THE EFFECT OF TRIS ALOE VERA (*ALOE BARBADENSIS MILLER*) GEL ON CHILLED AND FROZEN-THAWED BULL SPERMATOZOA QUALITY

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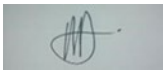
Signature:		Date: 27/02/2023
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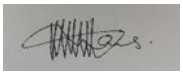
Signature:		Date: 27/02/2023
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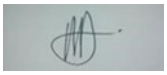
DECLARATION

I, Kenny Seshoeni, hereby declare that this dissertation for Master of Science Agriculture (MSCAGR) Animal science submitted to the Department of Animal Science, Faculty of Science, Engineering and Agriculture, at the University of Venda has not been submitted previously for any degree at this or another university. It is original in design and in execution, and all reference material contained therein has been duly acknowledged.

Signature:  Date: 27/02/2023

KENNY SESHOENI

DEDICATION

This dissertation is dedicated to my son, Jaden and my lover, Funanani, you both are my source of strength, you are the reason I wake up every day and put more effort on this work, for that, I appreciate you for providing me a sense of belonging.

To my mother, big thanks for being very patient with me, for believing in me, I really would have not done it without you, your love and support. I thank you MMA.

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ABSTRACT

Semen extenders protect sperm from cold shock, osmotic stress, and alterations in membrane fluidity and permeability plus provide energy substrates for sperm metabolism. Egg yolk is one of the most common additives of animal origin that are used for semen preservation, however, it has become a suspect for facilitating the transmission of diseases. Tris-aloe vera gel have several beneficial properties, such as anti-inflammatory, antioxidant, antiviral and antibacterial features. The aim of this study was to determine the effect of Tris-aloe vera gel (TAG) extender on the quality of bull spermatozoa. In experiment 1, the effect of Tris-*Aloe vera* gel extender was assessed after the semen was chilled at 5 ^{0}C in a refrigerator for different storage times up to 120 hours, and frozen in liquid nitrogen at -196 ^{0}C for 5 days in experiment two. Assessment was done for morphology, motility, and viability of bull spermatozoa. Semen samples were collected from four (4) Nguni bulls with proven fertility records, aged between 6 and 8 years kept at the University of Venda Experimental Farm under intensive farming practice. Collected semen samples were pooled and diluted at a ratio of 1:5 (semen to extender) in Tris-egg yolk (TEY) as a control and TAG at three different concentration levels 12%, 16% and 20% of *Aloe vera* gel (AVG). In experiment one, diluted semen samples were evaluated immediately after the extension using computer aided sperm analyzer (CASA) before storage at 5 ^{0}C, followed by sperm analysis after every 24 hours for 120 hours. In experiment two, freshly collected and diluted semen samples were cryopreserved and stored at (-196 ^{0}C) in LN2 for 5 days and evaluated thereafter. The results were subjected to the analysis of variance (ANOVA) for statistical analysis using a general linear model (GLM) procedures of Minitab 19 program. Tris-egg yolk extender (TEY) with 0% *aloe vera* gel was used as a control. In experiment one, it was found that TEY showed consistency in keeping motility at an average of 100% ($p<0.05$) after 120 h of chilling semen samples. Semen samples extended in Tris-*aloe vera* gel had a decrease in spermatozoa motility as storage time increased. Experiment two discovered that AVG can equally be used to substitute

egg yolk in tris-based extender and be able to cryopreserve Nguni bull semen for a period of 5 days, this is due to a fact that there was no significant difference ($P<0.01$) in spermatozoa progressive motility, from both TEY and TAGs post thaw. It was also found that morphological normal spermatozoa were above 80% post thaw. Semen extended in TEY shows a great deal of consistency in maintaining high spermatozoa viability throughout the chilling process.

Keywords: Aloe vera gel, Egg yolk, Chilling, Cryopreservation, Semen extenders, Spermatozoa.

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ABBREVIATIONS AND ACRONYMS

AI : Artificial insemination

ANOVA : Analysis of variance

ART : Assisted Reproduction Technique

AV : Artificial vagina

AVG : Aloe vera gel

EE : Electro ejaculator

ICSI : Intracytoplasmic sperm injection

GLM : General linear model

FPA : Fast progressive type A

h : Hours

IVF : *In vitro* fertilization

LDL : Low density lipoprotein

LN : Liquid nitrogen

MED : Medium velocity

min : Minutes

mL : Millilitres

NPM : Non progressive motility

PM : Progressive motility

ROS : Reactive oxidation species

SPB : Slow progressive type B

SLW : Slow velocity

STC : Static

ST : Storage time

TAG : Tris-Aloe vera gel

TEY :Tris-Egg yolk

TM : Total motility

CHAPTER 1

INTRODUCTION

1.1 Background

Preservation of spermatozoa is in great demand for the conservation of genetically elite males. In this regard, the development of artificial reproductive technologies, including artificial insemination (AI), *In-vitro* fertilization (IVF), and Intracytoplasmic sperm injection (ICSI) is of great interest (El-Sheshtawy and El-Nattat, 2017). Conventional extenders use different materials such as animal sources and plant sources which provide various features and diverse problems depending on the type of sperm extender and species (Bustani and Baiee, 2021). Although egg yolk has been used as a main component of bovine semen extenders, the use of egg yolk-based extenders has been restricted in other countries (Chaudhari *et al*., 2015). This is because reports allude that egg yolk represents a risk of bacterial or xenobiotic contamination (Aires *et al*., 2003; Singh *et al*., 2013; Dorji *et al*., 2014; Layek *et al*., 2016). Therefore, the potential for introducing exotic diseases through semen distribution domestically and internationally and that endotoxins produced by such contaminants reduce the potential fertilizing capacity of sperm. Consequently, the use of plant extracts as semen extenders has emerged as a cheap and natural source of additives to preserve and improve sperm function during semen preservation (Santaella and Pintus, 2021).

The *Aloe vera* gel has been observed to be rich in pharmacological components including anti-inflammatory, antifungal, antiviral, antidiabetic, antimicrobial, and anticancer. A high dose of *Aloe vera* gel was associated with decreased count of red cells and sperm damage in the spermatozoa of mice (Oyeyemi and Fayomi, 2011). Moreover, *the Aloe vera* plant contains a numerous vitamins including antioxidants, vitamins like A and C, vitamins B, such as thiamine, niacin, vitamin B2 (Riboflavin), and B12 as well as folic acid. Minerals such as sodium,

potassium, calcium, magnesium, manganese, copper, chrome, and iron are also found in *Aloe vera* (Shahraki *et al*., 2014). Biological activities, including anti-diabetic, anti-viral, anti-bacterial, anti-cancer, antibiotic, hypotensive, laxative, protection against radiation, anti-inflammation, immunostimulant, and blood purifying properties are associated with polysaccharides in the *Aloe vera* gel (Fakhrildin and Sodani, 2014; Singh, 2020). Nonetheless, there is a scarcity of information about the use of the *Aloe vera* plant as a semen extender for Bulls (Olugbenga *et al*., 2011).

1.2 Problem Statement

The valuable genetic material of genetically superior animals is lost due to the inability to mate which could result from a lack of libido or injury (Parmer *et al*., 2013). Semen extension and preservation are significant because many livestock breeders rely on semen obtained from animals that are unknown to them (Sokunbi *et al*., 2015). However, semen preservation may result in reduced fertility as cells might be damaged during freezing and thawing. According to Layek *et al*. (2016), egg yolk extenders are suspected of facilitating the transmission of diseases due to bacterial contamination such as *Salmonella, Listeria, Haemophilus,* and *Mycoplasma*. The presence of bacteria may harm sperm quality, either by directly competing with spermatozoa for nutrients supplied by the semen extender or by the production of toxic metabolic by-products and endotoxins. Moreover, bacteria may cause inflammation or disease in inseminated females (Morrell and Wallgren, 2014). Hence, there is a dire need for the elimination of animal products as semen extenders (Anzar *et al*., 2019). Tris-aloe vera gel has several beneficial properties, such as anti-inflammatory, antioxidant, antiviral, and antibacterial features (Shakib *et al*., 2019). Therefore, the gel can be used as a plant-based semen extender for the preservation of superior genetic material without exposure to bacterial contamination.

1.3 Justification of the study

When freshly ejaculated semen cannot be used for assisted breeding, excellent semen extender will be required for extension and preservation of elite bulls and for the exploitation of the genetic material of superior bulls to the possible maximum extent (Raheja *et al*., 2018). The development and application of reproductive techniques such as AI, IVF, ICSI, etc depends on the preservation of semen. It also allows the management and selection of breeding in domestic animals and result in advances in the livestock industry (Yoon *et al*., 2016). Furthermore, cryopreservation is also used to prevent and control the spread of venereal diseases through assisted reproductive techniques (ART) (Khalil *et al*., 2018). Growing concern over the susceptibility of the components of egg yolk as a semen extender to the transmission of diseases motivated consideration of alternate plant-based extenders that maintain spermatozoa fertility and viability effectively (Layek *et al*., 2016). Due to similarities in biological components of Aloe vera and Egg yolk, this study will generate knowledge on whether Aloe vera gel can be used as a plant-based semen extender for the preservation of superior genetic material without any exposure to bacterial contamination.

1.4 Research Objectives

1.4.1 Main Objective

The main objective of this study was to determine the effect of Tris-*aloe vera* gel extender on bull spermatozoa quality after storage in a liquid and frozen state.

1.4.2 Specific Objectives

I. To determine the effect of substituting egg yolk with *Aloe vera* gel of different concentration levels (12%, 16%, and 20%) in bull semen chilled at 5 ^{0}C and stored under six storage times (24, 48, 72, 96, and 120 hours) on the motility, morphology, and viability of bull spermatozoa.

II. To determine the effect of substituting egg yolk extender with *Aloe vera* gel of different concentration levels (12%, 16%, and 20%) in bull semen which is cryopreserved for 5 days, on motility, morphology, and viability of bull spermatozoa.

1.5 Research Null Hypothesis

I. There will be no significant effect on motility, morphology, and viability in bull spermatozoa extended in Tris-*aloe vera* gel at different concentration levels (12 %, 16 %, and 20 %) of aloe vera gel after chilling at 5 ^{0}C up to 120 hours.

II. There will be no significant effect of cryopreservation on motility, morphology, and viability of spermatozoa extended in Tris-aloe vera gel at different concentration levels (12%, 16%, and 20%) of *Aloe vera* gel.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

For centuries, *Aloe vera* plant has been known and used for its health, beauty, medicinal, and skin care properties. However, it has recently been used as an alternative cryoprotectant to egg yolk for the chilling and cryopreservation of semen. Egg yolk represents a risk of bacterial or xenobiotic contamination and therefore, the potential for introducing exotic diseases through semen distribution domestically and internationally, and that endotoxins produced by such contaminants compromise the potential of the spermatozoa to fertilize the ovum (Aires *et al*., 2003; Singh *et al.*, 2013; Layek *et al*., 2016). Despite the type of extender and the preservation methods used, preservation of semen negatively affects the quality of spermatozoa, as such, the two existing methods of semen preservation are chilling and cryopreservation (Baiee *et al*., 2017). Therefore, this chapter provides an overview of the existing plants and animal-based extenders, semen preservation methods and their effects on the quality of sperm, and finally, the effect of plant-based extenders on the quality of spermatozoa as the subject matter in this case.

2.2 Semen extenders

Semen extender or diluent is a chemical medium used for semen preservation, extension, and protection of spermatozoa against various shocks during processing, storage, and transportation as employed during artificial insemination (AI), *Invitro Fertilization* (IVF), Intracytoplasmic Sperm Injection (ICSI), and other assisted reproduction technologies (ART). Semen extenders constitutes of nutrients such as carbohydrates such as glucose, lactose, raffinose, saccharose, trehalose, and cryoprotectant agents for protection against cold shock, buffers, salts (sodium citrate, citric acid), antioxidants, and antibiotics (Barbas and Mascarenhas, 2019; Santaella and Pintus, 2021). These properties keep the sperm stored and transported and enable it to be used

in AI, *in vitro* fertilization, and intra-cytoplasmic sperm injection. Moreover, conventional extenders use different materials such as animal sources and plant sources which provide various features and diverse problems depending on the type of sperm extender and species (Bustani and Baiee, 2021).

2.2.1 Animal-based extenders

For the past decades, egg yolk has been used as a main component of semen extender for the preservation of bovine semen. Egg yolk is a non-penetrating cryoprotectant that is used in semen cryopreservation, it contains phosphatidylcholine (lecithin), lipoprotein fractions, lipid extracts and specific low-density lipoproteins (LDL) which protects spermatozoa from cold shock. Moreover, egg yolk-based extenders provide protection for both chilled and cryopreserved bull spermatozoa (Rauch, 2013; Raheja *et al*., 2018). Furthermore, it has been reported to be of excellent use for spermatozoa protection against cold shock. However, some researchers reported that due to substances present in egg yolk containing extenders, spermatozoa motility and respiration may be negatively affected (Chaudhari *et al*., 2015). Moreover, various reports (Aires *et al*., 2003; Singh *et al*., 2013; Dorji *et al*., 2014; Layek *et al.*, 2016) reported that egg yolk represents a risk of bacterial or xenobiotic contamination. As such, egg yolk has the potential to introduce exotic diseases through the distribution of semen both domestically and internationally and that the endotoxins produced by such contaminants potentially reduces the fertilizing capacity of the spermatozoa.

2.3.2 Plant-based extenders

Due to the problems of the transmission of diseases through the use of various animal protein sources in semen extenders, most of the plant species' extracts are being employed as semen preservatives for the preservation of semen for several animal species, including humans. The extracts are usually added to the semen extender and used both for sperm refrigeration and cryopreservation. Furthermore, plant extracts have recently emerged as a cheap and natural

source of additives to preserve and improve sperm function during semen storage. Most plant species are considered powerful sources of antioxidants, which can act as reactive oxidation species (ROS) scavengers for palliating the deleterious effects of oxidative stress on sperm function. Moreover, these natural compounds can have antimicrobial properties and increase the activity of several antioxidant enzymes (Santaella and Pintus, 2021). The *Aloe vera* gel has been observed to have pharmacological activities such as anti-inflammatory, antifungal, antiviral, antidiabetic, antimicrobial, and anticancer. In mice spermatozoa, a high dose was associated with decreased count of red cells and sperm damage (Oyeyemi and Fayomi, 2011). Moreover, *Aloe vera* plant contains many vitamins including antioxidants, vitamins like A and C, vitamins B, such as thiamine, niacin, vitamin B2 (Riboflavin), and B12 as well as folic acid. Minerals such as sodium, potassium, calcium, magnesium, manganese, copper, chrome, and iron are also found in *Aloe vera* (Shahraki *et al*., 2014). Biological activities, including anti-diabetic, anti-viral, anti-bacterial, anti-cancer, antibiotic, hypotensive, laxative, protection against radiation, anti-inflammation, immunostimulant, and blood purifying properties are associated with polysaccharides in the *Aloe vera* gel (Fakhrildin and Sodani, 2014; Singh, 2020). Studies (Souza *et al*., 2016; Santella and Pintus, 2021) reported that *Aloe vera* can be used as an alternative cryoprotectant to egg yolk for the chilling and cryopreservation of peccary semen.

2.2 Semen preservation

Effective semen preservation is important for effective genetic improvement and selection programs (Wusiman *et al*., 2012). However, irrespective of the semen extender and storage conditions, depending closely on the preservation period, semen preservation negatively affects sperm quality by destroying the integrity and function of a whole series of sperm cellular structures (Gaczarzewicz *et al*., 2015; Santaella and Pintus, 2021). There exist two primary methods of semen preservation and these are chilling and cryopreservation. For chilling, semen is stored in

temperatures between 4-5 degrees Celsius for a maximum of 3 days, while cryopreservation involves storing semen at sub-zero temperatures in liquid nitrogen for years (Baiee *et al*., 2017)

2.2.1 Semen chilling

For the chilling technique, semen is stored in temperatures between 4-5°C for 3 days at maximum for the best results (Bustani and Baiee, 2021). The main advantage of the process of a chilling process is that it prevents damage associated with freezing, thereby ensuring greater sperm viability and allowing for reduced sperm concentrations per insemination dose and consistent use of genetically superior breeding animals (Patricia *et al*., 2015). Semen can be diluted and chilled as an alternative to freezing when insemination is performed within a short time after collection (Debbarma *et al*., 2019).

2.2.2 Semen cryopreservation

Cryopreservation is the freezing technique of spermatozoa used to keep cells and tissues in a vital state at −196°C in liquid nitrogen, however, cryopreservation still causes sperm damage in humans and various animals (Bustani and Baiee, 2021). Cryopreservation of spermatozoa is an invaluable technique employed to preserve spermatozoa of endangered superior animal species; however, it may result in mechanical damage to the plasma membrane and acrosomal membrane, oxidative damage to membrane phospholipids, DNA, and cause disturbances on spermatozoa metabolism (Taşdemir *et al*., 2013; Layek *et al*., 2016). Semen cryopreservation enables an ease and affordable dissemination of genetic material of elite animals through ART which may include AI, IVF, cloning, Intracytoplasmic Sperm Injection (ICSI), etc. However, cryopreservation may damage sperm cells due to induced alterations in sperm structures and functions that may ultimately lead to a generation of ROS that can react with spermatozoa lipids (Mostek *et al*., 2017).

2.3 Determination of semen quality

The quality of semen and its relationship to fertility are of major concern in animal production. As much as no technique for semen evaluation has been proven to predict the fertilizing capacity of a certain bull (Januskauskas *et al*., 2001). The quality of semen is generally affected by factors such as the nutrition and temperature conditions a bull is exposed to as well as the environmental conditions spermatozoa is exposed to after ejaculation during preservation processes (Dorji *et al*., 2014). Semen evaluation remains an important diagnostic tool that is used to assess bull fertility and it is essential for the effective utilization of cryopreserved semen in animal breeding. Generally, semen is evaluated on a basis of parameters such as Motility, Morphology, and Viability (Kumar *et al*., 2014; Takeda *et al*., 2015; Zubair *et al*., 2015).

2.3.1 Sperm motility

Sperm motility is normally considered as the percentage of sperm that are progressively motile. As such, spermatozoa can exhibit three types of motility parameters, which are grouped into two main categories: progressive (types a and b) and non-progressive motility (type c). Therefore, total motility (TM) includes progressive and non-progressive motility (PM and NPM) (Palomar and Molina, 2017). A progressively motile sperm swims forward in an essentially straight line, whereas a non-progressively motile sperm swims, but with an abnormal path, such as in tight circles, therefore, the acceptable progressive motility is greater than 30%, while total motility is greater than 60% in bulls (Guaus, 2016). Concerning sperm quality and ability to fertilize, sperm motility is one of the indicators that is mostly evaluated before and after sperm cryopreservation. Moreover, sperm motility is believed to be of paramount important parameter in a set of characteristics for sperm transport within the female reproductive tract and oocyte penetration (Muiño *et al*., 2008, Simonik *et al*., 2015), because if gives information about the spermatozoa's sources of energy(Taşdemir *et al*., 2013). A fraction of sperm motility in semen is measured either manually or with an aid of a Computer Assisted Semen Analyzer (CASA) (Binsila *et al*., 2017).

2.3.2 Sperm morphology

An important part of any breeding soundness exam is an evaluation of sperm morphology. In the most fundamental case, the size and shape of the head, midpiece, and tail are examined. Abnormalities can be classified as affecting the head, midpiece, or tail. The results of a sperm morphology exam are reported as percent normal. It is always the case that some sperm from an ejaculate are morphologically abnormal, but when that fraction becomes excessive, fertility may decrease. To obtain a standard measure of sperm morphology, the percentage of sperm with normal shape and size is determined. Nevertheless, it is generally accepted that approximately 50% of spermatozoa are damaged during cryopreservation (Watson, 2000). A bull that is healthy and sound should have a morphologically normal sperm greater than 70% and will be designated Satisfactory Potential Breeder (Guaus, 2016). Evaluation of sperm morphology is a major component of routine sperm quality assessment, and the type of sperm abnormalities correlates significantly with the fertility of the bull (Januskauskas *et al*., 2001). To maximize reproduction function in both natural services and assisted reproduction, bulls should produce a great number of morphologically normal fertile sperm (Kastelic, 2014).

2.3.3 Sperm viability

Generally, sperm evaluation processes concern more about the viability and functional status of the spermatozoa because it influences the fertilization of gametes. Nevertheless, for sperm to produce viable and healthy offspring, vital factors such as genetic material content and quality are essential (Binsila *et al*., 2017). Regardless of the initial quality of the bull semen, lower fertility is generally accepted because of cryopreservation of spermatozoa and many efforts are being developed in cryopreservation techniques to improve sperm viability (Barbas and Mascarenhas, 2009). The viability of spermatozoa is essential for motility and fertilizing ability, therefore, once sperm viability is reduced, their ability to induce fertilization will be decreased (Kumaresan *et al*.,

2017). According to Watson (2000), approximately 40–50% of spermatozoa do not survive cryopreservation regardless of the best cryopreservation protocol used. In this regards, viable spermatozoa are generally less post-thaw than of fresh semen.

2.4 The effect of *Aloe vera* gel-based extenders on sperm quality

Despite the dearth of information on the effect of *Aloe vera* plant on the quality of bull sperm (Olugbenga *et al*., 2011). Various studies (Souza *et al*., 2016; Yong *et al*., 2017; Moreira *et al*., 2021;) reported on the use of *Aloe vera* gel as the main ingredient of semen extender on the quality of spermatozoa from a wide range of species (Collared peccary, Spix's yellow-toothed cavy, Tilapia fish) except for another study by Singh *et al*. (2020) which reported on the use of *Aloe vera* gel as an additive to Tris-egg yolk extender and its effect on the quality of bull sperm. It was found that the *Aloe vera* gel added extenders had a progressive motility of 69.0%, morphological abnormal spermatozoa of 7.9%, and viability of 76.2% post-thaw, the increased semen quality in bull semen was attributed to the antioxidant abilities of the aloe vera gel. According to Yong *et al*. (2017), the higher concentration of *Aloe vera* gel in *Aloe vera*-based extender had the best sperm motility score Tilapia fish sperm compared to the lower concentrations, this was reported to be due to the presence of various polysaccharides which served as a source of energy for the sperm and thus improved their movement. However, the lower concentration of *Aloe vera* gel showed higher sperm viability. Furthermore, the study suggested that folic acid and zinc served as antioxidants and reduced apoptosis in sperm cells and hence improved Tilapia sperm quality. Souza *et al*. (2016) reported that *Aloe vera* can be effectively used as a substitute for Egg yolk in the formulation of Tris extenders for chilling or freezing of collared peccary semen. This was noted after the realization that the higher concentration of *Aloe vera* gel (20%) showed an effective cryoprotective effect on peccary semen, providing results similar to the extender containing Egg yolk. *Aloe vera* extender produced means (40.2%, 20.2%, 9.7%, 10.2%, and 59.7%) after 36 hours of storage at 5 ^{0}C and (46.4%, 6.4%,

30,4%, 9.6%, 53,3%) post-thaw for motility parameters (TM, RAP, MED, SLW, and STC) respectively. Nonetheless, the higher morphologically normal mean was observed from semen extended in a low (10%) *Aloe vera* concentration extender compared to the one containing a high (20%) concentration while the viable sperms were found to be about 23.3% from the extender containing 20% *Aloe vera*. The successful preservation of peccary semen by *Aloe vera*-based extender is associated with the active biological substances, including vitamins, minerals, enzymes, sugars, anthraquinones, lignin, saponins, sterols, amino acids, and salicylic acid in which the components such as polysaccharides (pectin, hemicellulose, glucomannan, glucose, and mannose) are abundant and may have served as a source of energy for the peccary sperm. Moreover, the study demonstrates that the best semen preservation is achieved when this extract is used from a 20% to 50% concentration, but if the concentration is increased above 50%, it will present significant detrimental effects on semen quality. On the other hand, the study conducted by Moreira *et al*. (2021) reported no significant difference between the two concentration levels (10% and 20%) of *Aloe vera* in semen extender on the quality of Spix's yellow-toothed cavy epididymal sperm post-thaw. The motility and subpopulations parameters had average means (24.7%, 7.9%, 19,3%,0.5%, and 75.1%) for motility parameters (TM, RAP, MED, SLW, and STC) respectively, while the average morphologically normal mean percentage was found to be 76,7% for both concentration levels. Spix's yellow-toothed cavy spermatozoa was better cryopreserved with extenders containing egg yolk than *Aloe vera* gel.

2.5 Summary of literature review

The use of plant extracts as semen extenders' additives is emerging as a substitute to egg yolk. This has far been due to a fact that egg yolk has been suspected to be harmful to spermatozoa quality and spread diseases due to its bacterial contamination. Despite the death of information on the use of *Aloe vera* as semen extenders for bull semen, *Aloe vera* has been implied as plant-based semen extender additives for preservation of spermatozoa other animal species such as

fish, rodents, and pigs. Nonetheless, the effect of *Aloe vera*-based extenders on the quality of spermatozoa varies with different species.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Description of the study area

The study was conducted in the Centre of Excellence in Animal Assisted Reproduction (CEAAR) Biotechnology Laboratory in the Faculty of Science, Engineering and Agriculture, Department of Animal Science, University of Venda, Thohoyandou under Thulamela Municipality located in Vhembe district, Limpopo Province. Thohoyandou is based about 70 km east of Louis Trichardt.

3.2 Management of experimental Animals

Four (4) Nguni bulls with known fertility records were used in this study, the bulls were kept at the University of Venda experimental farm under an intensive feeding system. Feed and fresh water were available to animals *ad libitum*.

3.3 Preparation of Extenders

3.3.1 Preparation of Egg yolk

A day before semen collection, freshly laid chicken eggs were collected from a layer house in the experimental farm at the University of Venda. Eggs were washed roughly with clean running water and dried with a paper towel. Seventy percent (70%) alcohol was then used to spray the eggs to disinfect the eggs against any possible contamination. Each egg was tapped with a spatula and egg yolk was obtained using a sterile egg divider. The egg yolk was transferred to a clean filter paper and rolled against the paper to remove the remaining egg white. The egg yolk was punctured by a sterile needle and aspirated into a 20 ml syringe and finally transferred into a sterile glass beaker.

3.3.2 Collection and Preparation of *Aloe vera* gel

The *Aloe vera* (AV) plant (*Aloe barbadensis miller*) was harvested at Sereni village, Mashamba area, Vhembe district, Limpopo Province. The AV leaves were cut at the bottom of the AV plant and transported to CEAAR biotechnology laboratory. All the equipment was sterilized prior to gel extraction and processing. The Aloe vera leaves were washed with running water and rinsed with distilled water before drying them with a paper towel. The colorless gel was extracted from the parenchyma of the leaves by a means of an incision in the middle of the leaf and the gel was squeezed with a spatula into a sterile glass beaker.

3.3.3 Preparation of Tris-Egg yolk and Tris-*Aloe vera* gel

Extenders were prepared according to the methods and procedures described by Naz *et al*. (2018) with modifications.

Table 3.1 Constituents of Tris-Egg yolk or Tris-*Aloe vera* gel extenders

Ingredient	**TEY**	**$TAG_{12\%}$**	**$TAG_{16\%}$**	**$TAG_{20\%}$**
Tris (g)	1.21	1.21	1.21	1.21
Citric acid (g)	0.67	O.67	0.67	0.67
Fructose (g)	O.50	0.50	0.50	0.50
Egg Yolk (% v/v)	20	------	-----	-----
Aloe vera gel (% v/v)	-----	12	16	20
Glycerol (% v/v)	07	07	07	07
Penicillin-Streptomycin (% v/v)	1	1	1	1
Ph	7	7	7	7
Total volume (ml)	50	50	50	50

TEY: Tris-egg yolk with 0% *Aloe vera* gel (Control)

$TAG_{12\%}$: Tris-Aloe vera gel with 12% *Aloe vera* gel

$TAG_{16\%}$: Tris-Aloe vera gel with 16% *Aloe vera* gel

$TAG_{20\%}$: Tris-Aloe vera gel with 20% *Aloe vera* gel

3.4 Semen collection

Bulls were moved to the crush pen for semen collection. A pulsator IV-Auto Adjust ™ electro ejaculator (EE) was set ready while the bull was being calmed and settled in a standing position within a crush pen. A scissor was used to cut the prepuce hair and thoroughly cleaned with water and then dried with a clean paper towel. A rectal probe of the EE was lubricated with paraffin oil and gently and slowly inserted into the rectum of the bull with the metal electrodes facing down. EE was then turned on and the voltage automatically increase until the bull's penis erected and ejaculated. Semen was transferred into a sterile 37 ^{0}C pre-warmed graduated test tube. The tube was then submerged into a thermo-flask containing water at 37 ^{0}C to maintain the temperature until the samples reached the laboratory.

3.5 Methods for Experiment 1

3.5.2 Experimental Design

Using four Nguni bulls (n= 4), the present study investigated the effect of four levels (0%, 12%, 16%, 20%) of extender and six levels (0 h, 24 h, 48 h, 72 h, 96 h, 120 h) of storage time on the quality of bull spermatozoa stored in a refrigerator at 5 ^{0}C. The experiment was arranged in a 4 x 6 factorial design.

3.5.3 Treatments

Before semen arrival in the laboratory, four (4) 15ml sterile graduated tubes containing prepared semen extenders were placed inside the water bath at 37 ^{0}C and labeled TEY, $TAG_{12\%}$, $TAG_{16\%}$, $TAG_{20\%}$. Tube TEY was containing Tris-egg yolk with 0% aloe vera gel (AVG) (control extender), and tubes ($TAG_{12\%}$, $TAG_{16\%}$, $TAG_{20\%}$ contained Tris-aloe vera gel extenders with different aloe vera gel concentrations, 12%, 16%, and 20% respectively.

Semen was collected using Electro ejaculator from four mature bulls and pooled together to eliminate individual differences. Equal amount of semen was then diluted in each of the four 37 ^{0}C pre-warmed extenders at a ratio of 1:5 v/v (semen to extender) and sperms were evaluated. Afterward, the sample labeled tubes were stored at 5 ^{0}C for 120 h and evaluated at 0 h, 24 h, 48 h, 72 h, 96 h, and 120 h. The collection and preservation of semen was replicated five times, once a week.

3.5.4 Microscopic sperm evaluations

3.5.4.1 Evaluation of Sperm motility

Spermatozoa motility parameters (Table 4.1) were analyzed using a computer-aided sperm analyzer (CASA) system (Sperm Class Analyzer® [SCA] 5.3, Microptic, Barcelona, Spain), motility program, Ph1 phase contrast 10X magnification. During analysis, a 10 µL drop of extended semen was placed on a 37 ^{0}C pre-warmed glass slide of the CASA microscope thermo-plate and covered with a cover slip for evaluation. For spermatozoa motility parameters analysis, five fields were captured, analyzed and percentages were calculated automatically by the software (Raseona, 2015).

3.5.4.2 Evaluation of Sperm morphology

Spermatozoa structural abnormalities were evaluated using a computer-aided sperm analysis (CASA) system. Spermatozoa morphology was evaluated according to modified methods and procedures described by Sokunbi *et al*. (2015) where a 10 µL drop of the sample was placed on a sterile 37 ^{0}C warm glass slide, followed by a 10 µL drops of Eosin 1% and Nigrosin 5% stains (Kyron prescription CC, 21 New Goch Road, Benrose 2094) respectively and gently mixed and smeared on a slide with the edge of another clean slide then air dried. Morphological parameters (absence tail, twisted tail, and twisted neck) were viewed under 60X magnification, phase contrast A in the morphology program of the CASA. Sperms were manually counted and the ones with morphological defects were recorded, and percentages calculated.

3.5.4.3 Evaluation of Sperm Viability

The viability of spermatozoa was evaluated using the methods and procedures described by Raseona (2015) with modification. Vitality program of the Computer Class Analyser (CSA) was used to assess live and dead spermatozoa. Eiosin 1% and Nigrosin 5% fixed stained as described in 3.5.4.2 were placed on the microscope heat stage at 37 ^{0}C and a drop of immersion oil was placed on the stained slide and covered with a cover slip and therefore observed under 60X magnification, phase contrast A. 200 spermatozoa were counted, and live cells identified as clear (white) while dead cells identified as dark (pinkish) as shown in figure 2. Results were recorded and the percentage was calculated.

3.5.5 Statistical analysis

The data was statistically analyzed using Minitab 19 statistical software. The results were subjected to the analysis of variance (ANOVA) in a 4 x 6 factorial design using the General Linear Model (GLM) procedures of Minitab statistical package version 19 (Minitab, 2019). Means of different treatments were compared using Tukey's post hoc test. Significance was set at $P<0.05$.

Model

$$Y_{ijk} = \mu + D_i + T_j + (DT) + \varepsilon_{ijk}$$

Where y_{ijk} will be the observation.

μ = overall mean

D_i = the effect of i^{th} extender.

T_i = the effect of j^{th} storage time

$(DT)_{ij}$ = the interaction between the i^{th} extender and j^{th} storage time

ε_{ijk} = random error

3.6 Methods for Experiment 2

3.6.2 Experimental design

Using four Nguni bulls (n=4), this study investigated the effect of cryopreservation on the quality of spermatozoa extended in Tris-aloe vera gel at different concentration levels (0%, 12%, 16%, 20%) of *Aloe vera* gel. The experiment was set at a 4 x 2 factorial design.

3.6.3 Treatments

Similarly, to semen chilling, the same amount of semen was diluted at a ratio of 1:5 v/v (semen to extender) in four sterile 37 ^{0}C pre-warmed graduated tubes labeled TEY and $TAG_{12\%,}$ $TAG_{16\%,}$ $TAG_{20\%}$. Diluted semen samples were loaded into 0.25 ml French semen straws and labeled TEY and $TAG_{12\%,}$ $TAG_{16\%,}$ and $TAG_{20\%}$ respectively for identification. Straws were then sealed with polyvinyl powder and equilibrated for 2 hours at 5 ^{0}C.

Post equilibration, Liquid nitrogen (LN_2) was poured into a styrofoam box enough to cover the surface area of the box and semen straws were placed on a holding rack 5 cm above the surface of the LN_2 for freezing. After 10 minutes, semen straws were removed from the holding rack and instantly submerged into the LN_2 at -196 ^{0}C in the canister and stored for 5 days. Extended semen samples were evaluated prior to cryopreservation and post-thaw. The collection and preservation of semen was replicated five times, once a week.

3.6.4 Microscopic sperm evaluations

3.6.4.1 Sperm motility evaluation

Evaluation of spermatozoa motility parameters (total motility, progressive and non-progressive motility, rapid motility, medium velocity, Slow velocity, fast progressive type A, and slow progressive type B and static) as shown in table 4.4 were analyzed as described in 3.5.4.1.

3.6.4.2 Sperm morphology evaluation

Morphological parameters (absence tail, twisted tail, and twisted neck) were evaluated using CASA as described in 3.5.4.2.

3.6.4.3 Sperm viability evaluation

Spermatozoa viability (Live/Dead) was observed using CASA following the procedures used by Raseona (2015) as described in 3.5.4.3.

3.6.5 Statistical analysis

The data was statistically analyzed using Minitab 19 statistical software. The results were subjected to the analysis of variance (ANOVA) in a 4 x 2 factorial design using the General Linear Model (GLM) procedures of Minitab statistical package version 19 (Minitab, 2019). Means of different treatments were compared using Tukey's post hoc test. Significance was set at $P<0.05$.

Model

$$Y_{ijk} = \mu + D_i + T_j + (DT) + \varepsilon_{ijk}$$

Where y_{ijk} will be the observation.

μ = overall mean

D_i = the effect of i^{th} extender.

T_i = the effect of j^{th} storage time

$(DT)_{ij}$ = the interaction between the i^{th} extender and j^{th} storage time

ε_{ijk} = random error

3.7 Ethical Considerations

The University Research Ethics Committee granted the current project ethical approval for the use of animals to collect semen, and evidence of approval is attached. The project number is SARDF/19/ANS/08/2011. The study was conducted using semen that has been collected from Nguni bulls at the experimental farm of the University of Venda.

CHAPTER 4

RESULTS

4.1 Experiment 1

4.1.1 Sperm motility

From experiment 1, table 4.1 presents the effects of extender and storage time, on the motility of spermatozoa of a bull semen chilled at 5^0C. Extenders had significant effect (P<0.01) on all parameters under spermatozoa motility. The semen extended using TEY had higher percentages for TM and PM which differed from the lower percentages observed using $TAG_{12\%}$, $TAG_{16\%}$, and $TAG_{20\%}$. TAGs had an average mean percentage of (70%) TM and (47.9%) PM. Semen extended using TEY had the lowest NPM percentage (12.0%) compared to the TAGs. Semen extended using $TAG_{12\%}$ and $TAG_{16\%}$ had a similar NPM percentage (23.0%) which is higher than the lower (20.2%) produced by $TAG_{20\%}$. Semen extended in TEY had a higher percentage for RAP which differed from lower percentages observed using TAGs. $TAG_{12\%}$ and $TAG_{16\%}$ had higher mean percentages (16.6% average) for MED which differed from the lower percentages (13.1% average) observed using TEY and $TAG_{20\%}$. TEY had the lower mean percentages of (0.9%) SLW and (9.6%) FPA which differed from the higher mean percentages (8.3%) SLW average and (22.3%) average observed from the other TAGs. Semen extended in TEY had a higher percentage for SPB which differed from lower percentages observed using TAGs. TEY had the lower mean percentage of (0.3%%) SLW which differed from the higher mean percentage (30%) average observed from the other TAGs.

Storage time had significant effects (P<0.01) on percentages of TM, PM, NPM, RAP, MED, SLW, FPA, SPB, and STC as shown in Table 4.1. The mean percentages for TM, PM, and RAP differed among all storage time and decreased when storage time increased. Whereas differences in mean percentages for NPM, MED, SLW, and FPA had no pattern. The present study reports

lower mean percentages for NPM, MED, SLW, and STC at 0 hour and the higher percentages observed at 72 h for NPM and MED. However, a higher mean percentage was observed for SLW at 72 and 96 hours. SPB has the higher mean percentages (80.6%) 0 hour which differed from the rest of the storage times. The lowest mean percentage (20.7%) for SPB was observed at 120 hours. Differences in mean percentages for STC differed significantly throughout the storage time and increased with increase in ST.

The interaction between extender and storage time had significant ($P<0.01$) effects on percentage TM, PM, NPM, RAP, MED, SLW, FPA, SPB, and STC (Table 4.1). TEY consistently showed a higher TM percentage throughout the storage time, which differed from the mean percentages observed from TAGs, except after 24 hours where it showed a similar percentage to $TAG_{20\%}$. There were significant differences in TM percentages among the TAGs after 24, 48, 72, and 96 hours. A higher percentage (97.0%) of TM was observed for $TAG_{20\%}$ which differed from the lower percentages observed for $TAG_{16\%}$ after 24 hours, and a higher percentage of 55.8% which differed from the lower percentage observed for $TAG_{12\%}$ after 96 hours. However, after 48 and 72 hours $TAG_{12\%}$ and $TAG_{16\%}$ had the average TM percentage (86.7% and 79.6%) respectively higher than the ones observed for $TAG_{20\%}$. TAGs had a similar TM mean percentage (22.1%) average after 120 hours. TEY extender had a higher percentage PM compared to lower percentages observed for TAGs, after 24, 48, 72, 96, and 120 hours. There were significant differences in mean percentage PM among the TAGs after 24 hours. A higher percentage of PM (75.6%) was observed for $TAG_{20\%}$ after 24 hours which was higher than the other TAGs. Nonetheless, $TAG_{16\%}$ yielded the higher PM (64.4%, 47.0%, 11.6%) respectively after 48, 72, and 120 hours compared to the other TAGs. TEY had the lowest NPM mean percentages after 24, 48, 72, and 96 hours which differed from the higher ones observed for TAGs. $TAG_{12\%}$ had the higher NPM mean percentage (26.8%) which differed from the one observed for $TAG_{16\%}$, but similar to the one for $TAG_{20\%}$ after 24 hours, however, $TAG_{16\%}$ showed a higher percentage after 48 hours. TAGs had

similar NPM mean percentages after 72, 96, and 120 hours. Extender TEY had higher RAP mean percentages which differed from the lower percentages observed for TAGs, after 24, 48, 72, 96, and 120 hours. There were significant differences in mean percentage RAP amongst the TAGs after 24, 48, and 72 hours. $TAG_{20\%}$ had higher RAP mean percentage (74.0%) compared to the ones observed for $TAG_{12\%}$ and $TAG_{16\%}$ respectively after 24 hours. After 48 hours, $TAG_{16\%}$ had a higher mean percentage (59.8%) which differed from the lower (45.4%) average observed from $TAG_{12\%}$ and $TAG_{20\%}$. TAGs depicted a similar mean percentage from 72, 96, and 96 hours. The lower MED mean percentages observed for TEY after 24 hours and differed from those of TAGs, inversely, TEY had a higher mean percentage after 120 hours. Nonetheless, TEY had a higher mean percentage which differed from TAGs after 72 and 120 hours. $TAG_{12\%}$ depicted a higher MED mean percentage which differed from $TAG_{16\%}$ and $TAG_{20\%}$ after 48 hours. Similarities in mean percentages for MED were observed for TAGs after 24, 72, 96, and 120 hours. Extender TEY had the lower SLW mean percentages after 48, 72, and 96 hours. However, TEY had a mean percentage similar to that depicted by $TAG_{20\%}$ after 24 hours, and a mean percentage similar to TAGs after 120 hours. TAGs had similar SLW mean percentages after 48, 72, 96, and 120 hours. TEY depicted lower FPA mean percentages which differed from those depicted by TAGs after 24, 48, and 72 hours, however, similarities in mean percentages amongst all the extenders were observed after 96 and 120 hours. $TAG_{16\%}$ depicted a lower FPA mean percentage (31.4%) after 24 hours, and a higher mean percentage (41.6%) after 48 hours compared to the other TAGs. Extender TEY depicted higher SPB mean percentages compared TAGs after 24, 48, 72, 96, and 120 hours. $TAG_{20\%}$ depicted a higher mean percentage (30.8%) which differed from the lower ones (18.3%) average observed from $TAG_{12\%}$ and $TAG_{16\%}$ after 24 hours. The similar SPB mean percentages were observed for TAGs after 48, 72, 96, and 120 hours. TEY depicted lower STC mean percentages which differed from the ones depicted by TAGs after 48, 72, 96, and 120 hours. There were significant differences in STC mean percentages among the TAGs after 24, 48, 72, and 96 hours. The mean percentage STC was higher for $TAG_{20\%}$ (23.4%) than

$TAG_{16\%}$ after 48 hours and higher (34.4%) than those for the other TAGs after 72 hours. However, $TAG_{16\%}$ had a higher STC percentage (18.2%) than $TAG_{20\%}$ after 24 hours and $TAG_{12\%}$ had a higher STC percentage (53.8%) than $TAG_{20\%}$ after 96 hours. TEY was superior to TAGs in all motility parameters across storage times, except after 24 hours where it was comparable to $TAG_{20\%}$ for TM percentage. The TAGs did not show consistent performance across storage times, but the best options could be $TAG_{20\%}$ after 24 and 96 hours, and $TAG_{16\%}$ after 48 and 72 hours.

Table 4.1: The effects of extender and storage time, on the motility of bull spermatozoa chilled at 5 ^{0}C for 120 hours.

Extender	ST (h)	TM%	PM%	NPM%	RAP%	MED%	SLW%	FPA%	SPB%	STC%
TEY		99.7^{a}	87.7^{a}	12.0^{c}	86.6^{a}	12.2^{b}	0.9^{b}	9.6^{b}	78.2^{a}	0.3^{b}
TAG$_{12\%}$		69.9^{b}	46.9^{b}	23.0^{a}	44.9^{b}	17.0^{a}	8.2^{a}	22.0^{a}	24.9^{b}	30.1^{a}
TAG$_{16\%}$		71.0^{b}	48.0^{b}	23.0^{a}	46.3^{b}	16.2^{a}	8.5^{a}	21.8^{a}	26.2^{b}	29.0^{a}
TAG$_{20\%}$		69.1^{b}	48.9^{b}	20.2^{b}	47.0^{b}	14.0^{b}	8.2^{a}	23.0^{a}	25.8^{b}	30.9^{a}
SEM		0.665	0.702	0.725	0.755	0.545	0.393	0.678	0.658	0.665
	0	100.0^{a}	99.1^{a}	1.0^{e}	99.0^{a}	1.0^{d}	0.0^{d}	18.6^{c}	80.6^{a}	-0.0^{f}
	24	91.9^{b}	70.3^{b}	21.7^{c}	68.7^{b}	17.1^{b}	6.6^{c}	31.5^{a}	38.8^{b}	8.1^{e}
	48	87.5^{c}	62.0^{c}	25.5^{b}	58.7^{c}	20.9^{a}	8.0^{bc}	27.9^{b}	32.9^{c}	12.5^{d}
	72	81.2^{d}	52.0^{d}	29.3^{a}	50.9^{d}	20.6^{a}	9.7^{ab}	20.3^{c}	32.9^{c}	18.9^{c}
	96	62.7^{e}	37.5^{e}	25.2^{bc}	34.9^{e}	17.8^{b}	10.0^{a}	10.5^{d}	27.0^{d}	37.3^{b}
	120	41.3^{f}	26.7^{f}	14.7^{d}	25.1^{f}	11.9^{c}	4.4^{d}	6.0^{e}	20.7^{e}	58.7^{a}
SEM		0.815	0.860	0.888	0.925	0.665	0.481	0.831	0.805	0.815
TEY	0	100.0^{a}	99.0^{a}	1.0^{ij}	98.8^{a}	1.2^{hi}	0.0^{g}	17.2^{efg}	81.8^{ab}	-0.0^{h}
TAG$_{12\%}$		100.0^{a}	99.0^{a}	1.0^{ij}	98.8^{a}	1.2^{hi}	0.0^{g}	21.0^{def}	78.0^{bc}	0.0^{h}
TAG$_{16\%}$		100.0^{a}	98.8^{ab}	1.2^{ij}	98.8^{a}	1.2^{hi}	-0.0^{g}	16.8^{efgh}	82.2^{ab}	-0.0^{h}
TAG$_{20\%}$		100.0^{a}	99.6^{a}	0.8^{ij}	99.6^{a}	0.4^{i}	-0.0^{g}	19.4^{def}	80.2^{abc}	-0.0^{h}
TEY	24	100.0^{a}	94.6^{ab}	5.4^{ij}	94.0^{ab}	6.0^{ghi}	0.0^{g}	7.0^{ij}	87.6^{a}	-0.0^{h}
TAG$_{12\%}$		88.8^{bc}	62.0^{e}	26.8^{bcde}	60.0^{f}	20.8^{abcd}	9.2^{bcde}	42.6^{a}	19.2^{fgh}	11.2^{fg}
TAG$_{16\%}$		81.8^{cd}	48.8^{fg}	33.0^{abc}	46.6^{g}	23.2^{abc}	12.0^{abc}	31.4^{bc}	17.4^{fgh}	18.2^{ef}
TAG$_{20\%}$		97.0^{ab}	75.6^{d}	24.4^{defg}	74.0^{e}	18.2^{cd}	5.0^{efg}	44.8^{a}	30.8^{e}	3.0^{gh}
TEY	48	100.0^{a}	89.8^{bc}	10.0^{hij}	84.0^{cd}	15.2^{def}	0.8^{fg}	7.2^{ij}	78.2^{bc}	-0.0^{h}
TAG$_{12\%}$		85.2^{cd}	49.0^{f}	36.2^{ab}	45.4^{g}	27.8^{a}	11.8^{abc}	30.6^{bc}	18.2^{fgh}	14.8^{ef}
TAG$_{16\%}$		88.2^{c}	61.4^{e}	26.8^{bcde}	59.8^{f}	20.2^{bcd}	8.2^{cde}	41.6^{a}	19.8^{fg}	11.8^{f}
TAG$_{20\%}$		76.6^{d}	47.6^{fg}	29.0^{abcd}	45.4^{g}	20.2^{bcd}	11.0^{abc}	32.0^{b}	15.4^{fgh}	23.4^{e}
TEY	72	99.8^{a}	85.4^{c}	14.6^{fghi}	88.6^{bc}	10.4^{efg}	0.6^{fg}	8.2^{hij}	81.8^{ab}	0.2^{h}
TAG$_{12\%}$		76.6^{d}	39.8^{gh}	36.8^{a}	37.4^{gh}	26.6^{ab}	12.6^{abc}	22.6^{cdef}	17.2^{fgh}	23.4^{e}
TAG$_{16\%}$		82.6^{cd}	47.0^{fg}	35.6^{ab}	45.0^{g}	25.6^{ab}	12.2^{abc}	26.2^{bcd}	20.8^{f}	17.4^{ef}
TAG$_{20\%}$		65.6^{e}	35.6^{hi}	30.0^{abcd}	32.4^{hi}	19.8^{bcd}	13.4^{ab}	24.0^{bcde}	11.6^{ghi}	34.4^{d}
TEY	96	99.4^{a}	81.6^{cd}	17.8^{efgh}	79.6^{cde}	18.0^{cd}	1.8^{fg}	8.6^{ghij}	73.2^{cd}	0.6^{h}
TAG$_{12\%}$		46.2^{g}	20.6^{jk}	25.6^{cde}	17.8^{jk}	17.8^{cd}	10.4^{abcd}	9.4^{ghij}	11.2^{hi}	53.8^{b}
TAG$_{16\%}$		49.2^{fg}	20.6^{jk}	28.6^{abcd}	17.8^{jk}	18.0^{cd}	13.4^{ab}	9.2^{ghij}	11.4^{ghi}	50.6^{bc}
TAG$_{20\%}$		55.8^{f}	27.0^{ij}	28.8^{abcd}	24.4^{ij}	17.2^{cde}	14.4^{a}	14.8^{fghi}	12.2^{ghi}	44.2^{c}
TEY	120	98.8^{a}	75.8^{d}	23.0^{def}	74.4^{de}	22.4^{abc}	2.0^{fg}	9.2^{ghij}	66.6^{d}	1.2^{h}
TAG$_{12\%}$		22.8^{h}	11.2^{l}	11.6^{hi}	9.8^{kl}	8.0^{gh}	5.0^{efg}	5.8^{j}	5.4^{i}	77.2^{a}
TAG$_{16\%}$		24.2^{h}	11.6^{kl}	12.6^{ghi}	10.0^{kl}	8.8^{fg}	5.4^{def}	5.8^{j}	5.8^{i}	75.8^{a}
TAG$_{20\%}$		19.4^{h}	8.0^{l}	11.4^{hi}	6.0^{l}	8.2^{fgh}	5.2^{ef}	3.2^{j}	4.8^{i}	80.6^{a}
SEM		1.630	1.720	1.781	1.850	1.330	0.962	1.660	1.610	1.630
Significance										
Extender		**	**	**	**	**	**	**	**	**
ST		**	**	**	**	**	**	**	**	**
Extender*ST		**	**	**	**	**	**	**	**	**

ST= storage time, TM= total motility, PM= progressive motility, NPN= non-progressive motility, RAP= rapid velocity, MED= medium velocity, SLW= slow velocity, FPA= fast progressive type a, SPB= slow progressive type b, STC= static, TEY= tris-egg yolk, TAG$_{12\%}$= tris plus 12% *aloe vera* gel, TAG$_{16\%}$= tris plus 16% *aloe vera* gel, TAG$_{20\%}$= tris plus 20% *aloe vera* gel, SEM= standard error means, **= significant (P<0.01), ^{abcdefghijkl} within a column, means with different superscripts are significantly different (P<0.05).

4.1.2 Sperm morphology

Table 4.2 presents the effects of extender and storage time, on the morphology of spermatozoa chilled at 5^0C. Semen extenders had a significant effect (P<0.01) on total abnormal, absence tail, and twisted tail. Tris-egg yolk depicted a lower total abnormal mean percentage (7.1%) which differed from the higher (9.3%) average observed from TAGs. Tris-egg yolk had a lower absence tail mean percentage (1.6%) which differed significantly from the (2.2%) average yielded by $TAG_{12\%}$ and $TAG_{16\%}$, however, it was similar to the mean percentage yielded by $TAG_{20\%}$. Tris-egg yolk depicted a lower twisted tail mean percentage (4.6%) which differed from the mean percentages (6.2%) average observed from $TAG_{16\%}$ and $TAG_{20\%}$. Extender had no significant effect (P.0.05) on the twisted neck.

Storage time had a significant effect (P<0.01) on total abnormal, absence tail, and twisted tail. A higher mean percentage (9.7%) at average for total abnormal was observed after 48, 72, 96, and 120 hours, which differed from the low (6.4%) average observed at 0 hour and after 24 hours. However, a higher absent tail mean percentage (3.6%) was observed after 120 hours which differed from the mean percentages observed after 24, 48, 72, and 96 hours. Nonetheless, the mean percentage (2.1%) observed after 72 hours was higher than the average (1/1%) observed at 0 hour and after 24 hours. A higher twisted tail mean percentage (6.8%) observed differed from the means observed (4.4%) average at 0 hour and after 24 hours, however, it was similar to those observed after 72, 96, and 120 hours. Storage time had no significant effect (P>0.5) on twisted neck.

The interaction between extender and storage time had significant (P<0.01) effects on the mean percentage for total abnormal, absence tail, and twisted tail (Table 4.2). There were no significant differences in total abnormal mean percentages between TET and TAGs after 24, 48, and 120 hours. However, TEY depicted a lower total abnormal mean percentage (5.4%) compared to the higher ones (11.5%) average after 72 hours. $TAG_{20\%}$ had a higher mean percentage (13.3%) for

total abnormal which differed from the ones (8.5%) average yielded by $TAG_{12\%}$ and $TAG_{16\%}$. There were no significant differences in absence tail mean percentages between TEY and TAGs after 24, 48, 96, and 120 hours. $TAG_{12\%}$ depicted a higher absence tail mean percentage (3.5%) which differed from the mean percentage (1.0%) observed for TEY after 72 hours. TEY depicted twisted tail mean percentages that are similar to those depicted by TAGs after 24, 72, and 120 hours. The twisted tail mean percentage (9.1%) observed was higher than the mean percentage (4.4%) depicted by TEY after 48 hours. TEY had a mean percentage (9.1%) higher than the one (4.7%) depicted by TEY after 96 hours. The interaction between the extender and storage time had no significant effect ($P>0.05$) on twisted neck.

Table 4.2: The effects of extender and storage time on morphology of bull spermatozoa chilled at 5 ^{0}C for 120 hours.

Extender	ST (h)	Total Abnormal%	Absent Tail%	Twisted tail%	Twisted Neck%
TEY		7.1^{b}	1.6^{ab}	4.6^{b}	0.9
$TAG_{12\%}$		8.7^{a}	2.2^{a}	5.5^{ab}	1.1
$TAG_{16\%}$		9.4^{a}	2.2^{a}	5.9^{a}	1.3
$TAG_{20\%}$		9.0^{a}	1.3^{b}	6.5^{a}	1.2
SEM		0.386	0.184	0.319	0.130
	0	6.0^{b}	1.2^{c}	3.8^{c}	1.0
	24	6.7^{b}	1.0^{c}	4.9^{bc}	0.8
	48	9.5^{a}	1.4^{bc}	6.8^{a}	1.3
	72	9.1^{a}	2.1^{b}	5.7^{ab}	1.3
	96	9.2^{a}	1.7^{bc}	6.3^{ab}	1.2
	120	10.8^{a}	3.6^{a}	6.0^{ab}	1.2
SEM		0.473	0.225	0.391	0.159
TEY	0	8.2^{bcdef}	2.1^{cde}	4.8^{bc}	1.3
$TAG_{12\%}$		4.3^{f}	0.7^{e}	3.3^{c}	0.3
$TAG_{16\%}$		5.6^{ef}	1.2^{cde}	3.1^{c}	1.3
$TAG_{20\%}$		5.9^{def}	0.7^{e}	4.2^{bc}	1.0
TEY	24	6.4^{cdef}	0.9^{de}	4.9^{bc}	0.6
$TAG_{12\%}$		6.3^{cdef}	0.9^{de}	4.6^{bc}	0.8
$TAG_{16\%}$		8.0^{bcdef}	1.2^{cde}	5.8^{abc}	1.0
$TAG_{20\%}$		6.0^{def}	0.9^{de}	4.1^{bc}	1.0
TEY	48	6.7^{cdef}	1.5^{cde}	4.4^{bc}	0.9
$TAG_{12\%}$		9.4^{abcde}	1.8^{cde}	5.7^{abc}	1.9
$TAG_{16\%}$		10.9^{abcd}	1.3^{cde}	8.2^{ab}	1.3
$TAG_{20\%}$		11.2^{abc}	1.1^{de}	9.1^{a}	1.0
TEY	72	5.4^{ef}	1.0^{de}	3.5^{c}	0.9
$TAG_{12\%}$		11.9^{ab}	3.5^{abc}	6.8^{abc}	1.6
$TAG_{16\%}$		11.1^{abc}	2.6^{bcde}	7.1^{abc}	1.4
$TAG_{20\%}$		7.9^{bcdef}	1.4^{cde}	5.4^{abc}	1.1
TEY	96	6.5^{cdef}	1.1^{de}	4.7^{bc}	0.9
$TAG_{12\%}$		8.1^{bcdef}	1.5^{cde}	5.8^{abc}	0.8
$TAG_{16\%}$		8.9^{abcdef}	1.6^{cde}	5.7^{abc}	1.6
$TAG_{20\%}$		13.3^{a}	2.6^{bcde}	9.1^{a}	1.6
TEY	120	9.1^{abcdef}	3.1^{abcd}	5.3^{abc}	0.7
$TAG_{12\%}$		12.1^{ab}	4.7^{ab}	6.5^{abc}	0.9
$TAG_{16\%}$		12.0^{ab}	5.3^{a}	5.3^{abc}	1.4
$TAG_{20\%}$		10.0^{abcde}	1.3^{cde}	7.0^{abc}	1.7
SEM		0.946	0.450	0.781	0.318
Significance					
Extender		**	**	**	Ns
ST		**	**	**	Ns
Extender*ST		**	**	**	Ns

ST= storage time, TEY= tris-egg yolk, $TAG_{12\%}$= tris plus 12% *aloe vera* gel, $TAG_{16\%}$= tris plus 16% *aloe vera* gel, $TAG_{20\%}$= tris plus 20% *Aloe vera* gel, SEM= standard error means, **= significant (P<0.01), ns= not significant, ^{abcdef} within a column, means with different superscripts are significantly different (P<0.05).

4.3. Sperm viability

Table 4.3 presents the effects of extender and storage time, on the viability of spermatozoa chilled at 5^0C. Extenders had significant effect (P<0.01) on the percentage of live and dead spermatozoa. We observed a high mean percentage (83.0%) for live spermatozoa for TEY which differed significantly from low percentages (43.9% average) yielded by TAGs.

Storage time had a significant effect (P<0.01) on the mean percentage of live and dead sperm cells. Mean percentages for live sperm cells decreased when time increased. A high mean percentage was observed at 0 h while a lower mean percentage was observed after 120 hours. Live sperm cells had a similar mean percentage (36.2%) average after 72 and 96 hours.

The interaction between extender and storage time had a significant effect (P<0.01) on the mean percentage of live sperm cells and dead sperm cells. TEY depicted a higher mean percentage for live sperm cells compared to those depicted by TAGs after 24, 48, 72, 96, and 10 hours. There were no significant differences in live sperm cells' mean percentage depicted by TAGs when the time changed. TAGs had the average mean percentage of (67.1%) after 24 hours, (42.9%) after 48 hours, (23.0%) after 72 hours, (19.9%) after 96 hours, and (13.7%) after 120 hours. There was a decrease in bull sperm viability when storage time increased.

Table 4.3: The effects of extender and storage time on viability of bull spermatozoa chilled at 5 ^{0}C for 120 hours.

Extender	ST (h)	Live sperm cells%	Dead sperm cells Tail%
TEY		83.0^{a}	17.0^{b}
$TAG_{12\%}$		42.3^{b}	58.1^{a}
$TAG_{16\%}$		44.2^{b}	55.8^{a}
$TAG_{20\%}$		45.3^{b}	54.7^{a}
SEM		1.190	1.200
	0	96.3^{a}	3.7^{e}
	24	75.3^{b}	25.1^{d}
	48	54.6^{c}	45.4^{c}
	72	38.1^{d}	61.8^{b}
	96	34.1^{d}	65.9^{b}
	120	23.8^{e}	76.2^{a}
SEM		1.460	1.470
TEY	0	95.0^{ab}	5.0^{hi}
$TAG_{12\%}$		94.5^{ab}	5.5^{hi}
$TAG_{16\%}$		99.5^{a}	0.5^{i}
$TAG_{20\%}$		96.3^{ab}	3.7^{hi}
TEY	24	99.8^{a}	0.2^{i}
$TAG_{12\%}$		70.6^{de}	31.4^{def}
$TAG_{16\%}$		64.6^{ef}	35.4^{de}
$TAG_{20\%}$		66.2^{ef}	33.8^{de}
TEY	48	89.5^{abc}	10.5^{ghi}
$TAG_{12\%}$		40.4^{gh}	59.6^{bc}
$TAG_{16\%}$		42.2^{g}	57.8^{c}
$TAG_{20\%}$		46.2^{g}	53.8^{c}
TEY	72	83.5^{bcd}	16.5^{fgh}
$TAG_{12\%}$		19.8^{i}	80.2^{a}
$TAG_{16\%}$		24.0^{i}	75.8^{a}
$TAG_{20\%}$		25.2^{hi}	74.8^{ab}
TEY	96	76.5^{cde}	23.5^{efg}
$TAG_{12\%}$		15.6^{i}	84.4^{a}
$TAG_{16\%}$		20.9^{i}	79.1^{a}
$TAG_{20\%}$		23.3^{i}	76.7^{a}
TEY	120	53.9^{fg}	46.1^{cd}
$TAG_{12\%}$		12.6^{i}	87.4^{a}
$TAG_{16\%}$		13.9^{i}	86.1^{a}
$TAG_{20\%}$		14.7^{i}	85.3^{a}
SEM		2.920	2.940
Significance			
Extender		**	**
ST		**	**
Extender*ST		**	**

ST= storage time, TEY= tris-egg yolk, $TAG_{12\%}$= tris plus 12% *aloe vera* gel, $TAG_{16\%}$= tris plus 16% *aloe vera* gel, $TAG_{20\%}$= tris plus 20% *aloe vera* gel, SEM= standard error means, **= significant (P<0.01), ^{abcdefghi} within a column, means with different superscripts are significantly different (P<0.05).

4.2 Experiment 2

4.2.1 Sperm motility

Table 4.4 presents the effects of extender and freezing on the motility of spermatozoa of bull semen cryopreserved at -196 ^{0}C for 5 days. Semen extender had significant effects (P<0.01) on TM, PM, NPM, RAP, MED, SLW, FPA, SPB, and STC. TEY had the higher mean percentage (76.6%) for TM which differed from that of $TAG_{12\%}$. An average TM mean percentage (77.2%) was observed for $TAG_{16\%}$ and $TAG_{20\%.}$ TEY had a lower PM mean percentage (62.2%) which differed from the higher mean percentage (69.8%) depicted by TAGs. A higher NPM mean percentage (14.3%) was observed for TEY which differed from the lower mean percentage (5.9%) average for TAGs. TEY had a lower RAP mean percentage (59.2%) which differed from the higher mean percentage (68.6%) average for TAGs. TEY had a higher MED mean percentage (10.5%) which differed from the lower mean percentage (4.3%) average for TAGs. TEY had a higher SLW mean percentage (7.9%) which differed from the lower (2.5%) average for TAGs. A lower FPA mean percentage was observed for TEY which was similar to that of $TAG_{12\%}$ on average (7.9%). $TAG_{16\%}$ and $TAG_{20\%}$ depicted a higher average mean percentage (13,1%). TEY had a lower SPB mean percentage (54.3%) similar to that (54.1%) of $TAG_{16\%}$. $TAG_{12\%}$ and $TAG_{20\%}$ had an average mean percentage (60.1%) for SPB. TEY had a lower mean percentage (23.5%) for STC which differed from the higher mean percentage (28.0%) observed for $TAG_{12\%}$. $TAG_{16\%}$ and $TAG_{20\%}$ had an average mean percentage of 22.3% (Table 4.4).

Freezing had significant effects (P<0.05) on all spermatozoa motility parameters (TM, PM, NPM, RAP, MED, SLW, FPA, SPB, and STC) as shown in Table 4.4. The higher mean percentages (100% TM, 100% PM 100, 100% RAP 12.0% FPA and 88.0% SPB) were observed for FE which differed significantly from low mean percentages (50.6% TM, 34.6% PM, 31.8% RAP, 8.3% FPA, and 25.8% SPB) observed for FT. Conversely, NPM, MED, SLW, and STC had low percentages when semen was fresh and high post-thaw.

Table 4.4 depicts that the interaction between the extender and freezing had a significant effect (P<0.01) on TM, PM, NPM, RAP, MED, SLW, FPA, SPB, and STC. An average percentage of (100%) TM was observed for all extenders when semen was fresh. TEY had a TM higher mean percentage which differed from the lower mean percentage (45.0%) depicted by $TAG_{12\%}$ post-thaw. However, $TAG_{16\%}$ and $TAG_{20\%}$ depicted an average TM mean percentage of 54.4% for FT. We observed a PM average mean percentage of 100% for all the extenders for FE. TEY had the lower mean percentage (24.4%) for PM which differed from the higher mean percentage (39.5%) yielded by TAGs post-thaw. There were no significant differences in NPM mean percentages depicted by extenders for FE, however, a higher mean percentage (28.6%) which differed significantly from the mean percentages yielded by TAGs was observed. An average NPM mean percentage (14.1%) was observed for $TAG_{16\%}$ and $TAG_{20\%}$ compared to the lower mean percentage (7.0%) for $TAG_{12\%}$. There were no significant differences in RAP mean percentage (100% average) for all extenders for FE. A lower RAP mean percentage was observed for TEY which differed from the higher mean percentage (37.2%) average observed for TAGs post-thaw. There were no significant differences between MED mean percentages in all the extenders for FE. A higher MED mean percentage (21.0%) observed for TEY was significantly different from the means depicted by TAGs. $TAG_{16\%}$ and $TAG_{20\%}$ had a MED higher mean percentage (10.4%) average compared to the lower mean percentage (5.0%) depicted by $TAG_{12\%}$. There were no significant differences between SLW mean percentages in all extenders for FE. A higher SLW mean percentage (15.8%) observed for TEY was significantly different from the mean percentages depicted by TAGs. There were differences in SLW mean percentages depicted by TAGs post-thaw. We observed a higher mean percentage (7.0%) for $TAG_{16\%}$ which differed significantly from the lower mean percentage (3.0%) for $TAG_{12\%}$. TEY had a lower FPA mean percentage (10.2%) which differed from the higher mean percentage (16.6%) observed for $TAG_{16\%}$. A lower FPA average mean percentage (10.6%) was observed for $TAG_{12\%}$ and $TAG_{20\%}$

for FE. TEY had a lower FPA mean percentage (5.4%) which differed from the average higher mean percentage (12.6%) for $TAG_{16\%}$ and $TAG_{20\%}$ post-thaw.

Table 4.4: The effects of extender and freezing on the motility of bull spermatozoa cryopreserved at -196 ^{0}C for 5 days.

Extender	Freezing	TM%	PM%	NPM%	RAP%	MED%	SLW%	FPA%	SPB%	STC%
TEY		76.5^{a}	62.2^{b}	14.3^{a}	59.2^{b}	10.5^{a}	7.9^{a}	7.8^{c}	54.3^{b}	23.5^{b}
$TAG_{12\%}$		72.5^{b}	69.0^{a}	3.5^{c}	68.0^{a}	2.5^{b}	1.5^{c}	7.9^{c}	60.6^{a}	28.0^{a}
$TAG_{16\%}$		76.6^{a}	68.8^{a}	7.8^{b}	67.4^{a}	5.3^{b}	3.5^{b}	14.6^{a}	54.1^{b}	23.6^{b}
$TAG_{20\%}$		77.8^{a}	71.5^{a}	6.3^{bc}	70.3^{a}	5.2^{b}	2.5^{bc}	11.5^{b}	59.6^{a}	22.2^{b}
SEM		0.908	0.923	0.741	1.080	0.757	0.443	0.790	0.599	0.884
	FE	100.0^{a}	100.0^{a}	0.0^{b}	100.0^{a}	0.1^{b}	0.0^{b}	12.0^{a}	88.0^{a}	0.0^{b}
	FT	51.7^{b}	35.8^{b}	16.0^{a}	32.5^{b}	11.7^{a}	7.7^{a}	8.9^{b}	26.3^{b}	48.7^{a}
SEM		0.642	0.652	0.524	0.762	0.535	0.313	0.558	0.424	0.625
TEY	FE	100^{a}	100^{a}	0.0^{d}	100^{a}	-0.0^{d}	-0.0^{d}	10.2^{bc}	89.8^{a}	0.0^{c}
$TAG_{12\%}$		100^{a}	100^{a}	0.0^{d}	100^{a}	0.0^{d}	0.0^{d}	10.8^{b}	89.2^{a}	-0.0^{c}
$TAG_{16\%}$		100^{a}	100^{a}	-0.0^{d}	99.8^{a}	0.2^{cd}	0.0^{d}	16.6^{a}	83.4^{b}	0.0^{c}
$TAG_{20\%}$		100^{a}	100^{a}	0.0^{d}	100^{a}	0.0^{d}	0.0^{d}	10.4^{bc}	89.6^{a}	0.0^{c}
TEY	FT	53.0^{b}	24.4^{c}.	28.6^{a}	18.4^{c}	21.0^{a}	15.8^{a}	5.4^{cd}	18.8^{e}	47.0^{b}
$TAG_{12\%}$		45.0^{c}	38.0^{b}	7.0^{c}	36.0^{b}	5.0^{c}	3.0^{c}	5.0^{d}	32.0^{c}	56.0^{a}
$TAG_{16\%}$		53.2^{b}	37.6^{b}	15.6^{b}	35.0^{b}	10.4^{b}	7.0^{b}	12.6^{ab}	24.8^{d}	47.2^{b}
$TAG_{20\%}$		55.6^{b}	43.0^{b}	12.6^{b}	40.6^{b}	10.4^{b}	5.0^{bc}	12.6^{ab}	29.6^{c}	44.4^{b}
SEM		1.280	1.300	1.050	1.520	1.070	0.626	0.120	0.847	0.250
Significance										
Extender		**	**	**	**	**	**	**	**	**
Freezing		**	**	**	**	**	**	**	**	**
Extender*Freezing		**	**	**	**	**	**	**	**	**

FE= freshly extended semen, FT= frozen-thawed semen, TM= total motility, PM= progressive motility, NPN= non-progressive motility, RAP= rapid velocity, MED= medium velocity, SLW= slow velocity, FPA= fast progressive type a, SPB= slow progressive type b, STC= static, TEY= tris-egg yolk, $TAG_{12\%}$= tris plus 12% *aloe vera* gel, $TAG_{16\%}$= tris plus 16% *Aloe vera* gel, $TAG_{20\%}$= tris plus 20% *Aloe vera* gel, SEM= standard error means, **= significant (P<0.01), ^{abcde} within a column, means with different superscripts are significantly different (P<0.05).

4.5. Sperm morphology

Table 4.5 presents the effects of extender and the effect of freezing on spermatozoa morphology of bull semen cryopreserved at -196 ^{0}C for 5 days. Extender had a significant effect (P<0.05) on mean percentages for total abnormal, absence tail, and twisted tail. Tris-egg yolk had a mean percentage (9.8%) which was similar to the mean percentages depicted by TAGs. Nonetheless, $TAG_{12\%}$ had a higher mean percentage (10.3%) which significantly differed from the low mean percentage (6.9%) depicted by $TAG_{16\%}$ for total abnormal. TEY had a mean percentage (1.7%)

for absence tail which was similar to TAGs, however, $TAG_{12\%}$ had a higher mean percentage (2.1%) which significantly differed from the mean percentage (1.1%) depicted by $TAG_{20\%}$ for absence tail. A higher twisted tail mean percentage (7.9%) observed for TEY differed from the lower mean percentage (4,3%) depicted by $TAG_{16\%}$. There were no significant differences in mean percentages (5.4%) average for TAGs. Extender had no significant effect ($P>0.05$) on twisted neck.

Freezing had a significant effect ($P<0.01$) on total abnormal, absent tail, and twisted neck. A higher mean percentage (10.3%, 2.1%, and 1.9%) respectively observed for total abnormal, absent tail, and twisted neck FT significantly differed from the lower mean percentages (7.3%, 1.0%, and 1.0%) respectively observed for FE. Freezing had no significant effect ($P>0.05$) on twisted neck on both FE and FT.

There were no significant effects ($P>0.05$) observed for interaction between extender and freezing on total abnormal, absence tail, and twisted tail. The average mean percentages observed for morphology parameters were (8.8%) total abnormal, (1.6%) absence tail, (5.8%) twisted tail. The interaction between the extender and freezing had significant effects ($P<0.05$) on the mean percentage for the twisted neck. TEY had a twisted neck mean percentage (1.2%) similar to the mean percentage (1.0%) average observed for TAGs for FE. TEY had a lower twisted neck mean percentage (0.8%) which differed from the higher mean percentage (2.6% and 2.5%) for $TAG_{12\%}$ and $TAG_{20\%}$ respectively post-thaw.

Table 4.5: The effects of extender and freezing on the morphology of bull spermatozoa cryopreserved at -196 ^{0}C for 5 days.

Extender	Freezing	Total abnormal%	Absence tail%	Twisted tail%	Twisted neck%
TEY		9.8^{ab}	1.7^{ab}	7.1^{a}	1.0
$TAG_{12\%}$		10.3^{a}	2.1^{a}	6.4^{ab}	1.8
$TAG_{16\%}$		6.9^{b}	1.2^{ab}	4.3^{b}	1.4
$TAG_{20\%}$		8.1^{ab}	1.1^{b}	5.4^{ab}	1.7
SEM		0.807	0.254	0.691	0.244
	FE	7.3^{b}	1.0^{b}	5.3	1.0^{b}
	FT	10.3^{a}	2.1^{a}	6.4	1.9^{a}
SEM		0.571	0.180	0.489	0.173
TEY	FE	10.0	0.8	8.0	1.2^{ab}
$TAG_{12\%}$		8.8	1.7	6.0	1.1^{ab}
$TAG_{16\%}$		5.3	0.8	3.5	1.0^{ab}
$TAG_{20\%}$		5.2	0.8	3.6	0.8^{b}
TEY	FT	9.7	2.7	6.2	0.8^{b}
$TAG_{12\%}$		11.9	2.6	6.8	2.6^{a}
$TAG_{16\%}$		8.5	1.6	5.2	1.7^{ab}
$TAG_{20\%}$		11.1	1.4	7.2	2.5^{a}
SEM		1.140	0.360	0.977	0.345
Significance					
Extender		*	*	*	ns
Freezing		**	**	ns	**
Extender*Freezing		ns	Ns	ns	*

FE= freshly extended semen, FT= frozen-thawed semen, TEY= tris-egg yolk, $TAG_{12\%}$= tris plus 12% *aloe vera* gel, $TAG_{16\%}$= tris plus 16% *Aloe vera* gel, $TAG_{20\%}$= tris plus 20% *Aloe vera* gel, SEM= standard error means, **= significant (P<0.01), *= significant (P<0.05), ns= not significant (P>0.05), ^{ab}within a column, means with different superscripts are significantly different (P<0.05).

4.6. Sperm viability

Table 4.6 presents the effects of extender and the effect of freezing on spermatozoa viability of bull semen stored at -196 ^{0}C. Semen extenders had no significant effect (P>0.05) on both live and dead spermatozoa and the average mean percentage observed for live spermatozoa was 55.5%. Freezing had significant effect (P<0.01) of freezing on the viability of spermatozoa. Live sperm cells depicted a high mean percentage (99.6%) when semen was freshly extended before freezing, which differed significantly from the lower mean percentage (24.8%) observed post-thaw. Inversely, a low mean percentage (0.4%) observed for dead sperm cells was significantly different from the high percentage (75.2%) post-thaw. There was no significant difference (P>0.05) in the mean percentages for the interaction between extender and freezing under both

live and dead spermatozoa. The average mean percentage observed for live sperm cells was 99.6% for FE and 24.8% for FT.

Table 4.6: The effects of extender and freezing on the viability of bull spermatozoa cryopreserved at –196 ^{0}C for 5 days.

Extender	Freezing	Live sperm cells%	Dead sperm cells%
TEY		36.9	36.1
$TAG_{12\%}$		60.0	40.1
$TAG_{16\%}$		62.3	37.7
$TAG_{20\%}$		62.6	37.4
SEM		1.520	1.520
	FE	99.6^{a}	0.4^{b}
	FT	24.8^{b}	75.2^{a}
SEM		1.080	1.080
TEY	FE	100.0	-0.0
$TAG_{12\%}$		99.1	0.9
$TAG_{16\%}$		99.3	0.7
$TAG_{20\%}$		99.8	0.2
TEY	FT	27.8	72.2
$TAG_{12\%}$		20.8	79.2
$TAG_{16\%}$		25.2	74.8
$TAG_{20\%}$		25.4	74.6
SEM		2.150	2.150
Significance			
Extender		Ns	ns
Freezing		**	**
Extender*Freezing		Ns	ns

FE= freshly extended semen, FT= frozen-thawed semen, TEY= tris-egg yolk, $TAG_{12\%}$= tris plus 12% *aloe vera* gel, $TAG_{16\%}$= tris plus 16% *Aloe vera* gel, $TAG_{20\%}$= tris plus 20% *Aloe vera* gel, SEM= standard error means, **= significant (P<0.01), ns= not significant (P>0.05), ^{ab} within a column, means with different superscripts are significantly different (P<0.05).

CHAPTER 5 DISCUSSION

5.1. Experiment 1

5.1.1. The effect Tris-*Aloe vera* gel on the quality of bull semen after 120 h storage at 5 ^{0}C.

The present study evaluated the effect of different concentration levels of aloe vera gel (12%, 16%, and 20%) in Tris-based extenders named $TAG_{12\%}$, $TAG_{16\%}$, and $TAG_{20\%}$ respectively on Nguni bull semen stored at 5 ^{0}C for 120 hours. Tris-egg yolk extender (TEY) with 0% *Aloe vera* gel was used as a control. The present study revealed a significant difference ($P<0.01$) in the TM, and PM on semen extended with TEY and TAGs observed from 24h throughout the 120h of chilling. The highest motility means were observed with TEY throughout the storage time which could be attributed to protective agents' low-density lipoprotein (LDL) present in egg yolk (Rauch, 2013). However, the present study showed that the Tris-*Aloe vera* gel extenders can successfully be used as Nguni bull semen extenders when chilled for 72 hours. This is derived from its ability to keep the total and progressive motility rates greater than 60% and 30% respectively at average as declared acceptable (Guaus, 2016). Nonetheless, the present study depicts a non-significant (P.0.05) in the mean percentages of NPM, MED, SLW, and FPA produced by the three levels of *Aloe vera* gel after 72 hours. Furthermore, bull semen extended in Tris-*Aloe vera* gel spermatozoa motility decreased with an increase in storage time at 5 ^{0}C (Raseona *et al*., 2017).

Morphological characteristics showed consistency amongst all three concentration levels of AVG throughout the chilling period. Moreover, the mean percentages of the total abnormalities in bull spermatozoa (table 4.2) were found to be less than 15%, and ultimately denote greater than 85% morphologically normal spermatozoa, thereof, the present study suggests that TAG extended and chilled for 120 h semen can be used effectively and possibly reach the highest fertility. This is because the highest possible fertility can be reached when morphologically normal spermatozoa

is 70% (Rauch, 2013 and Guaus, 2016). These results agree with those reported by Souza *et al*. (2016) who found that collared peccary semen extended in TEY, or TAGs had a non-significant different in sperm morphological characteristics. As such, the effective cryoprotective effect of *Aloe vera* gel on bull spermatozoa could be attributed to biological components such as polyphenols, indoles, and alkaloids as they have been reported to preventors of sperm oxidative damage (Souza *et al*., 2016). The present study suggest that Nguni bull semen extended in Tris-Aloe vera gel losses morphological qualities when storage tie time increases and hence could results in bull fertility impairment.

Semen extended in TEY showed consistency in maintaining high spermatozoa viability throughout the chilling period at 5 ^{0}C. However, there was no significant difference (P>0.05) amongst the viability mean values revealed by TAGs. It was found that semen samples extended in Tris-aloe vera gel extender (TAGs 12%, 16%, and 20%) had a greater than 50% viability after 24 h storage, which makes *Aloe vera* gel a potential tris-base extender additive because Watson (2000) reported that a good spermatozoa viability should be about 50% and greater after preservation. In contrast, Yong *et al*. (2017) reported that only the lower concentration level of *Aloe vera* gel had higher sperm viability and further alluded that the folic acid and zinc served as antioxidants and reduced apoptosis in sperm cells and hence improved Tilapia sperm quality.

5.2. Experiment 2

5.2.1. The effect of Tris-*aloe vera* gel on the quality of bull semen after cryopreservation for 5 days.

The experiment two of the current study revealed a significant (P<0.01) difference in all the motility parameters post-thaw. Although the TM motility was below the acceptable percentage (60%). The performance of TEY and TAGs 16%, and 20% showed no significant difference (P>0.05) on the motility of bull spermatozoa. Despite the effect of an extremely low temperature of -196 ^{0}C in

Liquid nitrogen, TAGs outperformed TEY in improving PM and RAP post-thaw. These results contradict the findings of Moreira *et al*. (2021) which indicated that most spermatic parameters of Spix's yellow-toothed cavy sperm were better cryopreserved with extenders containing egg yolk than *Aloe vera* gel. In spite of the Nonetheless, these findings suggest that *Aloe vera* gel can be used to substitute egg yolk in Tris-based extender and cryopreserve Nguni bull semen for 5 days. This is due to the minimized oxidative stress caused by *aloe vera* gel (Singh *et al*., 2020).

As it was alluded by Indriastuti *et al*. (2020) that abnormalities in spermatozoa might occur due to disturbances in the epididymis during maturation processes. The present study revealed that Nguni bull semen extended in TEY, or TAGs had no significant difference (P>0.05) in the spermatozoa abnormalities characteristics post-thaw. The present study found that morphological normal spermatozoa were above 85% post-thaw. This may be because bull spermatozoa are morphologically compact, and they are less susceptible to injury from mechanical manipulations during cryopreservation (Long, 2006). Signh *et al*. (2020) reported normal abnormalities of (85.8%) post-thaw similar to the present study's when cross-bred bull semen was extended using *aloe vera* as semen additive in Tris-egg yolk-based extender. Moreover, these results are in accordance with the results found in the study of Morrell *et al*. (2018) who reported normal morphology for beef and dairy bulls of 76 % and 87 % respectively post-thaw.

The present study revealed that there was no significant difference (P>0.05) in viability mean values between TEY and TAGs post-thaw. As result, this implies that *Aloe vera* gel can successfully substitute egg yolk in Tris-base extenders to cryopreserve Nguni bull semen. The ability of TAGs to keep the spermatozoa viable could be attributed to the presence of antioxidants, vitamins like A and C, and vitamins B, such as thiamine, niacin, vitamin B2 (Riboflavin), and B12 as well as folic acid and zinc in the *aloe vera* gel (Shahraki *et al*., 2014).

In general, the successful preservation of Nguni bull semen by Tris-*Aloe vera* gel extender is associated with the active biological substances, including antioxidants, Anti-inflammatory,

Antimicrobial, which includes vitamins and minerals, sugars, enzymes, anthraquinones, lignin, saponins, sterols, amino acids, and salicylic acid in which the components such as polysaccharides (pectin, hemicellulose, glucomannan, glucose, and mannose) are abundant and may have served as a source of energy for the bull semen.

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

Based on the findings from experiment one and two of the present study, it can be concluded that 12%, 16%, and 20% of *Aloe vera* gel can successfully be used as an egg yolk substitute in Tris-base extender to preserve Nguni bull semen at 5 ^{0}C for 72 h and cryopreserve for 120 h. The ability of *Aloe vera* gel to improve the quality of bull spermatozoa post-thaw could be attributed to biological components such as antioxidants, anti-inflammatory, antifungal, antiviral, antidiabetic, antimicrobial, and anticancer due to their observed potential in fighting against apoptosis and acting as reactive oxidative species scavengers which prevented the effects of oxidative stress on the spermatozoa function. *Aloe vera* gel-containing extenders showed no significant effect on the morphology of bull spermatozoa after the storage of extended semen at 5 ^{0}C or post-thaw. Nonetheless, the recommendations are that research be done on the use of *Aloe vera* gel with inclusion of low-density lipoproteins (LDL) for both chilling and freezing of bull semen to evaluate the effect of these LDL as cryoprotectants as they are absent in *Aloe vera* gel.

REFERENCES

Aires, V.A., Hinsch, K.D., Mueller-Schloesser, F., Bogner, K., Mueller-Schloesser, S. and Hinsch, E., 2003. In vitro and in vivo comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of bovine semen. *Theriogenology*, *60*(2), pp.269-279.

Anzar, M., Rajapaksha, K. and Boswall, L., 2019. Egg yolk-free cryopreservation of bull semen. *PLoS One*, *14*(10), p.e0223977.

Barbas, J.P. and Mascarenhas, R.D., 2009. Cryopreservation of domestic animal sperm cells. *Cell and Tissue Banking*, *10*, pp.49-62.

Binsila, B.K., Selvaraju, S., Somashekar, L., Archana, S.S., Arangasamy, A., Ravindra, J.P. and Bhatta, R., 2017. Molecular advances in semen quality assessment and improving fertility in bulls—a review. *Indian Journal of Animal Reproduction*, *39*, pp.1-10.

Chaudhari, D.V., Dhami, A.J., Hadiya, K.K. and Patel, J.A., 2015. Relative efficacy of egg yolk and soya milk-based extenders for cryopreservation (− 196 C) of buffalo semen. *Veterinary World*, *8*(2), p.239.

Dorji, P., Pattarajinda, V. and Vongprolub, T., 2014. Cryopreservation of semen of mithun and siri bulls. *Bangladesh Journal of Veterinary Medicine*, *12*(2), pp.147-153.

El-Sheshtawy, R.I., Gamal, A. and El-Nattat, W.S., 2016. Effects of pomegranate juice in Tris-based extender on cattle semen quality after chilling and cryopreservation. *Asian Pacific Journal of Reproduction*, *5*(4), pp.335-339.

Gaczarzewicz, D., Udala, J., Piasecka, M., Blaszczyk, B. and Stankiewicz, T., 2015. Storage temperature of boar semen and its relationship to changes in sperm plasma membrane integrity,

mitochondrial membrane potential, and oxidoreductive capability. *Turkish Journal of Biology*, *39*(4), pp.582-594.

Indriastuti, R., Ulum, M.F., Arifiantini, R.I. and Purwantara, B., 2020. Individual variation in fresh and frozen semen of Bali bulls (Bos sondaicus). *Veterinary World*, *13*(5), p.840.

Januskauskas, A., Johannisson, A. and Rodriguez-Martinez, H., 2001. Assessment of sperm quality through fluorometry and sperm chromatin structure assay in relation to field fertility of frozen-thawed semen from Swedish AI bulls. *Theriogenology*, *55*(4), pp.947-961.

Kastelic, J.P., 2014. Understanding and evaluating bovine testes. *Theriogenology*, *81*(1), pp.18-23.

Khalil, W.A., El-Harairy, M.A., Zeidan, A.E., Hassan, M.A. and Mohey-Elsaeed, O., 2018. Evaluation of bull spermatozoa during and after cryopreservation: Structural and ultrastructural insights. *International Journal of Veterinary Science and Medicine*, *6*, pp.S49-S56.

Kumar, D., Kumar, P., Singh, P., Yadav, S.P., Sarkar, S.K., Bhardwaj, A. and Yadav, P.S., 2014, April. Characteristics of frozen thawed semen in predicting the fertility of buffalo bulls. ICAR.

Layek, S.S., Mohanty, T.K., Kumaresan, A. and Parks, J.E., 2016. Cryopreservation of bull semen: Evolution from egg yolk based to soybean-based extenders. *Animal Reproduction Science*, *172*, pp.1-9.

Long, J.A., 2006. Avian semen cryopreservation: what are the biological challenges?. *Poultry Science*, *85*(2), pp.232-236.

Moreira, S.S.J., da Silva, A.M., Souza, A.L.P., Praxedes, E.C.G., de Souza Junior, J.B.F., Pereira, A.F. and Silva, A.R., 2021. Cryopreservation of Spix's yellow-toothed cavy epididymal sperm using Tris-and coconut water-based extenders supplemented with egg yolk or Aloe vera. *Cryobiology*, *99*, pp.40-45.

Morrell, J.M. and Wallgren, M., 2014. Alternatives to antibiotics in semen extenders: A review. *Pathogens*, *3*(4), pp.934-946.

Morrell, J.M., Valeanu, A.S., Lundeheim, N. and Johannisson, A., 2018. Sperm quality in frozen beef and dairy bull semen. *Acta veterinaria scandinavica*, *60*(1), pp.1-10.

Mostek, A., Dietrich, M.A., Słowińska, M. and Ciereszko, A., 2017. Cryopreservation of bull semen is associated with carbonylation of sperm proteins. *Theriogenology*, *92*, pp.95-102.

Muiño, R., Tamargo, C., Hidalgo, C.O., and Peña, A.I., 2008. Identification of sperm subpopulations with defined motility characteristics in ejaculates from Holstein bulls: effects of cryopreservation and between-bull variation. *Animal Reproduction Science*, *109*(1-4), pp.27-39.

Naz, S., Umair, M. and Iqbal, S., 2018. Comparison of Tris egg yolk based, Triladyl® and Optixell® extender on post-thaw quality, kinematics, and in vivo fertility of Nili Ravi buffalo (Bubalus bubalis) bull spermatozoa. *Andrologia*, *50*(8), p.e13063.

Olugbenga, O.M., Olukole, S.G., Adeoye, A.T. and Adejoke, A.D., 2011. Semen characteristics and sperm morphological studies of the West African Dwarf Buck treated with Aloe vera gel extract. *Iranian Journal of Reproductive Medicine*, *9*(2), p.83.

Oyeyemi, M.O. and Fayomi, A.P., 2011. Gonadosomatic index and spermatozoa morphological characteristics of male wistar rats treated with graded concentration of Aloe vera gel. *International Journal of Animal and Veterinary Advances*, *3*(2), pp.47-53.

Palomar Rios, A. and Molina Botella, I., 2017. Sperm parameters that play a major role in the assessment of semen quality after cryopreservation. *Journal of Assisted Reproduction and Genetics*, *34*(10), pp.1271-1276.

Parmar, M.S., Pant, C., Karuppanasamy, K., Mili, B., Upadhyay, D. and Kant, V., 2013. Intracytoplasmic sperm injection (ICSI) and its applications in veterinary sciences: An overview. *Science International*, *1*(8), pp.266-270.

Raheja, N., Choudhary, S., Grewal, S., Sharma, N. and Kumar, N., 2018. A review on semen extenders and additives used in cattle and buffalo bull semen preservation. *Journal of Entomology and Zoology Studies*, *6*(3), pp.239-245.

Raseona, A.M., 2015. *Comparative evaluation of different extenders of bull semen stored under different conditions* (Doctoral dissertation, University of Venda).

Raseona, A.M., Ajao, O.A., Nethengwe, L.D., Madzhie, L.R., Nedambale, T.L. and Barry, D.M., 2017. Viability of bull semen extended with commercial semen extender and two culture media stored at 24 C. *South African Journal of Animal Science*, *47*(1), pp.49-55.

Rauch, A., 2013. *Cryopreservation of bovine semen in egg yolk-based extenders* (Doctoral dissertation, University of Saskatchewan).

Shahraki, A., Mojahed, A.S. and Afshar-Goli, J., 2014. The effects of hydroalcoholic extract of Aloe vera gel on spermatogenesis of adult male rats. *International Journal of Biosciences*, *5*(7), pp.158-165.

Shakib, Z., Shahraki, N., Razavi, B.M. and Hosseinzadeh, H., 2019. Aloe vera as an herbal medicine in the treatment of metabolic syndrome: A review. *Phytotherapy Research*, *33*(10), pp.2649-2660.

Simonik, O., Sichtar, J., Krejcarkova, A., Rajmon, R., Stadnik, L., Beran, J., Dolezalova, M. and Biniova, Z., 2015. Computer assisted sperm analysis–the relationship to bull field fertility, possible errors, and their impact on outputs: a review. *Indian Journal of Animal Sciences*, *85*(1), pp.3-11.

Singh, P., Agarwal, S., Singh, H., Verma, P.K., Pandey, A. and Kumar, S., 2020. Antioxidant effects of Aloe vera as semen additive in cryopreservation of cattle bull semen. *International Journal of Current Microbiology and Applied Sciences*, *9*(9), pp.1625-1635.

Singh, V.K., Singh, A.K., Kumar, R. and Atreja, S.K., 2013. Development of soya milk extender for semen cryopreservation of Karan Fries (crossbreed cattle). *CryoLetters*, *34*(1), pp.52-61.

Sokunbi, O.A., Ajani, O.S., Lawanson, A.A. and Amao, E.A., 2019. Antibiotic potential of Moringa leaf (Moringa oleifera Lam.) crude extract in bull semen extender. *European Journal of Medicinal Plants*, *9*(2), pp.1-8.

Souza, A.L.P., Lima, G.L., Peixoto, G.C.X., Silva, A.M., Oliveira, M.F. and Silva, A.R., 2016. Use of Aloe vera–based extender for chilling and freezing collared peccary (Pecari tajacu) semen. *Theriogenology*, *85*(8), pp.1432-1438.

Takeda, K., Uchiyama, K., Kinukawa, M., Tagami, T., Kaneda, M. and Watanabe, S., 2015. Evaluation of sperm DNA damage in bulls by TUNEL assay as a parameter of semen quality. *Journal of Reproduction and Development*, *61*(3), pp.185-190.

Tarig, A.A., Wahid, H., Rosnina, Y., Yimer, N., Goh, Y.M., Baiee, F.H., Khumran, A.M., Salman, H. and Ebrahimi, M., 2017. Effect of different concentrations of egg yolk and virgin coconut oil in Tris-based extenders on chilled and frozen-thawed bull semen. *Animal Reproduction Science*, *182*, pp.21-27.

Taşdemir, U., Büyükleblebici, S., Tuncer, P.B., Coşkun, E., Özgürtaş, T., Aydın, F.N., Büyükleblebici, O. and Gürcan, I.S., 2013. Effects of various cryoprotectants on bull sperm quality, DNA integrity and oxidative stress parameters. *Cryobiology*, *66*(1), pp.38-42.

Üstüner, B., Alçay, S., Nur, Z., Sağırkaya, H. and Soylu, M.K., 2014. Effect of egg yolk and soybean lecithin on tris-based extender in post-Thaw Ram Semen quality and in vitro fertility.

Watson, P.F., 2000. The causes of reduced fertility with cryopreserved semen. *Animal Reproduction Science*, *60*, pp.481-492.

Woelders, H., 1997. Fundamentals and recent development in cryopreservation of bull and boar semen. *Veterinary Quarterly*, *19*(3), pp.135-138.

Wusiman, A., Wang, Y., Ren, K., Zhou, G., Fu, X., Suo, L., Fan, Z., Wang, L. and Zhu, S., 2012. Semen storage at 23, 4 or-196° C and its application to artificial insemination in small-tail Han sheep. *Asian Journal of Animal and Veterinary Advances*, *7*(4), pp.299-308.

Yong, S.Y., Nguang, S.I. and Kari, A., 2017. Comparison between the effect of egg yolk-based extender and *Aloe vera* (Aloe barbadensis) based extender on red tilapia (Oreochromis niloticus) sperm quality. *Journal of Fundamental and Applied Sciences*, *9*(2S), pp.813-820.

Yoon, S.J., Rahman, M.S., Kwon, W.S., Park, Y.J. and Pang, M.G., 2016. Addition of cryoprotectant significantly alters the epididymal sperm proteome. *PLoS One*, *11*(3), p.e0152690.

Zubair, M., Ahmad, M. and Jamil, H., 2015. Review on the screening of semen by hypo-osmotic swelling test. *Andrologia*, *47*(7), pp.744-750.

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THE EFFECT OF TRIS ALOE VERA (*ALOE BARBADENSIS MILLER*) GEL ON CHILLED AND FROZEN-THAWED BULL SPERMATOZOA QUALITY

By

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A dissertation submitted in fulfilment of the requirements for the degree of

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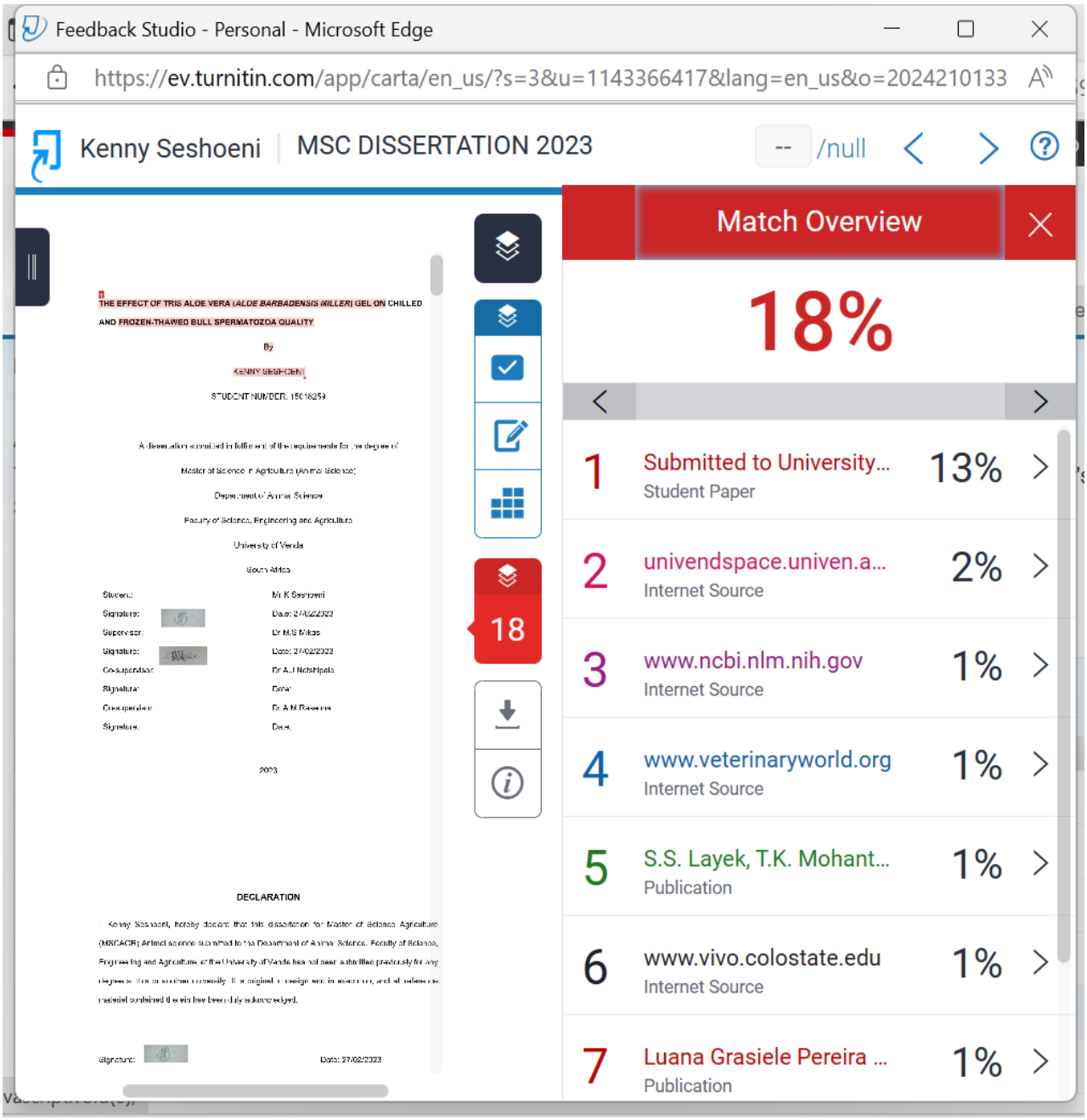

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Please don't hesitate to contact me for any enquiry.

Kind regards

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