agriculture and Rural Development). (2014). Reported on the international conference on enhancing economic growth and public health through livestock development and one health approach, Tigray, Ethiopia.

Brito, L. F., A. E. Silva, L. H. Rodrigues, F. V. Vieira, L. A. Deragon, and J. P. Kastelic. 2002a. Effect of age and genetic group on characteristics of the scrotum, testes and testicular vascular cones, and on sperm production and semen quality in AI bulls in Brazil. Theriogenology. 58:1175– 1186. doi:10.1016/S0093-691X(02)00921-4

Brito, L. F., A. E. Silva, L. H. Rodrigues, F. V. Vieira, L. A. Deragon, and J. P. Kastelic. 2002b. Effects of environmental factors, age and genotype on sperm production and semen quality in bos indicus and bos taurus AI bulls in Brazil. Anim. Reprod. Sci. 70:181–190. doi:10.1016/ S0378-4320(02)00009-X

Cannon, Bryant (2009). *Investment Opportunities in Mekelle, Tigray State, Ethiopia*. New York: Millennium Cities Initiative

Casady, R.B., R.M. Meyers, and J.E. Legates. 1953. The effect of exposure to high ambient temperature on spermatogenesis in the dairy bull. J. Dairy Sci. 36:14.

CHANG, M. C. & WALTON, A. (1940). The effects of low temperature and acclimatization on the respiratory activity and survival of ram spermatozoa. Proc. Roy. Soc. B, 139, 517

characteristics and sexual activity of beef bulls with a comparison to dairy bullsJournal of Animal Science, 36 (1973), pp. 331-336

Chenoweth P, Chase C, Risco C, et al. Characterization of gossypol-induced sperm abnormalities in bulls. Therio 2000;53:1193-1203.

Chenoweth PJ, Risco CA, Larsen RE, et al. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. Therio 1994;42:1-13.

Chenoweth PJ, Spitzer JC and Hopkins FM (1992). A new bull breeding soundness evaluation form. Proc. Society for Theriogenology AGM, pp63-70.

Coulter, G. H. (1986): Puberty and post puberal development of beef bulls. In: Morrow, D. (ed): Current Therapy in Theriogenology. 2nd ed. Philadelphia: W. Sounders Company. pp. 142-152.

CSA (2021/2022): Agricultural sample survey, report on livestock and livestock characteristics for the year 2021/2022, AdisAbaba, Ethiopia.

CSA. 2020a. Agricultural Sample Survey 2019/20 [2012 E.C.]. Volume II report on livestock and livestock characteristics (private peasant holdings). Central Statistical Agency (CSA): Addis Ababa, Ethiopia.

Das, H. N., Verma, R. P., Ansari, M. R., Munjumdar, A. C. (1999): Assessing of the fertilizing potential in crossbreeding bulls by some current diagnostic approach invitro. Journal of Animal Sciences. 69 (1), 27-31.

Desalegn, G., Medhin, Bekana, Merga, Tegegne, Azage, Belihu, Kelay, 2008. Status of Artificial Insemination Service in Ethiopia. Ethiopian Society of Animal Production.

Dmitriev, N. G., Zhigachev, A. I., & Chemisova, E. F. (1989). Breeding of farm animals with the basics of private zootechny and industrial livestock. Agropromizdat, Leningrad, 118-119 (Ru).

Evans, G., Maxwell, W.M.C., 1987. Salamon's Artificial Insemination of Sheep and Goats. Butterworth, Sydney, Australia.

FAO. 2019. FAOSTAT database. www.fao.org/faostat/en/.

Faulkner, L. C. and Pineda, M. H. (1980): Artificial insemination. In: Donald, M.C. (ed): Veterinary Endocrinology and Reproduction. 3rd ed. Philadelphia: Lea and Febiger, pp. 330-366.

Feradis MP. 2010. Bioteknologi Reproduksi pada Ternak. Alfabeta. Bandung.

Fiaz, M., R. Usmani, M. Abdullah, and T. Ahmad. 2010. Evaluation of semen quality of Holstein Friesian and Jersey bulls maintained under subtropical environment. Pak. Vet. J. 30:75–78.

Fuerst-Waltl, B., H. Schwarzenbacher, C. Perner, and J. Sölkner. 2006. Effects of age and environmental factors on semen production and semen quality of Austrian simmental bulls. Anim. Reprod. Sci. 95:27–37. doi:10.1016/j. anireprosci.2005.09.002.

Gamborg C, Sandøe P. (2005) Sustainability in farm animal breeding: a review. Livestock Production Science: 221-31.

Garner DL and Johnson LA (1995). Viability assessment of mammalian sperm using SYBR14 and propidium iodide. Biology of Reproduction 53:276-284.

Garner DL, Hafez ESE. 2000. Spermatozoa and Seminal Plasma. In: Hafez B, Hafez ESE (eds). Reproduction In Farm Animals. 7th ed. Lippincott Williams and Wilkins. South Carolina. p96-109.

Garner, D. L. (1991): Artificial Insemination. In: Cupps, P. T. (ed.): Reproduction in Domestic Animals. 4 th ed. San Diago, California: Academic Press Inc., pp. 251-274.

Garner, D. L. (1991): Artificial Insemination. In: Cupps, P. T. (ed.): Reproduction in Domestic Animals. 4 th ed. San Diago, California: Academic Press Inc., pp. 251-274.

Gebremedhin, D. (2008) Assessment of Problems/Constraints Associated with Artificial Insemination Service in Ethiopia. M.Sc. Thesis, Addis Ababa University, Debre Zeit.

Godfrey, R.W. and R.E. Dodson. 2005. Breeding soundness evaluations of Senepol bulls in the US Virgin Islands. Theriogenology 63:831- 840.

Gomes, W. R. (1977): Artificial Insemination. In: Cole, H. H. and. Cupps, P. T. (ed): Reproduction in Domestic Animals. 3rd ed. New York: Academic Press, pp. 257-284.

Goyal H. Histo quantitative effects of orchiectomy with and without testosterone enanthate treatment

on the bovine epididymis. Am J. of Vet Res 1983; 44:1085-1090.

Graffer, T. Solbu, H. and Filseth, O. (1988): Semen production in artificial insemination bulls in Norway. Theriogenology. 30 (5), 1011-1021.

Graham EF, Schmehl Nelson DS, (1980). Problems with laboratory assays. Proc Eighth NMB Tech Conf Al Reprod:59-66.

Hafez, E. S. E. (1993): Reproduction in Farm Animals. 6 th ed. Philadelphia: Lea and Febiger, pp. 405-439.

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

Hancock, J.L., 1952. The morphology of bull spermatozoa. J. Exp. Biol. 29, 445–453.

Hernandez E, Galina CS, Orihuela A, Navarro-Fierro R. Observation of freezing capability and seminal charecteristics in four breeds of Bos indicus cattle. Animal Reproduction Science. 1991; 25:23-29.

Himanen, A. and Tegegn, A. (1998) A Proposal for Establishment of a National Milk Recording and Herd Registration Scheme in Ethiopia. Ministry of Agriculture, Addis Ababa.

Hoflack, G., A. Van Soom, D. Maes, A. De Kruif, G. Opsomer and L. Duchateau. 2006. Breeding soundness and libido examination of Belgian Blue and Holstein Friesian artificial insemination bulls in Belgium and The Netherlands. Theriogenology 66:207 216.

Hopkins, F.M. and J.C. Spitzer 1997. The new society for theriogenology breeding soundness evaluation system. Bull infertility 13:283-293.

Hudison, R. S. (1972): Symposium of reproduction in cattle and horse, Anim. vet. Soc. for study of breeding Soundness. American College of Theriogenology, Mimeo, Hastings.

Ismaya. 2014. Bioteknologi Inseminasi Buatan Pada Sapi Dan Kerbau. Gadjah Mada University Press. Yogyakarta.

Kavak, A, N. Lundeheim, M. Aidnik and S. Einarsson. 2004. Sperm morphology in Estonian and Tori breed stallions. Act.Vet. Scan. 45:11-18.

L.Mason Aworld Dictionary of Livestock Breeds,Types and Varieties,(1996)

Lasley, J. F., Easley, G. T. & Mckenzie, F. F. (1942). A staining method for the differentiation of live and dead spermatozoa. Anat. Rec. 8a, 167.

Lin, C. Y., & Togashi, K. (2005). Maximization of Lactation Milk Production without decreasing persistency. J. Dairy Sci., 88, 2975–2980.

Lindasay, D. R., Entwistle, K. W., Winantea, L. (1982): Reproduction in Domestic Livestock in

Indonesia. Australian Universities of International Development Program (AUIDP), Australia.

Makhzoomi, A, N. Lundeheim, M. Haard, and H. Rodriguez-Martinez. 2007. Sperm morphology and fertility of progeny-tested AI Swedish dairy bull. J. Anim. Vet. Advances 8: 975-980.

Mathevon, M., M. M. Buhr, and J. C. Dekkers. 1998. Environmental, management, and genetic factors affecting semen production in holstein bulls. J. Dairy Sci. 81:3321–3330. doi:10.3168/jds.S0022-0302(98)75898-9

Mayer DT, Squiers CD, Bogart K, and Oloufa MM. (1951). The technique for characterizing mammalian spermatozoa as dead or living by differential staining. J Anim Sci 10:226-35

Mekonnen, T., Bekana, M. and Abayneh, T. (2010). Reproductive performance and efficiency of artificial insemination smallholder dairy cows/heifers in and around Arsi-Negelle, Ethiopia. Livestock research for rural development 22(3), Retrivedjune 10, 2014, from http://www.lrrd.org./lrrd22/3//meko22061.htm.

Mekuriaw G and Kebede A, (2015). A review on indigenous cattle genetic resources in Ethiopia: adaptation, status and survival. Online Journal of Animal Feed Resources. 5(5): 125-137.

Mukhopadhyay CS, Gupta AK, Yadav BR, Mohanty TK. Exploitation of Y-chromosome specific markers to discover SNP associated with sub fertility traits in dairy bulls. Indian Journal of Biotechnology. 2011; 10:178- 182.

MulugetaFtiwi Gebreyohanes,( 2015 )Production System and phenotypic characterization of Begait cattle, and effects of supplementation with concentrate feeds on milk yield and composition of Begait cows in Humera ranch, Western Tigray, Ethiopia. PhD thesis Department of Animal Production studies. Addis Ababa University.

Mureda,E.and Mekuriaw ,Z. (2007). "Reproductive performance of crossbred dairy cows in Eastern lowlands of Ethiopia."Livestock research for rural development. **19** (161)Retrived July 15, 2014, from http://www.lrrd.org./lrrd19/11//mure19161.htm.

Nath R 1988; cryopreservation of buffalo semen. An MVSc. Thesis Department of Animal Reproduction ,Gynecology and Obstetrics, Govind Ballabh Plant University of Agricultural and Technology,Pantnagar,India.

Ndambi, A., Zijlstra, J., van Eupen, M., Berhanu, T., Ceccarelli, T., & Vernooij, A. (2018). *Mapping and assessing high potential areas to increase milk production in Ethiopia* (No. 1128). Wageningen Livestock Research.

Nuraddis, I. & Ahmed, S. (2017). Review on Reproductive Performance of Crossbred Dairy Cattle in Ethiopia. Journal of Reproduction and Infertility, 8(3), 88–94.

Okomo-Adhiambo M (2002) Characterisation of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques. ILRI, Nairobi, Kenya.

Ott, R. S. (1986): Breeding soundness examination of bulls. In: Morrow, A. (ed.): Current Therapy in Theriogenology. 2ed. Philadelphia: W. B. Sounders Company, pp. 125-135.

Pan, Q., Z. Ju, J. Huang, Y. Zhang, C. Qi, Q. Gao, L. Zhou, Q. Li, L. Wang, J. Zhong, M. Liu, and C. Wang. 2013. PLCz functional haplotypes modulating promoter transcriptional activity are associated with semen quality traits in Chinese Holstein bulls. PLoS One 8:e58795. https://doi.org/10.1371/journal.pone.0058795.

Parker, R., Mathis, C. and Hawkins, D. 1999. Evaluating The Breeding Soundness in Beef Bulls. Dept. of Animal and Range Sciences. New Mexico State University.

Publishing Inc., 1989.

Randel RD, Chase CC, Jr., Wyse SJ. Effects of gossypol and cottonseed products on reproduction of mammals. J Anim Sci 1992;70:1628-1638.

Rege, J.E., Toel, F., Mukasa-Mugerwa, E., Tembely, S., Amindo, D., Baker, R.L., LahlouKassi, A., 2000. Reproductive characteristics of Ethiopian highland sheep. II. Genetic parameters of semen characteristics and their relationships with testicular

Rekwot PI, Oyedipe EO, Akerejola OO, Kumi-Diaka J. The effect of protein intake on body weight, scrotal circumference and semen production of Bunaji bulls and their Friesian crosses in Nigeria. Animal Reproduction Science 1988; 16:1-9.

Rezene Teweldemedhine. (2007). Production and economics of Arado and Begait cattle in Eritrea.MSc Thesis. University of Kwazulu-natal Pietermaritzburg.

Roberts, S. J. (1971): Veterinary Obstetrics and Genital Disease, 2 ed ed. Indian: CBS publishers and Distributors, pp. 612-750.

Salisbury, G. W., Van Demark, N. L., Lodge, J. R. (1978): Physiology of Reproduction and Artificial Insemination of Cattle. 2ed. ed. San Francisco: W. H. Freeman, pp. 385-479.

Sankhi, KR Sapkota, B Regmi-International Journal of Applied..,2019-researchgate.net

Sarastina, Susilawati, T. and Ciptadi, G. 2006. Analysis of multiple parameters sperm motility in different breed of cattle using computer assisted Semen analysis (CASA). J. Livestock Tropica. 6 (2): 1-12. (Indonesian).

Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, Klinefelter GR, Makris SL, Perreault SD, Schrader S, Seyler D. (1996). Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. Reproductive toxicology. 1;10 (3):237-44.

Setchell, B. P. (1991): Male reproductive organs and semen. In: Cupps, P. T. (ed): Reproduction in Domestic Animals. 4th ed. San Diago: Academic Press, pp. 221-249.

Shapiro, B.I., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Aboset G. and Mechale. H. 2017. Ethiopia livestock sector analysis. ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Skinner, J.D. and G.N. Louw. 1966. Heat Stress and spermatogenesis in Bos indicus and Bos Taurus cattle. J. Appl. Physiol. 21:1784-1790.

Snoj, T., S. Kobal, and G. Majdic. 2013. Effects of season, age, and breed on semen characteristics in different bos taurus breeds in a 31-year retrospective study. Theriogenology. 79:847–852. doi:10.1016/j.theriogenology.2012.12.014.

Sorensen, Jr. A. M. (1979): Animal Reproduction Principles and Practices. New York: McGraw-Hill Publishing Company, pp. 85-233.

Stalhammar, E. M. (1995): Prospects of selecting Artificial Insemination bulls for fertility. Uppsala: Swedish University of Agricultural Science. No.116.

Statham, Jonathan, Katie Burton, and Mark Spilman. (2019). "Looking after the bull: guide to management and assessment of fertility." *In Practice* 41.2, (2019): 69-83.

Sullivan, J. J. (1978): Morphology and motility of spermatozoa. In: Salisbury, G. W., Van Demark, N. L., Lodge, J. R. (ed): Physiology of Reproduction and Artificial Insemination of Cattle. 2ed ed. San Francisco: W. H. Free man, pp. 286-328.

Tadesse, B. (2002). Reproductive performances of zebu (Fogera) breed in the central highlands of Ethiopia. DVM. Thesis, Addis Ababa University, Ethiopia.

Tadesse, B. (2005) Calf Sex Ratios in Artificially Inseminated and Natural Mated Female Crossbred Dairy Herd. Proceedings of the 13th Annual Conference of the Ethiopian Society of Animal Production, Addis Ababa, 225-230.

Tanga, Bereket Molla, Ahmad Yar Qamar, Sanan Raza, Seonggyu Bang, Xun Fang, Kiyoung Yoon, and Jongki Cho.(2021). "Semen evaluation: Methodological advancements in sperm quality-specific fertility assessment—A review." *Animal bioscience* 34, no. 8 1253.

Tegegn, F. & Zelalem, A. (2017). Evaluation of oestrus synchronization and mass artificial insemination service of dairy cattle in Mizan Aman area, Bench Maji zone, South West Ethiopia. International Journal of Livestock Production, 8(1), 1−4. DOI: 10.5897/ IJLP2016.0338

Tekeev, M. E. (2015). Perfection of dairy breeds of the North Caucasus using the genofond of Holstein cattle. Doctoral dissertation, North Caucasian state Humanitarian and Technological academy. Cherkessk, Russia (Ru).

Ulfina G, Zelalem B, Jemal D, Gemeda D, Chala M, Jiregna D, Diriba G, Lemma G, Workneh A and Adam D (2005) Survey of cattle production and marketing practices in Danno District, western Ethiopia, using PRA tools. PRA Report

Webb D.W. (2003): Artificial Insemination in Cattle. University of Florida, Gainesville. IFAS Extension, DS 58. Pp. 1-4.

Webb, D.W. (2003) Artificial Insemination in Cattle. University of Florida, Gainesville, IFAS Extension, DS 58, 1-4.

Williams, W.W. and Pollak, O.J. (1950) Study of sperm vitality with the aid of Eosin-Nigrosin stain. Fertil. Steril., 1, 178-181.

Yemane B., Chernet T., Shiferaw T. (1993): Improved Cattle Breeding. National Artificial Insemination Centre. Addis Ababa, Ethiopia. Pp. 15.

Zerabruk, M., Vangen ,O. and Haile ,M.,(2007): The status of cattle genetic resources in North Ethiopia : On farm characterization of six major cattle breeds. Animal genetic Resources /Resources

genetiques animals / Recursos geneticos animals,40,pp,15-32.

Zewdie E (2007) Artificial insemination and its implementation. Ethiopian Society of AnimalProduction ESAP Addis Ababa, Ethiopia: 7-45.

## 8. ANNEXES

### Annex 1: Procedure for the preparation of the artificial vagina (Salisbury et al., 1978)

A. Insert the inner liner into the cylinder
B. Turn the ends of the liner back over the cylinder
C. Fit the collection receptacle into the ends of the rubber funnel
D. Place the funnel over one end of the cylinder and the liner
E. Secure the connections between the receptacle and the funnel and between the ends of the liner and the cylinder with string or elastic bands or heavy cords.
F. Once the artificial vagina is assembled in the above manner, fill it with water at the proper Temperature and pressure (Note the temperature of the water will vary with the air Temperature, with delay between filling and collection, and with individual bulls. At the time of collection the temperature of AV between 42 0C and 44 0C is usually effective). If the external temperature is cold or if there is a chance of delay between filling of warm water and collection of the semen a temperature of 55 0C or higher is recommended.
G. Following addition of warm water to the water jacket at the proper temperature and pressure, the first 3 to 5 inches of the interior of the liner should be thinly coated with sterile lubricating jelly that is harmless to the spermatozoa.

Preparation of Bovine Artificial Vagina

**Annex 2: Evaluations & Processing**

A. The tube containing the freshly collected semen should be capped with aluminum foil as Soon as it is placed in the dynamic pass box before transferring to the laboratory. The collection tube should preferably remain capped until processed.
B. As soon as the neat semen is received, it shall be kept in a thermo- controlled water bath at 34$^{o}$C under Laminar Air Flow Unit, after recording the volume of semen.
C. After examination of sperm concentration and initial motility as soon as possible, the semen samples shall be primarily diluted (1 1) with dilutor maintained at 34°C. After dilution of semen in the ratio of 1:1, it shall be extended further (final) in appropriatelyized flasks after with dilutor / flasks maintained at the same temperature (34°C), under the Laminar air flow unit. The neat semen samples should not be allowed to get accumulated for long time in water bath to minimize adverse effects on viability. After the final dilution the flask should be kept at room temperature for 10-15 minutes so that temperature reaches 20-22oC.
D. Sperm concentration shall be checked by a digital photometer with auto dilutor, manufactured by a reputed company. Semen samples having concentration of less than 500 million / ml sperm concentration shall be discarded. The dilution unit of photometer should be placed under laminar air flow unit. The volume of straws should be checked as it may vary between manufacturer/ type of straw. While determining the dilution rate as per the photometric reading, the correct volume of the French mini straw should be fed to the photometer. The volume of randomly drawn straws from a day's production should be checked as part of quality assurance and be documented. Each straw (dose) should possess a minimum of 20 million spermatozoa.
E. Semen samples selected for processing should have a minimum of 70% initial progressive motility.
F. Filling and sealing of semen shall be done under Laminar Air Flow Unit using sterile straws, filling nozzles and disposable rubber tubing's. Rubber tubing's shall not be reused in any case.
G. Unused straws shall be repacked (air-tight) under Laminar Air Flow Unit before storage. Immediately after use, all the glass ware and other re-usable articles shall be immersed in lukewarm neutral detergent solution (in a plastic tub near the Laminar Air Flow Unit).

H. The freezing should be carried out as per the recommended protocols for freezing cattle and buffalo semen. After freezing gets over, the straws should be collected from the racks using scoop tongs. The operator should wear appropriate protective gloves to avoid frost injury

**Preparation to Mount**

**During Semen Collection**

**Annex 3: Williams stain, staining technique**

1. Make fresh semen smear (thin) on clean glass and allow it to dry,

2. Fix the dried smear on the flame,

3. Flood the fixed smear with absolute alcohol for 3-4 minutes,

4. Flood the smear with chloramine solution, for 1-2 minutes,

5. Wash the smear with distilled water and flood it (finally) with carbolfuchsin for 8 to 10 minute,

6. Wash with tape water, air dry, and examine under oil immersion objectives of the bright field microscope.

**Annex 4. Eosin-Nigrosine Staining Procedure**

1. Obtain a semen sample from the bull, ensuring it is collected in a sterile manner.
2. Preparation of the Staining Solution:
    - Eosin Y: A synthetic dye that stains dead spermatozoa.
    - Nigrosine: A dye that provides a contrasting background, allowing for better visualization of the sperm.
3. Typical ratio for the staining solution is:1 part eosin to 5 parts nigrosine.
4. Dilution of Semen sample with a suitable buffer or saline solution to achieve an optimal concentration for microscopy.
5. Mix a small volume (e.g., 10 µL) of the diluted semen with an equal volume (10 µL) of the eosin-nigrosine staining solution on a clean glass slide.
6. Use another slide to spread the mixture into a thin smear.
7. Allow the smear to air dry.
8. Examine the dried smear under a light microscope.
    - Live spermatozoa will appear white or pale (unstained),
    - Dead spermatozoa will be stained pinkish-red due to the eosin dye.


Semen Evaluation

**CURRICULUM VITAE**

## I. Personal Identification

| | |
|---|---|
| Name | Tesfay Gebremariam |
| Birth Place | Tigray ,Eastern Zone, Adigrat |
| Kebele | Adigrat 06 |
| Birth date | 07/11/1965 |
| Sex | Male |
| Marital status | Married |
| Religion | Christian |
| Denomination | Orthodox |
| Nationality | Ethiopian |
| Profession | Veterinarian |
| Occupation | Field veterinary service |

**II. Contact Address** Tigray Bureau of Agriculture and Rural development

Email: tesfaygebremariam77@ gmail.com, Mobile 0938768541

**III. Education**

**Elementary School** - Adigrat ,Agiazi, elementary school from 1969 - 1974

**Secondary school -** Adigrat ,Agiazi comprehensive high School from 1975 - 1980

**Diploma** - Faculty of Veterinary Medicine, Debre Zeit, Ethiopia. From 1981 -1982

**University** -My first degree program Join in Mekelle University from 1996 -1998

IV. Work Experience

From 1983 until I joined the MSc program in the Faculty of Veterinary Medicine in 2013, I served as a field veterinarian and worked at the artificial insemination center in the Tigray Regional State.

V. Language Skill

Tigrigna language ……. Read, write and speak

Amharic language…….. Read, write and speak

English language ……...Read, write and speak