Effect of Avocado (*Persea americana Mill*) oil inclusion in the Tris-based extender on the quality of Boer goat semen stored at different temperatures

By

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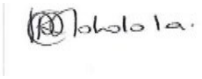
Signature: 

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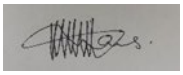
Signature: 

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DECLARATION

I, **Khomotso Cathrine Mohololа**, the undersigned, hereby declare that this dissertation for Master of Science in Agriculture in Animal Science (MSCAGR) 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 execution, and all reference material contained therein has been duly acknowledged.

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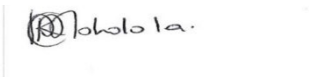

K. C Mohololа

DEDICATION

This dissertation is dedicated to my family.

To my parents, thank you for all your patience, love, and support throughout. I would not have achieved this if it was not for your guidance, advice, and support. A big “THANK YOU” to you and the rest of the family.

To my big brother and his wife, you guys have offered tremendous support throughout this study. My greatest gratitude to you and your family.

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Above all, I thank God for the courage and strength He gave me during the trying times faced by our country. I alone would not have been able to put this project together and accomplish it.

ABSTRACT

Semen extenders are chemical mediums used for preserving, and protecting spermatozoa against different shocks while processing, storing and transportation for use in artificial insemination (Raheja *et al*., 2018). There are challenges with the preservation of goat semen for Artificial Insemination (AI). Spermatozoa with poor viability were observed when goat semen was extended with egg yolk and skimmed milk because of the seminal plasma secreted by the bulbourethral gland (Cabrera *et al*., 2005). This study aimed to evaluate the effect of Avocado oil on Boer goats semen quality parameters during liquid storage (5˚C,17 ˚C and 24˚C). Semen samples collected from four matured Boer goats were pooled and Tris-egg yolk extenders were supplemented with Avocado oil at 0, 1, 2 and 3%. The samples were then stored at 5˚C,17 ˚C, 24˚C and several sperm parameters (motility, viability, morphology, and DNA fragmentation) were assessed at 0, 24, 48 and 72 h intervals. Results showed that the supplementation of Tris egg yolk with 1%, 2% and 3% Avocado oil improved total motility, progressive motility, morphology, and viability when stored at 5˚C for up to 72 hours and when stored at 17˚C for 24 hours. However, non-fragmented DNA improved when 3% of Avocado oil was added to the Tris egg yolk when kept at 5˚C from 24 hours. Therefore, it was concluded that the Boer goat spermatozoa quality could be preserved for 72 hours at 5˚C when adding 2% and 3% of Avocado oil to the Tris egg yolk extender. Semen with inclusions of Avocado oil in Tris-egg yolk can preserve Boer goat semen for 24 hours or less when stored at 17°C.

Keywords: Semen extender, Avocado oil, sperm quality and storage temperature

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ABBREVIATIONS AND ACRONYMS

AI	: Artificial Insemination
AV	: Artificial Vagina
CASA	: Computer-Assisted Semen Analysis
EE	: Electro Ejaculator
GLM	: General linear Model
H	: Hour
MED	: Medium
MIN	: Minutes
M	: Meter
NPM	: Non-Progressive Motility
PM	: Progressive Motility
TAO	: Tris Egg Yolk with Avocado Oil
TEY	: Tris Egg Yolk
TM	: Total Motility
SLW	: Slow

STC : Static

CHAPTER 1: INTRODUCTION

1.1 Background

Semen extenders are chemical mediums used for preserving, and protecting spermatozoa against different shocks while processing, storing and transportation used for artificial insemination (Raheja *et al*., 2018). Extenders must have the following qualities critical for semen preservation be isotonic, can buffer (regulate pH), protect against cold shock, provide energy for sperm metabolism, regulate microbial contamination, and preserve fertility of spermatozoa. Two methods used to preserve semen are chilling, and cryopreservation, and these methods are used with most of the semen extension media (Borges-Silva *et al*., 2016; Baiee *et al*., 2018; Yang *et al*., 2018). Chilled semen yields spermatozoa of good quality which are viable for three days, whereas cryopreserved semen yielding spermatozoa of moderate quality which are viable for years (Bustani and Baiee, 2021).

Egg yolk has been recognized as a powerful semen extender for sperm preservation due to its ability to withstand lipid-phase transition and cold shock (Aboagla and Terada, 2007). However, the variations in egg yolk from different periods of egg yolk storage and its harmful effects on the motility and respiration of spermatozoa have led researchers to look for alternatives. Some studies have shown that egg yolk can have a harmful effect on the motility and respiration of spermatozoa (Aurich *et al*.,1997). Although egg yolk has been used as a main component of semen extenders, some studies have shown that it can have a harmful effect on the motility and respiration of spermatozoa (Amirat *et al*., 2004). Hence that led researchers to concentrate on discovering at least a desirable supplement or cryoprotectant to solve these constraints (Tarig *et al*., 2017).

The two commonly used extenders to preserve semen in liquid storage are egg yolk and skim milk. However, these extenders cannot totally prevent sperm parameters such as motility changes from happening in *in vitro* conditions. Over time, especially when the period of liquid storage exceeds 12 hours, a decrease in motility has been observed (Paulenz *et al*., 2002). According to researchers

(Del Maestro,1980; Alvarez and Storey,1984; Sikka, 1996), this decrease in motility to be caused by the effect of reactive oxygen species, the superoxide anion radical (O^{2-}), hydrogen peroxide (H_2O_2), nitric oxide, and peroxynitrite anion, produced by the cellular components of semen, as well as the lipid hydroperoxides produced by the lipid peroxidation of the spermatozoa membranes. Kelly *et al,* (1998) reported that different macromolecules, for instance proteins and lipids, can be attacked by the reactive oxygen species which can cause oxidative damage. To combat these issues, numerous natural antioxidants are added to prevent or slow down this process and maintain the quality of liquid ram semen (Mart *et al*., 2003; Ashrafi *et al*., 2011; Câmara *et al*., 2011). ((

In this study, Avocado oil was added as a supplement to the tris-based extender to evaluate its potential to preserve the viability of goat semen. Avocado oil consists of monosaturated (60% at minimum) and poly-saturated fatty acids of approximately 10% (Forero-Doria *et al*., 2017). In addition, antioxidant-rich pigments, namely chlorophylls and carotenoids (which reflect in green color) are relatively abundant (between 11-19mg/kg). These pigments are believed to help with disease prevention and protection from pathogenic bacteria because they function as antioxidants (Krinsky and Johnson, 2005). The primary fatty acids in avocado oil are linolenic, linoleic acids, palmitoleic, oleic acids, palmitic and stearic acids (Thomas *et al*., 2000). Avocado oil appears to be a potentially desirable supplement due to the mentioned benefits.

Buck semen can be preserved using egg yolk extenders up to three days with motility maintained above 50% (Shamsuddin *et al*., 2000). The components of the buck's bulbourethral gland secretion, egg yolk coagulating enzyme and BUSgp60 protein fraction, caused poor spermatozoa viability (Pellicer-Rubio *et al*., 1997; Cabrera *et al*., 2005). Seminal plasma could be washed from the spermatozoa before semen dilution, but it is a complicated process. Hence, there is a need to identify additives which could counter the effects of the buck's seminal plasma to improve semen viability.

1.2 Statement of the Research Problem

There are challenges with preserving goat semen for Artificial Insemination (AI). Spermatozoa with poor viability were observed when buck semen was extended with an egg yolk and skimmed milk because of the seminal plasma secreted by the bulbourethral gland (Cabrera *et al*., 2005). The components of the bulbourethral gland secretion, egg yolk coagulating enzyme and BUSgp60 protein fraction, caused poor spermatozoa viability (Pellicer-Rubio *et al*., 1997; Cabrera *et al*., 2005). Removal of seminal plasma by washing the spermatozoa before semen dilution is possible, but it is a complicated process which requires time. Hence, there is a need to identify additives to counter the effects of the buck's seminal plasma to improve semen viability (Xu *et al*., 2009; Batista *et al*., 2011; Sadeghi *et al*., 2020; Bustani and Baiee, 2021). Most small-scale farmers in the developing world lack money to buy bucks of superior genetic quality, and they often cannot afford resources to cryopreserve the semen like liquid nitrogen tank (Vishwanath and Shannon, 2000; Bester *et al*., 2009).

Egg yolk is commonly added as an extender in liquid storage of ruminant semen, but it cannot completely prevent changes in sperm parameters under in vitro conditions. After more than 12 hours of storage, the motility of sperm reduces because of reactive oxygen species. These include the superoxide anion radical, hydrogen peroxide, nitric oxide, and peroxynitrite anion, which are produced by the cellular components of semen, as well as the lipid hydroperoxides produced by lipid peroxidation of spermatozoa membranes. These reactive species can attack proteins and lipids, causing oxidative damage that leads to fragmentation of DNA and acrosome integrity. Studies by Del Maestro (1980), Alvarez and Storey (1984), Sikka (1996), Mammoto *et al*. (1996), Gosalvez *et al*. (2007), and Lopez-Fernandez *et al*. (2008) have confirmed these findings.

Antioxidants could be supplemented in the semen extenders to prevent deleterious membrane changes during the chilling, freezing, and thawing processes (Maxwell and Watson, 1996). Avocado oil contains vitamin E in a form of α-tocopherol, and minimal concentrations of β-tocopherol, δ-

tocopherol, and γ-tocopherol (Woolf *et al*., 2009). The α –tocopherol's key biochemical role is to provide polyunsaturated fatty acids with protection from peroxidation (Beringer and Dampert, 1976; Kamal-Eldin and Anderson, 1997; Drissi *et al*., 2004). Thus, this study aims to investigate the effect of adding avocado oil in tris egg yolk extender on goat semen parameters during liquid storage.

1.3 Justification/Rationale of the Study

Extracts from some of the plants were proven to protect spermatozoa from pathogenic bacteria or serve as key sources of antioxidants that act as reactive oxygen species scavengers or do both. In addition, Al-Daraji (2012a) highlighted that the positive impact of oil's high antioxidant concentration on spermatozoa motility may be due to the oil's high antioxidant content. Supplementing Tris-based extender with avocado oil will help farmers in developing areas to be able to preserve goat spermatozoa in their homes or farms using refrigerators or preserving at a controlled temperature successfully for artificial insemination since avocado oil contains antioxidant-rich pigments, chlorophylls, and carotenoids, that are relatively abundant. These pigments are believed to help with disease prevention and protection because they function as antioxidants.

1.4 Research Objectives

1.4.1. Main objective

The aim of this study was to evaluate the effect of Avocado oil inclusion levels in a Tris-based on sperm quality of goat semen stored at different temperature for 72 hours.

1.4.2 Specific objectives

I. To evaluate the effect of Avocado oil inclusion levels (0%, 1%, 2% and 3%) in a Tris-based extender on sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

II. To evaluate the effect of storage temperatures (5˚C, 17˚C and controlled room temperature 24˚C) on sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

III. To evaluate the effect of storage times (0 hour, 24 hours, 48 hours, 72 hours) on sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

IV. To evaluate the effect of interaction among Avocado oil inclusions, storage temperatures and storage times on sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

1.5 Research Hypothesis

1.5.1 Null hypothesis

i. Different concentrations of Avocado oil inclusion in a Tris-based extender will not have a significant effect on sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa at different storage temperatures

ii. Different storage temperatures conditions will not have a significant effect on different concentrations of avocado oil in a Tris-based extender on the sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

iii. Storage time will not have a significant effect on different concentrations of avocado oil in a Tris-based extender on the sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

iv. Interaction among avocado oil inclusion, storage temperature and storage time will not have a significant effect on sperm motility, morphology, viability, and sperm DNA fragmentation of goat spermatozoa

CHAPTER 2: LITERATURE REVIEW

2.1 General introduction

Plant extracts may be used as additives for spermatozoa preservation to improve semen quality of animals. These plant extracts include a range of plant materials like juice, powder and oil extracted from diverse components of a plant including leaves, stems, seeds, and fruit pulp (Ros-Santaella; Pintus, 2021; Sun *et al*., 2020; Allai *et al*., 2017). Extracts from some of the plants were proven to protect spermatozoa from pathogenic bacteria or serve as key sources of antioxidants which act as reactive oxygen species (ROS) scavengers or do both (Ros-Santaella and Pintus, 2021). Avocado oil contains antioxidant-rich pigments, namely chlorophylls and carotenoids that are relatively abundant. These pigments are believed to help with disease prevention and protection because they function as antioxidants (Krinsky and Johnson 2005). For this reason, this chapter gives an overview of effect of inclusion of plant extracts as additives to combat oxidative stress and formation of endogenous free radicals associated with preserved semen and the possibility that animal-based extension media like egg yolk and skimmed milk could contaminate the semen and transmit diseases to the recipients on semen quality.

2.2 Semen extension and preservation

According to Mahesh e*t al*. (2017), a semen extender implies a diluent or aqueous solution for expanding the volume of the ejaculates to the necessary dose or quantity while protecting the usefulness of the spermatozoa. Extenders are used to maintain the fertility of spermatozoa by providing them with an energy source and protecting them against cold shock while keeping them in an environment that is suitable for temporal survival of the spermatozoa (Gadea, 2003; Purdy, 2006). The success of artificial insemination depends partly on semen preservation to ensure that its spermatozoa can fertilize the ova. Semen preservation requires good extension media which increases semen volume and allows it to be stored using various methods for future use (Raheja *et*

al., 2018; Bustani and Baiee, 2021). Plant extracts were investigated by various researchers as additives for spermatozoa preservation to combat oxidative stress and formation of endogenous free radicals associated with stored semen and the possibility that animal-based extension media like egg yolk and skimmed milk could contaminate the semen and transmit diseases to the recipients (Baiee *et al*., 2018; Anzar *et al*., 2019; Bustani and Baiee, 2021; Ros-Santaella and Pintus, 2021). These plant extracts include a range of plant materials like juice, powder and oil extracted from diverse components of a plant including leaves, stems, seeds, and fruit pulp. Extracts from some of the plants were proven to protect spermatozoa from pathogenic bacteria or serve as key sources of antioxidants that act as reactive oxygen species (ROS) scavengers or do both (Ros-Santaella and Pintus, 2021). Among other things, plant oils are known to improve sperm quality in bovine, human, and poultry (Aboua *et al*., 2009; Al-Daraji, 2012a; Towhidi and Parks, 2012). Cryopreservation and chilling are the two techniques used for preserving semen, these techniques are mostly used with extenders or semen extension media (Borges-Silva *et al*., 2016; Baiee *et al*., 2018; Yang *et al*., 2018).

2.3 Determination of semen quality

According to Tévar *et al*. (2022), sperm quality is assessed by a diagnostic procedure known as a semen analysis. The procedure enables the specialist to evaluate several microscopic and macroscopic parameters, such as sperm morphology, motility, and count, in addition to the semen pH and volume, as determinants of semen quality (Tévar *et al*.,2022). Total motility, progressive motility, viability, and abnormality have been evaluated as parameters of sperm quality by other researchers (Al-Daraji, 2012a; Raseona, 2015; Allai *et al*., 2015). Sperm morphology evaluation is a significant part of standard sperm quality evaluation, and the varieties of sperm abnormalities correlates significantly to the fertility of the male animal (Januskauskas *et al*., 2001). There are correlations between viability and fertility, and morphology and fertility, provided that the quality of the evaluated parameters and the fertility obtained with the semen vary widely (Januškauskas & Žilinskas, 2002). According to Ott and Memon (1980) and Hildalgo *et al*. (2006), the acceptable

standard for normal morphology is 70% and is considered excellent when it is greater than 90%. At least 40% of the sperm in an ejaculate sample must be motile for the semen to be deemed (Cooper *et al*., 2010). This may also involve non-progressive motion. A minimum of 32% of the sperm must exhibit progressive motility (Cooper *et al*., 2010) which is line with Ott and Memon's (1980) findings that the standardized acceptable percentage for progressive motility of buck semen is 30% and is considered excellent when it is greater than 50%.

The sperm evaluation process pays more attention to the sperm's viability and functional status which will affect the gamete's ability to fertilize the oocytes. However, the content and quality of the genetic material are the major factors which are essential for sperm to produce viable and healthy offspring (Binsila *et al.,* 2017).

According to Bungnum *et al*. (2011), sperm DNA Fragmentation (SDF) can be defined as the physical breakdown of one or two DNA strands in the chromosomes of sperm. Fragmentation happens when one or two strands of DNA undergo a base change or physical break (Bungum *et al*., 2011). If this happens in genes necessary for embryo growth and is not repaired, the result may be embryo death. The SDF has been used as an indicator of male fertility (Bungum *et al*., 2011). The assessment of sperm SDF is similar to the commonly used method of semen analysis (Woodruff, 2012). Detection of Sperm DNA Fragmentation in semen is influenced by various factors, including oxidative stress, apoptosis, and histone-protamine replacement failure (Sakkas *et al*., 1999; Agarwal *et al*., 2008). Also, it has been shown that temperature variations affect the speed of Sperm DNA Fragmentation (López-Fernández *et al*., 2007, Toro *et al*., 2009).

2.4 Effects of storage temperature, storage time and including plant oils in extenders on spermatozoa quality

Allai *et al*. (2015) reported that adding 2% and 5% of argan oil Tris-egg yolk extenders could preserve the quality of liquid ruminant (i.e., ram) sperm at 5 ˚C and 15 ˚C for up to 48 hours. According to Tarig

et al. (2017) adding 1, 1.25, 1.5, and 1. 75% concentrations of soybean lecithin to a tris-based extender containing 2% virgin coconut oil had a favourable impact on motility, morphology, viability, membrane integrity, and acrosome integrity of chilled semen. In addition, their outcomes showed that, the addition of 1.5% soybean lecithin with 2% virgin coconut oil in a Tris-based extender had considerably higher quality parameters of chilled semen after 24, 72, and 144 hours.

According to Allai *et al*. (2015), the inclusion level of 1, 2 and 5% of argan oil in tris egg yolk extender improved the progressive motility of sperm when preserved at 5˚C after 8 hours. Furthermore, including 1% and 2% of argan oil in tris egg yolk extender improved the progressive motility for semen kept at 15 ˚C after 24 and 48 hours. (Allai *et al*.,2015). Allai *et al*. (2015) further observed that 1% of argan oil in tris egg yolk extender increased the viability of sperm after 24 and 48 hours of preservation at 5 ˚C. It was then concluded that the sperm quality of the ram could be preserved at 5 ˚C and 15 ˚C for up to 48 hours of storage due to the addition of argan oil to skim milk and tris egg yolk extenders.

In the study conducted by Al-Daraji (2012a), it was concluded that olive oil can be used efficiently for an improvement of semen quality during the liquid storage of diluted semen. The study further reported that the motility consistently increased with olive oil inclusions when evaluating the effects of diluting semen and preserving sperm motility. Moreover, it was observed that the inclusion of olive oil improved the quality of the semen extended with olive oil-based extender.

When cactus seed oil of 1% and 2% were added to Tris egg, total and progressive motility and viability did not differ between 24 and 48 hours of preservation at 5˚C. The most significant decline in those parameters after 48 hourst of storage were observed. However, total motility and progressive motility recorded higher sperm motility (±84% and ±42% respectively) when supplemented with 1% and 2% of cactus seed oil compared to extenders supplemented with 0% and 5% of cactus seed oil in Tris egg yolk during 8, 24, 48, and 72 hours of storage. Furthermore, tris egg yolk supplemented with 1%

and 2% of cactus seed oil other preserved greater total motility than when supplemented with 0,5% and 10% of cactus seed oil, according to the interaction of extenders and cactus seed oil concentrations (Allai *et al*. 2017; Allai *et al.,* 2015).

According to the study conducted by Allai *et al*. (2017), from 24 hours of preservation forward sperm viability increased with the inclusion of 1% and 2% of the cactus seed oil to Tris egg yolk in comparison to the inclusion level of 0% of cactus seed oil in Tris egg yolk at 5˚C. The authors observed percentages of sperm viability were higher in Tris egg yolk extender with 1% and 2% cactus seed oil compared to inclusion levels of 0, 5 and 10% of cactus seed oil in Tris egg yolk, due to the interaction between extender and cactus seed oil. Sperm motility, viability and abnormalities were dramatically decreased over storage period (especially from 48 h to 72 h) when 1% and 2% of the cactus seed oil were added in skim milk and 1% added in tris egg yolk, at 5 ˚C , respectively (Allai *et al*. 2017).

2.4. Properties of plant oils useful to preserve semen

Avocado oil consists of monosaturated (60% at minimum) and polysaturated fatty acids of approximately 10% (Forero-Doria *et al*., 2017). In addition, it contains antioxidant-rich pigments, namely chlorophylls and carotenoids (which reflect a rich green color), that are relatively abundant. These pigments are believed to help with disease prevention and protection because they function as antioxidants (Krinsky and Johnson, 2005). The primary fatty acids in avocado oil are linolenic and linoleic acids, palmitoleic and oleic acids, and palmitic and stearic acids (Woolf *et al*., 2009). Hass avocado oil contains minimal concentration of β-tocopherol, δ-tocopherol, and γ-tocopherol in addition to α-tocopherol, which happens to be the primary vitamin E type found in Avocado oil (Woolf *et al*., 2009). When evaluating how α-tocopherol and ascorbic acid affect the quality of cryopreserved equine semen, Franco *et al*. (2013) drew a conclusion that α -tocopherol is an effective antioxidant since it minimizes the oxidative stress caused by cryopreservation and horse spermatozoa's lipid peroxidation.

Al-Daraji, (2012a) have done a similar study using olive oil into diluents to improve the quality of semen and its storage ability of rooster's semen stored at 5˚C. The author further highlighted that the high antioxidant concentration of olive oil, which was found to have a favorable effect on spermatozoa motility, could be attributable to the oil's high content of antioxidants. When samples that were preserved using Al – Daraji 2 diluent plus 2, 4, 6, and 8 ml of olive oil, and those without were assessed every 24 hours for three days after the commencement of *in-vitro* storage, samples with olive oil had higher ($p<0.05$) viability of sperm, normality and acrosomal integrity. Sample with 8 ml of olive oil performed best when evaluated for viability compared to the treatments with 2 ml, 4 ml and 6 ml of olive oil that were included in the study. AL-Daraji (2012a)'s findings are consistent with those of Al-Daraji (2012b) who discovered that adding substances rich in antioxidants to the semen diluent improved spermatozoa integrity, vitality, freezing ability, and acrosomal integrity of rooster semen kept at 4 ˚C for 24 hours.

The improvement in spermatozoa viability is thought to be due to antioxidants inhibiting or minimizing the adverse effects of *in vitro* lipid peroxidation. Furthermore, it was reported that olive oil can infiltrate the plasma membrane of spermatozoa and decrease free radical damage due to its lipid solubility. Donoghue and Donoghue (1997) also confirmed that shelf-life of rooster semen could be improved by additional antioxidants as the antioxidant activities in seminal plasma and sperm are insufficient to prevent lipid peroxide damage after extension and in vitro storage. Hence, enhancing the antioxidant capacity of sperm by adding olive oil to the semen diluent could be a great opportunity to improve the shelf-life of rooster semen.

2.5. Summary of literature Review

To my knowledge, no study was done to assess the effectiveness of adding avocado (Persea Americana Mill) oil in semen diluents to improve the quality and the storage of goat spermatozoa. Nonetheless, several plants extracts have been reported to serve as key sources of antioxidants that scavenge reactive oxygen species (ROS), or do both functions, as such, they protect spermatozoa

against pathogenic bacteria, Avocado oil has a favorable antioxidant capacity because mono-unsaturated fats are dominant, it has several phytochemicals that could improve sperm quality, and it also contains chlorophyll and carotenoids that are antioxidant rich and are believed to help with disease prevention and protection.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Description of the Study Area

The experiment was conducted at the Biotechnology laboratory of the Centre of Excellence in Animal Assisted Reproduction (CEAAR) at the University of Venda. The University is situated in Thohoyandou, under the Thulamela Municipality, Vhembe District of the Limpopo Province of South Africa.

3.2 Animals and management

Four Boer bucks aged 3 to 4 years, with scrotal circumference of approximately 25cm and an average weight of 78.41 ± 5.29 kg were used in this study. These animals were given a minimum of 3 days of sexual rest after a day of semen collection. These bucks were kept in the pens and provided with pellets and fresh drinking water *ad libitum*. The bucks were treated in a humane manner and without any cruelty. The bucks used for semen collection were separated a night before from the herd to prevent them from mating with does.

3.3 Experimental design

Using four Boer bucks (n=4), the present study investigated the effect of four levels (0, 1, 2 and 3%) of extenders and four storage times (0,24,48 and 72 hours) and temperature levels (5 ˚C, 17 ˚C and 24 ˚C) on the quality of goat spermatozoa. The collection and preservation of semen was replicated five times, once a week. The study was conducted in a 4 x 3 x 3 factorial design.

3.4 Preparation of Tris-egg yolk extender and inclusions of levels of avocado oil

Fresh eggs from red and white amberlink breed were collected from a layer house in the experimental farm at the University of Venda. These eggs were washed thoroughly with clean running water and dried up with a paper towel. Ethanol (70 % alcohol) was then used to spray these eggs to disinfect them against any possible contamination. Eggs were tapped using a spatula and egg yolk was

obtained using a sterile egg divider to separate the yolk from the egg white. After preparations, different levels of avocado oil from the market were added to produce Tris-egg yolk with the concentration of avocado oil labelled as follows: TEY- Tris-egg yolk with no avocado oil, TAO_A -Tris-egg yolk plus 1% avocado oil, TAO_B - Tris-egg yolk plus 2% avocado oil and TAO_C - Tris-egg yolk plus 3% avocado oil.

During the day of semen collection, semen samples were collected from the bucks. These samples were incubated in the water bath at 37˚C. Semen samples were then extended in the biotechnology laboratory and evaluated before they could be subjected to different temperatures (5 ˚C, 17 ˚C and 24 ˚C). Collected data were evaluated and recorded before it could be subjected to 5 ˚C, 17 ˚C and 24 ˚C so that it assists in checking if the sperm cells were viable, mobile, and normal. Every 24 hours, samples were analyzed for the following spermatozoa parameters; motility, viability, morphology, and DNA Fragmentation using Computer-Assisted Semen Analysis (CASA). Table 3.1 illustrates the constituents of Tris egg yolk used in the experiment as described by (Qureshi *et al*., 2013).

Table 3. 1: Composition of Tris-egg yolk and inclusions levels of avocado oil

Ingredient	**TEY**	**TAO_A**	**TAO_B**	**TAO_C**
Tris (g)	1.802	1.802	1.802	1.802
Citric acid (g)	1.012	1.012	1.012	1.012
Fructose (g)	O.50	0.50	0.50	0.50
Egg Yolk (ml)	5	5	5	5
Avocado oil (%)	0	01	02	03
Penicillin Stryptomycin (ml)	0.25	0.25	0.25	0.25
Ph	6.50	6.50	6.50	6.50
Added up to 50ml with distilled water	**50**	**50**	**50**	**50**

TEY=Tris-egg yolk, TAO_A= Tris-egg yolk with the inclusion of 1% avocado oil, TAO_B= Tris-egg yolk with the inclusion of 2% avocado oil and TAO_C= Tris-egg yolk with the inclusion of 3% avocado oil

3.5 Semen collection and processing

Semen samples were collected from Boer bucks four times per week to give bucks a minimum of three days of sexual rest for a period of 5 weeks. An electro-ejaculator of minimum of 2 volts was used to collect semen into 15ml graduated tubes. During the electro-ejaculation technique, the rectal probe was lubricated using Liquid paraffin oil and inserted into the rectum which was cleaned prior the insertion. The probe's three brass electrodes were pointed downward and firmly placed on the

rectum's floor while the buck was restrained. The electro-ejaculator was then switched on and the voltage increased gradually and automatically until the goat maintained an erection.

The end tip of the erected penis was directed into the graduated tube for the goat to ejaculate inside. Immediately after semen collection, the volume of each semen samples was measured because low volume can be accompanied by low sperm cell concentration (Ajao, 2015), and their color was visually observed. Graduated tubes were quickly put into a thermos flask filled with warm water at 37 degrees Celsius after the semen was visually observed for color and to check if the sample was not contaminated. These samples were then taken to the biotechnology laboratory for further evaluations and analyses. Upon the arrival at the laboratory in less than 2 minutes, the semen samples were pooled in a 15 ml sterile, pre-warmed Cellstar tube to remove individual differences.

3.6 Microscopic evaluations of semen parameters

3.6.1 Sperm motility

The CASA was used for analyzing both progressive and non-progressive motility to attain total motility. The spermatozoa analysis was done using Sperm Class Analyzer® 5.0 (Microscopic, S.L,Barcelona, Spain) under 10× magnification (Nikon, China) at phase contrast Ph1 on the microscope wherein a drop 10µl of semen sample was placed on the microscopic slide and covered with a coverslip. Five different fields on the slide were analyzed with each slide having about 100 to thousands of sperm cells depending on the concentration of the sample. The CASA was also used to measure other parameters of motility like rapid, medium, slow, and static movement.

3.6.2 Sperm Viability

Live and dead spermatozoa were determined by using Eosin-Nigrosin stain (Onderstepoort Faculty of Veterinary Sciences Pharmacy, South Africa). Pipette tip was used to place 10µl of the semen sample on the microscopic slide and mixed with 10 µl of eosin and nigrosine stain on a warm glass stage of the microscope at 37 °C. A clean slide held about an inclination of roughly 20° from the

horizontal plane and pushed forward to smear the cocktail across the slide. The smeared slide was placed immediately on a hot Buehler® slide warmer (Buehler Ltd., 42 Waukegan Road, lake Bluff, Illions, United States of America) to allow it to dry. The slide was then allowed to cool down before assessing. The CASA vitality program was then used at 60X magnification (Olympus Corporation BX 51FT, Tokyo, Japan) at phase contrast A to count the number of live and dead spermatozoa per 200 sperm cells minimum. The dead spermatozoa absorbed the stain and appeared dark while live sperm cells did not absorb the stain and appeared clear. All these data were recorded and calculated into percentages.

3.6.3 Sperm morphology

The spermatozoa morphology was determined the same way as viability using the same magnification size used while analyzing viability but under the sperm morphology program. Acrosome, head, and mid-piece of the spermatozoa are automatically detected by the system (CASA). The system automatically detects the acrosome, head, and mid-piece of the spermatozoa. All the spermatozoa with twisted or double tails, damaged mid-piece and damaged or detached heads were considered abnormal (Sokunbi *et al*., 2015). A minimum of 200 sperm cells were counted and all these data were recorded and calculated to percentages.

3.6.4 DNA Fragmentation

To determine the DNA sperm fragmentation, a Sperm-halomax kit (Halotech DNA, Madrid, Spain) was used. An Eppendorf tube containing agarose was floated inside a thermos flask containing hot water at 95-100 ˚C for 5 minutes up until it is fully melted. Incubated lysis solution was set at room temperature. After 5mins, the tube containing the agarose was transferred to a 37˚C water bath for another 5 minutes. While waiting, 25µl of each extended sample was transferred into empty Eppendorf tubes and were combined gently with 50µl of that melted agarose at 37˚C. From each mixture that has been prepared, a drop of 2 µl was withdrawn and placed on the microscope slide on

the horizontal plane and covered with a coverslip. Coverslips on the slide were gently pressed down to avoid the formation of bubbles (Raseona,2015). These slides were placed on the pre-cooled metal tray at 4 ˚C and then kept in a refrigerator at 4 ˚C to solidify agarose for a time frame of 5 minutes. The microscope slides were then gently removed from their coverslips and taken out of the refrigerator. Uncovered slides were then placed horizontally inside the floating boat and a lysis solution was applied to them. These slides were then incubated in the water bath at 37 ˚C for 5 minutes. After being incubated, the applied solution was drained by tilting the slides and were placed horizontally.

The slides were subjected to washing using distilled water with the horizontal position maintained for 5 minutes. distilled was then drained by tilting the slide and then the slide was placed horizontally, and 70% ethanol was applied to the slide using a disposable pipette for 2 minutes. The slides were tilted for draining the alcohol and placed horizontally again and 99.9% ethanol was applied on the slides for another 2 minutes. Processed slides were then allowed to air dry before staining. After drying, the slides were stained using the Spermac-Blue stain and the CASA was used to evaluate the slides using the DNA fragmentation program under the 60X magnification at phase contrast A. Two hundred sperm cells were counted per slide. Fragmented and non-fragmented cells were recorded and calculated to percentages.

3.7 Statistical Analysis

The study was conducted in a 4 x 3 x 3 factorial in a completely randomised design with ten replicates. Analysis of variance (ANOVA) was conducted using General Linear Model (GLM) procedures of the Minitab statistical package (Minitab 2019). The means of different treatments were compared using Tukey's post hoc test. Significance was set at $P<0.05$. The following model was used:

$$Y_{ijk} = \mu + O_i + T_j + H_K + O_i T_i + O_i H_K + T_j H_K + O_i T_j H_K + \varepsilon_{ijK}$$

Where: μ = the overall mean

O_i = the effect of i^{th} level of avocado oil on extended semen (I = 4: 0, 1, 2 and 3%)

T_j = the effect of storing extended semen j^{th} temperature (j = 3: 1= 5 ˚C,2 = 17 ˚C and 3=24 ˚C)

H_K = the effect of evaluating extended semen after the k^{th} time of evaluation in hours (k = 4: 1= 0,2= 24,3=48,4=72)

$O_i T_j$ = the interaction between the i^{th} level of avocado oil and j^{th} level of temperature

$O_i H_K$ = the interaction between the i^{th} level of avocado oil and k^{th} level of evaluation time

$T_j H_K$ = the interaction between the j^{th} level of temperature and the k^{th} level of evaluation time

$O_i T_j H_K$ = the interaction among the i^{th} level of avocado oil, j^{th} level of temperature and the k^{th} level of evaluation time

ε_{ijl} = the random error

3.8 Ethical Considerations

The Higher Degrees Committee granted its ethical approval for the use of animals to collect semen, and evidence of approval is attached. The project number is SARDF/19/ANS/12/0612.

CHAPTER 4: RESULTS

The main effects of extender, storage temperature and storage time on percentage motility (total motility-TM, progressive motility- PM, non-progressive motility- NPM, rapid motility-RAP, medium motility- MED, slow motility- SLW, and static motility- STC) of Boer goat spermatozoa are presented on **Table 4.1** below. Avo oil inclusion level affected ($p<0.01$) all parameters which explained spermatozoa quality except for the rate of TM ($p>0.05$). The rates of PM and RAP were higher for TEY andTAO_C (42% and 41%, respectively) than for TEY (30% and 24%, respectively) and TEY and TAO_A (36% and 34%, respectively). TEY had higher rate of MED (16%) than those for TAO_s (i.e., TAO_A, TAO_B, TAO_C), and the MED for TAO_A was higher (10%) than that for TAO_C (8%). Live spermatozoa were higher for TAO_C (39%) than for other inclusion levels (average of 30%). Extender TAO_C yielded spermatozoa with a slightly higher rate of non-fragmented DNA (98%) than the average 97% observed for the TEY and TAO_C. However, all Avocado oil inclusion levels yielded spermatozoa with a high rate, of >90%, for non-fragmented DNA. The rate for TA was higher for TAO_A (5%) than the <4% observed for other inclusion levels. Storage temperature affected ($p<0.01$) all parameters, which explained the spermatozoa quality of extended goat semen. The parameters which explained the motility and viability of spermatozoa decreased when storage temperature increased. Most rates for TM (97%), PM (81%), RAP (79%), MED (17%) and live spermatozoa (75%) were for semen stored at 5 ˚C and the least of 12% TM, 4% PM, 1% RAP, 4% MED and 1% live spermatozoa were for semen stored at 24 ^{0}C. Spermatozoa with non-fragmented DNA increased when storage temperature increased, the most of 99% was at 24 ˚C and the least of 95% was at 5 ˚C. The effects of storage temperature on total abnormal spermatozoa had no pattern, and a higher rate of 6% was at 5 ˚C, and a lower rate of 2% was at 17 ˚C and 24 ˚C. Storage time affected ($p<0.01$) all parameters which explained the spermatozoa quality of extended Boer buck semen, though the effects had no pattern (Table 3.1). After 24 h, buck spermatozoa had higher rates for TM (65%), PM (51%), RAP (49%), MED (13%) and live spermatozoa (47%) that differed from the lower rates observed after 48 and 72

h of an average 41% TM, 29% PM, 27% RAP, 9% MED and 25% live spermatozoa. Spermatozoa with non-fragmented DNA were higher after 48 h (98%) than after 24 and 72 h (97%). Storage time had no effect (p>0.05) on the total abnormal spermatozoa.

Table 4. 1: Main effects of extender, storage temperatures and storage times on motility rates of Boer goat spermatozoa

Factor	TM%	PM%	NPM	RAP%	MED%	SLW%	STC%
Extender							
TEY	60.55	44.89^{c}	15.66^{a}	40.39^{c}	14.09^{a}	4.39^{a}	41.11^{a}
TAO_A	61.25	50.35^{b}	10.89^{b}	49.15^{b}	9.09^{b}	2.78^{b}	37.19^{b}
TAO_B	62.18	54.13^{ab}	8.06^{c}	53.12^{a}	6.74^{c}	2.31^{b}	37.84^{b}
TAO_C	63.11	55.94^{a}	7.18^{c}	54.87^{a}	5.55^{c}	2.68^{b}	36.90^{b}
SEM	0.967	1.170	0.641	0.969	0.568	0.202	1.050
Temperature (˚C)							
5	98.76 ^{a}	82.91^{a}	15.85^{a}	81.53^{a}	15.42^{a}	1.77^{b}	1.25^{c}
17	52.50 ^{b}	44.12^{b}	8.68^{b}	42.03^{b}	7.03^{b}	3.59^{a}	46.04^{b}
24	33.77 ^{c}	26.95^{c}	6.82^{b}	24.59^{c}	4.14^{c}	3.77^{a}	67.48^{a}
SEM	0.838	0.010	0.555	0.839	0.492	0.175	0.907
Storage time (hrs)							
0	99.73^{a}	94.85^{a}	4.88^{c}	94.39^{a}	4.90^{c}	0.39^{c}	0.28^{c}
24	65.11^{b}	51.11^{b}	14.01^{a}	48.79^{b}	12.95^{a}	3.34^{b}	34.90^{b}
48	42.64^{c}	29.87^{c}	12.77^{a}	27.95^{c}	9.61^{b}	4.78^{a}	57.45^{a}
72	39.61^{c}	29.48^{c}	10.13^{b}	26.40^{c}	8.00^{b}	3.65^{b}	60.41^{a}
SEM	0.967	1.170	0.641	0.969	0.568	0.202	1.050
Significance							
Extender	ns	**	**	**	**	**	*
Temperature	**	**	**	**	**	**	**
Storage time	**	**	**	**	**	**	**

^{a,b,c} Means with a different superscript in a column differ significantly (P<0.05). TM = total motility, PM = progressive motility, RAP = rapid motility, MED = medium motility, SLW = slow motility, STC = static motility; TEY = tris-egg yolk extender, TAO_A = tris-egg yolk extender with 1% Avocado oil, TAO_B = tris-egg yolk extender with 2% Avocado oil, TAO_C = tris-egg yolk extender with 3% of Avocado oil; SEM = standard error mean; ns = not significant (P >0.05), * = significant (P<0.05), ** = significant (P<0.01).

Table 4.2 presents the effects of interaction amongst the extender, storage temperature and storage time on motility rates (percentage TM, PM, NPM, RAP, MED, SLW, and STC) of Boer goat spermatozoa. The effects of interactions amongst these factors were significant (P>0.05). The spermatozoa of extended fresh semen (with TEY, TAO_A, TAO_B and TAO_C) that were subsequently stored at 5 ˚C, 17 ˚C and 24 ˚C did not differ (P>0.05) in percentage TM, PM and STC. The means for these variables were: TM of 98% at average, PM of (80%) at average and STC being less than 2%. However, the NPM, RAP, MED and SLW differed (P<0.01) extended for fresh extended semen. The NPM and MED were higher for TEY (18.35%) and TAO_A (16.78%) extended semen that were

subsequently stored at 5 ˚C than for semen extended with TAO_C (0.98%) that was subsequently stored at the same temperature, and those subjected to all the extenders that were subsequently stored at 17 ˚C and 24 ˚C. The fresh semen extended with TEY and TAO_A that were subsequently stored at 5˚C had lower RAP% (80%) at average compared to the higher values of 96% at average observed for semen extended with TAO_C that was subsequently stored at the same temperature and those subjected to all extenders that were subsequently stored at 17 ˚C and 24 ˚C. The TM and STC did not differ significantly (P<0. 05) for spermatozoa of all extended semen stored at 5 ˚C for 24, 48 and 72 hours, and their average rates were TM >90% and STC <10% (Table 4.2).

The semen extended with TEY stored at 5 ˚C for 24 and 48 hours had high PM of 65% and 53%, and RAP of 63% and 50% which differed from lower to higher rates observed for the TAOs of 73% - 95%. However, the PM% of 74% and RAP% of 69% was observed for TEY for semen stored at 5 ˚C for 72 hours differed from high rates (83% - 91%) observed for TAO_B and TAO_C that were subjected to the same conditions. The PM% did not differ significantly (p>0.05) between the TAOs for semen stored at 5 ˚C for different periods, but the RAP% did differ between the TAO_A (75%) and higher TAO_C of 94%, for semen stored at 5 ˚C for 48 hours. The NPM for TEY extended semen stored at 5 ˚C were higher for semen stored for 24 hours (35%) and 48 hours (41%) than those for TAOs extended semen stored under the same conditions, of <23%. The semen extended with TEY extender and stored at 5 ˚C had significant difference (p <0.05) higher NPM (35% and 41%) after 24 and 48 hours, respectively. Moreover, the NPM for TEY was higher (25%) than those for TAO_B and TAO_C of ≤10%, at 5 ˚C and 72 hours of storage. In addition, the NPM for TAO_A was higher (22%) than those of TAO_C (<10%) stored at 5 ˚C for 24 and 48 hours. The MED of TEY extended semen stored at 5 ˚C for 24 hours was higher (30%) than those for TAO_B and TAO_C under the same conditions, of 12% at average. Furthermore, the MED of TEY were higher at 5 ˚C for semen stored for 48 hours (36%) and for 72 hours (27%) compared to the low values observed for the TAOs of 21% at average under the same conditions. It was observed that TAO_A had higher MED (21.4%) for semen stored for 24 hours and 20.65% when stored for 48 hours than TAO_C (9.18%) for 24 hours and 5.20 % for 48 hours, at 5

˚C. The SLW for TEY extended semen stored at 5 ˚C were higher only for semen stored for 48 hours (8%) than those for TAOs of 3% at average extended semen stored under the same conditions. At 5 ˚C storage temperature and all storage times, all TAOs had similar rates for SLW.

Table 4.2 also presents the results of extended semen stored at 17 ˚C and 24 ˚C for 24 up to 72 hours. The TAOs had higher TM of 97% at average, PM of 82% at average and RAP of 81% at average than those of TEY (70%, 45% and 28%, respectively) after being stored at 17 ˚C for 24 hours. However, TEY had higher NPM (25%) than TAO_C (9%), and higher MED (30%), SLW (11%) and STC (30%) than those of TAOs of 14, 2 and 2%, respectively, when subjected to the same condition. The TAOs had similar rates for the motility variable at 17 ˚C for 24 hours. All motility variables except STC had rates of 35% at average for all extenders when semen was stored at 17 ˚C for 48 and 72 hours, and when stored at 24 ˚C for all storage times. The highest value of 35% was observed for TEY at 24 ˚C for 48 hours. Overall, these results suggest that semen extended with extenders used in this research will yield acceptable motility parameters (>60% TM and >30% PM) when stored at 5 ˚C for up to 72 hours and when stored at 17 ˚C for 24 hours.

The percentage of TM and STC did not differ ($P<0.05$) for spermatozoa of all extended semen stored at 5 ˚C for 24, 48 and 72 hours, and their average rates were TM >90% and STC <10% (Table 4.2). The semen extended with TEY that was stored at 5 ˚C for 24 and 48 hours had moderate PM of 65% and 53%, respectively and moderate RAP of 63% and 50%, respectively, which differed from higher rates observed for the TAOs of 73% - 95%, for both. The PM% of 74% and RAP% of 69% observed for TEY for semen stored at 5 ˚C for 72 hours differed from high rates (83% - 91%, for both) observed for TAO_B and TAO_C that were subjected to the same conditions. The PM% did not differ between the TAOs for semen stored at 5 ˚C for different periods, but the RAP% did differ between the TAO_A of 75% and the high TAO_C of 94%, for semen stored at 5 ˚C for 48 hours. The NPM for TEY extended semen stored at 5 ˚C was higher for semen stored for 24 hours (35%) and 48 hours (41%) than those for TAOs extended semen stored under the same conditions, of <23%. However, the NPM for TEY was higher (25%) than those for TAO_B and TAO_C of 10% at average, at 5 ˚C and 72 hours of storage.

In addition, the NPM for TAO_A was higher (22%) than those of TAO_C (<10%) stored at 5^0C for 24 and 48 hours. The MED of TEY extended semen stored at 5 ˚C for 24 hours was higher (30%) than those for TAO_B and TAO_C under the same conditions, of 12% at average. Furthermore, the MED of TEY were higher at 5 ˚C for semen stored for 48 hours (36%) and for 72 hours (27%) compared to the low values observed for the TAOs (average of 21%) under the same conditions. We observed that TAO_A had higher MED (21.40%) than that TAO_C (9.18%), at 5˚C for semen stored for 24 and 48 hours. The percentage SLW for TEY extended semen stored at 5˚C were higher only for semen stored for 48 hours (8%) than those for TAOs extended semen stored under the same conditions, of ≤3%.

Table 4. 2: Effects of interactions amongst extenders, storage temperatures and storage times on the motility of Boer goat spermatozoa

Temperature (˚C)	Storage time (hrs)	Extender	TM%	PM%	NPM	RAP%	MED%	SLW%	STC%
Fresh semen	0	TEY	98.53^{a}	80.18^{abcde}	18.35^{cdefgh}	78.78^{bcdef}	17.58^{cdef}	1.88^{bc}	1.50^{d}
		TAO_A	98.98^{a}	82.20^{abcde}	16.78^{cdefghi}	80.78^{bcdef}	16.25^{cdefg}	1.93^{bc}	1.05^{d}
		TAO_B	99.87^{a}	93.80^{abcd}	6.07^{fghijklm}	93.03^{abcd}	6.70^{fghijk}	0.15^{c}	0.13^{d}
		TAO_C	99.96^{a}	98.98^{a}	0.98^{m}	99.80^{a}	1.01^{k}	0.03^{c}	0.06^{d}
Fresh semen		TEY	99.98^{a}	99.80^{a}	0.18^{m}	99.80^{a}	0.18^{k}	0.03^{d}	0.00^{d}
		TAO_A	100.00^{a}	99.38^{ab}	0.63^{lm}	99.25^{a}	0.65^{k}	0.05^{d}	0.03^{d}
		TAO_B	99.98^{a}	99.68^{a}	0.30^{m}	99.65^{a}	0.35^{k}	0.00^{d}	0.00^{d}
		TAO_C	99.83^{a}	97.48^{abc}	2.35^{jklm}	97.20^{ab}	2.55^{ijk}	0.01^{d}	0.18^{d}
Fresh semen		TEY	99.88^{a}	91.43^{abcd}	8.45^{fghijklm}	90.58^{abcd}	9.00^{fghijk}	0.30^{fg}	0.13^{d}
		TAO_A	100.00^{a}	98.88^{ab}	1.13^{lm}	98.78^{a}	1.23^{jk}	-0.00^{g}	0.00^{d}
		TAO_B	99.93^{a}	98.10^{abc}	1.83^{klm}	97.88^{ab}	1.93^{jk}	0.08^{g}	0.08^{d}
		TAO_C	99.85^{a}	97.00^{abc}	2.85^{jklm}	96.75^{ab}	2.83^{jk}	0.02^{g}	0.18^{d}
5	24	TEY	97.58^{a}	65.55^{efg}	32.03^{ab}	63.53^{fg}	29.83^{ab}	4.23^{ab}	2.43^{d}
		TAO_A	97.53^{a}	74.98^{cdef}	22.55^{bcd}	73.70^{def}	21.40^{bcd}	2.45^{bc}	2.43^{d}
		TAO_B	99.30^{a}	87.10^{abcde}	12.20^{cdefghijklm}	86.27^{abcde}	12.00^{defghik}	0.75^{bc}	0.83^{d}
		TAO_C	99.22^{a}	90.08^{abcd}	9.14^{fghijklm}	89.22^{abcde}	9.18^{fghijk}	0.93^{bc}	0.78^{d}
	48	TEY	93.75^{a}	53.15^{fg}	32.03^{ab}	50.33^{g}	35.78^{a}	7.65^{a}	6.23^{d}
		TAO_A	98.30^{a}	76.45^{bcde}	21.85^{bcde}	74.88^{def}	20.65^{bcde}	2.75^{bc}	1.68^{d}
		TAO_B	99.83^{a}	87.27^{abcde}	12.57^{cdefghijklm}	86.27^{abcde}	13.17^{defghij}	0.35^{c}	0.17^{d}
		TAO_C	99.88^{a}	94.86^{abcd}	5.02^{ijklm}	94.38^{abc}	5.20^{ijk}	0.35^{c}	0.12^{d}
	72	TEY	98.75^{a}	73.80^{def}	24.95^{bc}	69.15^{efg}	27.00^{abc}	2.65^{bc}	1.13^{d}
		TAO_A	99.75^{a}	85.28^{abcde}	14.48^{cdefghij}	83.80^{abcde}	15.15^{defghi}	0.73^{bc}	0.28^{d}
		TAO_B	100.00^{a}	90.97^{abcd}	9.03^{defghijklm}	90.30^{abcd}	9.60^{defghijk}	0.03^{c}	0.10^{d}
		TAO_C	99.12^{a}	88.58^{abcd}	10.54^{efghijklm}	87.66^{abcd}	10.24^{efghijk}	1.48^{bc}	0.92^{d}
17	24	TEY	69.80^{b}	44.65^{gh}	25.15^{bc}	28.20^{h}	30.48^{ab}	11.08^{a}	30.28^{c}
		TAO_A	94.63^{a}	75.65^{cdef}	18.98^{cdefg}	74.63^{def}	15.80^{defgh}	4.18^{bcd}	5.38^{d}
		TAO_B	97.85^{a}	78.70^{abcde}	19.15^{cdef}	77.00^{cdef}	18.33^{cdef}	2.60^{cd}	2.13^{d}
		TAO_C	99.80^{a}	91.13^{abcd}	8.68^{fghijklm}	90.23^{abcd}	9.20^{fghijk}	0.40^{d}	0.20^{d}
	48	TEY	7.63^{d}	1.68^{i}	5.95^{hijklm}	0.85^{i}	3.03^{ijk}	3.75^{bcd}	92.40^{a}
		TAO_A	11.40^{d}	4.15^{i}	7.25^{fghijklm}	0.95^{i}	4.05^{ijk}	4.15^{bcd}	69.75^{b}
		TAO_B	10.63^{d}	2.03^{i}	8.60^{fghijklm}	0.68^{i}	4.78^{hijk}	5.15^{bc}	89.38^{ab}
		TAO_C	9.35^{d}	1.83^{i}	7.53^{fghijklm}	0.45^{i}	3.63^{ijk}	5.28^{bc}	90.65^{a}
	72	TEY	7.65^{d}	1.25^{i}	6.40^{ghijklm}	0.33^{i}	3.10^{ijk}	4.18^{bcd}	93.38^{a}
		TAO_A	12.48^{d}	2.70^{i}	9.78^{efghijklm}	1.05^{i}	5.70^{ghijk}	5.73^{bc}	87.55^{ab}
		TAO_B	7.55^{d}	1.75^{i}	5.80^{hijklm}	0.08^{i}	3.00^{ijk}	3.75^{bcd}	92.58^{a}
		TAO_C	16.20^{cd}	4.08^{i}	12.13^{defghijklm}	1.43^{i}	7.75^{fghijk}	7.03^{ab}	83.85^{ab}
24	24	TEY	7.00^{d}	1.23^{i}	5.78^{hijklm}	0.50^{i}	2.38^{ijk}	4.15^{cde}	93.00^{a}
		TAO_A	5.57^{d}	1.05^{i}	4.70^{ijklm}	0.53^{i}	2.18^{ijk}	2.83^{ef}	94.25^{a}
		TAO_B	7.00^{d}	1.40^{i}	5.60^{ijklm}	0.55^{i}	2.95^{ijk}	3.50^{e}	93.00^{a}
		TAO_C	5.95^{d}	1.03^{i}	4.93^{ijklm}	0.40^{i}	2.53^{ijk}	3.00^{e}	94.08^{a}
	48	TEY	34.75^{c}	24.00^{hi}	10.75^{defghijklm}	2.78^{i}	6.05^{ghijk}	6.65^{abc}	85.20^{ab}
		TAO_A	10.43^{d}	2.58^{i}	7.85^{fghijklm}	1.30^{i}	3.83^{ijk}	5.30^{bcde}	89.55^{ab}
		TAO_B	17.33^{cd}	4.18^{i}	13.15^{cdefghijkl}	1.40^{i}	8.20^{fghijk}	7.75^{ab}	82.68^{ab}
		TAO_C	18.40^{cd}	4.38^{i}	14.03^{cdefghijk}	1.18^{i}	8.95^{fghijk}	8.28^{a}	81.63^{ab}
	72	TEY	11.38^{d}	2.00^{i}	9.38^{efghijklm}	0.55^{i}	4.70^{hijk}	6.18^{abcd}	88.60^{ab}
		TAO_A	5.75^{d}	0.98^{i}	4.78^{ijklm}	0.18^{i}	2.25^{ijk}	3.30^{e}	94.30^{a}
		TAO_B	6.75^{d}	1.13^{i}	5.63^{ijklm}	0.30^{i}	2.78^{ijk}	3.68^{de}	93.28^{a}
		TAO_C	10.20^{d}	1.90^{i}	8.30^{fghijklm}	0.50^{i}	4.60^{ijk}	5.08^{cde}	89.83^{ab}
SEM			3.350	4.050	1.280	3.360	1.970	0.701	3.630
Significance									
Extender × Storage time			*	*	*	**	*	**	Ns

Storage time × Temperature	**	**	**	**	**	**	**
Extender × Temperature	**	**	**	**	**	**	*
Extender × Temperature × Storage time	*	**	**	**	**	**	**

[a,b,c,d,e,f,g,h,I,j,k,l,m] Means with a different superscript in a column differ significantly (P<0.05). TM = total motility, PM = progressive motility, RAP = rapid motility, MED = medium motility, SLW = slow motility, STC= static motility; S5 = extended semen subsequently subjected to 5^0C, S17 = extended semen subsequently subjected to 17^0C, S24 = extended semen subsequently subjected to 24^0C; TEY =tris-egg yolk extender, TAO_A =Tris-egg yolk extender with 1% Avocado oil, TAO_B = tris-egg yolk extender with 2% Avocado oil, TAO_C = Tris-egg yolk extender with 3% of Avocado oil; SEM = standard error mean; ns= not significant (p>0.05), * = significant (P<0.05), ** = significant (P<0.01).

The main effects of extender, storage temperature and storage time on morphological parameters (absent tail = AT, twisted neck = TN, coiled tail = CT, and TA = total abnormality) of Boer goat spermatozoa are presented on **Table 4.3**. Extender had significant effects (P<0.01) on the percentage of TN, CT, and TA. The least TN of 0.14% was observed for TEY and was different from values observed for TAO_A, TAO_B, and TAO_C (0.38%, 0.16% and 0.38% respectively). The percentage CT for TAO_A was higher (4.27%) than those of TAO_B and TAO_C. A high percentage of TA were observed for TAO_A (5.35%) and differed from the lower percentages observed for TAO_B and TAO_C. The percentage CT differed between TAO_A, TAO_B, and TAO_C.TAO_A was higher (4.27%) than TAO_C (0.80%).

Table 4.3 shows also that storage temperature had a significant effect (P<0.01) on the percentage of AT, TN, CT, and TA. The AT percentage increased as the temperature increased. Higher total abnormality was found at 5˚C (5.71%) which differed from the percentage TA at 17 ˚C (1.96%) and 24 ˚C (2.54%). No significant difference was detected in the percentage TA amongst storage hours. Storage time had no significant effects (P>0.05) on the percentage of AT, TN, CT, and TA.

Table 4. 3: Main effects of extenders, storage temperatures and storage times on morphological parameters of Boer goat spermatozoa

Factor	AT%	TN%	CT%	TA%
Extender				
TEY	0.75	0.14[a]	2.27[a]	3.16[b]
TAO_A	0.70	0.38[a]	4.27[b]	5.35[a]
TAO_B	0.96	0.16[a]	1.81[b]	2.94[b]
TAO_C	0.93	0.38[a]	0.80[c]	2.11[b]
SEM	0.128	0.077	0.245	0.249
Temperature (˚C)				
5	0.39[b]	0.09[b]	5.23[a]	5.71[a]
17	0.64[b]	0.43[a]	0.89[b]	1.96[b]
24	1.48[a]	0.28[ab]	0.74[b]	2.54[b]
SEM	0.111	0.067	0.213	0.288
Storage time (hrs)				
0	0.78	0.17[a]	2.55	3.50
24	0.91	0.22[a]	1.73	2.87
48	0.63	0.43[a]	2.47	3.53
72	1.02	0.23[a]	2.41	3.66
SEM	0.129	0.077	0.245	0.288
Significance				
Extender	n	ns	**	**
Temperature	**	**	**	**
Storage time	ns	ns	ns	ns

[a,b,c] Means with a different superscript in a column differ significantly (P<0.05). AT= absent tail, TN = twisted neck, CT = coiled tail, TA = total abnormality; TEY =Tris egg yolk extender, TAO_A =tris-egg yolk extender with 1% Avocado oil, TAO_B= tris-egg yolk extender with 2% Avocado oil, TAO_C = tris-egg yolk extender with 3% of Avocado oil; SEM = standard error mean; ns = not significant (p>0.05), * = significant (P<0.05), ** = significant (P<0.01).

The effects of interactions amongst extenders, storage temperatures, and storage times on the morphological parameters of Boer goat spermatozoa are presented on **Table 4.4** below. The impact of these interactions was significant (P<0.05) for TN, CT, and TA. The significant difference (P<0.05) among the extended semen for percentage TN and TA was observed during storage temperature 5˚C. TEY, TAO_B, and TAO_C extended semen for percentage TN had lower percentages (4.82%, 3.48% and 0.37% respectively) than TAO_A (6.60%) at 24 hours. At 48 hours, TEY, TAO_B, and TAO_C had a lower percentage of 3.53% at average than TAO_A (12.44%), and at 72 hours, TEY, TAO_B, and TAO_C had a lower percentage of 2.95% at average than TAO_A (14.97%). Furthermore, during the same storage temperature, the percentage for TA differed significantly amongst extenders. TEY, TAO_B, and TAO_C had a lower percentage of 4.08% at average than TAO_A (12.44%) at 48 hours. TEY, TAO_B, and TAO_C had lower percentages (4.50%, 4.51% and 0.75% respectively) than TAO_A (14.97%) at 72 hours. We observed no significant difference in percentage TN when extenders were stored at 17 ˚C for 24, 48, and 72 hours and stored at 24 ˚C for 24, 48 and 72 hours.

Table 4. 4: Effects of interactions amongst extenders, storage temperatures and storage times on the morphology of Boer goat spermatozoa.

Temperature (˚C)	Storage time (hrs)	Extender	AT%	TN%	CT%	TA%
Fresh semen	0	TEY	0.37	4.84^{cde}	0.25^{bc}	5.46^{cdef}
		TAO_A	0.25	10.30^{ab}	0.38^{bc}	10.92^{abc}
		TAO_B	0.38	5.96^{bcd}	0.24^{bc}	6.57^{cde}
		TAO_C	1.10	0.48^{e}	0.00^{c}	1.57^{def}
Fresh semen		TEY	0.25	2.22^{cde}	0.00^{c}	2.48^{def}
		TAO_A	0.70	0.48^{e}	0.38^{bc}	1.55^{def}
		TAO_B	0.50	0.22^{e}	-0.00^{c}	0.72^{f}
		TAO_C	0.38	1.75^{cde}	0.00^{c}	2.13^{def}
Fresh semen		TEY	0.70	0.48^{e}	0.36^{bc}	1.53^{def}
		TAO_A	1.96	1.85^{cde}	0.12^{bc}	3.93^{def}
		TAO_B	1.49	0.99^{e}	0.00^{c}	2.48^{def}
		TAO_C	1.36	0.99^{e}	0.37^{bc}	2.72^{def}
5	24	TEY	0.13	4.82^{cde}	-0.00^{c}	4.95^{def}
		TAO_A	0.36	6.60^{bc}	0.00^{c}	6.97^{bcd}
		TAO_B	0.87	3.48^{cde}	0.25^{bc}	4.59^{def}
		TAO_C	0.61	0.37^{e}	0.00^{c}	0.98^{ef}
	48	TEY	0.00	6.49^{bc}	-0.00^{c}	6.49^{cde}
		TAO_A	0.00	12.44^{a}	-0.00^{c}	12.44^{ab}
		TAO_B	0.37	3.84^{cde}	0.25^{bc}	4.46^{def}
		TAO_C	0.91	0.25^{e}	0.12^{bc}	1.28^{def}
	72	TEY	-0.00	4.50^{cde}	-0.00^{c}	4.50^{def}
		TAO_A	0.00	14.97^{a}	0.00^{c}	14.97^{a}
		TAO_B	0.24	4.23^{cde}	-0.00^{c}	4.51^{def}
		TAO_C	0.62	0.12^{e}	0.00^{c}	0.75^{f}
17	24	TEY	11.12	0.48^{e}	0.25^{bc}	1.85^{def}
		TAO_A	0.58	0.68^{e}	0.10^{bc}	1.35^{def}
		TAO_B	0.31	0.74^{e}	0.25^{bc}	1.30^{def}
		TAO_C	0.60	0.97^{e}	0.12^{bc}	1.69^{def}
	48	TEY	1.35	0.87^{e}	0.00^{c}	2.22^{def}
		TAO_A	0.75	1.13^{de}	2.38^{a}	4.25^{def}
		TAO_B	0.58	0.36^{e}	0.00^{c}	0.94^{ef}
		TAO_C	0.87	1.24^{de}	1.23^{abc}	3.34^{def}
	72	TEY	1.58	1.22^{de}	0.13^{bc}	2.92^{def}
		TAO_A	0.27	0.49^{e}	0.25^{bc}	1.00^{ef}
		TAO_B	0.25	0.12^{e}	0.25^{bc}	0.61^{f}
		TAO_C	0.13	1.25^{de}	1.58^{ab}	2.96^{def}
24	24	TEY	1.12	0.50^{e}	0.25^{bc}	1.86^{def}
		TAO_A	0.78	0.78^{e}	0.73^{bc}	2.29^{def}
		TAO_B	3.12	0.50^{e}	0.61^{bc}	4.22^{def}
		TAO_C	1.37	0.86^{e}	0.12^{bc}	2.35^{def}
	48	TEY	0.37	0.49^{e}	0.49^{bc}	1.35^{def}
		TAO_A	1.49	1.24^{de}	0.25^{bc}	2.98^{def}
		TAO_B	0.50	0.75^{e}	0.00^{c}	1.24^{def}
		TAO_C	0.37	0.05^{e}	0.50^{bc}	1.37^{def}
	72	TEY	2.00	0.38^{e}	0.00^{bc}	2.37^{def}
		TAO_A	1.25	0.25^{e}	0.00^{c}	1.50^{def}
		TAO_B	2.97	0.50^{e}	0.13^{bc}	3.59^{def}
		TAO_C	2.89	0.85^{e}	0.49^{bc}	4.23^{def}
SEM			0.445	0.266	0.850	0.998
Significance						
Extender × Storage time			ns	*	Ns	ns
Storage time × Temperature			ns	*	**	ns
Extender × Temperature			**	*	**	**

Extender × Temperature × Storage time	ns	**	**	*

[a,b,c] Means with a different superscript in a column differ significantly (P<0.05). S5 = extended semen subsequently subjected to 5˚C, S17 = extended semen subsequently subjected to 17 ˚C, S24 = extended semen subsequently subjected to 24 ˚C; TA = total abnormality, TN = twisted neck, CT = coiled tail, AT = absent tail; TEY = tris-egg yolk extender, TAO_A = tris-egg yolk extender with 1% Avocado oil, TAO_B = tris-egg yolk extender with 2% Avocado oil, TAO_C = tris-egg yolk extender with 3% of avocado oil; SEM = standard error mean; ns = not significant (p>0.05); * = significant (P<0.05), ** = significant (P<0.01).

Table 4.5 presents the main effects of extender, storage temperature, and storage time on the viability (percentage of live and dead) of Boer goat spermatozoa. Extenders had a significant effect (P <0.01) on live and dead spermatozoa. The extender TAO_C had higher live spermatozoa (48%) that differed from the lower of 41% at average observed for TEY, TAO_A, and TAO_B had high dead spermatozoa (59.38%) that differed from a lower percentage of (51,54%) observed for TAO_C. Storage temperature had a significant effect (P<0.01) on live and dead spermatozoa. We observed that live percentage decrease when storage temperature increases, with the most live spermatozoa of 76.84% observed at 5ºC and the least of 14.25% observed at 24 ˚C. There was a significant effect (P<0.01) on live and dead spermatozoa. Live spermatozoa were observed for semen stored for 24 hours (47%) that differed from the higher 73% at 0 hours and the lower percentage of 25% at average for 48 and 72 hours.

Table 4.5 also presents the main effect of extenders, storage temperatures, and storage times on DNA fragmentation (percentage fragmented and non-fragmented) of Boer goat spermatozoa. Extender had a significant effect (P<0.01) on DNA fragmentation spermatozoa. Extender TEY, TAO_A and TAO_B differed from TAO_C with lower non-fragmented of 97.30% at average than TAO_C with 98.49%. Storage temperature had a significant effect (P<0.01) on the DNA fragmentation of spermatozoa. We observed that the non-fragmented spermatozoa percentage increased as the storage temperature increased with a lower percentage of 95.22% observed at 5 ˚C and a higher 99.44% at 24 ˚C. Storage time had a significant effect (P<0.01) on the DNA fragmentation of

spermatozoa. We also observed that non-fragmented spermatozoa percentage was the same at 0, 24, and 72 hours with (97.44%) and 98.07% at 48 hours, like non-fragmentation at 0, 24, and 72 hours. Overall, all extenders performed the same across storage times.

Table 4. 5: Main effects of extenders, storage temperatures and storage times on viability and DNA fragmentation rates of Boer goat spermatozoa

Factor	Viability (%)		DNA fragmentation (%)	
	Live	Dead	**Fragmented**	**Non-fragmented**
Extender				
TEY	41.56^{b}	58.40^{a}	2.77^{a}	97.23^{b}
TAO_A	41.66^{b}	58.46^{a}	2.74^{a}	97.26^{b}
TAO_B	38.94^{b}	61.29^{a}	2.58^{a}	97.42^{b}
TAO_C	48.38^{a}	51.54^{b}	1.51^{b}	98.49^{a}
SEM	0.873	0.910	0.183	0.183
Temperature (°C)				
5	76.84^{a}	23.29^{c}	4.78^{a}	95.22^{a}
17	36.82^{b}	63.26^{b}	1.86^{b}	98.14^{b}
24	14.25^{c}	85.72^{a}	0.56^{c}	99.44^{c}
SEM	0.756	0.788	0.158	0.158
Storage time (hrs)				
0	73.43^{a}	26.47^{c}	2.24^{ab}	97.76^{ab}
24	47.02^{b}	53.06^{b}	2.68^{a}	97.32^{b}
48	25.62^{c}	74.61^{a}	1.93^{b}	98.07^{a}
72	24.47^{c}	75.54^{a}	2.76^{a}	97.24^{b}
SEM	0.873	0.910	0.183	0.183
Significance				
Extender	**	**	**	*
Temperature	**	**	**	**
Storage time	**	**	**	**

^{a,b,c} Means with a different superscript in a column differ significantly (P<0.05).TEY = tris-egg yolk extender, TAO_A = tris-egg yolk extender with 1% avocado oil, TAO_B = tris-egg yolk extender with 2% avocado oil, TAO_C = tris-egg yolk extender with 3% of avocado oil; SEM = standard error mean; ns = not significant (p>0.05); * = significant (P<0.05), ** = significant (P<0.01).

Table 4.6 presents the effects of interactions amongst the extender, storage temperature and storage temperature on the viability of Boer goat spermatozoa. The effect of interaction amongst extender, storage temperature and storage temperature a had significant effect (P<0.01) on the viability (live cells) of spermatozoa on fresh Boer goat semen. Extender TEY had higher live spermatozoa of 87% for extended fresh semen that was subsequently stored at 5^{0}C than those observed for TAO_B of 63%.

Furthermore, TAO_A and TAO_C had higher live spermatozoa of 91% at average than TAO_B under the same conditions. During storage temperature of 5 ˚C, at 24 hours, extender TEY had lower live spermatozoa (79.28%) than those for TAO_C (93.65%). TAO_C had higher live spermatozoa than those observed for TAO_A and TAO_B of 72.20% at average. At 48 hours under the same condition, TEY had lower live spermatozoa (72.67%) than those for TAO_A and TAO_C. However, TAO_B and TAO_C had higher live spermatozoa (70.97% and 93.65% respectively) than those observed for TAO_A (48.49%). Extender TEY at 72 hours under 5 ˚C had lower live spermatozoa (71.77%) than TAO_C (85.10%). TAO_A and TAO_B had lower percentage of 69.01% at average than TAO_C (85.10%). During storage temperature of 17 ˚C extender, TEY had lower live spermatozoa (44.04%) when stored at 17˚C. AT 24 hours than those observed for TAO_A of 75.95%. Furthermore, TAO_A and TAO_C had higher live spermatozoa of 76.58% at average than TAO_B under the same condition. Overall, these results suggest that semen extended (TEY, TAO_A, TAO_B, and TAO_C) used in this research yield moderate to high viability when stored at 5 ˚C for up to 72 hours and decreased viability when stored at 17˚C for 24 hours only. Furthermore, they suggest that extended semen should not be stored for >24 hours at 17 ˚C and should not be stored at 24 ˚C.

The effect of interactions amongst extenders, storage temperatures, and storage times on the DNA fragmentation of Boer goat spermatozoa are presented on **Table 4.6**. Extender, storage temperature, and storage time had a significant effect ($P<0.01$) on the non-fragmented DNA of Boer goat spermatozoa. TEY, TAO_A, TAO_B, and TAO_C showed a significant difference in non-fragmented DNA percentage before they were subjected to 5 ˚C. TEY had a higher percentage (98.25%) than TAO_B (92.13%). TAO_A and TAO_C had a higher percentage of 97.13% at average than those observed in TAO_B (92.13%). A significant difference was observed when extenders were subjected to 24 ˚C at 24 hours. Extender TEY had a lower percentage (90.63%) of non-fragmented DNA than observed in TAO_B. TAO_B and TAO_C had higher non-fragmented DNA percentage of 98.4% at average than observed in TAO_A (92.25%).

Table 4. 6: Effects of interactions amongst extenders, storage temperatures and storages time on the viability and DNA fragmentation of Boer goat spermatozoa

Temperature (˚C)	Storage time (hrs)	Extender	Viability (%)		DNA fragmentation (%)	
			Live	Dead	Fragmented	Non-fragmented
Fresh semen	0	TEY	87.48^{abc}	12.52^{klm}	1.75^{fghi}	98.25^{abcde}
		TAO_A	88.55^{ab}	11.54^{klm}	3.88^{defghi}	95.38^{defghi}
		TAO_B	63.04^{efgh}	37.13^{cdefgh}	7.88^{ab}	92.13^{ij}
		TAO_C	94.25^{a}	4.63^{m}	1.38^{ghi}	98.88^{abcd}
Fresh semen		TEY	81.67^{abcd}	18.33^{ijklm}	1.88^{efghi}	98.13^{abcdef}
		TAO_A	87.88^{abc}	12.38^{klm}	1.75^{fghi}	98.25^{abcde}
		TAO_B	79.08^{abcde}	20.93^{hijklm}	1.13^{ghi}	98.88^{abcd}
		TAO_C	84.04^{abcd}	15.96^{ijklm}	1.88^{efghi}	98.13^{abcdef}
Fresh semen		TEY	49.81^{ghi}	50.19^{bcd}	0.38^{i}	99.63^{ab}
		TAO_A	51.79^{ghi}	48.21^{bcde}	0.25^{i}	99.75^{a}
		TAO_B	60.34^{fghi}	39.67^{bcdefg}	0.38^{i}	99.63^{a}
		TAO_C	53.28^{ghi}	46.73^{bcdef}	0.63^{hi}	99.38^{ab}
5	24	TEY	79.28^{abcd}	20.72^{hijklm}	1.88^{efghi}	98.13^{abcdef}
		TAO_A	73.42^{bcdef}	26.58^{ghijk}	2.13^{efghi}	97.88^{abcdef}
		TAO_B	70.97^{cdef}	29.04^{fghijk}	1.88^{efghi}	98.13^{abcdef}
		TAO_C	93.65^{a}	6.35^{lm}	2.13^{efghi}	97.88^{abcdef}
	48	TEY	72.67^{bcdef}	27.33^{ghijk}	0.88^{ghi}	99.13^{abc}
		TAO_A	48.49^{hi}	51.62^{bc}	0.88^{ghi}	99.13^{abc}
		TAO_B	74.17^{bcdef}	25.83^{ghijk}	0.75^{ghi}	99.25^{abc}
		TAO_C	88.57^{ab}	11.43^{klm}	1.00^{ghi}	99.00^{abcd}
	72	TEY	71.77^{bcdef}	28.23^{ghijk}	7.64^{abc}	92.37^{hij}
		TAO_A	66.87^{defg}	33.39^{defghi}	5.38^{bcdef}	94.63^{efghi}
		TAO_B	71.15^{cdef}	31.35^{efghij}	6.75^{bcd}	93.25^{ghij}
		TAO_C	85.10^{abc}	14.90^{jklm}	4.13^{cdefgh}	95.88^{bcdefgh}
17	24	TEY	44.04^{i}	55.96^{b}	0.50^{hi}	99.50^{ab}
		TAO_A	75.95^{bcdef}	24.81^{ghijk}	0.50^{hi}	99.50^{ab}
		TAO_B	45.56^{i}	54.45^{bc}	0.25^{i}	99.75^{a}
		TAO_C	77.21^{abcdef}	22.96^{ghijk}	0.38^{i}	99.63^{a}
	48	TEY	4.61^{j}	95.40^{a}	5.13^{bcdef}	94.88^{efghi}
		TAO_A	3.34^{j}	96.66^{a}	5.50^{bcde}	94.50^{fghi}
		TAO_B	0.75^{j}	99.25^{a}	4.38^{bcdefg}	95.63^{cdefghi}
		TAO_C	2.00^{j}	98.01^{a}	1.88^{efghi}	98.13^{abcdef}
	72	TEY	0.00^{j}	100.00^{a}	1.38^{ghi}	98.63^{abcd}
		TAO_A	1.75^{j}	98.25^{a}	1.25^{ghi}	98.75^{abcd}
		TAO_B	0.25^{j}	99.76^{a}	1.00^{ghi}	99.00^{abcde}
		TAO_C	0.97^{j}	99.03^{a}	1.00^{ghi}	99.00^{abcde}
24	24	TEY	0.88^{j}	99.13^{a}	9.38^{a}	90.63^{j}
		TAO_A	1.13^{j}	98.88^{a}	7.75^{abc}	92.25^{hij}
		TAO_B	1.13^{j}	98.88^{a}	2.45^{efghi}	97.55^{abcdef}
		TAO_C	1.00^{j}	99.00^{a}	0.75^{ghi}	99.25^{abc}
	48	TEY	0.38^{j}	99.63^{a}	2.13^{efghi}	97.88^{abcdef}
		TAO_A	0.50^{j}	99.50^{a}	1.88^{efghi}	98.13^{abcdef}
		TAO_B	0.63^{j}	99.38^{a}	3.88^{defghi}	96.13^{abcdefg}
		TAO_C	0.38^{j}	99.63^{a}	2.63^{efghi}	97.38^{abcdef}
	72	TEY	6.17^{j}	93.83^{a}	0.38^{i}	99.63^{a}
		TAO_A	0.25^{j}	99.75^{a}	1.00^{ghi}	99.00^{abcd}
		TAO_B	0.20^{j}	99.80^{a}	0.25^{i}	99.75^{a}
		TAO_C	0.13^{j}	99.88^{a}	0.63^{hi}	99.38^{ab}
SEM			3.020	3.150	0.633	0.634
Significance			**	**		
Extender × Storage time			**	**	**	**
Storage time × Temperature			**	**	**	**
Extender × Temperature			**	**	**	**
Extender × Temperature × Storage time			**	**	**	**

$^{a,b,c,d,e,f,g,h,\ i,j,k,l,m}$ Means with a different superscript in a column differ significantly (P<0.05). S5 = extended semen subsequently subjected to 5˚C, S17 = extended semen subsequently subjected to 17 ˚C, S24 = extended semen subsequently subjected to 24˚C; TEY = tris-egg

yolk extender, TAO_A = tris-egg yolk extender with 1% Avocado oil, TAO_B = tris-egg yolk extender with 2% Avocado oil, TAO_C = tris-egg yolk extender with 3% of Avocado oil; SEM = standard error mean; ns= not significant ($p>0.05$); * = significant ($P<0.05$), ** = significant ($P<0.01$).

CHAPTER 5: DISCUSSION

In this study, the effects of adding avocado oil in a Tris-egg yolk extender on sperm quality stored at different storage temperatures for three days were investigated. Boer goat semen was subjected to Tris-egg yolk supplemented with 0% (control), 1%, 2% and 3% avocado oil and stored at 5 ˚C, 17 ˚C and 24 ˚C until 72 hours. The motility, morphology, viability, and DNA fragmentation of the Boer goat spermatozoa were assessed every 24 hours.

5.1. Effects of avocado oil inclusion in Tris-egg yolk extender on the quality of Boer goat spermatozoa

The present study observed that extenders had significant differences ($P<0.05$) in progressive motility (PM), morphology, viability and DNA fragmentation but did not affect total motility (TM). The results showed high PM when 1,2 and 3% of avocado oil were added to Tris-egg yolk. Treatment with 3% of avocado oil had higher PM (56%) than the one with 1% of avocado oil. These findings agree with the study conducted by (Allai *et al*., 2015). The author observed an increase in sperm quality of ram semen when inclusion of 2% or 5% and 1% or 2% of argan oil was added to Tris-egg yolk when stored at 5 ˚C or 15 ˚C. It was reported by (Venegas *et al*., 2011; El Kharrassi *et al*., 2014) that the increase in sperm motility may be caused by the fatty acid content of argan oil, as well as sterols, tocopherols, phenolic compounds, and coenzyme Q10. High total abnormality (TA) was observed in the treatment with 1% of avocado oil and lower percentages in 2% and 3% avocado oil inclusion. These findings contradict the findings observed by (Allai *et al*., 2017) that inclusion of 1% of cactus seed oil in a Tris egg yolk decreased the rate of abnormality in ram sperm quality. The inclusion level of 3% of avocado oil in Tris-egg yolk was found to have a higher percentage of live spermatozoa and higher non-fragmented DNA than the inclusion level of 0,1 and 2% of avocado oil. The findings agree with the study conducted by Allai *et al*. (2017) that when Tris egg yolk was supplemented with the cactus seed oil at 1% and 2%, total and progressive motility and viability did not differ between 24 and 48 hours of preservation at 5°C but the difference was observed when 5% and 10% of the cactus

seed were added to Tris egg yolk to improve sperm quality of the small ruminant. According to Towhidi and Parks (2012), fatty acids increase the viability and progressive motility of bovine sperm.

5.2. Effects of storage temperature on the quality of Boer goat spermatozoa

The storage temperature had a significant difference in PM, TM, TA, viability, and DNA fragmentation of Boer goat spermatozoa. The results showed a decrease in PM, TM, TA, and live spermatozoa when the storage temperature increased. These findings agree with the study conducted by Allai *et al*. (2015) that total motility was observed to be higher when semen was stored at 5 ˚C than when stored at 15 ˚C. Furthermore, Xu *et al*., 2009 observed that TM of goat spermatozoa was higher at 5 ˚C than when kept at 15 ˚C and 20 ˚C. Peterson *et al*. (2007) observed that spermatozoa motile percentage of goat semen stored in liquid form for 72 hours (either at storage temperature 4 ˚C or 18 ˚C) progressively declines over time. Total abnormality was found at 5 ˚C (5.71%) which differed from the percentage TA at 17 ˚C (1.96%) and 24 ˚C (2.54%). Live percentage decrease when storage temperature increases, with the most live spermatozoa of 76.84% observed at 5 ˚C and the least of 14.25% observed at 24˚C. Non-fragmented spermatozoa percentage increased as the storage temperature increased with a lower percentage of 95.22% observed at 5 ˚C and a higher 99.44% at 24 ˚C. According to (Acharya *et al*., 2020; Allai *et al*., 2015; Iusupova *et al*., 2022; O'Hara *et al*., 2010; Xu *et al*., 2009), 4-5 ˚C yield the best TM in goat and rams because it reserves energy by maintaining a low metabolic rate and a rapid decrease in that rate when the temperature increases.

5.3. Effects of storage time on the quality of Boer goat spermatozoa

The storage time had a significant difference in sperm motility (progressive and total motility), viability and DNA fragmentation however did not affect the sperm morphology of Boer goat spermatozoa. Previous studies indicated that as storage duration increased, the quality of cooled ram spermatozoa

drastically decreased (Acharya *et al*., 2020; Sadeghi *et al*., 2020; Allai *et al*., 2017). The authors observed a decrease in total motility between 0 hours and 24 hours when 0%,1%, 5%, and 10% of argan oil concentration when added to the Tris egg yolk (Allai *et al*., 2015) This concurs with the results from present results which showing the decrease of TM and PM when the storage time increased. According to Allai *et al*. (2017), sperm motility was found to remain the same between 24 hours and 72 hours of chilling when cactus seed oil was added to Tris egg yolk (Allai *et al*., 2015). Findings from the present study showed that total motility and progressive motility remained high after 24 hours (48-65%) and lower after 48 hours and 72 hours. Moderate live spermatozoa were observed for semen stored for 24 hours (47%) and differed from the higher 73% at 0 hours and the lower ± 25% at 48 and 72 hours. Non-fragmented spermatozoa percentage was found the same for 72 hours. These differences could be due to the presence of oxygen in the refrigerator which can cause oxidative stress to the spermatozoa and further decreasing their motility.

5.4. Effect of avocado oil inclusion in a Tris egg yolk extender subjected to different storage temperatures (5 ˚C, 17 ˚C and 24 ˚C) for 72 hours on the quality of Boer goat spermatozoa.

There was a significant difference ($P<0.05$) amongst extenders (TEY, TAO_A, TAO_B and TAO_C) at the interaction of extender, storage time and storage temperature for total motility (TM) and NPM stored at either 5 ˚C, 17 ˚C and 24 ˚C for 72 hours. The improvement was observed in TM when semen samples were kept at 17 ˚C for 72 hours. Semen samples supplemented with 1, 2 and 3% of Avocado oil performed better than TEY at 24 hours and performed the same after 24 hours. These findings agree with the study conducted by Allai *et al,* (2017) who reported that TM of ram spermatozoa was improved by adding 1% and 2% cactus oil to tris egg yolk preserved at 5 ˚C for 72 hours. All these extenders show the best results (≥50%) of total motility of sperm cells from 0 hours to 48 hours at 5 ˚C. The total motility was found to be less than the standardized acceptable motility range (<30%) from 24 to 72 hours when kept at 24 ˚C (Ott and Memon, 1980). The addition of 1, 2 and 3% of

Avocado oil improved progressive motility from 48 hours (higher than TEY) at 5 ˚C. When stored at 17 ˚C inclusions of avocado oil used in the present study improved progressive motility compared to TEY at 24 hours. The inclusion of 1% and 2% of argan oil in Tris-egg yolk improved the PM of ram spermatozoa at 15 ˚C after 24 h (Allai *et al*., 2015). Furthermore, it was indicated that 1% and 2% argan oil inclusions improved PM for TEY at 15 ˚C after 48 h. Progressive motility is greatly reduced from 24 hours to 72 hours when stored at 24 ˚C. Findings from the present study reveal that storage temperature and time had a significant effect on sperm motility. Sperm motility percentages of Boer goat semen stored at either 5 ˚C, 17 ˚C or 24 ˚C decreased with time. These results are in line with the findings that were observed by Peterson *et al*. (2007) that where motile spermatozoa stored in liquid form for 72 hours (either at storage temperature 4 ˚C or 18 ˚C) progressively decline overtime. The addition of 2% and 3% Avocado oil to TEY improved the quality of goat spermatozoa by improving their progressive motility without affecting total motility, live and normal spermatozoa at 5 ˚C until after 72 h. These findings are in line with literature showing that adding plant oils to commercial extenders could improve the quality of liquid goat and ram spermatozoa (Allai *et al*., 2015: 2017). It has been observed that the interaction amongst extenders, storage time and storage temperature on the quality of Boer goat spermatozoa have a significant effect ($P< 0.05$) on PM. The improvement of the sperm quality observed could be due to α-tocopherol and carotenoids present in Avocado oil that serves as antioxidants that hinder autoxidation by preventing the initiation and the spread of free radicals by scavenging on the free radicals resulting from oxidative reactions (Woolf *et al*.,2009). Findings from the present study agree with the study conducted by Allai *et al*. (2015) that by adding argan oil Tris-egg yolk extenders the quality of liquid ram sperm could be preserved at 5 ˚C and 15 ˚C for up to 48 hours. Progressive motility recorded greater sperm motility ±42% when supplemented with 1% and 2% of cactus seed oil compared to extenders supplemented with 0% and 5% of cactus seed oil in tris egg yolk during 8, 24, 48, and 72 hours of storage (Allai *et al.,* 2015). PM of the extenders supplemented with 1,2 and 3% Avocado oil showed excellent PM, and this is in line

with Ott and Memon's (1980) findings that the standardized acceptable percentage for progressive motility of goat semen is 30% and it is considered excellent when it is greater than 50%. Results from the current study suggest that semen extended with extenders used in this research yields acceptable motility parameters (>60% TM and >30% PM) when stored at 5 ˚C for up to 72 hours and when stored at 17 ˚C for 24 hours. Although goat semen can be stored in liquid form, it is evident that at 5 ˚C, motility rates are better with time than it is at storage temperature of 17 ˚C and 24 ˚C. Progression motility percentages are better during the first few hours of storage at 17 ˚C and 24 ˚C, extended goat semen's motility is greatly reduced than in 5 ˚C. These findings agree with the study conducted by Allai *et al*. (2015) and (2017) showing that adding plant oils to extenders could improve the quality of liquid goat and ram spermatozoa.

The present study also investigated the effect of different inclusions of avocado oil in Tris-egg yolk extender, storage temperature and storage time on morphological parameters of Boer goat semen. These three factors influenced the sperm morphological parameters of Boer goat semen. Spermatozoa quality of Boer goat performed better when 2% and 3% of avocado oil were added to Tris-egg yolk at 5 ˚C for 72 hours and at 17 ˚C for 24 hours because normal spermatozoa were greater than 90% (Hidalgo *et al*., 2006; Tibary *et al*., 2018). However, adding 1% of Avocado oil to Tris-egg yolk reduced the abnormality of the spermatozoa at 5 ˚C when stored for more than 24 hours. These findings are in line with the study conducted by Allai *et al*. (2017) who reported that adding 1% of cactus seed oil to Tris-egg yolk decreased the abnormal ram spermatozoa for Tris-egg yolk at 5 ˚C after 48 hours. Adding 1% of avocado oil decreased the rate of normal spermatozoa after 24 hours when stored at 5 ˚C whereas the percentage of normal goat spermatozoa at 5 ˚C did not change when stored for 72 hours and at 17 ˚C after 24 hours when adding 2 to 3% Avocado oil to TEY.

The present study reveals that semen extenders used in this research yield high viability when stored at 5 ˚C for up to 72 hours and moderate viability when stored at 17 ˚C for 24 hours only. An inclusion

level of 3% Avocado oil performed better than the inclusion levels of 1%, 2 % and TEY at 24, 48 and 72 hours. At 17 ˚C, high viability was observed when 1% and 3% of Avocado oil than Tris-egg yolk and 2% of Avocado oil when stored up to 48 hours. Live percentage greatly reduced from 48 hours to 72 hours at 24 ˚C. This is in line with the study conducted by Allai *et al*. (2017). During the interaction of extender and cactus seed oil, Allai *et al*. (2017) observed a high percentage of sperm viability in tris egg yolk with 1% and 2% cactus seed oil as compared to tris egg yolk with 5% and 10% of cactus oil when stored at 5 ˚C for 72 hours which also agrees with the study conducted by Allai *et al*. (2017) when liquid storing ram semen. The improvement in sperm viability when adding 1% and 2% of cactus seed oil to Tris-egg yolk from 24 hours when kept at 5 ˚C for 72 hours. This could be due to the fact that cactus seed oil is known to have high levels of antioxidants, such as tocopherols and phenolic compounds, which can scavenge free radicals and reduce oxidative stress in the spermatozoa. Their study observed that 1% of argan oil in Tris-egg yolk extender increased the viability of sperm after 24 and 48 hours of preservation at 5˚C. The sperm quality of the ram could be preserved at 5˚C and 15 ˚C for up to 48 hours of storage due to the addition of argan oil to tris egg yolk extenders. From the present study, the findings suggest that adding 3% Avocado oil to TEY could improve the percentage of live spermatozoa (ranging from 72 - 79% of TEY) of goat at 5 ˚C until 72 h, as this addition had the highest overall numerical increase of about 13% for this parameter. According to Raseona (2015), the DNA integrity of sperm is crucial for the transmission of genetic information to future generations. The DNA integrity has become a significant maker of fertile sperm. It was suggested that sperm DNA integrity is a target marker for the functionality of sperm rather than the sperm parameters such as motility (Love *et al*., 2005). Cold shock triggers DNA damage and deteriorates the functional parameters of sperm, (Gandini *et al*., 2006).

The present results indicate that the effect of different levels of avocado oil stored at different temperatures for 72 hours influenced sperm DNA Fragmentation as there was a significant difference. The improvement of non-fragmented DNA was observed when 3% of Avocado oil was

added to the Tris egg yolk when kept at 5 ˚C from 24 hours. However, at 17 ˚C and 24 ˚C, the DNA fragmentation was not affected when adding 1,2 and 3% of avocado oil to the Tris egg yolk. This agrees with the study conducted by Allai *et al*. (2015). The author found that adding 1% argan oil to Tris-egg yolk improved the DNA membrane integrity of ram spermatozoa. Allai *et al*. (2017) also reported similar results when preserving ram spermatozoa for 72 hours at 5 ˚C when adding cactus seed oil to tris egg yolk extender. The author reported decreased DNA fragmentation when 1% of cactus seed oil was added to the Tris egg yolk extender when ram spermatozoa were preserved at 5 ˚C.

Overall, it has been observed from the present study that adding 2% and 3% avocado oil to Tris-egg yolk improved the quality of goat spermatozoa by improving their progressive motility without affecting total motility, live and normal spermatozoa and their DNA membrane integrity at 5 ˚C until 72 h. These findings are in line with literature showing that adding plant oils to commercial extenders could improve the quality of liquid goat and ram spermatozoa (Allai *et al*., 2015, 2017).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

The results from this study showed that adding 2% and 3% of avocado oil in Tris-egg yolk extender to Boer goat semen and storing it at 5 ˚C until 72 hours improved the quality of Boer goat spermatozoa and adding 1%,2%, and 3% and storing it at 17 ˚C for 24 hours. Therefore, it can be concluded that the addition of avocado oil in a Tris egg yolk extender can safely be used to preserve Boer goat semen and it remains viable for up to 72 hours at 5 ˚C and for only 24 hours at 17 ˚C and 24 ˚C. Any alteration in avocado oil inclusions leads to a drastic decline in sperm motility and viability during higher storage temperatures. However, it is not recommended to preserve Boer goat semen with inclusions of Avocado oil in a tris egg yolk extender at 24 ˚C and not more than 24 hours at 17 ˚C.

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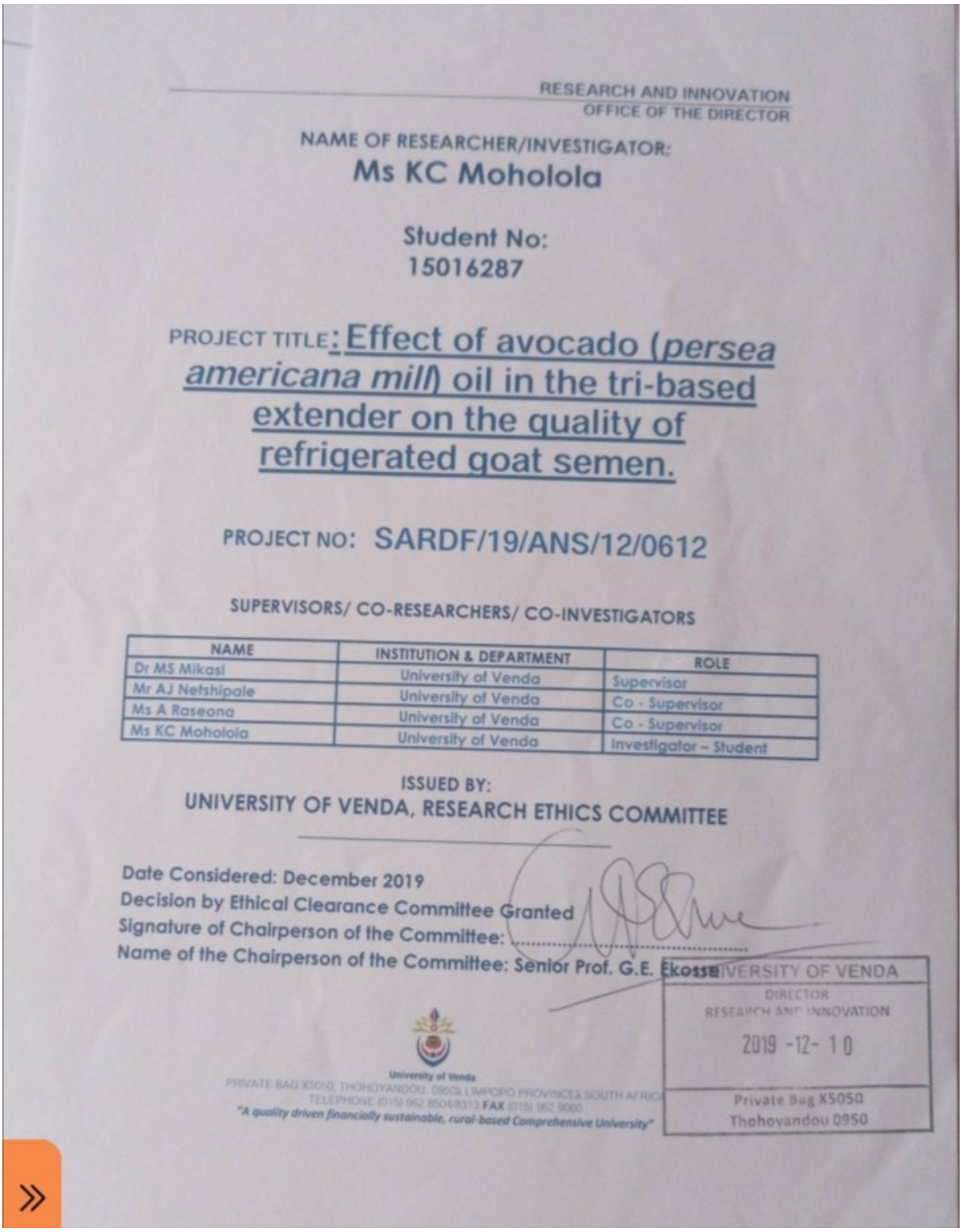

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Student No:
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PROJECT TITLE: Effect of avocado (*persea americana mill*) oil in the tri-based extender on the quality of refrigerated goat semen.

PROJECT NO: SARDF/19/ANS/12/0612

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Effect of Avocado (*Persea americana Mill*) oil inclusion in the Tris-based extender on the quality of Boer buck semen stored at 5 °C, 17 °C and 24 °C

By

Mohololа Khomotso Cathrine

15016287

A dissertation submitted in fulfilment of the degree Master of Science in Agriculture (Animal Science)

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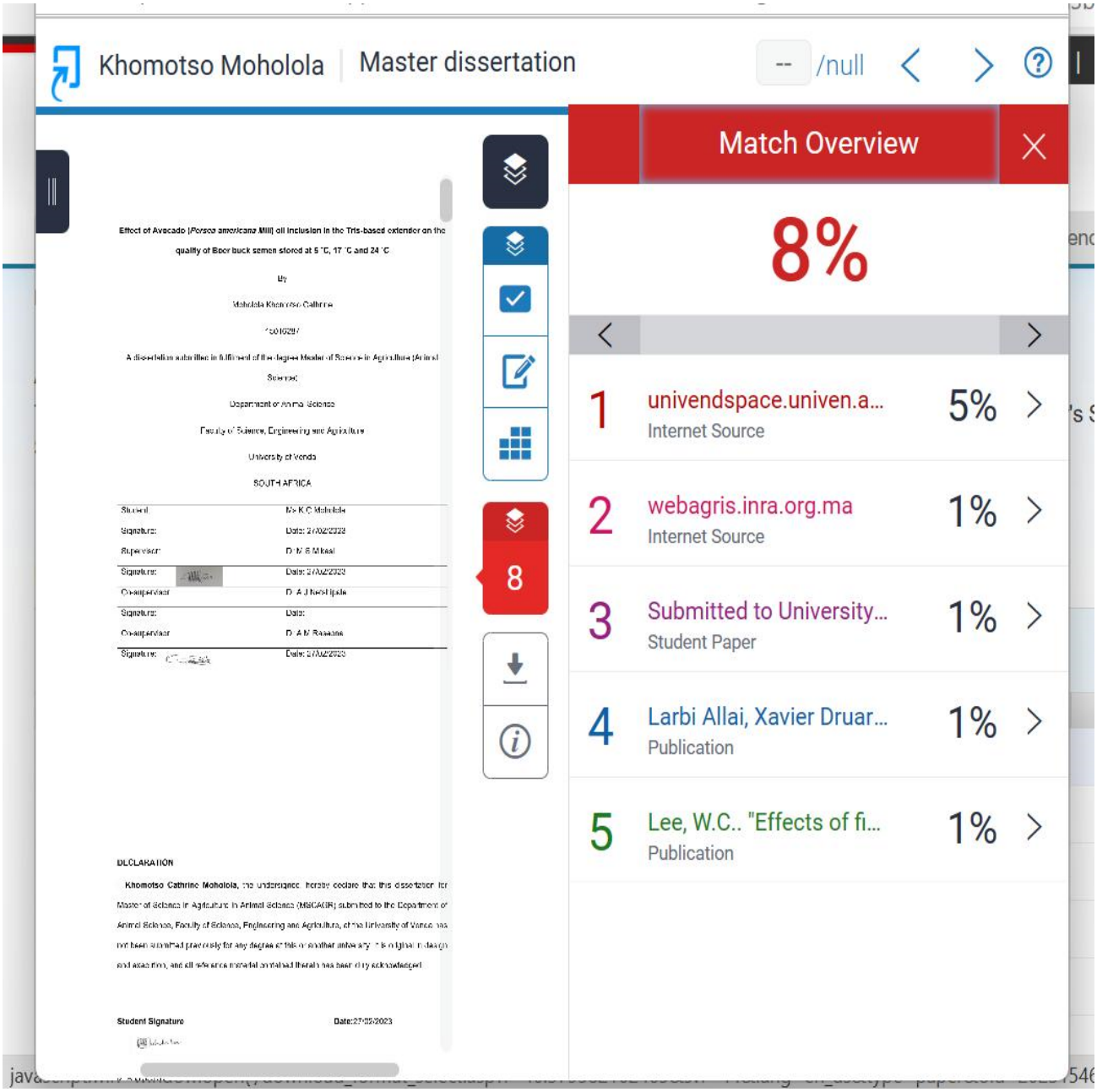

LANUAGE EDITOR LETTER

P.O BOX 663
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Dear Sir/Madam

This is to certify that the dissertation entitled "Effect of Avocado (Persea americana Mill) oil inclusion in the Tris-based extender on the quality of Boer buck semen stored at 5 ˚C, 17 ˚C and 24 ˚C" by Mohololа Khomotso Cathrine, student number 15016287 has been edited and proofread for grammar, spelling, punctuation, overall style and logical flow. The edits were carried out using the "Track changes" feature in MS Word, giving the author final control over whether to accept or reject effected changes prior to submission, provided the changes I recommended are effected to the text, the language is of an acceptable standard.

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

Kind regards

Dr. Hlaviso Motlhaka (BEDSPF-UL, BA Hons-UL, MA-IUP: USA, PhD-WITS, PGDiP-SUN)

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