



**MEKELLE UNIVERSITY  
COLLEGE OF VETERINARY SCIENCE**



**EVALUATION OF BULL SEMEN QUALITY IN MEKELLE ARTIFICIAL  
INSEMINATION CENTER, TIGRAY, ETHIOPIA**

**By**

**Hadish Ghidey**

**A Thesis Submitted to the, College of Veterinary Science, Mekelle University in  
Partial Fulfillment of the requirements for the Degree of Master of science in  
Veterinary Reproduction and Obstetrics**

**February, 2024**

**Mekelle, Ethiopia**

## Declaration

*I acknowledge the information provided. This certification confirms that the thesis titled "Evaluation of Bull Semen Quality in Mekelle Artificial Insemination Center Tigray" was submitted by Mr. Hadish Ghidey as part of the requirements for the degree of Master in Veterinary Reproduction and Obstetrics. It further states that the work presented in the thesis is original and has not been submitted previously for any other degree, diploma, or publication. The thesis was conducted under the close guidance and supervision of the principal advisor.*

Name            Hadish Ghidey

Signature        \_\_\_\_\_

Date of submission \_\_\_\_\_

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

Destalem Gebretsadkan (Dvm, Msc, Assistant Professor)

Gebregiorgis Ashebre (Dvm, Msc, Associate Professor)

**EVALUATION OF BULL SEMEN QUALITY IN MEKELLE ARTIFICIAL  
INSEMINATION CENTER AND DISTRICTS TIGRAY, ETHIOPIA**

**By**

**Hadish Ghidey**

**BOARD OF EXTERNAL EXAMINERS**

1. \_\_\_\_\_

2. \_\_\_\_\_

**Advisors:**

**Signature:**

**1. Dr. Destalem Gebretsadkan (DVM, MSC)**

\_\_\_\_\_

**2. Dr. Gebregiorgis Ashebre (DVM, MSC)**

\_\_\_\_\_

## TABLE OF CONTENTS

CONTENTS	PAGES
TABLE OF CONTENTS .....	I
ACKNOWLEDGEMENTS .....	III
LIST OF FIGURES .....	IV
LIST OF TABLES .....	IV
LIST OF ABBREVIATIONS .....	V
ABSTRACT .....	VI
CHAPTER I: .....	1
<b>1.1. Introduction</b> .....	<b>1</b>
1.1.1. Statement of the problem .....	3
1.1.2. General objective .....	5
1.1.3. Specific objectives .....	5
1.1.4. Significance of the study .....	5
1.1.5. Scope of the study .....	6
<b>1.2. Literature Review</b> .....	<b>6</b>
<b>1.2.1. Cattle Production in Ethiopia</b> .....	<b>6</b>
1.2.3. Semen collection .....	9
1.2.4. Semen quality assessment .....	10
1.2.5. Semen collection and processing .....	10
1.2.5.1 Appearance or color .....	10
1.2.6. Factors affecting bovine semen quality .....	16
<b>2. CHAPTERII: MATERIAL AND METHODS</b> .....	<b>20</b>
<b>2.1. Study Area</b> .....	<b>20</b>
<b>2.2. Study Animals</b> .....	<b>21</b>
<b>2.3 Study Design and sampling</b> .....	<b>23</b>
2.3.1 Sample size determination .....	23
2.3.2 Sample Collection and Procedure .....	23
2.3.3 Physical examination of the semen .....	24
2.3.4 Microscopically examination .....	24
2.3.5. Sperm concentration .....	24
2.3.6. Sperm morphological examination .....	25

2.3.7. The Live and cell of spermatozoa .....	25
2.3.8. Semen PH.....	26
2.3.9. Sperm Acrosome integrity Test .....	26
2.3.1. OHOST .....	27
<b>2.4. Dilution and cryopreservation of semen .....</b>	<b>27</b>
<b>2.5. Production of the bulls.....</b>	<b>28</b>
<b>2.6. Data analysis .....</b>	<b>28</b>
<b>3. CHAPTER III: RESULTS.....</b>	<b>29</b>
<b>3.1. Physico-Morphological Characteristics and Functional Attributes of Semen</b>	<b>29</b>
<b>3.2 Physico-Morphological Characteristics and Functional Attributes of post thawed Semen .....</b>	<b>30</b>
<b>3.3 Physico-Morphological Characteristics and Functional Attributes of post thawed Semen at districts .....</b>	<b>30</b>
<b>3. 4. Assessment of the semen result between Districts.....</b>	<b>31</b>
<b>3. 5 Semen production potential of Mekelle artificial insemination center .....</b>	<b>32</b>
<b>3.6. Production of bull semen during study season .....</b>	<b>33</b>
<b>4. CHAPTER IV: DISCUSSION.....</b>	<b>34</b>
<b>4.1 Result of fresh semen at MAIC .....</b>	<b>34</b>
<b>4.2. Result of post thawed semen in MAIC .....</b>	<b>36</b>
<b>4.3. Result of post thawed semen at District .....</b>	<b>37</b>
<b>4.4 production of the bulls .....</b>	<b>38</b>
<b>5. CONCLUSIONS AND RECOMMENDATIONS.....</b>	<b>39</b>
<b>6. REFERANCE.....</b>	<b>40</b>
<b>7. ANNEXES .....</b>	<b>58</b>

## **ACKNOWLEDGEMENTS**

I would like to express my gratitude to God and my parents for their unwavering support throughout my academic journey. I am also grateful to the Mekelle University Veterinary College and the Tigray Bureau of Agriculture and Rural Development Bureau for providing me with financial assistance and granting me permission for a study leave.

I extend my heartfelt thanks to my advisor, Dr. Destalem Gebretsadkan (DVM, MSc, Assistant Professor), for his invaluable guidance, scholarly counsel, constructive criticism, references, thorough editing, and assistance in finalizing my thesis. I am also grateful to my co-advisor, Dr. Gebregiorgs Ashebre (DVM, MSc, Associate Professor), for his ongoing support, guidance, technical assistance, and valuable contributions to my research.

I would like to acknowledge and thank Tadese Gugsu for his advice and technical support throughout my study. I am also grateful to Amdework Zekarias for his continuous counsel, assistance, and technical support. Furthermore, I express my appreciation to Bisrat Mesfin for his assistance with the semen work at the Tigray Regional Animal Laboratory. I am indebted to all the individuals and institutions mentioned above for their contributions to my academic and research endeavors. Their support and guidance have been instrumental in the successful completion of my thesis.

## **LIST OF FIGURES**

Figure 1: map of study area .....	21
Figure 2: Bulls at exercise.....	22
Figure 3: semen collection MAIC.....	22

## **LIST OF TABLES**

Table 1: Physico-morphological quality of fresh semen .....	29
Table 2: Mean of post thawed semen physico-morphological parameters.....	30
Table 3: physico-morphological quality of post thawed semen at field .....	31
Table 4: Assessment of the semen result between Districts .....	31
Table 5 : Semen production potential of MAIC .....	32
Table 6: Liquid nitrogen production potential of MAIC .....	32
Table 7: production of bull semen during study season .....	33

## **LIST OF ABBREVIATIONS**

AI	Artificial insemination
AV	Artificial vagina
HF	Holstein-Friesian
HOST	Hypo osmotic swelling test
MAIC	Mekelle Artificial Insemination Center
NAGII	National Animal Genetic Improvement Institute
NAIC	National artificial insemination center
SQPs	Semen Quality Parameters
TAI	Timed-artificial insemination

## ABSTRACT

The study aimed to assess the quality characteristics of fresh and post-thaw semen from 10 artificial insemination (AI) bulls of different breeds (5 Holstein-Friesian, 2 Jersey, and 3 Begait) at the Mekelle Artificial Insemination Centre (MAIC). The research also compared semen quality among the breeds and evaluated the potential of the bull semen at the center. Additionally, semen samples from districts including Ganta Afeshum, Kiltawlaelo, Enderta, and Mekelle City were examined. A total of 400 semen samples were analyzed for various parameters, including volume, mass motility, individual motility, sperm concentration, normal morphology, live and dead cell counts, pH, acrosome integrity, and HOST (Hypo-Osmotic Swelling Test). Semen was collected once per week from each bull using an artificial vagina and diluted with the optixcell2 extender. Statistical analyses such as one-way analysis of variance (ANOVA) and paired t-tests were performed using SPSS 20.0 to determine the significance levels. The results showed that the mean values of fresh semen were as follows: volume 6.8 ml, mass motility 3.4 (on a scale of 1-4), individual motility 77.5%, concentration 0.7 billion/ml, normal morphology 69%, live cells 68.2%, and pH 6.2. According to the results obtained at MAIC, there were no significant differences between breeds ( $p>0.05$ ) in terms of fresh semen volume, pH, concentration, individual and mass motility, and morphology, except for live cell count. In the post-thawing evaluation, the following parameters were observed individual motility - 59.9%, normal morphology - 64.4%, total live cell count 62.3%, acrosome integrity 70.7%, and HOST 61.5%. There were significant differences between the breeds ( $p<0.05$ ) in terms of individual motility, normal morphology, and live and dead cell counts. However, no significant differences were found in acrosome integrity and HOST sperm quality ( $p>0.05$ ). In the field study, the post-freezing motility was recorded as 42.7%, normal morphology 50.4%, live cell count 53.7%, acrosome integrity 49.3%, and HOST  $51.6\pm 0.6\%$ . Overall, the results indicated that the semen quality of the bulls at the center was satisfactory, except for the lower morphology results. However, the districts' results were unsatisfactory, highlighting the need for further investigation at both the center and district levels.

**Keywords:** *AI, Breed, Evaluation, physico-morphological parameters, Quality, Semen*

## **CHAPTER I:**

### **1.1. Introduction**

Livestock production plays a vital role in Ethiopia's agricultural economy, the livelihoods of farmers, and the country's food security. Livestock contributes approximately 16-17% of Ethiopia's foreign exchange earnings and 35-49% of the agricultural GDP (Hamid, 2021). With an estimated population of 70 million cattle, Ethiopia possesses a significant genetic resource, making it the most populous country in Africa and the fifth most populous in the world. Cattle are widely raised in Ethiopia, with crossbred and exotic breeds accounting for only 2.3% and 0.31% of the total cattle population, respectively (CSA, 2021).

Ethiopia is home to five classes of native cattle breeds, including Zebu, Sanga, intermediate Sanga, Hamatic Longhorn, and Humpless Brachyceros, as reported by EBI (2016). The genetic diversity of animal genetic resources is of utmost importance for current and future research and development projects. According to Delgado Bermejo *et al.* (2019), genetic diversity allows native genetic resources to adapt and thrive in diverse agro-ecological environments.

Tigray, one of the regions in Ethiopia, is the fourth most cattle-populated region, accounting for approximately 8% of the country's genetic resources (CSA, 2018). Within Tigray, there are five indigenous cattle populations: Arado, Begait, Abergelle, Erob, and Raya cattle. A study by Zegeye *et al.* (2021) identified four distinct clusters among these populations, with Abergelle and Erob grouped together.

Dairy production in Ethiopia faces several constraints, as highlighted by Getabalew *et al.* (2019). These constraints include diseases and parasites affecting cattle, poor cattle performance, inadequate veterinary care, and a lack of artificial insemination (AI) services. These factors contribute to the challenges faced in the dairy sector. The total production of cow milk in Ethiopia is approximately 4.7 billion liters, resulting in a low average daily milk production per cow of only 1.48 liters. These challenges and limitations in the dairy sector were also reported in a recent CSA (Central Statistical Agency) report from the 2020-21 period.

It is evident that despite the potential for dairy development, the productivity of indigenous livestock genetic resources, including cattle, remains low in Ethiopia. The direct contribution of indigenous cattle to the national economy is limited, as indicated by the low average daily milk production per cow, which is reported to be only 1.48 liters/day out of a total cow milk production of approximately 4.7 billion liters (CSA, 2020-21) approximately 50% of the annual milk production is used for household consumption, 10% is sold, 0.56% is used for wages in kind, and the remaining 39% is utilized for other purposes such as butter, cheese, and yogurt production.

Improvement in indigenous cattle can be achieved through a comprehensive assessment of the production system, evaluation of cattle performance, identification of production constraints, and the design of appropriate breeding strategies that align with the existing production system. Artificial insemination (AI) technology is a valuable tool in maximizing the utilization of superior males, facilitating the transmission of superior genetic material, enhancing the rate and effectiveness of genetic selection, and introducing new genetic material (Shanku, 2022; Vikram, 2023).

Indeed, the low productivity of indigenous livestock in Ethiopia can be attributed to poor genetic potential and nutritional status. However, there is ample opportunity for improvement through the application of various scientific approaches, including upgrading or crossbreeding programs. Artificial insemination (AI) is a significant biotechnological advancement that has played a crucial role in the genetic improvement of livestock. AI involves the transfer of superior genetic material from selected males to females. It allows for the controlled and strategic mating of animals to improve desirable traits such as milk production, growth rate, and disease resistance. Prior to insemination, the fundamental characteristics of sperm can be accessed through macroscopic or microscopic inspection (Agriris *et al.*, 2018). These assessments help ensure the quality of the semen used in the AI process.

According to Ashebir *et al.* (2016), AI services were first introduced in the Tigray region 19 years ago, specifically in the capital city of Mekelle and Adigrat. A sub-center was established in 2001 to provide consumables and liquid nitrogen to the breeding units in the region. However, it was only in 2016 that the center began production by collecting

semen from the center bulls. It is worth noting that there has not yet been any research conducted on the quality of semen generated at the Mekelle artificial insemination center.

The success of AI relies on high-quality semen, which is determined by various parameters that may or may not be determinable. The focus of the mentioned thesis is on the semen quality of bulls, particularly emphasizing field quality. The investigations in the thesis are based on laboratory evaluations and field trials. The semen quality of each breed, including Begait (a native breed), Jersey, and Holstein Frisian (foreign breeds), are evaluated as part of the study. The center is in charge of coordinating AI operations in the area and is the sole facility in the region that produces semen for usage. The Center kept ten bulls during the research periods, mostly of the HF, Jersey, and Begait breeds. The center provides 80% of the semen production from exotic breeds, with the remaining 20% coming from Begait's native breeds. The Center fills semen into 0.25 ml straws; the normal amount of spermatozoa per straw is 30 million.

#### **1.1.1. Statement of the problem**

Semen quality is the main convention for using of the Artificial insemination .AI technology has indeed proven to be a valuable tool in animal breeding, including in Ethiopia. It allows for the efficient utilization of superior males by enabling the widespread distribution of their genetic material. This, in turn, facilitates the transmission of superior genetic traits, enhances the rate and effectiveness of genetic selection, and provides opportunities to introduce new genetic material into breeding programs (Shanku, 2022; Vikram, 2023). In Ethiopia, promising advancements in AI technology have been observed primarily in dairy farms surrounding the country's largest towns, as mentioned by Hassen et al. (2007). These advancements likely include improvements in semen production, evaluation, and storage techniques. Semen evaluation is a critical step in the use of semen for artificial insemination. Various parameters are assessed to determine the quality of semen produced in artificial insemination centers. These parameters typically include volume, sperm motility (the ability of sperm to move), sperm morphology (the size and shape of sperm), the presence of live and dead cells, acrosome integrity (the structural integrity of the sperm's acrosome, which plays a role in fertilization), and the use of HOST (Hypo-Osmotic Swelling Test) to assess the

functional integrity of sperm membranes. It's important to note that the process of freezing and storing sperm samples can have an impact on semen quality. Sperm cells are sensitive to freezing and thawing, and their viability and functionality can be affected during the cryopreservation process. Therefore, it is crucial to optimize freezing protocols and storage conditions to maintain the highest possible semen quality. Overall, the advancements in AI technology in Ethiopia, particularly in dairy farms near major towns, have likely contributed to the improvement of breeding programs by maximizing the utilization of superior males and enhancing the transmission of desirable genetic traits. Continued research and development in this field will further refine AI techniques and contribute to the genetic progress of livestock populations in Ethiopia.

According to the research conducted by Mekonnen and Berhe (2023) and Ashebiret *al.* (2016), the west zone of Tigray and the eastern and south-eastern zones of Tigray have shown relatively low conception rates at first insemination, specifically 20.4%. To understand the potential causes of the low fertility, semen quality was assessed. The evaluation focused on motility and livability of the semen samples. However, the percentage of progressive motile spermatozoa (sperm cells showing forward movement) and the percentage of live spermatozoa collected did not meet the standard values for semen. This suggests that there may be issues with the semen quality being used for artificial insemination in the Tigray region, which could be contributing to the low fertility rates observed. Further investigations and analysis would be needed to determine the specific factors affecting semen quality and to identify potential solutions to improve fertility rates.

The purpose of the study was to assess the quality of bull semen in the Mekelle artificial insemination center and in the districts of Tigray. This study is motivated by the significance of dairy cattle farming in providing food (milk) and revenue for resource-poor farmers in the area. Despite the importance of the dairy industry, it is facing various issues that hinder its ability to contribute effectively. In order to address these challenges and improve the dairy industry, it is believed that the use of artificial insemination (AI) services in milk shade areas with easy access to transport can play a crucial role. As a result, the entire study area was purposefully chosen to evaluate the quality of bull semen

in the mentioned districts and the Mekelle artificial insemination center. By assessing the quality of bull semen, the study aims to provide valuable insights and recommendations to enhance the dairy industry in the region.

### **1.1.2. General objective**

The general objective of the study was to evaluate the quality of semen produced in Mekelle artificial insemination center and after the semen distributed to Eastern & South eastern zones of Tigray.

### **1.1.3. Specific objectives**

- To evaluate physico-morphological characteristics of bulls semen quality
- To compare the semen quality between breeds
- To evaluate physico-morphological characteristics of bulls semen quality in districts.
- To estimate semen production potential of Mekelle artificial insemination center

### **1.1.4. Significance of the study**

The study's significance lies in its contribution to infertility assessment, fertility evaluation, empirical research, planning and implementation of interventions, and providing a baseline for future studies. It has the potential to improve reproductive health outcomes and guide further investigations in the Mekelle AI center.

- Semen examination has great value in determining the cause, severity, and degree infertility, as well as, being of value in estimating the fertility of the male
- The study identifies the major semen parameters in the Mekelle AI center.
- This study will have significant contribution, particularly important due to the limited empirical studies on MAI center.
- It helps in planning and put into effect of the semen quality in the semen production center.
- The study should be serving as a baseline for the next research in the Mekelle AI center.

### **1.1.5. Scope of the study**

This research focus on studying evaluation of bull semen quality in Mekelle artificial insemination center and quality of semen in the collaboration with four selected districts in Tigray.

## **1.2. Literature Review**

### **1.2.1. Cattle Production in Ethiopia**

A cattle rising is a significant agricultural sector in Ethiopia, contributing to the country's economy. The annual production of beef surpasses 1 million tons, while milk production exceeds 3.8 billion liters. The estimated values of beef and milk production are USD 5.1 billion and USD 2.5 billion, respectively (FAO, 2018). Ethiopia is known for its rich diversity of cattle breeds, with 28 indigenous breeds recognized based on the characterization and identification of the country's cattle genetic resources. However, it is important to note that this number may not be exhaustive, and there might be additional indigenous breeds yet to be identified (EBI, 2016).

The native cattle breeds in Ethiopia have evolved and adapted to the tropical climate and local management practices through natural selection. These breeds are known for their hardiness, heat tolerance, disease resistance, adaptability, low feed requirements, and minimal maintenance needs. These characteristics make them well-suited for the local environment and the challenges associated with cattle farming in Ethiopia.

Given the growing demand for milk and milk products, there is a need to enhance the genetic potential of the native cattle breeds. Genetic improvement programs have been suggested as a means to address this demand. These programs aim to selectively breed indigenous cattle to enhance their milk production and overall productivity while preserving their valuable traits such as hardiness and adaptability. By strategically improving the genetic makeup of native cattle, it is possible to achieve higher milk yields and enhance the overall productivity of the dairy sector in Ethiopia. However, it is crucial to implement these genetic enhancement efforts carefully, taking into consideration the preservation of the unique characteristics and genetic diversity of the indigenous cattle breeds.

There are incredibly few exotic and hybrid cattle in Ethiopia. This shows that the overall number of exotic and hybrid female cattle generated in the nation during several decades of crossbreeding efforts is relatively small, showing that the crossbreeding efforts were unsuccessful. Once more, this indicated that Ethiopia must make a concerted effort to enhance the reproductive and productive capabilities of cattle through appropriate breeding and related work. This is because improving cattle performance through an appropriate dairy production system and influencing productivity breeding and related activities is a prerequisite for long-term performance improvements in cattle (Nuraddis and Ahmed, 2017).

### 1.2.2. Cattle breeds in Ethiopia

Ethiopia is the home of a wide variety of cattle breeds because of its diverse topography, agro-ecology, and proximity to Asia, home to the majority of Africa's domesticated animals. The mass of Ethiopia's livestock population consists of indigenous cattle, which produce the majority of the country's milk in both urban and peri-urban areas. The 28 indigenous breeds of cattle in Ethiopia are divided into five classes: intermediate Sanga/Zebu, Hamatic Longhorn, Zebu, Sanga, and Humpless Brachyceros (EBI, 2016).

According to Fasil and Workneh (2014), Ethiopia's indigenous livestock breeds are a valuable source of genetic material because of their ability to adapt to harsh climates, make better use of the scarce and low-quality feed resources, and tolerate a variety of diseases common in these areas. In addition to having sociocultural value, most rearing zebu cattle traditionally prefer those with horns. In addition to being able to protect themselves against predatory attacks, cattle with big horns are seen to be attractive and have aesthetic value (Mulugeta, 2015). With practically all livestock species being indigenous and a very small number of hybrid animals, Ethiopia's livestock industry hardly ever adapts to new technologies, which lowers average productivity (Hagos, 2016).

#### 1.2.2.1. *Begait*

Begait cattle are identified by their well-developed udders, long teats, and increased milk output. Adaptable in hot and dry conditions, able to walk great distances in search of food

and drink, with an energetic personality, long legs, and huge, typically cervical thoracic, humps. Reductions brought on by feed scarcity, disputes, and unchecked breeding could jeopardize certain ranch improvement initiatives and stimulate conservation (EBI, 2016).

The Begait cattle are visually appealing due to their variety of colors, and their tameness belied their aggression toward outsiders and predators while they were with their owners. Begait cattle are valued by the rural population as draught animals because they have larger skeletal frames than Arado cattle. Begait cattle can generate an average of 12–15 liters of milk per day, but they lose body condition more quickly than Arado cattle, making them more prone to disease (Mekuriaw and Kebede, 2015), (Mezgebe, *et al.*, 2017)

Begait cattle are predominant in the warm, humid lowlands of the Kafta-Humera district. In comparison to other native Ethiopian cattle, Begait cattle have a comparatively higher productivity potential, a larger body size, a well-developed udder, and long teats (Zerabruk: Gebretnsae. *et al.*, 2017). It was discovered that the Tahtay-adiabo, Kafta-Humera, Welqait, and Asgede-tsimbla districts have the greatest potential for Begait breeds. For Begait livestock to be properly conserved and used, accurate categorization is necessary. Except for the work of Ftiwi and Tamir (2015), Begait cattle are registered as one of Ethiopia's indigenous cattle breeds; nonetheless, both quantitative and qualitative data on these cattle reared in on-farm and ranch situations is currently sparse. (Zerabruk *et al.*, 2007)

#### 1.2.2.2. Jersey

Jersey cows are highly tolerant to heat, in contrast to many breeds that originated in temperate regions. They also adapt well to a variety of habitats and temperatures. It has been exported to many nations; in a few of them, such as the United States, Denmark, France, and New Zealand, it has become its own breed. According to Valerie *et al.*, (2016), bulls might be unpredictable or aggressive, while cows are peaceful and submissive. According to Imbayarwo-Chikosi (2010), Jersey is the perfect breed to crossbreed with *Bos indicus* to create a resilient, disease-tolerant dairy cow that can be raised in community settings and doesn't require a high nutritional level to deliver a

respectable amount of milk. Currently, a growing number of dairy farmers are interested in switching from Holsteins to Jerseys again because of Jersey breed make more profit per acre than other larger breeds (Kumar *et al.*, 2015).

#### 1.2.2.3. *Holstein Friesian*

The most widely distributed breed of cow in the world, found in over 150 nations Dairy breeders looked to the Netherlands for their animals as the New World expanded and milk markets grew in North and South America. Disease issues in Europe forced the export of Friesians (black pied Germans) to stop after roughly 8,800 had been brought in (FAO, 2020).

The characteristic markings of Holsteins are commonly red and white or black and white, with piebald patterns (Fontanesi, *et al.*, 2011). Rarely, some have red and black colors combined with white. This particular coloring is caused by red factor. Another well-known color is "blue." The cow's bluish tinge is created when black and white hairs mingle to form this color. In certain farming circles, this hue is also referred to as "blue roan." With an average annual milk production of 22,530 pounds (10,220 kg), they are well known for their prolific dairy output. 719 pounds (3.1%) and 858 pounds (3.7%) of this milk comprise protein. Holstein bulls are biological machinery that can produce frozen semen at a productive age(Lestari *et al.*, 2013).

#### 1.2.3. Semen collection

After being cleaned and sterilized, every piece of equipment required collecting and process semen was prepared for use. In addition, a properly constructed space devoid of stressors and hygienic conditions was used for the semen collection process. Each bull was completely bathed throughout the process of collecting semen. Washing, however, places more emphasis on the copulatory organ and related tissues. The same care was taken with mount animals, or "teaser bulls." The regions that came into contact with the erected penis and the fluids in the posterior quarter were also cleaned before each subsequent mount. Before being collected, bulls were used to two or three false mounting after being sexually stimulated by a handler with whom they were familiar .artificial

vagina that had been lubricated and filled with water and air was then used to collect semen (45°C).

Evaluations of the collected sample included volume, color, mass motility, individual motility, PH, concentration, living and dead count, and, upon freezing, assessment of the semen's acrosome integrity and hypo-osmotic swelling (HOST). Samples of the district (mother containers) were being thawed at 37°C. In a similar manner, individual motility, living and dead count, acrosome integrity and HOST were assessed.

#### 1.2.4. Semen quality assessment

Assessing a male's potential fertility through clinical and scientific testing is crucial when selecting him for breeding, particularly AI. One of the key elements that can affect the effectiveness of artificial insemination is the quality of frozen semen (Lemma, 2011). With the use of semen analysis, male animal fertility may be assessed based on the quantity of sperm generated that have the capacity to fertilize the egg both in vivo and in vitro (Wang , 2014). Fertility is positively impacted by high-quality frozen semen (Kebede, 2018).

#### 1.2.5. Semen collection and processing

Bull semen has different appearances depending on the age, breed, and ejaculate of the bull. The color of normal bull semen varies. The color of semen may alter due to contaminants and feed. If the semen was contaminated, it was rejected for further processing. Generally speaking, typical bull semen can have a variety of colors and textures, including milky, yellowish, and creamy (Sarder, 2005).

##### *1.2.5.1 Appearance or color*

Bull semen has different appearances depending on the age, breed, and ejaculate of the bull. The color of normal bull semen varies. The color of semen may alter due to contaminants and feed. If the semen was contaminated, it was rejected for further processing. Generally speaking, typical bull semen can have a variety of colors and textures, including milky, yellowish, and creamy (Sarder, 2005).

#### *1.2.5.2. Volume*

Semen volume refers to the amount of ejaculate produced during ejaculation. Normal values typically range from A graded test tube that was fastened to the prosthetic vagina's tip was used to measure the volume of semen. Semen volume is decreased when semen is obtained via repeated or insufficient ejaculations. Therefore, with the right ejaculation techniques, semen obtained from adult individuals is devoid of systemic and reproductive system disorders. Individual bulls have different volumes of semen per ejaculate Semen Volume the minimum acceptable semen volume for a bull is typically around 0.5 milliliters (ml). Anything below this volume may indicate a potential issue with semen production or collection (Zewdie *et al.*, 2005).

#### *1.2.5.3 Sperm Mass Motility and Individual Motility*

The collective movement of spermatozoa is known as mass motility, and under low magnification (100X), the mass activity was measured in a drop of semen without a cover slip. Good-quality semen is indicated by rapid wave motion and the creation of eddies at the end of the waves. The method of scores was fairly basic, ranging from one (very poor) to four (very good). For this examination, semen exhibiting bulk activity 3 and 4 was assessed.

Individually motility was examined Small drops of semen under a cover slip at a greater magnification to assess motility. Sperm cells are the only ones that travel ahead in a straight line. When bull semen is collected, it should be at least 70% motile (Kathiravan *et al.*, 2011).

#### *1.2.5.4 Sperm Concentration*

It is crucial for AI stations to precisely and precisely determine the goal of sperm concentration in an ejaculate in order to provide insemination doses that contain an adequate amount of sperm. AI stations frequently employ a certain safety scope to guarantee that each insemination dose contains a minimum quantity of sperm. This suggests that individuals with high genetic value are not exploited effectively and that some insemination dosages contain an excessive amount of sperm.

In order to achieve the best possible fertility during insemination, sperm concentration is crucial. After freezing, viability is decreased when insemination dosages are low. Following dilution, seminal plasma levels, fresh sperm volume, and sperm count may all have an impact on the decline of sperm viability in dosages. One of the key factors for insemination is the quantity of sperm needed to achieve optimal fertility (Mohanty et al., 2018). The morphometry variables of spermatozoa have been linked to the total number of spermatozoa in the ejaculate. According to (Górski *et al.*, 2017), ejaculates with low sperm concentration had smaller, shorter, and narrower head sizes as well as a smaller head area than those with high sperm counts.

#### *1.2.5.5. Sperm Morphology*

Evaluation of sperm morphology is a critical component of complete male breeding soundness examinations in all species. Through the development of spermograms,

**Sperm Morphology:** refers to the size, shape, and structure of sperm cells. Abnormalities in sperm morphology can affect their ability to penetrate and fertilize an egg. The morphology is often assessed by examining a stained semen sample under a microscope, and a certain percentage of normal-shaped sperm is considered optimal.

Given the knowledge that the process of freezing and thawing precipitates cryogenic damage, either morphological or biochemical, which leads to sperm dysfunction and membrane alterations, the assessment of sperm morphology is an essential component of any semen analysis and offers clinical insights into the potential fertility of the semen sample. When combined with other objective characteristics, sperm morphometry can be helpful in creating a fertility index. Male fertility in the dairy sector is defined as the proportion of conceived females that do not conceive again within a certain number of days following the initial insemination, as artificial insemination is the norm (Kumar and Krupakaran, 2014). The ability of a particular sperm cell can be impacted by anomalies in the head, midpiece, or tail of the sperm.

Sperm experience a number of transformations, such as the condensation of the nucleus and arrangement of the genetic material, the acquisition of the acrosome in the sperm head, and the arrangement of the mitochondria in the sperm midpiece. These

modifications are essential for the sperm to ejaculate normally and fulfill its role as a penetrating, self-propelled enzyme male genome delivery vehicle (Staub and Johnson, 2018). The methodology for estimating spermatozoa viability was carried over to the computation of anomaly. The 400x magnification of the microscope was used to view the anomaly. After that, the following formula is used to calculate it: (Barek *et al.*, 2020)

$$\text{Abnormalities (\%)} = \frac{\text{total abnormal spermatozoa}}{\text{total spermatozoa}} \times 100\%$$

over the total sperm

#### 1.2.5.6. Semen Live and Dead Cell

The Eosin-Nigrosin staining method was used to evaluate the viability of spermatozoa. From the mixture, sperm smear preparations were created and dried on a heating table. A light microscope with a 40x objective was used to examine two hundred cells per slide of non-stained live sperm (transparent head) and dead sperm (redhead) (Salisbury *et al.*, 1978 ;) from a stock solution containing 10% Nigrosin and 5% Eosine. Three and five drops, respectively, were combined in a tiny glass test tube that was kept in a water bath at 37 degrees Celsius. On a slide that had been warmed up beforehand, two drops of mixed stain and a tiny drop of semen were collected, gently mixed, and two smears were made. These were then left to dry in the air. To provide a representative figure, the fields were counted across the slide. 200 sperms were counted to find out the percentage of live sperm using the following formula:

$$\frac{\text{No of live sperm counted}}{\text{No of total sperm counted}} \times 100 = \text{ \_\_\_\_\_\_ \%}$$

No of total sperm counted

Part of the sperm was stained, such as the nucleus, it was counted as dead.

#### 1.2.5.7. Sperm PH

When an ejaculate loses carbon dioxide, its pH rises right away. This is followed by a drop in pH because of the buildup of lactic acid, which is mostly caused by fructose breakdown. The increased generation of lactic acid by more spermatozoa in ejaculates with a higher sperm concentration will cause the pH to fall more quickly and dramatically. Sperm fertility may be harmed by a pH shift that is too significant, either in

an acidic or alkaline direction. (Storey, 2004) suggests that the capacitating process may be negatively impacted by a low intracellular pH.

#### *1.2.5.8. Acrosome integrity of spermatozoa*

**Acrosome Integrity:** The acrosome is an important structure on the head of the sperm cell that contains enzymes necessary for fertilization. The minimum acceptable percentage of sperm cells with intact acrosomes is generally around 70%. The three types of membranes found in spermatozoa are the acrosome membrane, the mitochondrial membrane, and the plasma membrane. Due to the presence of polyunsaturated fatty acids, the membranes are highly vulnerable to oxidative stress, particularly when freezing processes are involved (Chelucci *et al.*, 2015). One of the elements that determine the success of fertilization is acrosome integrity. Spermatozoa without acrosomes are the only ones that can pass through the zona pellucida and merge with the plasma membrane of the oocyte (Celeghini *et al.*, 2010). The Giemsa staining approach was utilized to test the spermatozoa's acrosome integrity and assess its integrity (Almadaly, 2014).

#### *1.2.5.9 HOST*

**HOST (Hypo-Osmotic Swelling Test):** The HOST is a test that assesses the functional integrity of the sperm cell membrane. The minimum acceptable percentage of sperm cells showing hypo-osmotic swelling (indicating functional membrane integrity) is typically around 60%. Semen plasma is made up of a variety of biochemical substances and metabolites that are crucial for fertilization, sperm interaction with the oviduct, sperm protection and metabolism, membrane remodeling, and acrosome preparation and reaction. Sperm include cholesterol, which has a significant role in controlling the permeability and fluidity of sperm membranes (Memili, 2020)

The processing techniques or specific characteristics of the bull may be to blame for the important harm that deep freezing of fresh bull semen can have to the functional integrity of the sperm membranes. Several livestock species' sperm membrane integrity has been assessed using the HOS test. Predicting post-thaw semen quality parameters' reproductive potential and making efficient use of it in artificial breeding programs was the primary goal of the current study. The HOST may be completed quickly and affordably in almost

any laboratory. It has been proposed that the HOST yields more accurate results on spermatozoa's ability to fertilize than traditional semen analysis criteria.

To produce 150 mOsmol/l hypo- osmotic solution (Jeyendran *et al.*, 1984)

Extended semen (0.1 ml) was combined with a hypo-osmotic solution (0.735 g sodium citrate, 1.351 g fructose, and dissolved in 1000 ml of distilled water; 150mOsmo/L) in a test tube. In a separate test tube, 0.1 ml of the same semen was added concurrently with the preparation of a control solution (1 ml; 300mOsm/l). In order to preserve the shape of the spermatozoa, the test tubes were incubated for 60 minutes at 37°C and combined with 0.1 milliliter of 10% formaldehyde. Using a 400X magnification, a drop of incubated semen was examined to look for signs of sperm tail curling. Semen samples containing 40% coiled spermatozoa are deemed abnormal for fertility, while 60% coiled spermatozoa are considered normal.

#### *1.2.5.10. Laboratory Analysis of frozen semen*

The straw was taken out of the liquid nitrogen (LN<sub>2</sub>) and thawed for approximately 30 seconds in heated water at 37<sup>0</sup>cin preparation for the laboratory analysis. As soon as the straw thawed, it was removed and gently cleaned. To prevent cold shock, the grease-free, spotless glass slide was ready, heated, and brought to body temperature. Next, using a light microscope with a cover slide and 400 x magnifications, a drop of semen was placed on a pre-warmed slide in order to subjectively determine the proportion of sperm individual progressive motility. The frozen straws were broken right away, and a drop of semen was placed on a heated slide and stained with a mixture of eosin and Nigrosin to assess the population of live and dead sperm. Semen analysis was done based on the procedures reported by (Bearden *et al.*, 2004)

#### *1.2.5.11. Production of the bulls*

the productive semen of a bull plays a crucial role in cattle reproduction by providing spermatozoa necessary for fertilization and facilitating genetic improvement in the cattle population The minimum semen straw production in a bull can vary depending on several factors, including the age, health, and breed of the bull.

During a typical semen collection, a bull can produce between 2 to 5 milliliters of semen. Therefore, the total number of semen straws that can be produced from a single collection can range from 10 to 100 straws. It's important to note that not all of the ejaculate is used for semen straws, as the semen is often evaluated and processed to ensure the highest quality before it is packaged for use in artificial insemination it's worth mentioning that these numbers are estimates and can vary depending on individual factors. The semen production in bulls can also be influenced by factors such as genetics, nutrition, and overall reproductive health. Consulting with a veterinarian or a specialized reproductive expert can provide more accurate information regarding the specific bull's semen production capabilities.

#### 1.2.6. Factors affecting bovine semen quality

Sperm production and quality are among the variables that the AI industry and animal breeding programs need to take into account. Age (Prastowo *et al.*, 2018), season (Soren and Singh, 2016), temperature, and interval collection (Sankhi *et al.*, 2018) have all been linked to variations in semen quality in fresh. Genetic and individual variation (Mohammed and Ahmed, 2018) the act of cryopreservation and subsequent thawing for artificial intelligence has been shown to negatively impact sperm survival, even with the most advanced techniques now in use. The quality of the semen produced and its fertility during application are thought to be influenced to varying degrees by factors ranging from the bulls' selection process to the processing and storage of the semen (Lemma and Shemsu, 2015).

The quality and fertility of semen, knowledgeable technicians, a rise in AI coverage, and farmers' understanding of AI's benefits are the determining elements for the success of AI initiatives. According to (Akhteret *et al.*, 2013), the ability of semen to fertilize depends on its quality (motility, live concentration, and shape) as well as its amount (ejaculated volume). Both spermatogenesis and epididymal function may be impacted by stress in the form of pain, hunger, or cold since high levels of cortex in the blood inhibit the necessary testosterone concentrations for both organs. The publication by Callaghan (Callaghan *et al.*, 2016) does a great job of illustrating this. Where a single acidosis episode was followed in turn by decreased FSH and testosterone raised cortical and increased

abnormalities in sperm in the weeks that followed. And similarly, the stress associated with transport and relocation (Perry, 2017).

#### *1.2.6.1. Age*

The bull's age can have a big impact on the sperm generation, motility, volume, and concentration. Up until the age of seven, the volume of sperm grows as the bulls get older. Conversely, starting at age three and as one gets older, the bull's sperm concentration drops (Agririset *al.*, 2018).

#### *1.2.6.2. Bull Libido and mating ability*

Bull libido is a significant factor in natural mating farms. Because there is a lower chance of detecting a single cow in heat during brief mating times, the effect of libido is typically more noticeable. This is because there is a greater likelihood that cows may miss the first cycle if they are not in heat during the brief mating phase. When there is a clear hierarchy among the herd and a low workload that is, a high bull-to-cow ratio bulls perform at their best. Making sure that all bulls run together before to mating can help develop an established hierarchy (Parkinson, 2004). Bulls' physical characteristics and the quality of their semen undergo notable changes during their sexual maturation from puberty to sexual maturity. The likelihood of fertilization is increased as sperm concentration, motility, vigor, and wave motion rise with maturity before plateauing (Neto *et al.*, 2011).

#### *1.2.6.3. Nutrition*

Improving the nutritional status of bull calves in their first six months of life will cause gonadotropin secretion and testicular growth to rise, which will cause puberty to begin earlier. According to recent data, this is most likely mediated by specific neuron peptides acting as messengers for peripherally produced metabolites and metabolic hormones to neuron endocrine centers in the brain. Increased gonadotropin production and secretion as a result regulates testicular growth and function (Kenny and Byrne, 2018). Minerals are necessary for all animal physiological functions, including reproduction. Microminerals are crucial for preserving breeding bulls' reproductive health. Sperm production and fertility have improved after receiving more micronutrients, such as cobalt, zinc and manganese (Singh *et al.*, 2018).

#### *1.2.6.4. Temperature and season*

When assessing the performance of an animal raised in tropical or subtropical climates, the temperature humidity index is a commonly used measure of heat stress in animals. Due to their immature thermoregulatory systems and small number of sweat glands, buffaloes have weak thermal tolerance power (Marai and Haebe, 2010). Cattle reproduction can be significantly impacted by the season as well. Hot weather can harm spermatogenic cells, which can cause testicular degeneration and lower spermatogenesis efficiency, ultimately leading to low-quality sperm (Hirwa *et al.*, 2017). Temperature and humidity variations can lead to thermal discomfort, which can interfere with proper spermatogenesis and cause a drop in food intake and semen quality features. There was a positive link observed between biochemical characteristics and both ambient temperature and the season's temperature and THI have a major impact on the biochemical characteristics of the semen in Bhadawari bulls (Pandey *et al.*, 2014).

#### *1.2.6.5. Scrotal circumference*

Measurements of the scrotal circumference are taken during reproductive and general exams. Scrotal circumference measurement evaluates testicular volume and has a strong correlation with sperm production, making it a useful indicator for evaluating breeding soundness. This measurement can be utilized for the early selection of bulls because it is precise and simple to use (Menegassi *et al.*, 2011). Scrotal circumference can be measured from the side or back of the bull, depending on the person, the facilities, and the bull's temperament. After gently pressing both testes to the bottom of the scrotum, the most important things to remember are to keep the testes from spreading apart and to make sure that there is enough pressure applied to the measuring tape so that its top surface is level with the skin.

Take a measurement of the bull's scrotal circumference. If the bull's circumference is less than the minimum, the inspection doesn't need to proceed (Alexander, 2008). The results of multiple experiments using bulls of different ages and breeds have demonstrated that SC has a moderate-to-high heritability. Remarkably, SC heritability was evaluated at 6, 12, 18, and 24 months of age in a research of tropical composite bulls, and reported results were almost identical (Corbet *et al.*, 2013).

#### *1.2.6.6. Disease of male bull accessory glands*

Various clinical categories, including prostatitis, prostate-vesiculitis, and prostate-vesicular-epididymitis, are associated with male accessory gland infection. These categories include the following: they are common diseases, typically involving a chronic course, infrequent obstruction of seminal pathways, potential for unpredictable intracanicular spread to one or more sexual accessory glands of the reproductive tract, and the ability to affect either side or both (Vicari *et al.*, 2006)

#### *1.2.6.7. Other factors*

Certain factors, such as the kind of extender, permeability and non-permeable cryoprotectants, packaging technique, or freezing and thawing duration, might alter the motility, plasma membrane integrity, morphology, and survivability of spermatozoa during storage (Andrabi, 2009). The success rate of artificial insemination services is also impacted by poor inseminators' skills and motivation, as well as incorrect bull selection (Samreet *et al.*, 2015).

## 2. CHAPTERII: MATERIAL AND METHODS

### 2.1. Study Area

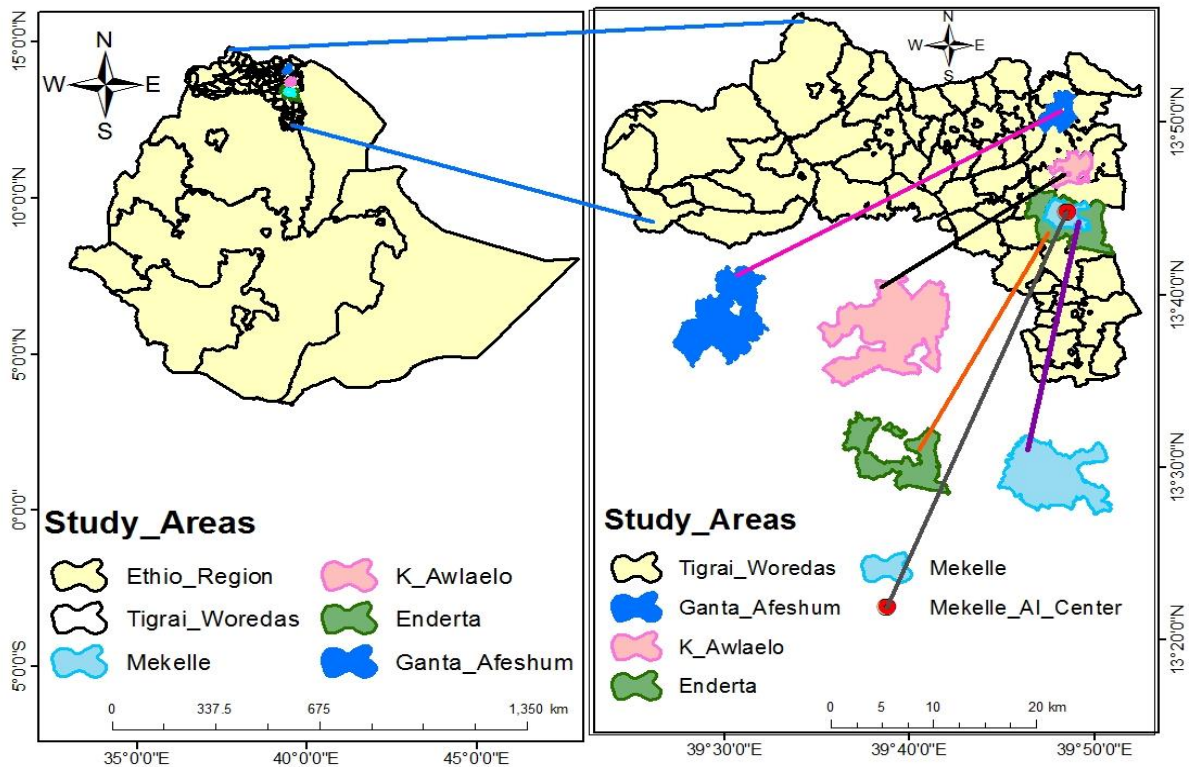
The present study was conducted from December 2013 to September 2014 in MAIC and four district areas namely G/afeshum, K/Awlaelo, Enderta and Mekelle city

**Mekelle City** is the capital of regional state and located approximately 780 kilometers north of Addis Ababa, the capital of Ethiopia. It is situated in the semi-arid highlands of Tigray with warm subtropical climate characterized by distinct wet and dry seasons. The warmest months in Mekelle City are April and May, with temperatures reaching up to 28°C. The coolest months are December and January, with temperatures dropping down to around 15° C On average, the yearly temperature in the city is approximately 22°C. The summer months from July through September receive the most rainfall in Mekelle City, with an average annual precipitation of about 500 mm. The city's topography places it at an elevation of 2,083 meters above sea level. (*Haltom et al.*, 2019)

**Ganta-Afeshum** is found in Eastern zone of Tigray region, in north Ethiopia about 117 km far away to the north from Mekelle. It is located geographically at the longitude range of 39°10' 0"E to 39°40'0" E and Latitude 14°10' 0"N to 14°30'0" N, (CSA Tigray, 2019).

**Kilte-Awlaelo** is situated in Eastern administrative zone of Tigray and one of the seventh rural wereda of the eastern zone found in the south of the eastern administrative zone. It is found at distance of 45 km to north of Mekelle, capital city of the region geographically, the longitude range 39°20' 0"E to 39°40'0" E and Latitude 13°30' 0" N to 14°0'0" N, (CSA Tigray, 2019).

**Enderta** is found in south east of Tigray state and its geographical location is longitude range 39°10' 0"E to 39°50'0" E and Latitude 13°10' 0" N to 14°40'0" N (CSA Tigray, 2019).



**Figure 1: map of study area**

## 2.2. Study Animals

The study animals used for this study were bulls from the Mekelle Artificial Insemination Center (N= 10) mainly HF (5), Jersey (2) and Begait (3). During the study period the age of the bulls were (2.2, 2.3, 2.4, 6 and 6.2 years for HF; 3.7, 4.7, 4.7 years for Begait and 5.2 and 5.8 years for Jersey) the average body weights were (292, 302, 349, 823 and 896 kg for HF; 343, 379 and 474 kg for Begait and 588 and 594 kg for Jersey). All the bulls were in good body condition score, healthy and vaccinated against black quarter and foot and mouth disease. They were fed with Hay, alfalfa, elephant grass, and when available, the quantity of concentrate according to their body weight.



**Figure 2: Bulls at exercise**



**Figure 3: Semen collection MAIC**

## 2.3 Study Design and sampling

### 2.3.1 Sample size determination

The study was conducted to assess the quality of semen produced in Mekelle artificial insemination center and in districts of G/ Afeshum, K/Awlaelo, Enderta, and Mekelle City.

In this study the sample size (the number of straws) were determined considering 95% confidence level and using the following formula developed by Yamane (1967:886). Accordingly, 306 samples were needed. However, for the accurateness and presetions, 400 samples were collected.

$$n = \frac{N}{1 + N(e)^2}$$

Where,

n= sample size; N= total population; e= sampling error (e=0.05)

### 2.3.2 Sample Collection and Procedure

The semen collection schedule was Monday every week. The bulls to be ejaculated went through preparatory measures including showering, drying and cleaning, brushing, grooming, and half-hour exercise. The bulls were then allowed to mount a teaser bull, and the semen was collected with the help of a pre-warmed (45°C) artificial vagina. Ejaculate volume was recorded directly from the graduated semen collection tube. Each ejaculate was examined for mass motility, individual motility, sperm concentration, morphology, live and dead cells, and PH, as described earlier, and ejaculates with less than 70% sperm motility were discarded. Fresh semen was diluted depending upon the evaluation of the sperm concentration in the Bioxcell extender. 0.25 ml straws (semen volume) were filled with diluted semen and sealed at the house temperature, and the semen was equilibrated at 4°C for 4 hours; finally, the processed semen was frozen and stored in liquid nitrogen at -196 °C. (Fiaz *et al.*, 2010) And post-thawing semen

individual motility; live/dead cell morphology normal Acrosome integrity and HOST were evaluated.

### 2.3.3 Physical examination of the semen

It was measured with a scaled collecting test tube and stored at 37<sup>0</sup>c in an IMV water bath. When processing semen, all procedures were completed in a closed space. A thorough check was done as soon as possible after collection to check Appearance and color, volume and for foreign objects of the semen for each animal in each breed. Based only on visual inspection, the color of the semen was determined to be creamy and yellowish. Additionally, the collected semen was examined to make sure it was clear of impurities such as blood, faces, hair, urine, and dirt. Utilizing a graduated test tube placed at the AV tip, the volume of ejaculates was measured. Bulls that produced more than 3 milliliters of ejaculate per ejaculate, as per the protocol of the Mekelle Artificial Insemination Center, were assessed in this investigation.

### 2.3.4 Microscopically examination

*2.3.4.1 Mass motility:* The mass motility of semen was measured by dropping undiluted semen at a field (100 x) magnification level on a warm slide (37<sup>0</sup>C) of an Optika microscope Italy stage phase contrast microscope. As a result, the intensity of the wave motion determined how much a mass motility score was assigned, ranging from 1 to 4. Scales of three and four were deemed appropriate for the research.

*2.3.4.2. Individual motility:* By analyzing undiluted semen placed on a warmed slide covered by a warmed cover slip, the percentage of individual sperm motility was calculated. Next, under a stage-warmed (37<sup>0</sup>C) microscope at a magnification of 200 x, sperm cells that demonstrated progressive movements were scored 0–100% based on the estimated percentage of spermatozoa that move in a progressive forward direction. Throughout the course of the trial, more than 70% of the motility was passed.

### 2.3.5. Sperm concentration

A spectrophotometer (IMV, Technologies France) was used to measure the concentration of semen. In order to dilute a neat semen sample, the foot paddle of an auto-dilution

device was first pressed to draw in 0.02 ml (20 $\mu$ l) of air, and then it was pressed once more to draw in 0.04 ml (40 $\mu$ l) of neat semen using a pipette from a semen collection tube. Lastly, it was repelled to simultaneously release the semen and 3.96 ml (3960 $\mu$ l) of normal saline in the cuvette. The photometer's cuvette holder was used to hold this cuvette. The ejaculate information was entered in order to obtain the outcome. Using a bovine photometer .the concentration of spermatozoa (million/ml) in the neat semen was measured.

#### 2.3.6. Sperm morphological examination

In order to preserve the spermatozoa for morphological analysis, one milliliter of Hancock solution (buffered formal saline) was heated to 37<sup>0</sup>C in a water bath. To this, one drop of fresh semen was added using a warm Pasteur pipette, and the mixture was gently stirred. A little drop of the material was placed on a spotless, grease-free glass slide, and it was covered with a cover slide under a phase contrast microscope (200x) to study any later morphological anomalies, primarily head, mid-piece, and tail defects, proximal, and distal droplets (Hancock's approach). This method allowed for the counting of 500 spermatozoa and the recording and visualization of anomalies (Salisbury *et al.*, 1978).

#### 2.3.7. The Live and cell of spermatozoa

Using Eosin-Nigrosin staining, the viability of the sperm was evaluated. From the mixture, sperm smear preparations were created and dried on a heating table. Using a light microscope with a 40 $\times$  objective, 200 cells per slide of non-stained live sperm (transparent head) and dead sperm (redhead) were counted using the Eosin–Nigrosin staining method .From the stock mixture of 10% Nigrosin and 5% Eosin. Three and five drops, respectively, were combined in a tiny glass test tube that was kept in a water bath at 37<sup>0</sup>C. On a slide that had been warmed up beforehand, two drops of mixed stain and a tiny drop of semen were collected, gently mixed, and two smears were made. These were then left to dry in the air. Fields were tallied.

To provide a representative figure, the fields were counted across the slide. 200 sperm were counted in order to determine the percentage of live sperm. If any part of the sperm

such as the nucleus was stained, it was counted as dead using the formula below:  
(Salisbury & associates, 1978 ;)

$$\frac{\text{No of live sperm counted}}{\text{No of total sperm counted}} \times 100 = \text{ \_\_\_\_\_\_ } \%$$

No of total sperm counted

### 2.3.8. Semen PH

The pH level of semen is an indicator of its acidity or alkalinity. A slightly alkaline pH reading of 6.0 to 7.0. The collected semen pH was evaluated by using an automatic PH-meter whether it ranges between 6 to 7 Normal bull semen PH reading (Storey, 2008).

### 2.3.9. Sperm Acrosome integrity Test

The spermatozoa's acrosome integrity was assessed using Giemsa staining, a more straightforward technique that included methanol fixation and Giemsa staining. The study's objective was to assess the quality and acrosome integrity of frozen spermatozoa from MAIC bulls, and since acrosome integrity plays a crucial role in fertilization processes. Straws (0.25 ml) were kept in liquid nitrogen-filled containers for a month before being tested for acrosome integrity and stained by dropping semen onto a glass item. As a result, a warming plate was used to prepare a smear at 37 °C. Following smear preparation, the smear was rinsed under running water and fixed with methanol for ten minutes.

After fixing, the sample was immersed in a staining jar for three hours and stained using a Giemsa solution consisting of three milliliters of absolute Giemsa, two milliliters of PBS, and thirty-five milliliters of distilled water. It was then washed under running water and dried once more. Ultimately, 200 cells were viewed at 400x magnification using a light microscope. The purple head of spermatozoa indicated that their acrosome was intact, but the pale head indicated that their acrosome was damaged. The number of spermatozoa with intact acrosomes divided by the total number of spermatozoa and multiplied by 100% was used to determine the acrosome status. (Almadaly & colleagues, 2014)

$$\text{Acrosome status} = \frac{\text{Acrosome-intact spermatozoa}}{\text{Total spermatozoa}} \times 100$$

## Total number of spermatozoa

### 2.3.1. OHOST

Using a solution made by dissolving 1.35 grams of fructose and 0.735 grams of sodium citrate in 1000 milliliters of distilled water, the hypo-osmotic swelling test (HOST) was used to assess the membrane integrity of spermatozoa (HOST Solution (150mosm/L)). The solution was kept cold until it was needed. To preserve the shape of the spermatozoa for further examination, 1 ml of hypo-osmotic solution was combined with 0.1 ml of semen, and the mixture was incubated at 37 °c for an hour. The mixture was then fixed with formaldehyde (10% formalin 0.1 ml). On a dry, sterile glass slide, a drop of diluted semen was inserted and covered with a cover slip. Under a phase contrast microscope, 200 spermatozoa were counted in various fields at 400X, and the proportion of spermatozoa with coiled tails that tested positive for the HOS test was calculated. A hypo-osmotic swelling test was performed on the spermatozoa (Jeyendran. *et al.*, 1984).

### 2.4. Dilution and cryopreservation of semen

By diluting the semen with appropriate extenders which serve as a source of nutrients and energy sperm survival can be increased. shields against the damaging effects of freezing and cooling, which occur at -196°C in liquid nitrogen, provide buffers to stop damaging pH changes and to maintain the right osmotic and electrolyte pressure. In order to use the semen for many inseminations, increase its volume .Extenders optics cell2, containing antibiotics (gentamicin, tyrosine, lincomycin, and spectinomycin) according to European Directive, and a 250 ml composition of carbohydrates, minerals, salts, buffer, antioxidants, glycerol, and phospholipids. It creates an isotonic environment (sperm swell and rupture in hypotonic solutions, while they become dehydrated in hypertonic solutions).Extenders stop fluid loss or gain by resisting changes in salinity and acidity.

French straws (0.25 ml) were used for each insemination dose, and an automatic filling-sealing device (IMV Technology MX4, France) was used to seal the straws' open ends. The relevant information was previously written on the straws. The straws were sealed and then allowed to get used to for three hours at 4<sup>0</sup>c. A cold handling cabinet (IMV,

France) was used for the cooling and calibration processes. A mechanical semen freezer (Digit Cool IMV Technology, France) was used to freeze the semen. Regulating frozen semen One to three days after freezed, the frozen semen underwent its first control test. Three straws were selected at random from a batch of semen, and the bull's previously frozen semen was tested under the following conditions: >40% motility of the spermatozoa, >500 million spermatozoa concentration/ml, 80% spermatozoa without morphological changes.

## **2.5. Production of the bulls**

When collecting and processing bull semen, the number of bull semen straws and the volume of extender used depend on the volume and concentration of the ejaculated semen. The concentration of the semen determines the number of straws required, as each straw typically contains a specific volume of semen. The extender is used to dilute the semen to achieve the desired concentration before it is filled and sealed into the straws. To maintain proper record-keeping and identification, a domino printer is often used to print important information on the semen straws. This information typically includes details such as the breed of the bull, batch number, bull number, printed date, and the name of the center or facility where the semen was collected. By printing this information on the straws, it becomes easier to track and identify the semen samples, ensuring accurate record-keeping and traceability throughout the process.

## **2.6. Data analysis**

Microsoft Excel was used to modify, code, input, and store the acquired data. The statistical package for social sciences (SPSS) software, version 20, SPSS, was used for analysis. The presentation of all the data was as mean  $\pm$  standard error of the mean (SE). ANOVA was utilized to determine the significant difference (SD) between the three breeds and sites. Additionally, a pair sample T-test was employed to compare the semen before and after thawing. To key was used to perform mean multiple mean comparisons at ( $P < 0.05$ ).

### 3. CHAPTER III: RESULTS

#### 3.1. Physico-Morphological Characteristics and Functional Attributes of Semen

The overall mean of ejaculation volume, mass motility (0-4), individual motility%, sperm concentration(109/ml, present of morphological normal%, live & dead sperm and semen% PH for HF, Jersey and Begait were(6.8±0.5<sup>a</sup>,7±0.6<sup>a</sup>and6.5±0.5<sup>a</sup>,ml)(3.6±0.2<sup>a</sup>,3.3±0.3<sup>a</sup> and3±0.0<sup>a</sup>), (78±2.5<sup>a</sup>,76.7±1.7<sup>a</sup> and77.5±2.5<sup>a</sup>)%,(0.76±0.0<sup>a</sup>,0.65±0.0<sup>a</sup> and 0.62±0.0<sup>a</sup>109/ml), (70.4±1.3<sup>a</sup>,69.3±2.3<sup>a</sup>and65±2.0<sup>a</sup>)%(73.6±2.7<sup>a</sup>,63.7±2.0<sup>b</sup>and61.5±.5<sup>b</sup>)and (6.2±0.2<sup>a</sup>,6.3±0.3<sup>a</sup> and 6±0.0<sup>a</sup>)respectively (Table 1)in this study the number of live and dead spermatozoa has a significant difference (p< 0.05). However the other physico-morphological parameters such as ejaculation volume, , mass motility (0-4), individual motility, sperm concentration and present of morphological normal sperm of sperm and semen PH quality has no significant difference(p> 0.05).

**Table 1:** Physico-morphological quality of fresh semen

Breeds	Physico-morphological parameters						PH
	Ejacu. volume(ml)	Mass mot. (0-4 scale)	Ind. Mot. (%)	Sperm conc. (109/ml)	Morpho. (%)	live & dead	
<b>HF (N=5)</b>	6.8±0.5 <sup>a</sup>	3.6±0.2 <sup>a</sup>	78±2.5 <sup>a</sup>	0.76±0.0 <sup>a</sup>	70.4±1.3 <sup>a</sup>	73.6±2.7 <sup>a</sup>	6.2±0.2 <sup>a</sup>
<b>Jersey(N=2)</b>	7±0.6 <sup>a</sup>	3.3±0.3 <sup>a</sup>	76.7±1.7 <sup>a</sup>	0.65±0.0 <sup>a</sup>	69.3±2.3 <sup>a</sup>	63.7±2.0 <sup>b</sup>	6.3±0.3 <sup>a</sup>
<b>Begait (N=3)</b>	6.5±0.5 <sup>a</sup>	3±0.0 <sup>a</sup>	77.5±2.5 <sup>a</sup>	0.62±0.0 <sup>a</sup>	65±2.0 <sup>a</sup>	61.5±.5 <sup>b</sup>	6±0.0 <sup>a</sup>
<b>P-value</b>	<b>0.869</b>	<b>0.415</b>	<b>0.93</b>	<b>0.662</b>	<b>0.213</b>	<b>0.032</b>	<b>0.737</b>

### 3.2 Physico-Morphological Characteristics and Functional Attributes of post thawed Semen

The overall mean of post thawed individual motility, morphological normal sperm, live & dead sperm, acrosome integrity and HOST were  $59.5 \pm 1.6\%$ ,  $64 \pm 1.2\%$ ,  $61.3 \pm 3.0\%$ ,  $70.7 \pm 0.5$  and  $61.9 \pm 1.6\%$  respectively (Table 2). In this study the individual motility, morphological normal sperm and number of live and dead spermatozoa has a significant difference ( $p < 0.05$ ). However the acrosome integrity and HOST sperm quality has no significant difference ( $p > 0.05$ ).

**Table 2: Mean of post thawed semen physico-morphological parameters**

Breeds	Physico-morphological parameters				
	Ind. Mot. (%)	Morpho. (%)	live & dead	Acrosome Integrity	HOST
HF (N=5)	$63 \pm 1.2^a$	$66.2 \pm 1.2^a$	$66.4 \pm 2.0^a$	$71.2 \pm 0.6^a$	$58.8 \pm 2.2^a$
Jersey (N=2)	$57.5 \pm 2.5^{ab}$	$65.5 \pm 0.5^a$	$67 \pm 6.0^a$	$69.5 \pm 2.5^a$	$62.5 \pm 2.5^a$
Begait (N=3)	$55 \pm 2.9^{bc}$	$59.3 \pm 1.2^b$	$49 \pm 1.2^b$	$70.7 \pm 0.7^a$	$66.7 \pm 0.9^a$
P-value	<i>0.046</i>	<i>0.011</i>	<i>0.003</i>	<i>0.547</i>	<i>0.084</i>
Total	$59.5 \pm 1.6$	$64 \pm 1.2$	$61.3 \pm 3.0$	$70.7 \pm 0.5$	$61.9 \pm 1.6$

### 3.3 Physico-Morphological Characteristics and Functional Attributes of post thawed Semen at districts

The mean of districts (field) Individual motility ( $42.8 \pm 1.2^a$ ,  $43.7 \pm 6.4^a$  and  $41.7 \pm 5.6^a$ ), the normal morphology of spermatozoa ( $50.6 \pm 1.0^{ab}$ ,  $53.4 \pm 2.8^a$  and  $47.2 \pm 4.3^{bc}$ ) live & dead ( $55.1 \pm 4.7^a$ ,  $58.0 \pm 4.8^a$  and  $46.8 \pm 5.1^b$ ), acrosome integrity ( $51.1 \pm 2.5^a$ ,  $47.6 \pm 2^b$  and  $46.9 \pm 1.2^b$ ) and HOST ( $52.5 \pm 0.8$ ,  $52.01 \pm 0.7$  and  $49.3 \pm 1.1$ ) in Holstein Frisian, Jersey and Begait breeds respectively. There is significant difference ( $P < 0.05$ ) in normal morphology of spermatozoa, live & dead and acrosome integrity between breeds.

**Table 3: physico-morphological quality of post thawed semen at field**

Breeds	Physico-morphological parameters				
	Ind. Mot. (%)	Morpho. (%)	live & dead	Ac. integrity	HOST
HF (N=5)	42.8±1.2 <sup>a</sup>	50.6±1.0 <sup>ab</sup>	55.1±4.7 <sup>a</sup>	51.1±2.5 <sup>a</sup>	52.5±0.8
Jersey (N=2)	43.7±6.4 <sup>a</sup>	53.4±2.8 <sup>a</sup>	58.0±4.8 <sup>a</sup>	47.6±2 <sup>b</sup>	52.01±0.7
Begait (N=3)	41.7±5.6 <sup>a</sup>	47.2±4.3 <sup>bc</sup>	46.8±5.1 <sup>b</sup>	46.9±1.2 <sup>b</sup>	49.3±1.1
<b>P-value</b>	0.753	0.020	0.001	0.001	0.71

### 3. 4. Assessment of the semen result between Districts

The mean of districts was Individual motility of Ganta-afeshum, Kile-awlaelo, Enderta, and Mekelle City (43.5±2.0<sup>a</sup>, 40.6±1.4<sup>a</sup>, 43.55±2.1<sup>a</sup>, and 43.6±1.8<sup>a</sup>) the result of normal morphology of spermatozoa was (50.6±1.3<sup>a</sup>, 49.1±1.7<sup>a</sup>, 50.4±1.8<sup>a</sup>, and 52.1.1<sup>a</sup>), the current result of live and dead (51.5±2.7<sup>a</sup>, 54.4±1.3<sup>a</sup>, 53.4±1.8<sup>a</sup>, and 56.4±2<sup>a</sup>), the mean of acrosome integrity was recorded (50.0±1<sup>a</sup>, 48.8±1<sup>a</sup>, 50.3±0.9<sup>a</sup>, and 50.3±1<sup>a</sup>) and the result of the HOST was (50.0±1.3<sup>a</sup>, 50.5±0.6<sup>a</sup>, 52.8±1.1<sup>a</sup> and 53.7±1.0<sup>a</sup>) respectively. There is no significant difference ( $P > 0.05$ ) between the districts, according to Table 4.

**Table 4: Assessment of the semen result between Districts**

No	Districts	Physico-morphological parameters				
		Motility %	Morphology%	Live and dead %	Acrosome Integrity %	Host %
1	Ganta-afeshum	43.5±2.0 <sup>a</sup>	50.6±1.3 <sup>a</sup>	51.5±2.7 <sup>a</sup>	50.0±1 <sup>a</sup>	50.0±1.3 <sup>a</sup>
2	Kile-awlaelo	40.6±1.4 <sup>a</sup>	49.1±1.7 <sup>a</sup>	54.4±1.3 <sup>a</sup>	48.8±1 <sup>a</sup>	50.5±0.6 <sup>a</sup>
3	Enderta	43.55±2.1 <sup>a</sup>	50.4±1.8 <sup>a</sup>	53.4±1.8 <sup>a</sup>	50.3±0.9 <sup>a</sup>	52.8±1.1 <sup>a</sup>
4	Mekelle City	43.6±1.8 <sup>a</sup>	52.1.1 <sup>a</sup>	56.4±2 <sup>a</sup>	50.3±1 <sup>a</sup>	53.7±1.0 <sup>a</sup>
	<b>P-value</b>	<b>0.593</b>	<b>0.679</b>	<b>0.453</b>	<b>0.624</b>	<b>0.060</b>

### 3. 5 Semen production potential of Mekelle artificial insemination center

The average semen production potential of MAIC was 51, 845 semen straw per year. The production was mainly from exotic breeds of Holstein Frisian and jersey but small amount from Begait breeds. Out of the total production 39,613 of semen straws were distributed to districts of Tigray region.

**Table 5 :** Semen production potential of MAIC

Year	Total semen straws produced	Distributed to districts No. (%)	Breeds semen production		
			Holstein Frisian No. (%)	Jersey No. (%)	Begaite No. (%)
2016	9,341	9,341(100)	9341(100)	0	0
2017	49,558	49,558(100)	21281(43)	28277(57)	0
2018	100,750	55,000(54.60)	37162 (37)	55844(55.4)	7744 (7.6)
2019	76,302	67,000(87.81)	22896 (30)	39240(51.4)	14166(18.6)
2020	38,034	38,034 (100)	18052(47.5)	8773(23)	11209(29.5)
2021	37,084	18,745 (50.55)	30603(82.5)	1550 (4)	4931(13.5)
<b>Total</b>	<b>311,069</b>	<b>237,678(76.4)</b>	<b>139335(44.8)</b>	<b>133684(43)</b>	<b>38050 (12.2)</b>
<b>Average</b>	<b>51,845</b>	<b>39,613</b>	<b>23,222.5</b>	<b>22,280.7</b>	<b>6,341.7</b>

No. = number

The average liquid production potential per year of Mekelle artificial insemination center is 36,867 liters and out of this an average of 86.19% (31,507 liters) was distributed to districts of the region.

**Table 6:** Liquid nitrogen production potential of MAIC

Year	Total liquid Nitrogen production Number	Distribution to districts	
		Number	Percent
2016	39,717	34,382	86.57
2017	67,901	53,129	78.24
2018	44,695	38,205	85.48
2019	44,916	42,722	95.12
2020	12,550	11,017	87.78
2021	11,420	9,587	83.95
<b>Total</b>	<b>221,199</b>	<b>189,042</b>	<b>85.46</b>
<b>Average</b>	<b>36,867</b>	<b>31,507</b>	<b>86.19</b>

### 3.6. Production of bull semen during study season

During the study of season the bulls of the MAIC produced of semen straws of HF, Jersey and Begait (363,347 and 335 straws) respectively. According to the below of the HF bulls 363 > Jersey 347 > Begait bulls 335 respectively. (Table 4)

**Table 7: production of bull semen during study season**

Bull Id	Production of semen									
	5016	5017	4658	4665	4684	5018	5019	5027	5028	5029
Breed	HF	HF	HF	HF	HF	JER.	JER	BEG.	BEG	BEG
Straw/ejaculation	413	472	386	241	304	393	300	272	301	335
Average	363			347			335			

HF = Holstein Friesian, JER= Jersey, BEG = Begait

## 4. CHAPTER IV: DISCUSSION

### 4.1 Result of fresh semen at MAIC

The mean volume of freshly ejaculated semen was found to be  $6.8\pm 0.3$  similar to  $6.48\pm 0.01$  as reported by Rahman *et al.* in 2014. This value was significantly greater ( $P < 0.01$ ) than the semen volume reported by Mandal *et al.* in 2023, which was  $4.61\pm 0.02$ . The study by Mandal *et al.* evaluated semen volume in India at the AIC. Several factors can contribute to the variability in reported semen volumes between studies. Some of these factors include body weight, scrotal circumference, seasons of the year covered by the study, method of semen collection procedure, and frequency of semen collection.

Based on the current mass motility values are reported as  $(3.4\pm 0.2)$ . This value is consistent with the result reported by Rahman *et al.* in 2014, who reported a value of  $3.59\pm 0.05$  at the National Artificial Insemination Center in Ethiopia. Additionally, Lemma *et al.* reported a value of  $3.47\pm 0.5$ , and Seyoum *et al.* reported a value of  $3.35\pm 0.06$  for HF (Holstein Friesian) in KAIC (Kaloty Artificial Insemination Center). It appears that the mass motility values obtained from different studies and sources are relatively close to each other, suggesting a similar level of motility in the samples tested. However, it's important to note that the specific experimental methods and conditions used in each study may vary, which could contribute to slight differences in the reported values.

The present individual motility was found to be  $77.5\pm 1.3\%$ . This percentage similar within the range of 75-80% reported by Sisay *et al.* in Amhara and is similar to the value of  $77.46\pm 0.55\%$  reported by Seyoum *et al.* However, it is lower than the individual motility reported in Indonesia, which was  $89.62\%\pm 0.51\%$  according to Indriastuti *et al.*, 2020. The semen concentration was  $0.7\pm 0.0$  billion/ml, is notably different from the reported value of  $1.084\pm 0.014$  billion/ml in Nepal by Sankhi *et al.* in 2018. Additionally, it is lower than the sperm concentration reported by Indriastuti *et al.* in 2020, which was  $1164.81\pm 9.10\times 10^6$ /ml. The variability in spermatozoa concentration reported in different studies can be attributed to several factors. These factors include genetic variations among individuals, differences in nutrition, age of the subjects, management practices,

semen collection frequency, and techniques employed during the collection process. (Salisbury et al., 1978; Blezinger, 1999; Andrabi et al., 2002)

The normal morphology value was reported as  $69 \pm 1.1$ . However, the findings of Sisay *et al.* (2012) reported an abnormality rate of 2.93%, and Alagaw (2017) in North Shoa reported a different abnormality rate. Additionally, Umesh Kumar *et al.* (2015) reported an abnormality rate of  $8.38 \pm 0.32$  specifically in pure jersey cattle. These differences in findings could be due to various factors, including variations in sample size, geographical location, methodology, or even genetic differences among the populations being studied. It is not uncommon to find variations in research findings, especially when studying complex biological phenomena.

The live percentage results obtained in the study indicate a value of  $68.2 \pm 2.3\%$ . This value is significantly lower than the  $77.62 \pm 0.63\%$  reported by Rahman *et al.* in 2014 and disagrees with the average live cell percentage of fresh spermatozoa reported by Gustina *et al.* in 2023, which was  $80.04 \pm 3.68\%$ .

Furthermore, during the initial evaluation of fresh semen in pre-service young bulls, a relatively higher percentage of dead sperm ( $34.2 \pm 19.5\%$ ) was observed. This indicates that a significant proportion of the sperm cells were not viable. The study also noted that both breed and age were identified as significant factors influencing semen parameters, as reported by Lemma and Shemsu in 2015. These factors can play a role in determining the quality of sperm and may contribute to the observed differences in live cell percentages. It is worth mentioning that the live cell percentage obtained ( $68.2 \pm 2.3\%$ ) is lower than the recommended guideline of  $>70\%$  as stated by Kality NAIC and the MAIC.

The pH result of  $6.2 \pm 0.1$ , as reported by Nongbua *et al.* in 2020, is similar to the pH result of  $6.2 \pm 0.1$  mentioned by the user. However, it is noted that the pH value of  $6.2 \pm 0.1$  reported by Nongbua *et al.* is lower than the pH value of  $6.64 \pm 0.50\%$  reported by Rahman *et al.* in 2014. There could be several factors contributing to these variations in the pH values, such as differences in sample size, breed, and management techniques.

#### 4.2. Result of post thawed semen in MAIC

The mean individual motility of post-thawed semen at MAIC (mean  $\pm$  standard deviation) was  $59.5 \pm 1.6$ . This value is higher than the reported motility of 50.83% in N. Showa by *Goshme et al.* in 2021 and superior to the mean motility of  $39.58 \pm 1.85$  reported in India by *Chaudhary et al.* in 2017. Additionally, the finding aligns with the result of  $58.22 \pm 1.71$  reported in India by *Pathak et al.* in 2020. The statement proposes that the observed differences in motility could be attributed to variations in equilibration time, as suggested by *Khan et al.* in 2021.

In the present study, the normal morphology result was found to be  $64 \pm 1.2\%$ . This value was significantly lower compared to the 92.17% reported by *Goshme et al.* in 2021. Additionally, in a study conducted in Bangladesh by *Das et al.* in 2020, the normal morphology percentages for crossbred local zebu bulls were reported as  $74.6 \pm 1.8\%$  and  $79.0 \pm 2.1\%$ , respectively

Based on these findings, the live percentage result obtained in the study mentioned ( $61.3 \pm 3.0\%$ ) is lower than the result reported in the Malaysia study (*Baiee et al.*, 2017) ( $89.37 \pm 0.62\%$ ). Furthermore, the live percentage result is significantly higher ( $P < 0.01$ ) than the average range of  $48.92 \pm 1.94\%$  to  $52.38 \pm 1.49\%$  reported (*Chaudhary et al.*, 2017) in the India study. the observed differences in live percentage could be attributed to Genetic Variation, Environmental Factors, Seasonal Variation, Handling and Storage.

The acrosome integrity was found to be  $70.7 \pm 0.5\%$ . This finding is consistent with the results of a study conducted by *Kefelegn and Alemayehu* in 2022 and a report from India by *Chowdhury et al.* in 2014, which reported acrosome integrity at 73.74%. Comparing these findings to other studies, the acrosome integrity exceeded the average range of 51.15% to 62.80% reported in Egypt by *Mohammed and Ahmed* in 2018. However, it fell below the range reported in Bangladesh, which averaged from  $82.3 \pm 2.5\%$  to  $92.0 \pm 1.5\%$  according to *Kumar et al.* in 2012.

The result of HOST current study was ( $61.9 \pm 1.6$ ). This result is greater than what was reported by *Mohammed and Ahmed* (2018) in Egypt. According to their study, the values

ranged from  $50.20 \pm 1.84$  to  $59.25 \pm 1.76$ . This suggests that the ( $61.9 \pm 1.6$ ) falls outside the range of values reported in the study by Mohammed and Ahmed.

### **4.3. Result of post thawed semen at District**

The post-thawed semen analysis results individual motility in the districts was  $42.7 \pm 0.9\%$ , which is lower than the 50.83% (17.23) reported by Tadesse *et al.* in West Gojjam Districts in 2012. However, it is similar to the 44.27% reported by Goshme *et al.* in 2021. The current result,  $42.7 \pm 0.9\%$ , is higher than the minimum suggested motility threshold of 40% proposed by the Food and Agriculture Organization (FAO) and (IAEA) in 2005.

The current result normal morphology of 50.4% with a standard deviation of 0.8%. This value is lower than the normal range reported in a study by Kanthiya *et al.* in 2020, which stated a normal morphology of 84.50% with a standard deviation of 1.62%. In healthy and fertile bulls, it is expected that around 90% of the sperm have normal morphology. This means that 90% of the sperm in fertile bulls should exhibit the typical shape and structure. This information indicates the extent of morphological integrity in sperm from healthy and fertile bulls. Furthermore, according to a report by Hafez in 1993, the total abnormal sperm defect in normal fertile bull spermatozoa does not exceed 20%. This suggests that the occurrence of abnormal sperm, including various morphological defects, should be below 20% in fertile bulls.

The result of  $53.7 \pm 1.0$  for live and dead cells is consistent with the findings reported by Kanthiya *et al.* in 2020, where they reported a value of  $59.25 \pm 3.90$ . The slight difference in the mean values can be attributed to natural variations that can occur in biological experiments. However, since the standard deviations overlap, it suggests that the results are within a similar range and are not significantly different. Furthermore, the values of 51.3% vs. 50.1% reported by Diansyah *et al.* in 2023 are also similar to the result of  $53.7 \pm 1.0$  for live and dead cells. Again, the small variations in the percentages can be attributed to experimental variability. The overlapping standard deviations indicate that the results are within a comparable range and do not show significant discrepancies.

The acrosome integrity result of  $49.3 \pm 0.5$ , as reported in the study, is consistent with the findings reported by Diansyah *et al.* in 2023. Diansyah *et al.* observed acrosome integrity percentages of 50.7% in Bali-polled bulls and 48.7% in horned bulls. The similarity in acrosome integrity percentages suggests that the results of the current study align with the findings of Diansyah *et al.* Furthermore, Kanthiya *et al.*, in a study conducted in 2020, reported an acrosome integrity result of  $56.83 \pm 3.90$ . While this result is higher than the  $49.3 \pm 0.5$  reported in the current study, it is important to note that the studies were conducted in different years and may have used different methodologies or involved different populations of bulls. These factors could contribute to the variation in acrosome integrity results between the studies.

The average HOST value of  $(51.6 \pm 0.6)$  suggests the possibility of breed differences related to this measurement. This finding contradicts the published value of  $(37.66 \pm 2.82)$  reported by Bahmid *et al.* in 2023 in Indonesia, specifically in the AIC. Additionally, there is a discrepancy with the result of  $41.06 \pm 2.09$  reported by Kanthiya *et al.* in 2020. These contradictions highlight the potential variability in HOST values across different studies and locations. It is important to carefully consider the methodologies used in each study, as well as any potential factors that could contribute to the observed differences, such as variations in sample size, breed composition, or environmental factors.

#### **4.4 production of the bulls**

The production of bull semen straws can indeed vary based on several factors. Here are some key factors that can influence the production of bull semen. Different breeds of bulls may have different levels of semen production. Some breeds are known for higher semen volume and sperm concentration, while others may have lower production levels. Age and Health of the bull younger bulls may have lower semen production compared to mature bulls. Additionally, the overall health and condition of the bull can affect semen production.

## **5. CONCLUSIONS AND RECOMMENDATIONS**

This study evaluated the semen physico-morphological in three different breed bulls kept at the MAIC. The physico-morphological fresh semen and post thawed analyzed from these breed bulls .the result of semen quality with different parameters and the results observed that most of the semen attributes mainly within the normal level set for normal fertile bulls in a different place in the AIC. The normal morphology result was relatively below the recommended MAIC standard of >70% for normal semen morphology. The quality semen of Holstein-Friesian (HF) bull semen being comparatively better than that of Jersey and Begait bulls, respectively. The production of the bull's straw was as much as necessary in their productivity. The result of semen at districts areas was found that low.

Considering the above conclusions, the following recommendations are put forth:

- The study identified abnormal sperm morphology as a primary issue, emphasizing and districts require for additional investigations strategies.

## 6. REFERANCE

- Abebe, A.S., Alemayehu, K., Johansson, A.M. and Gizaw, S., (2020): Breeding practices and trait preferences of smallholder farmers for indigenous sheep in the northwest highlands of Ethiopia: Inputs to design a breeding program. *PloS one*, 15(5), p.e0233040.
- Agca, Y, Gilmore. J, Byers M, Woods. E. J, Liu and J.K. (2002): Osmotic characteristics of mouse spermatozoa in the presence of extenders and sugars. *Biol Reprod*; 67:1493–501.
- Ahmadi .A, Soon-Chye, NG.(1992):The single sperm curling test a modified hypo-osmotic swelling test, as a potential technique for the selection of viable sperm for intracytoplasmic sperm injection. *FertilSteril*; 68:346-350.
- Ahmed, M. M., Bezabih Emanu, M.A. Jabbar, F. Tangka and Ehui S. (2003): Economic and nutritional impacts of market-oriented dairy production in the Ethiopian highlands.Socio-economic and Policy Research Working Paper 51.ILRI (International Livestock Institute), Nairobi, Kenya, 27p.
- Ahsan-ul-Haq, R.H. and Zahid, I.A., (2003): semen characteristics of crossbred (Friesian x sahiwal) and sahiwal young bulls maintained under sub-tropical conditions of Punjab. *Pakistan Veterinary Journal* .
- Akhter,S., Azad, A.K.,Rahman , Z.and Ashraf, A., (2013): Study on the quality of semen of different genetic groups of bull from Khulna region of Bangladesh. *International Journal of Pharmaceutical and Medical Research*, 1(1), pp. 19-23.
- Al Naib, A., Hanrahan, J.P., Lonergan, P. and Fair, S., (2011): In vitro assessment of sperm from bulls of high and low field fertility .*Theriogenology*, 76(1), pp.161-167.
- Alagaw, Ababayehu Demeke. (2017): "Physiomorphological Parameters of Semen Obtained from Selected Bulls raised At National Artificial Insemination Center

and Its Effect on Conception Rate of Cows Reared at North Shoa, Amhara Region, Ethiopia." Phd Diss.,

Alberts,B., Johnson, A., Lewis, J., Raff, M., Roberts, K. And Walter, P., (2002): The Molecular Genetic Mechanisms That Create Specialized Cell Types .in *Molecular Biology of the Cell. 4 th edition.* Garland Science.

Aldini, S.A., Isnaini, N., Yekti, A.P.A and Susilawati,T., (2022): Study of the quality and integrity of spermatozoa acrosome caps in frozen sexing semen Friesian Holstein cattle.Jurnal Ilmu-Ilmu Peternakan (Indonesian Journal of Animal Science), 32(2), pp.233-240.

Alexander, JH (2008): Bull breeding soundness evaluation: A practitioner's perspective. *Theriogenology*70 (3): 469-472.

Almadaly, E., Farrag, F., Shukry,M. and Murase, T., (2014): Plasma membrane integrity and morphology of frozen-thawed bull spermatozoa supplemented with desalted and lyophilized seminal plasma .*Global Vet*, 13, pp.753-766.

Amann, R.P. and Hammerstedt, R.H., (2002): Detection of differences in fertility. *Journal of andrology* , 23 (3) pp .317-325.

Amenu, K., Grace, D., Nemo, S. and Wieland, B., (2019): Bacteriological quality and safety of ready-to-consume milk and naturally fermented milk in Borana pastoral area, southern Ethiopia .*Tropical animal health and production*, 51, pp. 2079-2084.

Andrabi, S.M.H., (2009): Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. *Reproduction in domestic animals*, 44(3), pp. 552-569.Brito, L.F.C.D.,2006.Nutrition, metabolic hormones, and sexual development in bulls (Doctoral dissertation, University of Saskatchewan).

Argiris, A., Ondho, Y.S., Santoso, S.I. and Kurnianto, E.,(2018): February .Effect of age and bulls on fresh semen quality and frozen semen production of Holstein bulls in Indonesia .In *IOP Conference Series: Earth and Environmental Science* (Vol.119, No.1, p.012033).IOP Publishing.

- Ashebir, G., Birhanu, A. and Gugsu, T., (2016): Status of artificial insemination in Tigray Regional State, "Constraints and acceptability under field condition". *J Dairy Vet Anim Res*, 3(3), p.00078.
- Assefa, A. and Hailu, A., (2018): Ethiopian indigenous cattle breed's diversity, distribution purpose of keeping and their potential threats *Journal of Biotechnology*,7(5), pp. 770-789.
- Ax, R.L., Dally, B.A., Didion, R.W., Lenz, R.W., Love, C.C. and Varner, D.D., (2000): Semen evaluation In Hafez B, Hafez ESE (eds) *Reproduction in farm animals*.
- Bahmid, N.A., Jamil, N.I., Yusuf, O.D.P., Farida, S. and Gustina, S., (2023): May. Plasma membrane integrity and acrosomal integrity of fresh and frozen Bali bull semen based on different ejaculate volume. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1174, No. 1, p. 012034) IOP Publishing.
- Baiee, F.H., Wahid, H., Rosnina, Y., Ariff, O.M., Yimer, N., Salman, H., Tarig, A.A. and Khumran, A.M., (2017): Hypo-Osmotic Swelling Test Modification to Enhance Cell Membrane Integrity Evaluation in Cryopreserved Bull Semen. *Pertanika Journal of Tropical Agricultural Science*, 40(2).
- Baloch, A.H., Kunbhar, H.K., Memon, M.I., Sharif, S.M., Raiz Ahmed Leghari, H.R. and Rajput, Z.I., (2019): 80. Fresh and post thaw quality characteristics of Holstein Friesian bull semen maintained at semen production unit Quetta Balochistan, Pakistan. *Pure and Applied Biology (PAB)*, 8(1), pp. 780-789.
- Barek, M.E., Hine, T.M., Nalley, W. M. and Belli, H.L., (2020): Pengaruh Penambahan Sari Wortel Dalam Pengencer Sitrat Kuning Telur Terhadap Kualitas Spermatozoa Kambing Bligon (The Effect Of Carrot Juice Supplementation In Citrate-Egg Yolk Extender On Spermatozoa Quality Of Bligon Goat). *Jurnal Nukleus Peternakan*, 7(2), Pp.109-117.
- Barratt, C.L.R., Tomlinson, M.J. and Cooke, I.D., (1993): .Prognostic significance of computerized motility analysis for in vivo fertility. *Fertility and Sterility*, 60(3),

pp. 520-525.

Bearden, H.J., Fuquay, J.W. and Willard, S., (2004): Applied Animal Reproduction, 6th Edition. *Mississippi State University, USA*, 427.

Behnke, R.H., (2010): The contribution of livestock to the economies of IGAD member states: study findings, application of the methodology in Ethiopia and recommendations for further work. *IGAD LPI Working Paper 02–10*.

Biniová, Z., Ducháček, J., Stádník, L., Doležalová, M. and Hájek, R., (2017): Effects of climatic conditions on bovine semen characteristics. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 65(1).

BOANR.1999): Livestock census regional state of Tigray Bureau of agriculture and natural resource, Mekelle, Ethiopia .Vol.1 Cattle .Ansi. ok state .edu (2000-02-23) .Retrieved on 2011-11-03.

Budiyanto, A., Arif, M., Alfons, M.P.W., Fani, R.T., Hafid, A.F., Wicaksono, B., Insani, K.M. and Herdinta, M.,( 2021) :The Effect of Age and Breed on the Quality of Bull Semen in The Regional Artificial Insemination Centre .*Acta Veterinaria Indonesiana*, pp.132-136.

Cabrita, E., Alvarez, R., Anel, E. and Herraez, M.P., (1999): The hypoosmotic swelling test performed with coulter counter: a method to assay functional integrity of sperm membrane in rainbow trout. *Animal reproduction science*, 55 (3-4),pp. 279-287.

Callaghan, M.J.,P. McAuliffe, R. J.Rodgers, J. Hernandez-Medrano and Perry.V.E. A. (2016): Sub acute ruminal acidosis reduces sperm quality in beef bulls1. *Journal of Animal Science* 94: 3215-3228.

Celeghini, E.C.C., Nascimento, J., Raphael, C.F., Andrade, A.F.C.D. and Arruda, R.P.D., (2010): Simultaneous assessment of plasmatic, acrosomal, and mitochondrial membranes in ram sperm by fluorescent probes. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 62, pp. 536-543.

- Central statistics authority CSA (2020/21): Agricultural sample survey 2020/ 21 .Report on livestock characteristics .Statistical Bulletin 589.Vol.II.Addis Ababa, Ethiopia.
- Chaudhary, P.J., Dhami, A.J., Chaudhari, D.V and Parmar, S.C., (2017): Freezability of cattle and buffalo semen and association of fresh and frozen-thawed sperm quality parameters. *Int. J. Curr. Microbiol .App. Sci*,6 (12), pp.1445-1454.
- Chelucci, S., Pasciu, V., Succu, S., Addis, D., Leoni, G.G., Manca, M.E., Naitana, S. and Berlinguer, F., (2015): Soybean lecithin–based extender preserves spermatozoa membrane integrity and fertilizing potential during goat semen cryopreservation .*Theriogenology*, 83(6), pp.1064-1074.
- Chowdhury, S., Das, S., Gupta, T., Sana, D. and Bose, S., (2014): Evaluation of frozen semen by acrosomal integrity and sperm concentration-two vital quality parameters of male fertility in bovines *Explor. Anim. Med. Res*, 4 (1), pp. 101-107.
- Contri, A., Valorz, C., Faustini, M., Wegher, L. and Carluccio, A., (2010): Effect of semen preparation on casa motility result sincryo preserved bull spermatozoa .*Theriogenology*, 74 (3), pp. 424-435.
- Corbet, N. J., B. M. Burns, D. J. Johnston, M. L. Wolcott, D. H. Corbet, B. K. Venus, Y. Li, M. R. McGowan, and R. G. Holroyd,.(2013): Male traits and herd reproductive capability in tropical beef cattle. 2. Genetic parameters of bull traits. *Anim. Prod. Sci*. 53:101–113. Doi: 10.1071/ AN12163
- Coulter, G.H. and Foote, R.H., (1979): Bovine testicular measurements as indicators of reproductive performance and their relationship to productive traits in cattle: a review. *Theriogenology*, 11 (4), pp. 297-311.
- Coulter, G.H., Kastelic, J.P., Howard, J.and Smith, R., (1999): Management programs for developing bulls. *Current Veterinary Therapy-Food Animal Practices*, 4, pp. 127-136.

- CSA (2012/13): Agricultural sampling survey, report on livestock and livestock characteristics for the year 2012/13, Addis Ababa, Ethiopia.pp.194.
- CSA (2016/17): Agricultural sample survey of Ethiopia in 2016/17 [2009 E.C.], volume-2: Report on livestock and livestock characteristics (private peasant holdings). Statistical bulletin -585, April 2017, Addis Ababa, Ethiopia.
- CSA (2018): agricultural samples every (2017/2018(2010ec): volume II report on livestock and livestock characteristics.(private peasant holdings) Addis Ababa, Ethiopia;
- CSA (2021): Federal Democratic Republic of Ethiopia Central Statistical Agency Agricultural Sample Survey.Retrieved from Addis Ababa:
- Curry, M.R. and Watson, P.F., (1994): Osmotic effects on ram and human sperm membranes in relation to thawing injury. *Cryobiology*, 31(1), pp. 39-46.
- Delgado JV, Martínez MA, Rodríguez Galván G, Stemmer A, Navas González FJ , and Camacho Vallejo ME. (2019): Organization and management of conservation programs and research in domestic animal genetic resources. *Diversity*, 11: 235. <https://doi.org/10.3390/d11120235>
- Demeke, N. (2010): Study on The Efficiency of Conventional Semen Evaluation Procedure Employed at Kality National Artificial Insemination Center and Fertility of Frozen Thawed Semen. MSc. Thesis, Addis Ababa University, Debrezeit, Ethiopia.
- Diansyah, A.M., Yusuf, M., Toleng, A.L., Dagong, M.I.A., Maulana, T. and Hasrin, B.A.,( 2023): The sperms post-thawing quality and proteomic seminal plasma on fertility performance of Bali-pollled bull. *Adv. Anim. Vet. Sci*,11 (4), pp.517-525.
- DJ, L., (2010): Semen analysis in 21st century medicine: the need for sperm functioning testing. *Asian International journal of urology* .12, pp. 64-70.
- EBI (2016): Ethiopian National Strategy and Plan of Action for Conservation and Utilization of Animal Genetic Resources Ethiopian Biodiversity Institute (EBI)

Addis Ababa.URL: [http:// www.ebi.gov.et/wpcontent/ uploads /2013/01/ Final % 20 ENSAP\\_final\\_submitted %20 \(1\) pdf.](http://www.ebi.gov.et/wpcontent/uploads/2013/01/Final%20ENSAP_final_submitted%20(1).pdf)

Edea, Z., Dadi, H., Kim, S.W., Dessie, T. and Kim, K.S., (2012): Comparison of SNP variation and distribution in indigenous Ethiopian and Korean Cattle (Hanwoo) populations. *Genomics & informatics*, 10(3), p.200.

Eliasson, R., (2010): Semen analysis with regard to sperm number, sperm morphology and functional aspects. *Asian journal of andrology*, 12(1), p.26.

F.A.O (2005):improving artificial breeding of cattle in Africa Guidelines and recommendations. A manual prepared under the framework of an IAEA technical cooperation regional AFRA project on increasing and improving milk and meat production (No.IAEA-TECDOC--1437) .International Atomic Energy Agency.

FAO (2020):"Gateway to dairy production and products" Retrieved 20 July 2020.

Fasil Getachew and Workneh Ayalew.,(2014):On-farm phenotypic characterization of indigenous cattle populations of Awi,East and West Gojjam Zones of Amhara Region Ethiopia Research *Journal of Agriculture and Environmental Management*.Vol.3(4), pp. 227-237 April, 2014.Available online at [http:// www.Apex\\_journal.org](http://www.Apex_journal.org).

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

Fontanesi, L.; Scotti,E and Russo, V.(2011): "Haplo type variability in the bovine MITF gene and association with piebaldism in Holstein and Simmental cattle breeds". *Anima, Genetics*,3 (3): 250256.Doi:10.1111/j.13652052.2011.02242.xISSN/1365 2052PMID22486495.

Foote, R.H., (1999): Artificial insemination from the origins up to today. In Proceedings of the International Symposium .From the first artificial insemination to the

modern reproduction biotechnologies: traditional ways and the new frontiers of animal production. Reggio Emilia (Italy) (pp. 23-68).

France CIV, (2013): a tradition of animal husbandry. Animal husbandry and environment Archived 2013-04-12at the Wayback Machine. Civ-viande.org. Retrieved on 2011-11-03.

Ftiwi, M. and Tamir, B., (2015): Phenotypic characterization of indigenous cattle in Western Tigray, Northern Ethiopia. *The Journal of Agriculture and Natural Resources Sciences*,2(1), pp.343-354.

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 simmentalbulls. *Anim.Reprod.Sci.*95:27–37.doi:10.1016/j.anireprosci.2005.09.002

Gebremedhin, D.G., (200): Assessment of problems/constraints associated with artificial insemination service in Ethiopia (Doctoral dissertation, Addis Ababa University).

Getabalew, Alemneh & Akebergn, D. (2019): Dairy production in ethiopia-existing scenario and constraints. *Biomedical Journal of Scientific & Technical Research*, 16, 12304–12309

Ghanbari, L., Carter, R.E., Rynes, M.L., Dominguez, J., Chen, G., Naik, A., Hu, J., Sagar, M.A.K., Haltom, L., Mossazghi, N. and Gray, M.M., (2019): Cortex- wide neural interfacing via transparent polymer skulls. *Nature communications*, 10(1), p.1500.

Górski, K., Kondracki, S., Strachocka, K. and Wysokińska, A., (2017): Association of ejaculate sperm counts with their morphological and morphometric characteristics in Hypor boars. *Annals of Animal Science*, 17(4), p.1043.

Goshme, S., Asfaw, T., Demiss, C. and Besufekad, S., (2021): Evaluation of motility and morphology of frozen bull semen under different thawing methods used for artificial insemination in North Shewa zone, Ethiopia. *Heliyon*,7(10).

- Graham, E.F., (1978): Fundamentals of the preservation of spermatozoa. In: The Integrity of Frozen Spermatozoa. Proc. Conf. Natl. Acad. Sci., Washington, DC. pp 4–44.
- Haftom, H., Haftu, A., Goitom, K. and Meseret, H., (2019): Agroclimatic zonation of Tigray region of Ethiopia based on aridity index and traditional agro-climatic zones. *Journal of Agro meteorology*, 21(2), pp.176-181.
- Hagos, B., (2016): Ethiopian cattle genetic resources and unique characteristics under a rapidly changing production environment. A review. *International Journal of Science and Research*, 6 (12), pp.1959-1968.
- Hamid, S. Abduraman, and Tadesse, B. Hindawi. (2023): Veterinary Medicine International Volume, Article ID 6512010, 8 pages <https://doi.org/10.1155/2023/6512010>. “Risk Factors for the Efficiency of Artificial Insemination in Dairy Cows and Economic Impact of Failure of First Service Insemination in and Around haramaya town, oromia region, eastern Ethiopia,” *Veterinary medicine international*, vol. 2021, Article ID 6622487, 6 pages, 2021.
- Hancock, P. and Mayne, J.E.O., (1957): Anodic polarization as a possible rapid method of deciding whether a given solution is corrosive or inhibitive. *Journal of Applied Chemistry*, 7(12), pp.700-708
- Hassen, F., Bekele, E., Ayalew, W. and Dessie, T., (200): Genetic variability of five indigenous Ethiopian cattle breeds using RAPD markers. *African Journal of Biotechnology*, 6(19) .
- Hezavehei, M., Sharafi, M., Kouchesfahani, H.M., Henkel, R., Agarwal, A., Esmaili, V. and Shahverdi, A., (2018): Sperm cryopreservation: A review on current molecular cryobiology and advanced approaches. *Reproductive biomedicine online*, 37(3), pp.327-339.
- Hirwa CA, Kugonza DR, Amahoro E, Ingabire C, Niyiragira V, Myambi C, Manzi M, Murekezi T, Nyabinwa P, Nshimiyimana AM, and Kany and ekweCh, Gahakwa

- D. (2017): Influence of breed, season and age on quality bovine semen used for artificial insemination. *Int J Livest Prod.* 8:72–88. doi: 10.5897/IJLP2017.0368.
- Imbayarwo- Chikosi, V.E., (2010): Dairy cattle genetics and breeding module .Faculties of Agriculture and Veterinary, University of Zimbabwe, Harare, Zimbabwe.
- Isachenko, V., Maettner, R., Petrunkina, A.M., Sterzik, K., Mallmann, P., Rahimi, G., Sanchez, R., Risopatron, J., Damjanoski, I. and Isachenko, E., (2012): Vitrification of human ICSI/IVF spermatozoa without cryoprotectants: new capillary technology. *Journal of andrology*, 33(3), pp. 462-468.
- Jemberu, W.T., Li, Y., Asfaw, W., Mayberry, D., Schrobback, P., Rushton, J. and Knight-Jones, T.J., (2022): Population, biomass, and economic value of small ruminants in Ethiopia. *Frontiers in veterinary science*, 9, p.972887.
- Jeyendran, R.S., Van der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D., (1984): Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics *Reproduction*, 70(1), pp.219-228.
- Kanthiya, S., Jitborisutthipong, P., Teepatimakorn, S., Punyapormwithaya, V., Ito, J. and Sathanawongs, A., (2020): The effect of semen collection interval on quality in frozen bull semen production. *Veterinary Integrative Sciences*, 18(1), pp.53-60.
- Kebede, A., (2018): Review on factors affecting success of artificial insemination. *Int. J. Curr. Res. Aca. Rev*, 6(1), pp.42-49.
- Kefelegn and Alemayehu, L., (2022): Breeding soundness of semen producing bulls, their semen quality and inter relationship of functional tests with spermatozoa motility percentage. *Journal of Veterinary Medicine and Animal Health*, 14 (1), pp. 1-11.
- Kenny, D.A. and Byrne, C.J., (2018): The effect of nutrition on timing of pubertal onset and subsequent fertility in the bull. *Animal*, 12(s1), pp.s36-s44.

- Khan, A., Khan, R.U., Qureshi, M.S., Mobashar, M., Gohar, A., Ahmad, S., Khan, K., Khan, H. and Naz, S., (2021): Effects of different equilibration times on post-thaw cryopreserved semen quality of cattle and buffalo bulls. *Pakistan Journal of Zoology*, 53(2), pp. 613-618.
- Kjoestad, H., Ropstad, E. and Andersen Berg, K., (1993): Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. *Acta Veterinaria Scandinavica*, 34 (3), pp.299-303.
- Kumar, S., Kumar, A., Shahi, M.G. and Yeasmin, F.,(2012): Relationship between Semen Quality Parameters and Field Fertility of Bulls.
- Langlais, J. and Roberts, K.D.,(1985):A molecular membrane model of sperm capacitation and the acrosome reaction of mammalian spermatozoa .*Gamete research*, 12 (2), pp.183-224.
- Lemma,A. and Shemsu, T., (2015): Effect of age and breed on semen quality and breeding soundness evaluation of pre-service young bulls. *Journal of Reproduction and Infertility*, 6(2), pp. 35-40.
- Lemma,A., (2011): Effect of cryopreservation on sperm quality and fertility. *Artificial insemination in farm animals*, 12 , pp. 191-216 .
- Madeja, Z., Waroczyk, M., Strabel, T. and Lechniak, D., (2003): Use of the hypo-osmotic swelling test for evaluating bull and boar semen quality *Medycyna Weterynaryjna*, 59(12), pp.1115-1118.
- Mandal, D.K., Kumar, M. and Tyagi, S., (2023): Impact of seasons and management factors on seminal attributes and frozen semen doses production in Holstein Friesian crossbred dairy bulls. *Applied Veterinary Research*, 2 (1), pp. 2023004-2023004.
- Mann,T., (1951): Mammalian semen; composition, metabolism and survival. *The Biochemical journal*, 48(5), p.lxiii.

- Marai, I.F.M. and Haezeb, A.A.M., (2010): (Buffalo's biological functions as affected by heat stress—A review. *Livestock Science*,127(2-3), pp.89-109.
- Martins, L.F., Pinho, R.O., Siqueira, J.B., Costa, D.S., Guimarães, S.E.F., Neto, T.M. and Guimaraes, J.D.,(2018):Hypoosmotic swelling test in young Nelore bulls classified as sound and unsound for breeding.*Animal Reproduction (AR)*, 10(4), pp.684-688.
- Mathur, A.K., Kumar, S., Prabhakar, J.H., Mandal, D.K., Ingale, H.R. and James, J.O.S.E., (2014): Motility of frozen thawed semen after thermal resistance test. *Indian Journal of Animal Reproduction*, 35(1), pp. 18-20.
- Medeiros, C.M.O., Forell, F., Oliveira, A.T.D. and Rodrigues, J.L.,(2002): Current status of sperm cryopreservation: why isn't it better? *Theriogenology*, 57 (1) , pp.327-344.
- Mekuriaw, G. and Kebede, A., (2015): A review on indigenous cattle genetic resources in Ethiopia: adaptation, status and survival. *Online Journal of Animal and Feed Research*, 5(5), pp. 126-137.
- Memili E, Moura AA and Kaya A (2020): Anim. Reprod. Sci.22010 6355
- Menegassi, S.R.O., Barcellos, J.O.J., Peripolli, V., Pereira, P.R.R.X., Borges, J.B.S. and Lampert, V.D.N., (2011): Measurement of scrotal circumference in beef bulls in Rio Grande do Sul. *Arquivo Brasileiro de Medicina Veterináriae Zootecnia*, 63, pp. 87-93.
- Mezgebe, G., Gizaw, S., Urge, M. and Chavhan, A., (2017):Begait cattle production systems and production performances in northern Ethiopia. *International Journal of Life Sciences*, 5(4), pp. 506-516.
- Mohammed, A. and Ahmed, W.M.M., (2018): Pure Egyptian cattle bulls show both individual variation and different interaction with extender in the post-thawing sperm parameters. *Andrology*, 6(2), pp. 2-6.

- Mohanty, T.K., Lone, S.A., Kumaresan, A., Bhakat, M., Kumar, R., Baithalu, R.K., Sinha, R., Paray, A.R., Yadav, H.P., Sahu, S.K. and Mohanty, A.K.,(2018):Sperm dosage and site of insemination in relation to fertility in bovines. *Asian Pacific Journal of Reproduction*, 7(1), pp.1-5.
- Morrell, J.M.,(2011): Artificial insemination :current and future trends. *Artificial insemination in farm animals*, 1,pp. 1-14.
- Moura, A.A., Chapman, D.A., Koc, H. and Killian, G.J., (2007): A comprehensive proteomic analysis of the accessory sex gland fluid from mature Holstein bulls. *Animal reproduction science*, 98(3-4), pp.169-188.
- Nijs, M., Creemers, E., Cox, A., Janssen, M., Vanheusden, E., Castro-Sanchez, Y., Thijs, H. and Ombelet, W., (2009): Influence of freeze-thawing on hyaluronic acid binding of human spermatozoa. *Reproductive biomedicine online*, 19(2), pp. 202-206.
- Nongbua, T., Utta, A., Am-In, N., Suwimonteerabutr, J., Johannisson, A. and Morrell, J.M.,( 2020) Effects of season and single layer centrifugation on bull sperm quality in Thailand. *Asian-Australasian journal of animal sciences*, 33(9), p.1411. of Holstein Friesian and Jersey bulls maintained under subtropical environment. *Pak. Vet. J*,30(2), pp.75-78.
- Owen, R.D.(1999): Bull breeding soundness evaluation and venereal disease testing. Large animal clinical sciences college of veterinary medicine university of Florida.
- Pandey, V., Nigam, R., Singh, P., Sharma, A., Saxena, A., Swain, D.K., Sharma, L. and Dixit, S., (2014): Influence of season on biochemical attributes of Bhadawari buffalo bull semen: effect of temperature and humidity . *Journal of Animal Research*, 4(2), pp.201-209.
- Parkinson, T.J.,(2004): Evaluation of fertility and infertility in natural service bulls. *The Veterinary Journal*, 168(3), pp. 215-229.

- Patel, B.R. and Siddiquee, G.M., (2013): Physical and morphological characteristics of Kankrej bull semen. *Vet World*, 6(7), pp. 405-408.
- Perry, V.E.A., (2017): Increasing standardisation in sperm morphology Australian Cattle Veterinarians. *Sydney, ACT: AVA*.
- Peter Chenoweth, January (2010): James Cook University Guidelines for using the bull breeding soundness evaluation form, <https://www.researchgate.net/publication/281575349>
- Porter, V., Alderson, L., Hall, S.J. and Sponenberg, D.P., (2016): *Mason's world encyclopedia of livestock breeds and breeding, 2 Volume Pack (Vol. 2)*. Cabi.
- Prastowo, S., Dharmawan, P., Nugroho, T., Bachtiar, A. and Pramono, A., (2018): Kualitas semen segarsapi Bali (*Bos javanicus*) pada kelompok umur yang berbeda. *Jurnal Ilmu Ternak Universitas Padjadjaran*, 18(1), pp.1-7.
- Quintero-Moreno, A., Rigau, T. and Rodríguez-Gil, J.E., (2004): Regression analyses and motile sperm subpopulation structure study as improving tools in boar semen quality analysis. *Theriogenology*, 61(4), pp. 673-690.
- Rahman, M.A., Juyena, N.S., Ahmed, J.U., Ferdousy, R.N., Chakma, S., Rine, M.Z. and Tarif, A.M.M., (2014): Evaluation of semen for breeding soundness of four different breeds of bull used for artificial insemination. *Journal of Agriculture and Veterinary Science*, 7(9), pp.28-34.
- Said, T.M., Agarwal, A., Sharma, R.K., Thomas Jr, A.J. and Sikka, S.C., (2005): Impact of sperm morphology on DNA damage caused by oxidative stress induced by  $\beta$ -nicotinamide adenine dinucleotide phosphate. *Fertility and sterility*, 83(1), pp. 95-103.
- Salisbury, G.W., Van Demark, N.L. and Lodge, J.R., (1978): *Physiology of reproduction and artificial insemination of cattle (No. Ed.2)* WH Freeman and Company.

- Samre, M., Alemayehu, T. and Sisay Weldegebrie, S., (2015): Major Constraints of Artificial Insemination in and Around Alamata District, Tigray, Ethiopia. *African Journal of Basic & Applied Sciences*, 7(5), pp. 287-290.
- Sarder, M.J.U., (2005): Scrotal circumference variation on semen characteristics of artificial insemination (AI) bulls. *Journal of animal and Veterinary Advances*.
- Schenk, J.L., (2018): Principles of maximizing bull semen production at genetic centers. *Animal*, 12(s1), pp.s 142-s147.
- Senger, P. L.(2012): Pathways to pregnancy and parturition, 3rd ed. Redmond (OR): Current Conceptions, Inc
- Seyoum, K., Lemma, A. and Tera, A., (2021): Influence of breed on motility and motion characteristics of fresh, chilled and frozen bull spermatozoa. *International Journal of Livestock Production*, 12(1), pp. 37-42.
- Shah, M., Qureshi, M., Khan, R.U., Mobashar, M., Khaliq, M.A., Khattak, I., Tariq, A., Ahmad, I. and Naz, S., (2019): Semen quality of bulls as influenced by breed, body condition score and ascorbic acid under heat stress. *Pakistan Journal of Zoology*, 51(5), pp. 1699-1703.
- Shanku, E., (2022): Evaluation of artificial insemination (AI) after estrus synchronization of dairy cattle in Wondo Genet District, Sidama regional state of southern Ethiopia. *International Journal of Advanced Research in Biological Sciences*, 9, pp.109-117.
- Shukla ,m.k., saha, s., rathore, k.s., mishra, g.k., siddqui, m.u. and saxena, s.k., (2012): comparative study of plasma membrane integrity of i spermatozoa by using hos medium and distilled water and their relation with post thaw motility. *the indian journal of animal reproduction*, 33(2), pp.7-9.
- Singh, A.K., Rajak, S.K., Kumar, P., Kerketta, S. and Yogi, R.K.,(2018): Nutrition and bull fertility: A review. *J. Entomol. Zool. Stud*, 6(6), pp. 635-643.

- Sinshaw, W.,(2005): Study on semen quality and field efficiency of AI bulls kept at the National Artificial Insemination Center. *Debre Zei*, 53(2), pp. 135-138.
- Snoj, T., Kobal, S. and Majdic, G., (2013): Effects of season, age, and breed on semen characteristics in different *Bostaurus* breeds in a 31-year retrospective study. *Theriogenology*, 79(5), pp. 847-852.
- Soren, S., Singh, S.V. and Singh, P., (2016): Influence of season on seminal antioxidant enzymes in Karan Fries bulls under tropical climatic conditions. *Turkish Journal of Veterinary & Animal Sciences*, 40(6), pp.797-802.
- Sori, H.,( 2004): Evaluation of semen parameters in Ethiopian indigenous bulls kept at Kality, National Artificial Insemination Centre (Doctoral dissertation, MSc thesis. Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia).
- Stata (2001): Inter cooled stata7.0 for windows.702 University Drive East: Stata Corporation. [stata@stata.com](mailto:stata@stata.com)
- Storey, B.T., (2004): Mammalian sperm metabolism: oxygen and sugar, friend and foe. *International Journal of Developmental Biology*, 52 (5-6), pp.427-437.
- Suyadi, S., Herwijanti, E., Septian, W.A., Furqon, A., Nugroho, C.D., Putri, R.F. and Novianti, I.,( 2020): April. Some factors affecting the semen production continuity of elite bulls: reviewing data at Singosari National Artificial Insemination Center (SNAIC), Indonesia. In IOP Conference Series: Earth and Environmental Science (Vol. 478, No. 1, p. 012080).IOP Publishing.
- Tegegne, A., Kassa, T. and Mukasa-Mugerwa, E., (1995): Aspects of bull reproduction with emphasis on cattle in Ethiopia.II.Sperm production capacity and semen characteristics.*Ethiopian Society of Animal Production Proceedings*.
- Thornton,P.K.,(2010):Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1554), pp. 2853-2867.

- Thundathil, J.C., Dance, A.L. and Kastelic, J.P., (2016): Fertility management of bulls to improve beef cattle productivity. *Theriogenology*, 86(1), pp.397-405.
- Ugur, M.R., Saber Abdelrahman, A., Evans, H.C., Gilmore, A.A., Hitit, M., Arifiantini, R.I., Purwantara, B., Kaya, A. and Memili, E., (2019): Advances in cryopreservation of bull sperm. *Frontiers in veterinary science*, 6, p.268.
- Umesh Kumar, U.K., Gawande, A.P., Sahatpure, S.K., Patil, M.S., Lakde, C.K., Bonde, S.W., Borkar, P.L., Poharkar, A.J. and Ramteke, B.R., (2015): Assessment of semen quality in pure and crossbred Jersey bulls.
- Van der ven, h.h., jeyendran, r.s., al-hasani, s.a.f.a.a., perez-pelaez, m.a.r.i.a.n.o., diedrich, k. and zaneveld, l.j ., (1986): correlation between human sperm swelling in hypoosmotic medium (hypoosmotic swelling test) and in vitro fertilization. *Journal of andrology*, 7(3), pp.190-196.
- Vicari, E., La Vignera, S., Castiglione, R. and Calogero, A.E., (2006): Sperm parameter abnormalities, low seminal fructose and reactive oxygen species overproduction do not discriminate patients with unilateral or bilateral post-infectious inflammatory prostatico-vesiculo-epididymitis. *Journal of Endocrinological Investigation*, 29, pp. 18-25.
- Vikram, R., Perumal, P., Khan, M.H. and Girish, P.S., (2023): Assisted reproductive technologies (ARTs) in Mithun (*Bos frontalis*): what progress has been made so far? An overview. *Reproduction in Domestic Animals*, 58(5), pp.583-593.
- Voss, J. L., (1981): Stallion spermatozoal morphology and motility and their relationship to fertility. *J. Am. Vet. Med. Assoc.*, 178, pp.287-289.
- Wang, C. and Swerdloff, R.S., (2014): Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. *Fertility and sterility*, 102(6), pp.1502-1507.
- Webb DW. (2003): "Artificial Insemination in Cattle". University of Florida, Gainesville. IFAS Extension, DS 58: 1-4.

- World Health Organization, (2010): WHO laboratory manual for the examination and processing of human semen.
- Zegeye, T., Belay, G. and Hanotte, O., (2021): Multivariate characterization of phenotypic traits of five native cattle populations from Tigray, Northern Ethiopia. *Tropical Animal Health and Production*, 53(2), p.212.
- 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 génétiques animals/ Recursos genéticos animals*, 40, pp.15-32.
- Zewdie, E., Deneke, N., FikreMariam, D.,Chaka , E., HaileMariam, D. and Mussa, A., (2005):Guidelines and procedures on bovine semen production.*NAIC, Addis Ababa*.
- Zewdie, E., Mussa, A., Melese, G.M., Mariam, D.H. and Perera, B.M.A.O., (2007): Improving artificial insemination services for dairy cattle in Ethiopia. *Application of Radioimmunoassay in Improving the Reproductive Management of Smallholder Dairy Cattle*, p.17.

## 7. ANNEXES

### **I. Procedure for the preparation of the artificial vagina (Salisbury *et al.*, 1978)**

Insert the inner liner into the cylinder

II. Turn the ends of the liner back over the cylinder

III. Fit the collection receptacle into the ends of the rubber funnel

IV. Place the funnel over one end of the cylinder and the liner

V. 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.

VI. 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 45<sup>0</sup>C 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 <sup>0</sup>C or higher is recommended.

VII. 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.

### **II. Eosin –Nigrosin staining method is used to count live and dead sperm cells: (Salisbury *et al.*, 1978 ;)**

I. From stock solution of Eosine 5% and Nigrosine 10%

II.3 drops and 5 drops respectively are mixed in a small glass test tube maintained in a water bath at 34oC.

III. Two (2) drops of mixed stain and a small drop of semen are taken on a pre- warmed slide and mixed gently

IV. Two smears are prepared and allowed to dry in the air.

v. Random fields (diagonally) are counted over the slide to obtain a representative figure.

vi. A maximum of 300 sperms are counted to find out the percentage of live sperm using the following formula:

vii. No of live sperm counted X 100 = \_\_\_\_%

viii. No of total sperm counted.

ix. The death percentage per slide should not exceed 30%.

x. If part of the sperm is stained, such as the nucleus, it is counted as dead.

### **III. Procedure of Hypo osmotic swelling test (HOST) (Jeyendranet *al.*, 1984)**

- I. Maintain the control and HOS at 37°C for 5 min. before use
- II. Take 0.1ml. of semen and mix with 1ml. of HOS (Do for control too)
- III. Incubate at 37°C for 60 min.
- IV. Fix with formaldehyde (10% formalin 0.1ml.) so as to retain the shape of spermatozoa for subsequent observation.
- V. Place a drop (nearly 5 $\mu$ l) well mixed semen sample on a glass slide and cover it with a cover slip.
- VI. Count a total of 200 sperm cells in at least 5 different field of vision
- VII. Consider the number of sperms with coiled or swollen tail.
- VIII. The number of coiled or swollen tail spermatozoa in PBS should be deducted from the number in hypo-osmotic solution
- IX. The resultant figure will be taken as the HOS-reactive spermatozoa.

### **IV. Procedure of acrosome integrity (Almadaly E, Foad F, Mostafa S, and Tetsuma (M 2014)**

- I. The staining process was carried out by dripping semen on an object glass. Smear preparation was made and warmed using a warming plate at the temperature of 37 °C.
- II. The preparation was fixed using methanol for 10minutes and then rinsed in running water. It was stained using Giemsa solution of the composition of 3 ml absolute Giemsa, 2 ml PBS, and 35 ml distilled water for 3 hours by immersing it in a staining jar. It was rinsed in running water and dried again.
- III. Two hundred cells were examined under a light microscope at 400x magnification. Acrosome-intact spermatozoa were characterized by purple head, while those with damaged acrosome were characterized by pale lavender head.
- IV. Acrosomal status was established by counting the number of acrosome-intact spermatozoa divided by the total number of spermatozoa and multiplied by 100%.

**V. Procedure of morphology examination**

- I. one milliliter of Hancock solution (Buffered formal saline) was kept in 37 °C in water bath to which one drop of fresh semen was added with warm (37 °C) Pasteur pipette and gently mixed
- II. morphological abnormalities mainly head, mid-piece and tail defect, proximal and distal droplets were examined by placing small drop of the sample on clean grease free glass slide and covering by cover slide under phase contrast microscope (200x)
- III. count 500 spermatozoa were counted and the abnormalities were visualized and recorded as head, mid-piece (body) and tail abnormalities

**Hancock solution**

- 6.1232g disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4, 2\text{H}_2\text{O}$ ),
- 2.545g Potassium dihydrogen Phosphate ( $\text{KH}_2\text{PO}_4$ ),
- 5.412g sodium chloride (Nacl),
- 0.125ml (37%) or 0.116(40%) formaldehyde and
- 1000ml or 1 liter distilled water



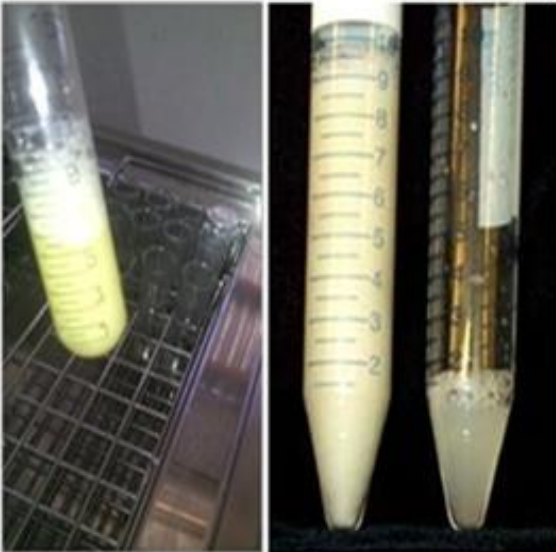
MAIC Mekelle Artificial Insemination Center



Photo meter (concentration)



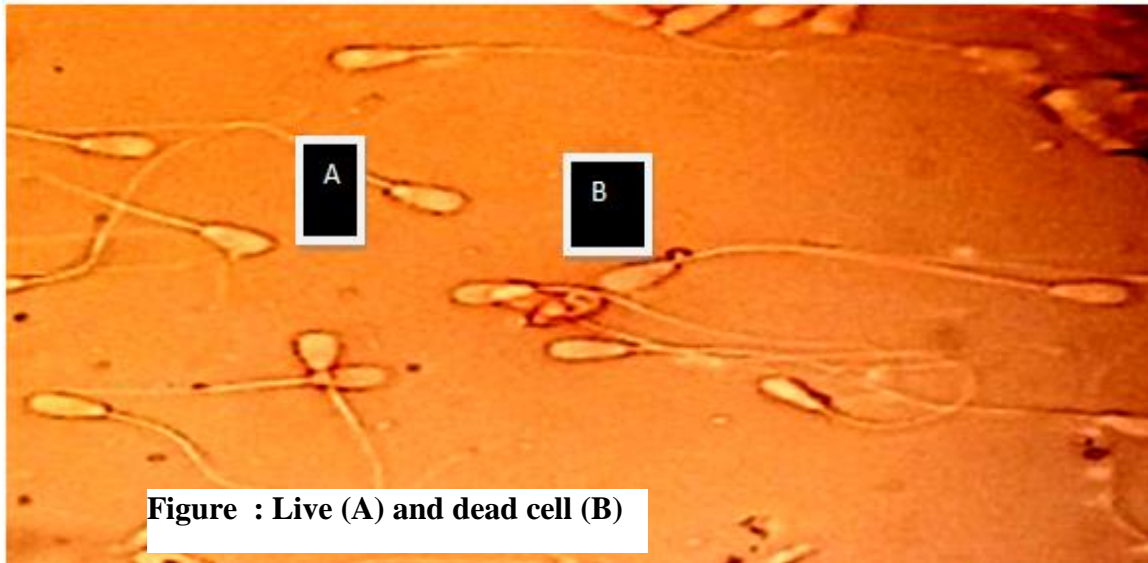
Evaluation of Semen concentration



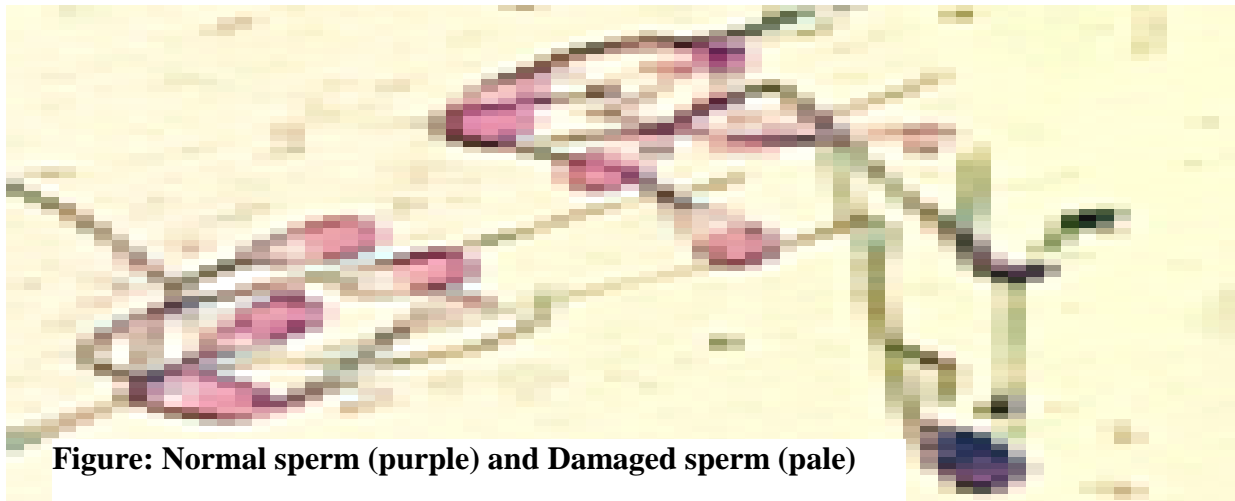
Semen volume , color



Acrosome and membrane integrity evaluation



**Figure : Live (A) and dead cell (B)**



**Figure: Normal sperm (purple) and Damaged sperm (pale)**



**Figure : membrane integrity curling tail of sperm**

## 10. CURRICULUM VITAE

### I. Personal Identification

Name Hadish Ghidey Kabsay

Birth Place Asmara Eritrea

Birth date 22/08/1970

Sex male

Marital status married

Religion Christian

Denomination orthodox

Nationality Ethiopian

Profession Veterinarian

Occupation Field veterinary service

II. Contact Address Mekelle, bureau of agriculture mail: [hadghi2012@gmail.com](mailto:hadghi2012@gmail.com).

Mobile= +251914195020

III. Education Elementary - Asmara-Eritrea from 1977 to 1983 where I learned from beginning to 6<sup>th</sup> grade, and 7-8<sup>th</sup> and High school Meda- Agame Senior Secondary School, Adigrat Tigray.

Secondary School Adigrat town where I continued my education from 9<sup>th</sup>- 12<sup>th</sup> grade

College Agrfa Bale Goba certificate –animal health

Dipolma Alage College diploma - animal health

University - In 2005/2007 I took my common course in Faculty of vet. Science Mekell University, Tigray, Ethiopia.

#### IV. Work Experience

Since 1993 until I joined the MSc. program in Faculty of Veterinary since in 2012, I was serving as field and AI center laboratory in Tigray Regional State.

#### V. Language Skill

Tigrigna language ..... Read, write and speak

Amhara language..... Read, write and speak

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

#### VII. REFERENCES

Mekelle University, Faculty of Science, Registrar office.

Mekelle University, Faculty of Veterinary Medicine, Mekelle, Registrar office.

Tigray Regional State Agricultural Beruea Mekelle.

Hadish Ghidey: Mekelle, Tigray.

Email      hadghi2012@gmail.com