IMPACT OF AN ARTIFICIAL INSEMINATION PROGRAMME ON REPRODUCTIVE EFFICACY AND THE CROSS-BREEDING OF INDIGENOUS SOUTH AFRICAN GOATS WITH BOER GOATS IN VHEMBE DISTRICT

By

Luvhengo Dakalo Nethengwe

(11571774)

A thesis submitted to the Institute for Rural Development (IRD) in fulfilment of the requirements of the Doctor of Philosophy in Rural Development

Faculty of Science, Agriculture and engineering

University of Venda

South Africa

March 2024

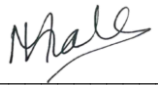
Promoter : **Dr. E. Bhebhe**

Co-promoter : **Prof. M. Manjoro**

Co-promotor : **Prof. F. Fushai**

DECLARATION

I, Luvhengo Dakalo Nethengwe, student number 11571774 the undersigned, hereby declare that the work contained in this thesis is my own original work, and that I have not previously in its entirety or in part submitted it at any university for a degree.

Student	**: L.D Nethengwe**		**26/01/2025**
		Signature	**Date**
Promoter	**: Dr. E. Bhebhe**		30-01-2025
		Signature	**Date**
Co-promoter	**: Prof. M. Manjoro**		**28/01/2025**
		Signature	**Date**
Co-promotor	**: Prof. F. Fushai**		**16/01/2025**
		Signature	**Date**

DEDICATION

This work is dedicated to my parents Mr. M.V Nethengwe and Mrs. F.H Nethengwe, my siblings Mbobvu, Muhassium and Lufuluvhi, my wife Tshianeo, my beloved daughters Zwavhudi and Zwitwaho.

AKNOWLEDGEMENTS

First and foremost, I would like to give my credit to the Almighty God who gave me power, strength, motivation, leadership, direction, and guidance throughout this research work. Without him I could not have achieved it.

My family for their encouragement, patience and support and assistance with many of the financial backing and their continuous love, confidence, and trust.

Professor Daniel Malan Barry for his indispensable guidance and mentorship through many years. I honestly appreciate your support and all the skills and training in assisted reproductive technology (ART).

I would like to thank my supervisory team Prof E Bhebhe, Prof M. Manjoro and Prof F. Fushai, for their profound inputs during the research, and toward production of the final thesis.

I would like to thank the following people from the Centre of Excellence in Animal Assisted reproduction (CEEAR) for their assistance in data collection and analysis: Marubeni Fannie, Mulaudzi Tshimangazo, Bopape Magdelin, Moloto Noko, Netshitomboni Tshimangadzo, Mkhari Xihlovo and my friends Joseph Monandi & Innocent Tshikovhi. My special acknowledgements go to Dr A.M Raseana and Dr M.W Lubisi for their unwavering support.

Abstract

To date, no attempt has been made to establish animal-assisted reproduction centres designed to facilitate controlled goat breeding, from which efficient oestrous synchronization and artificial insemination (AI) protocols can be administered in the communal farming areas of Vhembe district. The first study aimed to determine the socioeconomic dynamics associated with establishing assisted reproduction centres designed and equipped to facilitate effective artificial insemination (AI) service to improve South African indigenous goat production in the Vhembe district. A total of 140 communal goat farmers participated in the study. In-depth one-on-one interviews and focus group discussions were conducted to collect qualitative data. Questionnaires were used to collect quantitative data. Most (89.3 %) farmers accepted AI, and the use of the Boer goat to upgrade the indigenous goat, and to combat inbreeding through AI. Seventy per cent of the respondents showed interest in pen feeding and in separating does from bucks during oestrous synchronization and AI, while the remaining 30% could not afford to pen feed and separate their animals, though they were receptive to being part of the AI program. The farmer disposition to the proposed interventions, and the level of participation justified continuation of the research into animal assisted reproductive technology centres, to address the technical constraints to implementing AI, and advance the socio-economic development. The second study focused on affordable and effective spermatozoa extenders and preservation methods. Experiment 1 aimed to evaluate the motility, velocity, morphology properties and viability of Boer goat sperm stored at 5°C for 168 hours. A total of 48 ejaculates were collected from four Boer goat bucks twice a week for 6 weeks. Only uncontaminated ejaculates containing spermatozoa with >80% progressive motility rate and concentration >1.0 x 10^9 spermatozoa/ml were used. Tris extender-based bovine amniotic fluid of 10, 13 and 18 cm head-tail length fetus (60, 70 and 80 days pregnancy, respectively), denoted (BAF10, BAF13 and BAF18, respectively) were tested. Ejaculated spermatozoa samples were pooled and homogenized before dilution at a ratio of 1:4 with TEY, TBAF10, TBAF13 and TBAF18 extenders. Diluted spermatozoa samples were then stored at 5°C for 168 h and evaluated in a 4 (extenders) X 8 (storage time) factorial experiment replicated 12 times. Total motility was highest in sperm diluted with TBAF18 at 0 h, while progressive motility showed the lowest value in TEY at 96 h. In experiment 2, Boer goat semen was cryopreserved at -196 °C for 7 days in Tris extender-based bovine amniotic fluid and replicated 12 times. The semen samples were loaded into 0.25 ml France straws, sealed, and cooled at 5 °C for 4 h to equilibrate. After equilibration, the straws were frozen in liquid nitrogen (LN_2) vapour. The straw was immersed into LN_2 and kept at -196 °C for 7 days. After thawing at 37 °C for 60 seconds, the spermatozoa were evaluated for spermatozoa motility, viability, and morphology defects in a randomised design. The results revealed that the highest spermatozoa motility was observed with TBAF18

after the cryopreservation effect. No significant difference was observed between TEY and TBAF13 during refrigeration. Meanwhile, this was again observed for the spermatozoa motility parameters evaluated after freezing. In a third study, four animal-assisted reproduction centres were established to facilitate controlled animal breeding, efficient oestrous synchronization and artificial insemination (AI) with fresh/frozen semen extended and cryopreserved following the protocols which were developed in experiments 1 & 2 in the 2nd study. The inseminated does were released into their routine grazing in the communal land until 42 days, after which pregnancy diagnoses were conducted. The objective of this study was to compare the effects of oxytocin, human chorionic gonadotropin (hCG) and prostaglandin F2α (PGF2α) treatments at the time of AI using fresh/frozen semen. Oxytocin, PGF2α and hCG showed a significant effect on improving pregnancy rate. A significant effect (P>0.001) was observed in fresh semen diluted in BAF18, with the highest pregnancy rate. Oestrous ovulation-inducing drugs with frozen semen diluted in BAF13, BAF18 and TEY offered approximately equal pregnancy rates (68.8 %, 62.5% & 62.5 %), but the highest percentage was in BAF13. Therefore, there were acceptable beneficial effects of oxytocin, hCG and $PGF_{2\alpha}$ at the time of AI. In conclusion, animal-assisted reproduction centres established to facilitate controlled animal breeding, efficient oestrous synchronization and AI increased the pregnancy rate in South African indigenous goats in the Vhembe District. This will enable goat production to contribute towards generating household cash income and improve the livelihood of rural farmers.

Keywords: Artificial insemination; Bovine amniotic fluid, Inbreeding, Livelihood

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AH	: Absent head
AT	: Absent tail
AI	: Artificial Insemination
ARC	: Agricultural Research Council
ART	: Assisted reproductive technology
ANOVA	: Analysis of variance
ATP	: Adenosine triphosphate
BAF	: Bovine amniotic fluid
BCS	: Body condition score
BCF	: Beat cross-frequency
BSA	: Bovine serum albumin
BSE	: Breeding soundness examination
BT	: Bent tail
BUSgp60	: Bulbourethral secretion glycoprotein-60
Ca	: Calcium
CASA	: Computer-aided sperm analysis
CI&I	: Continuous improvement and innovation
CIDR	: Controlled internal drug release
CHO	: Chinese hamster ovary
DNA	: Deoxyribonucleic acid
EYCE	: Egg yolk coagulating enzyme
FBS	: Foetal bovine serum
FAO	: Food and national organisation
FSH	: Follicle Stimulating Hormone
GnRH	: Gonadotropin-releasing hormone
hCG	: Human chorionic gonadotropin
IFSS	: Integrated Food Security Strategy
IKS	: Indigenous Knowledge system
IU	International Unity
IVF	: *In vitro* fertilisation
IVM	: *In vitro* maturation
K	: Potassium
LIN	: Linearity
LN_2	: Liquid nitrogen

LPC : Lysophosphatidylcholine
LS : Live sperm
Na : Sodium
NADH : Nicotinamide Adenine Dinucleotide
NAMC : National agricultural marketing council
PC : Phosphatidylcholine
PM : Progressive motility
$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$
RAP : Rapid motility
ROS : Reactive oxygen species
SCA : Sperm class analyser
SCD : Sperm chromatin decondensation
SCSA : Sperm chromatin structure assay
SDF : Sperm DNA fragmentation
STR : Straightness
TCM-199 : Tissue culture medium 199
TEY : Tris egg yolk
VAP : Average path velocity
VCL : Curvilinear velocity
VSL : Straight line velocity
WOB : Wobble

CHAPTER 1: INTRODUCTION

1.1. Background

Livestock production is a major contributor to household food production and food security in rural areas, and there is a vast potential for increased production efficacy and economic activity (De Cock *et al*., 2013). Since 1994, many activities have been aimed at improving livestock production sectors, especially for communal goat farmers in South Africa (Hlomendlin, 2016). However, no interventions are designed to improve the reproductive management of communal goat farming systems in the Vhembe district of Limpopo province, South Africa (Raseona *et al*., 2017). Goats are particularly valuable in developing countries because of their ability to utilize limited grazing and stand unfavorable climate conditions (Mahanjana & Cronje, 2000). Goat meat is one of the most important African foodstuffs and is crucial to food security in many developing countries, including South Africa (Koster, 2004). About 1.3 billion people, including many of the world's poorest, consume goat products as their source of protein, iron and vitamin B12. Approximately 96% of the world's goats are owned by the poor, but the meat sold and consumed by the developed countries due to its health and quality meat (NAMC, 2005); productivity in communal goat production systems can be improved, goat production can be a major income generating sector for communal goat farmers in these countries (Syten, 2016).

Thus, livestock production in rural development does not only involve improving livestock production for its own sake, but rather, enabling rural people to use livestock as a source of survival, well-being and together with all the other activities which make up the livelihood systems of men, women and children who keep these animals (Walters-Bayer & Bayer, 1992). Livestock farming is a mainstay of South African agriculture. However, it is difficult for emerging black farmers to break into markets where a buck costs at least R 2500.00 (Nedambale & Nengovhela, 2011). The lack of new genetic material being introduced into the flocks of communal farmers leads to goat inbreeding, compromising their health and ability to reproduce (ARC, 2014). Given that communal goat farming is a pillar for the livelihoods of men, women and children who keep goats in the Vhembe district (Dara *et al.*, 2016), it is necessary to exercise The Diffusion of Innovation Theory and come up with innovative tools such as AI and measures like animal-assisted reproduction centres designed to facilitate controlled animal breeding that will ensure that all communal goat farmers improve their efficacy (Nethengwe *et al*., 2016). The diffusion of Innovations theory is an assumption or a proposed explanation of an idea outlining how new technological and other advancements

spread throughout societies and cultures, from introduction to widespread adoption (Dearing *et ai.,* 2018). It was developed by Everett Rogers in 1962 and, seeks to explain how and why new ideas and practices are adopted, including why the adoption of new ideas can be spread out over long periods (Green & Senge, 2011). Artificial insemination is the process of mechanical and unnatural depositing of semen into the female reproductive tract to achieve conception (Althouse, 2007). As the most frequently used ART procedure today, AI has had and continues to tremendously impact the various animal breeding industries (Althouse, 2007). This has been successfully achieved through AI in many African countries, and recently, at a research station, AI in Boer goats and Venda indigenous goats was combined with oestrous synchronization and a 78.6% conception rate was achieved (Dara *et al*., 2015). In communal goat farming systems, this can, however, only be achieved by providing an integrated support infrastructure that includes improved animal husbandry, access to markets, technology transfer and appropriate financial support (Red Meat Status Report, 2005). Centralised animal reproductive centres and AI techniques are complementary skills in communal livestock farming, which can provide rural farmers with unique opportunities to benefit progeny from animals with superior genetic makeup (Nethengwe *et al*., 2016). Assisted animal reproductive centre techniques can enable skills development for rural farmers to improve their livestock production and management skills, such as oestrous synchronization, heat detection and timing AI (Nethengwe *et al*., 2016; Delgado *et al*., 1999). Artificial insemination also ensures effectiveness in the use of semen. An increased number of offspring from a superior sire can be produced where AI is employed (Raseona *et al*., 2017). For example, a bull's ejaculate can be sufficient to inseminate 200 to 250 cows when split into doses instead of impregnating one cow (Heise, 2011). Buck ejaculates can also be split into about 15 or more fresh AI doses. Therefore, the overworking of males is prevented, and commercial distribution of superior buck semen is facilitated (Dara *et al*., 2016). Other important aspects are preventing venereal disease transmission, which plays a major role in the economic system of offspring production, and increased safety for valuable breeding animals as mating-related injuries are avoided (Ajao *et al*., 2015). The role of AI in rural areas is to combine adaptability, hardness, disease resistance, and heat tolerance in local goat breeds with high-quality buck semen that has a faster growth rate, higher milk production potential and feed conversion efficiency as well as short inter-kidding period of 234 days between the first and second kids (Barry & Godke 2005; Kebede, 1992).

The major drawback of these technologies is the need for experienced personnel with the appropriate equipment to achieve the desired success (Heise, 2011). The costs involved are most likely prohibitive for rural goat farmers (Munyai, 2012), although it has great potential for producers of breeding stock and propagating animals with outstanding production

characteristics (Kriel, 2016). In the fledgling indigenous goat farming, the recent introduction of the Boer goat is an excellent example (Roets, 2004). The need to apply assisted reproductive technologies to disseminate stock is viable with the support of rural-based universities, the Provincial Government, and the Department of Agriculture and Rural Development-cooperation between stakeholders in rural communities' livelihood and technological innovation unities (Syten, 2016).

1.2. Problem statement

Communal goats in Vhembe district show signs of inbreeding resulting from decades of uncontrolled breeding. The indications include petit body size, slow average daily weight gain, decreased fertility, loss of natural resistance against disease and parasites, and extended kidding intervals. In the communal farming system, female and male animals run together yearly. Typically, one or two male animals stay in a flock of 10-50 animals for up to five or more years, which increases inbreeding. Currently, no interventions are designed to improve reproductive management and genetics in communal goat farming systems.

A considerable volume of research has been published on the reproductive aspects of indigenous breeds of goats in the tropical areas of Africa, including South Africa. Most of the research was performed under controlled conditions at research stations. Consequently, results may not be directly applicable to the communal production systems. To date, no attempt has been made to establish animal-assisted reproduction centres designed to control animal inbreeding and from which efficient estrus synchronization and AI can be administered. Well-equipped animal-assisted reproductive technology centres can address the technical constraints to the implementation of ART, which include semen collection, semen processing, cryopreservation, and AI as well as embryo transfer (ET). The use of refrigerated spermatozoa is currently an area of active research in caprine, ovine, and equine species in response to poor sperm and consequent low fertility rates when sperm is cryopreserved. The latter option has been explored in bovine species but not in the caprine. Therefore, this study aims to establish animal-assisted reproduction centres designed to facilitate controlled goat breeding, from which efficient oestrous synchronization and AI protocols can be administered in the communal farming areas of Vhembe district.

1.3. Justification

First trimester bovine amniotic fluid (BAF) collected sterilely from pregnant cows after slaughter could be a valuable semen dilution to preserve the motility and viability of Boar goat semen for prolonged storage. This is a practical, low-cost alternative to conventional

cryopreservation, especially for farmers who are spread across wide geographic areas from the central service areas. This Is probably because BAF contains the necessary nutrients and growth factors needed for early embryonic development and semen dilution of sheep, goat and cow. Interestingly, due to the inexpensive collection methods and possibly supply/availability of BAF, it is recommended that BAF can replace TEY and many more commercial extenders that keep semen viable for 72 h of storage (Barry, 2005). The transmission of infectious micro-organisms such as viruses should, however, be considered as a possibly risk factor.

The Boer goat is a popular breed which originated from South Africa. it is well known for its excellent body conformation, fast growth rate, high adaptability to various environments and grazing conditions, and its meat is popular among consumers (Lu, 2003). Globally, Boer goat has a strong impact on goat meat industry (Tao et al., 2009). Establishing animal-assisted reproduction could be a valuable strategy that enables the exchange of desired indigenous genotypes within rural communities and facilitates the resource-poor farmer's access to superior exotic genotypes through AI. Improved goat production can significantly enhance the livelihoods of poor communal farmers. Therefore, the project sought to establish several animal-assisted reproduction sub-centres in strategic communal areas of the Vhembe district, to provide hands-on training sessions to emerging red meat producers in heat detection skills, pregnant diagnosis, vaccination programs and feed storage.

1.4.1. Broad objective

The broad objective of the study was to improve the reproductive management of indigenous goats for greater efficiency, and to facilitate the cross breeding of the indigenous goats with improved Boer goats in the communal farming area in the Vhembe district through artificial insemination at organized assisted reproduction centres.

1.4.2. Specific objectives

Specific objectives of the study were to:

(a) Establish pilot-assisted reproduction centres designed and equipped to facilitate effective AI service to improve reproductive management for greater efficiency and cross-breeding of indigenous goats with improved Boer goats in the Vhembe district of Limpopo province.
(b) Determine the efficacy of using fresh refrigerated semen extended using Tris extender-based bovine amniotic fluid as an alternative to frozen semen on doe's fertility measured

as pregnancy rates in an artificial insemination programme for communally managed indigenous goat does in Vhembe district.

(c) Evaluate the efficacy of different ovulation-inducing treatments on pregnancy rate in communally managed indigenous goat does in Vhembe district.

1.4.3. Hypotheses

(a) Assisted reproductive centres will improve the reproduction management and efficiency and facilitate cross breeding of indigenous goats with improved Boer goats in the communal farming area in the Vhembe district using artificial insemination with highly improved genetic buck semen in the Vhembe district.

(b) Fresh, chilled semen extended using tris extender-based bovine amniotic fluid will achieve the same pregnancy rates as frozen semen in synchronized doses in the Vhembe district.

(c) Ovulation-inducing treatments tailor-made for the Vhembe goat production environment will achieve the standard conception rates and, therefore, pregnancy rates when administered 12 hours before the time of artificial insemination.

1.5. Limitations of the Study

The following were limitations to the study:

i. *Economic Feasibility*: A comprehensive cost-benefit analysis was outside the scope of the research, which can provide in-depth insights into the financial sustainability of the proposed AI centres in resource-constrained settings.

ii. *Exclusion of Indigenous Knowledge Systems (IKS):* The study did not incorporate the IKS in relation to the application of AI, which could provide a critical cultural context for improving the adoption of AI technologies.

iii. *Limited Geographical Scope*: The study was restricted to the Vhembe District, which imposed a limit on the generalizability to other communal farming systems with different socio-economic and ecological conditions.

iv. *Short-Term Assessment:* The study evaluated technical innovations over a short period to measure the long-term impacts on goat productivity and on the sustainability of the project.

CHAPTER 2: LITERATURE REVIEW

2.1. The role of South African indigenous goats in promoting food security

Food security defined as when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (Peng & Berry, 2019). Due to the skewed per capita income in South Africa (about 5.8% of the population accounts for over 40% of total meat consumption), indigenous goats are regarded as an important source of protein and income (Baiphethi & Jacobs, 2009). Since a significant section of the South African population does not afford meat regularly and because approximately 63% of all goats (including Boer goats and Angora goats) are in black people's hands, the promotion of the indigenous goat industry should lead to the promotion of food security. Goats are deeply embedded in almost every African culture and are true friends to the poor rural people (Peacock, 2004). Goats can, therefore, play a vital role in ensuring food security for households. Often, it is the only asset possessed by poor households. In times of trouble, such as crop failure or family illnesses, goats can be sold, and food or medicine can be purchased (Roets, 2004).

In 1998, approximately 30% of the South African population was classified as ultra-poor (i.e. those who do not have sufficient food), and of those, approximately 80% are blacks living in rural areas. Due to their small size, adaptive feeding behaviour and low management (supervisory and time) requirements (although a study indicated that blacks regarded goats as unmanageable), goats are a viable option in improving household cash flows for rural people and resolving the issue of food security (Maarse, 2010).

Goat farming could enhance impoverished rural communities and improve primary and secondary industries that rely on goat farming enterprises. In rural, economically deprived regions, goats are a ready source of income, food, and social security (Phillips, 2016). Goats can be kept on one acre of land or even less. It is much easier for small farmers with no land or only little land to farm goats than cattle because 10 goats could be kept in the space of one cattle (Nedambale & Nengovhela, 2011).

2.2. Goat Spermatozoa

Spermatozoa are unique, specialised cells designed to accomplish a single objective: fertilising an ovum (Hafez, 1993). Spermatozoa are formed within the seminiferous tubules of the testes. After production, they are released into the rete testis and enter the epididymis.

During their transportation through the epididymis, spermatozoa obtain fertilising capacity and are stored at the cauda epididymis until ejaculation. The fully formed spermatozoa are elongated cells consisting of a flattened head containing the nucleus and a tail containing the apparatus necessary for cell motility. The main function of spermatozoa is to fertilise oocytes and to spread genetic material distant from where they are produced. Spermatozoa are one of the smallest cells in the body and do not possess energy storage (Varner & Johnson, 2007). Spermatozoa use adenosine triphosphate (ATP) as the basic energy source and require a constant supply for cell function and survival. Energy demands differ depending on spermatozoa activity, such as hyper-activation, acrosome reaction, sperm-oocyte fusion, and motility (Miki, 2007). To meet energy supply demands, spermatozoa rely on an extracellular substrate. Spermatozoa primarily use carbohydrates to meet their energy requirements, and the carbohydrates that are metabolisable cross the plasma membrane using protein transporters (ATP-dependent process). Spermatozoa can produce ATP through oxidative phosphorylation. However, glycolysis is the central metabolic pathway for energy supply (Miki, 2007; Varner & Johnson, 2007). Glucose metabolised through glycolysis produces two molecules of pyruvate (ATP and NADH). Mitochondria then oxidise pyruvate to produce 36 ATP molecules (Miki, 2007). Bull and ram spermatozoa readily metabolise fructose using protein transporters other than the glucose protein transporter (Hiipakka & Hammerstedt, 1978).

2.3. Characteristics and quality of buck semen.

It is commonly accepted that there is a correlation between semen fertility and its measurable properties. The ultimate goal of semen evaluation is to find one or a few parameters to predict the fertilising ability of the spermatozoa. Various methods have been evaluated, but only a few have been adopted for practical work. Most of these studies have used light microscopic evaluations of classical spermatozoa parameters, including sperm concentration, motility, morphology, and viability (Januškauskas & Žilinskas, 2002). The relationship between semen quality and fertility is the concept of compensable and un-compensable sperm quality traits and their interactions with the number of sperm per dose (Salisbury & Van Demark, 1961a; Saacke, 1998).

If the number of sperm per AI dose is increased based on the measurement of a compensable semen trait, fertility will increase. However, the increase in fertility is not proportionate over the entire range of sperm dosages. Compensable semen quality traits are generally believed to be associated with measures of spermatozoa viability (i.e., motility, acrosomal integrity, cell membrane integrity, etc.). Conversely, suppose the number of sperm per AI dose is increased

based on the measurement of an uncompensable semen trait. In that case, there is no increase in fertility with additional sperm beyond that associated with a compensable trait that might be added simultaneously (Januškauskas & Žilinskas, 2002).

2.3. Evaluation of buck semen

Male fertility is an imperative factor in farm animal reproduction because a single male can be used to breed numerous females, especially after the introduction of artificial insemination technology. Assessment of male animal fertility is primarily based on semen evaluation using parameters such as sperm motility, morphology, viability, biochemical estimations of enzyme release, membrane acrosome integrity (Kathiravan *et al.,* 2011) and DNA integrity (Januškauskas & Žilinskas, 2002).

2.4. Spermatozoa motility

Motility is considered the most important characteristic associated with the fertilising ability of spermatozoa, and it is an expression of their viability and structural integrity (Kathiravan *et al.,* 2011). Motility can be divided into quantitative (percentage of sperm cells with progressive motility) and qualitative. Qualitative motility involves several parameters: the speed of the moving sperm cells, altitude of head displacement and movement pattern (circular vs. linear movement, total distance vs. progression, etc.) (Januškauskas & Žilinskas, 2002). The traditional evaluation of spermatozoa motility using light microscopes has been very subjective because of the variability of assessment among observers. To overcome the subjective assessment, a computer-aided sperm analysis (CASA) system offers an objective and rapid approach to assess spermatozoa motility (Malmgren, 1997; Januškauskas & Žilinskas, 2002).

In addition to the total and progressive motility, the CASA system also measures other spermatozoa motion characteristics such as straight-line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), and beat cross frequency (BCF). The CASA system consists of an objective microscope attached to a video camera, a video frame grabber card, and a computer (Mortimer, 2000; Kathiravan *et al.,* 2011). The computer software identifies and follows all the spermatozoa in the video images and performs all the data calculations. The image of the microscope field is sent from the camera and converted into a digital image (Mortimer, 2000).

To perceive spermatozoa easily, the CASA system uses a dark field or a negative high-phase contrast image, which shows white sperm heads on a dark background. The brightness of the head image stays moderately consistent even when the spermatozoa move because the rotation of their heads does not change the intensity of the white image (Mortimer, 2000; Kathiravan *et al.,* 2011). The image of each sperm head is then digitised by the computer, determining the number of picture elements (pixels) the sperm head covers (Mortimer, 2000). In fluorescent optic microscopy, the sperm head is identified by staining it with a fluorescent dye that binds to the sperm DNA (Kathiravan *et al.,* 2011).

2.5. Sperm morphology

Concentration and morphology assessment of sperms is based on the direct relationship between the incidence of abnormal spermatozoa and the type of certain morphological defects with the *in vivo* fertility of the bull (Söderquist *et al.,* 1991). Morphological assessment of spermatozoa is accepted to be a more significant method of differentiating between the semen of high and low fertilizing capacity (Ball & Poters, 2004). Before male animals are used in a progeny testing program or semen preservation, accurate morphological evaluation of the ejaculates is performed to eliminate low fertility potential males, thus contributing to major savings for AI enterprises. There is certainly a correlation between motility and fertility, as well as morphology and fertility, provided there is a wide range of variation in the quality of the parameters assessed and fertility obtained with that semen. However, these correlations are concurrently reduced with an increase in the lower limit set to accept an ejaculate for further processing. When the acceptable range for these parameters is narrow, motility and gross morphology only have limited value for separating ejaculates concerning the expected fertility of the ejaculate (Januškauskas & Žilinskas, 2002).

Evaluation of sperm morphology using light microscopy by the human eye is very subjective, and different technicians often get different results on the same series of smears (Amann, 1981). Using computerized methods focused on evaluating the measurements to quantify and classify sperm morphology correctly offers repeatable and objective methods of assessing sperm head morphometry within and among technicians. Measurements of sperm head variables such as area, length, width, and perimeter have shown potential in the computerized analysis of sperm head morphology (Gravance *et al.,* 1999).

2.6. Sperm DNA Fragmentation

Sperm DNA fragmentation (SDF) is the physical breaking of one or both DNA strands in sperm chromosomes. It occurs when there is no alteration in the bases or a physical break in one or both DNA strands. If this occurs in a gene not repaired for embryo growth, the embryo may die. SDF has been advocated as a male fertility indicator (Bungum *et al.,* 2011). The evaluation of sperm DNA fragmentation is similar to the regular method of semen analysis. Tests used include the Halosperm kit based on the Sperm Chromatin Decondensation (SCD) technique, where approximately 100 sperms are characterised as to whether they have expanded nuclei, with five grades of expansion or no expansion (good sperm), the Tunel assay, the Comet assay, and the Sperm Chromatin Structure Assay (SCSA) (Woodruff, 2012).

The SDF detected in the ejaculate is caused by factors such as oxidative stress, apoptosis, and failures in the histone-protamine replacement (Agarwal *et al.,* 2008; Sakkas *et al.,* 1999). These natural processes observed in semen samples are amplified when and after ejaculation and when the semen samples are manipulated in the laboratory. It has also been proven that temperature fluctuations also affect the rate of SDF (Lopez-Fernandez *et al.,* 2007; Toro *et al.,* 2009).

2.7. Semen Diluents or extenders

The term diluent or extender refers to the aqueous solution that increases the ejaculate volume until the required concentration is reached. This needs to be done while preserving the functional characteristics of the sperm. The spermatozoa are found in the seminal plasma, which provides them with the required nutrients for the high metabolic activity demands of sperm transport through the female genital tract (Gadea, 2003); however, in the ejaculate, this high metabolic activity can only be maintained for a limited period (Lewis, 1911). To preserve spermatozoa for extended periods, their metabolic activity must be reduced by diluting them in an appropriate medium and lowered temperature. The medium should also supply the nutrients needed for the metabolic maintenance of the spermatozoa (Gadea, 2003). The extenders used for semen preservation of domestic animals must have an adequate pH and buffering capacity suitable for osmolarity and should protect sperm cells against cold shock (Salamon & Maxwell, 2000). To prevent microbial growth in stored semen, the diluents are supplemented with antibiotics. The extender should also provide an environment that inhibits the formation of harmful reactive oxygen species (ROS) or lipid peroxide (Orok *et al*., 2010).

Reactive oxygen species damage the sperm membrane and DNA, reducing the motility rate and the spermatozoan's capacity to fuse with the oocyte (Boonsorn *et al*., 2010).

2.8. Types of semen diluents

Diluents can be divided into two groups: those designed for short-term preservation (semen stored in a liquid state) and extenders for long-term semen preservation (semen stored in a frozen state). Short-term diluents are used for short-distance semen insemination, while long-term diluents are generally used in programmes where the semen production site is a long distance away from the insemination site (Gadea, 2003).

Different synthetic (e.g. fructose-tris, glucose-citrate, etc.) and non-synthetic-based extenders (e.g. skim milk) have been tested and recommended for liquid storage of semen in several species (Maxwell & Salamon, 1993). The early diluents for storing bovine semen at refrigerated temperatures were significantly influenced by the discovery of egg yolk as a useful additive, and the primary buffering component in this diluent is phosphate (Philips, 1939). Subsequently, citrate was found to have satisfactory buffering capacity with an improved period of survival of spermatozoa stored at 5^{o}C (Willett & Salisbury, 1942). Citrate became the salt of choice as its chelating properties improved the solubility of protein fractions in egg yolk. Egg yolk addition to skim milk extender improves the quality of spermatozoa during chilled storage (Salamon & Maxwell, 2000). It has also been found that egg yolk can be a detrimental component in synthetic extenders (Colas, 1975), or it is only detrimental at high concentrations, thus suggesting moderate amounts of egg yolk to improve results (Colas *et al*., 1968).

Egg yolk and skimmed milk are the most common cryopreservation diluents for goat semen, but the dilution of goat semen with extenders such as egg yolk or skimmed milk can be detrimental to spermatozoa. The harmful interactions between seminal plasma and egg yolk were first documented by Roy (1957) and with milk by Nunes *et al.* (1982). Egg yolk or skimmed milk extenders are widely used to store small ruminant semen (Salamon & Maxwell, 2000). The presence of enzymes (bulbourethral secretion glycoprotein-60 and egg yolk coagulating enzyme) in the seminal plasma caused damaging interactions between seminal plasma and egg yolk or milk (Nunes *et al.,* 1982; Leboeuf *et al.,* 2000). Bulbourethral secretion glycoprotein-60 (BUSgp60) has a triacylglycerol hydrolyse activity, which reduces sperm motility and movement quality by disrupting the cell membranes (Pellicer-Rubio & Combarnous, 1998). Phospholipase A_2 activity of egg yolk coagulating enzyme (EYCE),

catalyses the hydrolysis of egg yolk phosphatidycholine (PC) into fatty acids and lysophophatidycholine (LPC). LPC has a toxic effect on buck spermatozoa by acting as a detergent on biomembrane, which results in loss of motility, membrane integrity and consequently low fertility rates (Upreti *et al.,* 1999).

Generally, egg yolk has been recognised as an effective agent in semen extenders for sperm protection against cold shock and the lipid-phase transition effect (Aboagla & Terada, 2004). However, the use of chilled-stored semen diluted in an egg-based extender is limited by relatively short-time fertilization capacity (Aurich et al., 1997) and the individual differences in egg yolk due to different periods of egg storage. Removing chicken egg yolk from a semen extender would improve uniformity in the components of semen extenders and eliminate hygienic risks. It is, thus, sensible to develop synthetic semen extenders free of egg yolk. Several commercially available semen extenders containing a substitute for egg yolk like Andromeda® (Aires *et al*., 2003), Biochips plus® (Gill *et al*., 2000) and Bio cell® (Gil *et al*., 2003; Stradaioli *et al*., 2007) have been used for the preservation of ovine, bovine, and caprine semen, as well as maintaining better semen quality. Bovine spermatozoa preservation (Hansen *et al*., 2005; Stradaioli *et al*., 2007) and ram spermatozoa preservation (Gil *et al*., 2003a, b) in soya lecithin-based extender Bio cell® maintained the sperm quality and produced acceptable fertility rates (Gil *et al*., 2003b).

2.9. Temperature and dilution effect

The sperm plasma membrane separates the interior of the cell from the outside environment, and it is selectively permeable to ions and organic molecules. The sperm membrane also controls the movement of substances in and out of the sperm cell (Alberts *et al.,* 2002). These membranes consist of phospholipids bilayer with randomly embedded protein complexes. When these complexes are uninterrupted, the membrane remains in a fluid state. As the temperature is lowered, restriction of lateral movement of membrane phospholipids could result in a transition from a fluid to a gel phase. Once in a gel phase, lipids aggregate, forming micro-domains in the remaining fluid areas of the membrane. The edges of these micro-domains become fragile areas prone to fusion or rupture and more permeable to ions (Hammersted *et al*., 1990).

Spermatozoa are diluted with seminal fluids from the accessory glands at ejaculation, and their motility is retained for a few hours. To lengthen their survival *in vitro,* it is necessary to reduce the metabolic activity by chemical inhibitors or lowering the temperature, which also

requires dilution (Johnson *et al.,* 2000). The relationship between metabolic rate and temperature is often expressed as Q_{10}, which measures the rate increase for each 10°C rise in temperature. For example, if the metabolic rate of an animal at 0°C is x, then at 10°C the rate would be 2x, at 20°C 4x, etc. (ANON., 2012). The metabolic rate of a body cell (including spermatozoa) will thus decrease by a factor of 2x for every 10°C drop in temperature. In addition to a prolonged lifespan, mammalian spermatozoa respond to dilution with an initial increase in activity, followed by a loss of motility and an increase in membrane damage.

Excessive dilution results in a considerable loss of cell viability, especially when using pure electrolyte media (Johnson *et al.,* 2000). A reduction in the concentration of important seminal plasma components may contribute to the effect, which can be eliminated by the addition of albumin. Adding diluents in semen lowers the concentration of certain compounds in the seminal plasma, such as K^+ (Harrison *et al*., 1978) or the plasma proteins, changing sperm viability. These losses must be compensated by adding the necessary ingredients to the diluent's formulations. Also, minimal additions of K^+ may assist in maintaining the motility of spermatozoa since a high concentration of K^+ in the seminal fluid is essential for cell viability (Harrison *et al.,* 1978). Harrison *et al.* (1982) proposed that the dilution effect is due to the absence of proteinaceous motility stimulants from seminal plasma, and it showed that serum albumin could stimulate motility in a reversible manner. Moreover, bovine serum albumin (BSA) stimulated spermatozoan motility during a six-day storage test (Waberski *et al.,* 1989).

Buhr (1990) used BSA in an attempt to overcome the fluidity of the membrane, and consequently, the detrimental effect of lysophospholipids and free fatty acids produced by cooling. The Na^+ and Ca^{++} ions that enter the cell in spermatozoa are withdrawn by active transport. However, Ca^{++} permeability increases significantly at 5°C, accumulating within the cell and reaching toxic levels. Cold shock damage is partly determined by membrane cholesterol content and polyunsaturated fatty acid composition. Cholesterol/phospholipids ration has been revealed to have a key role in membrane fluidity. It was assumed that changes in membrane lipid fluidity could affect trans-membrane movement of Ca^{++}, which is essential in the capacitation process, but, detrimental during storage. However, BSA induced a temporal decrease in membrane fluidity, which was difficult to interpret. BSA improved fertility when semen was stored between 3 and 5 days (Waberski *et al.,* 1994).

2.10. Oestrous Synchronization

Synchronization of oestrous means manipulating the oestrous cycle of the female animals in a herd to allow breeding to occur at approximately the same time. Synchronization protocols use products that mimic natural hormones to alter the timing of oestrous by affecting structures on the ovaries (follicles and corpus luteum [CL]). Oestrous synchronization can be useful in both AI and natural service programs. By synchronizing oestrous, the producer can schedule labour for a concentrated time during the breeding and calving season (Heise, 2011).

For oestrous synchronization protocols to be successful, females must be healthy, on an adequate nutrition plan, and, for some protocols, exhibit oestrous cycles before the protocol begins. This means oestrous and ovulation occur during a normal 21-day (19-day for heifers) oestrous cycle. Some oestrous synchronization protocols can induce an oestrous cycle in anoestrous (non-cycling) females close to initiating an oestrous cycle. However, regardless of the oestrous synchronization program, the protocols are not a substitute for a good nutrition and herd health program (Rasby & Funston, 2010). Cows need to be at a body condition score (BCS) of 3 or better (out of 5) during the breeding season and be at least 50 days post-calving to help enhance the success of the synchronization protocol. If there are young, thin, or late calving cows in the herd, it is likely because they are not cycling. Adding a progestin or progesterone such as a CIDR® to the protocol will assist in jump-starting some of these non-cycling cows. However, caution needs to be taken, as the CIDR® or other progestin are not the “cure-all” for thin, young, and late calving cows, and one should consider whether it is cost-effective to synchronize these cows (Salverson, 2012). In the herd, non-pregnant cows or replacement heifers (12 to 16 months of age) will be at various stages of their 21-day oestrous cycle or in anoestrous. Under normal conditions, about 5 percent of the cycling females will be in oestrous on any given day. Cyclic females may be grouped into one of the three categories based on structures in their ovaries (Rasby & Funston, 2010). The first group consists of females with an active corpus luteum present. It includes females that are somewhere between day 6 and day 17 of the oestrous cycle. About 60 percent of cycling females will have an active CL in their ovaries. The remainder of the cyclic females will either develop a new CL (day 1 to 5) or regress a CL (day 18 to 21). Therefore, there will also be a group of females that are in anoestrous.

During the oestrous cycle, two to four waves of follicles will grow and regress in the ovary until one emerges as the dominant follicle from which the ova will be ovulated. This will occur when the progesterone secreted by the CL is regressing due to the death of the CL. Two hormones are produced in the ovulation process: estrogen and progesterone. The primary hormone produced by the follicle is estrogen. Progesterone is produced by CL in the ovary (Johnson *et al.,* 2010). Drugs have been developed to combat oestrus detection and minimize the labour

involvement in goat AI to synchronize oestrous cycles in females. In this manner, several females can be given the proper treatment to induce ovulation simultaneously (Cole & Garrett, 1980).

The majority of oestrous synchronization programs use one or a combination of the two basic methods that work with the physiology of the doe's normal oestrous cycle: prostaglandin ($PGF_{2\alpha}$) injections that cause CL regression and oestrous in 1 to 5 days after the injection unless the cow or heifer is in the first 4 to 5 days of her oestrous cycle when her CL is not responsive to $PGF_{2\alpha}$ (Rodning *et al*., 2012). The administration of PG alone is commonly used to synchronize an ovulatory oestrous in oestrous cycling. However, this method is ineffective in anoestrous females, and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in the onset of oestrous during the synchronized +period (Johnson *et al.*, 2010).

The progesterone or progestins, released from a controlled internal drug release inserts (EAZIBREED CIDR®) or ingested in feed by feeding melengesterol acetate (MGA®), mimic the effects of the doe's natural progesterone by preventing oestrous from occurring if they are present in the body. Once removed, the cow or doe typically enters oestrous in 1 to 3 days. However, a heifer is sub-fertile during the first oestrous following MGA treatment due to ovulation of an older oocyte, so the heifer should be bred on a subsequent, synchronized oestrous. Gonadotropin-releasing hormone (GnRH) injections promote and synchronize follicle growth and induce ovulation. A GnRH injection administered approximately 48 hours after a prostaglandin injection provides a more concise synchrony of ovulation (Rodning *et al.*, 2012).

2.11. Timing of Artificial Insemination for Maximum Conception

The timing of artificial insemination (TAI) is based on observed standing oestrous, and the actual times will vary depending on the length of standing oestrous. However, the goal is to inseminate near the end of the oestrous (Kasimanickam, 2008). Does ovulate approximately 4 to 16 hours after the end of standing oestrous. Inseminating near the end of oestrous provides time for the sperms to undergo capacitation, which gives sperms the ability to fertilize before they encounter the egg (Taminaude Agricultural University, 2008). It is generally better to have the sperms waiting for the ovum rather than the ovum waiting for the sperms because it has a shorter lifespan (Rodning *et al.,* 2012).

Some things to consider when timing AI include good oestrus detection, which is crucial for a successful AI. Observe standing oestrous for at least 30 minutes twice a day. Early morning, late afternoon, and evening are the best times for oestrus detection. Maximum conception rates for AI occur when animals are bred near the end of standing oestrous. Traditional AI has therefore followed the AM/PM rule. When an animal is first observed in oestrous in the AM, it should be inseminated during the PM of that day. When an animal is first observed in the PM oestrous, it should be inseminated in the next AM (Rodning *et al.,* 2012).

Some producers may consider using timed AI, where insemination occurs at a predetermined time, following an appropriate synchronization program. Timed AI allows for a more regimented schedule. Recent improvements in timed AI and oestrous synchronization protocols have increased pregnancy rates, allowing easier labour resource scheduling and fewer cattle handling. The need for oestrus detection can be eliminated if a timed AI program is used with oestrous synchronization. Another method is to combine AI and natural service with cleanup bulls to boost overall pregnancy rates and reduce the amount of oestrus detection and drug expense. Commonly, oestrous synchronization and AI are used for one AI service, and then clean-up bulls are turned out for the remainder of the breeding season (Rodning *et al.,* 2012).

2.12. Ovulation Inducing Drugs

The Prostaglandins (PG_S) was first isolated from accessory male sex gland fluids, were termed prostaglandins because of their association with the prostate gland. Almost all body tissues secrete them. The prostaglandins, derived from arachidonic acid, are short-acting. Some forms never appear in the blood, whereas others are degraded after circulating throughout the liver and lungs. $PGF_{2\alpha}$ is the natural luteolytic agent that terminates the luteal phase (corpus luteum) of the oestrous cycle and allows for initiating a new oestrous cycle without fertilization. $PGF_{2\alpha}$ is particularly potent in terminating early pregnancy (Reeves, 1987). PGs are found in the form of at least 6 parent compounds that induce various pharmacologic responses (Hafez, 1993).

All PGs are 20-carbon unsaturated hydroxyl fatty acids with a clopentane ring (cyclopentano perhydro phenanthrene ring structure). They are divided into PGE, PGF, PGA and PGB groups. Arachidonic acid, an essential fatty acid, is the precursor for PG most closely associated with reproduction, mainly prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2). PGs control blood pressure, lipolysis, gastric secretion, blood clotting, and renal and

respiratory function. Blood levels of PG's are generally low but are elevated in certain conditions such as parturition (DeSilva & Reeves, 1985; Murdock & Dunn, 1983). PGs are involved in ovulation in ewes and cows when administering indomethacin, an inhibitor of prostaglandin synthesis, blocks ovulation. LH release is unaffected, so PG's action and synthesis are at the ovarian follicle level involving $PGF_{2\alpha}$ and/ or $PGE_{2\alpha}$.

$PGE_{2\alpha}$ stimulates contraction of the uterus and dilates blood vessels but has no luteolytic action. $PGF_{2\alpha}$ stimulates the contraction of the uterus, aids in sperm transport in the male and female, causes constriction of blood vessels, and has luteolytic properties. Venoconstrictive effects of $PGF_{2\alpha}$ induce hypoxia, which in turn leads to luteolysis. If the uterus is removed, the CL will not regress for at least the length of the respective gestation. The mechanism by which $PGF_{2\alpha}$ transports from the uterus to the ovary is that PGE2α passes directly through the walls of the utero-ovarian vein into the ovarian artery and directly to the CL (McCracken, 1980). An increase in estrogen, which increases myometrium growth in the uterus, stimulates $PGF_{2\alpha}$ synthesis and release. If the female animal becomes pregnant, some signal (Btp1) is sent from the embryo to the uterus, preventing $PGF_{2\alpha}$ release, thus allowing CL of the cycle to become CL of pregnancy. $PGF_{2\alpha}$ will not cause regression of CL during its 5 days of life because the CL does contain $PGF_{2\alpha}$ receptors on the luteal cells.

In the sow, it will not cause regression until day 12 of cycle. Various treatments with PG are used to synchronize oestrous for AI. Cows are treated twice at 10-day intervals, after which they will all theoretically exhibit oestrous 3 days after the second injection. PG is used in timed breeding in mares and abortion in cattle. The trade names of these compounds are Lutalyse and Estrumate for the cows and mares. $PGF_{2\alpha}$ is used in treating the infected uterus of dairy cows. The mechanism of action is twofold: (a) Regression of CL, if present; induction of follicle growth and estrogen production; and (b) contraction of the uterus, under the action of estrogen (McCracken, 1980).

Oxytocin and vasopressin are synthesized in the hypothalamus and stored in the neurohypophysis (posterior pituitary). Arginine vasopressin, also called ADH. Lysine vasopressin is secreted in domestic pigs (Hafez, 1993). Oxytocin and ADH are synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and are only stored and released from the neurohypophysis. These hypothalamic hormones (neurohypophyseal hormones) are synthesized together with the carrier proteins called neurophysins. As with other neurosecretions, oxytocin and vasopressin are transported in small vesicles enclosed by a membrane down the neuron's axon. These secretory vesicles flow down into the hypothalamic-hypophyseal nerve axons by axoplasmic streaming. They are stored at the

nerve endings next to the capillary beds in the neurohypophysis until their release into the circulation (Hafez, 1993).

Oxytocin is also produced in the corpus luteum. Thus, oxytocin has two sites of origin: the ovary and the hypothalamus. Oxytocin has several functions: uterine muscle contraction and increased contraction frequency in the oviduct. Oxytocin transports both female and male gametes in the oviduct; it induces females to let down milk after parturition and aids delivery in young animals when the period of labour is extended (Hafez, 1993). Estrogen enhances the responsiveness of smooth muscle to oxytocin. The lactating female becomes conditioned to visual and tactile stimuli associated with suckling or milking. This conditioning induces the release of oxytocin into the circulation, acting on the myoepithelial cells (smooth cells) surrounding the alveoli in the mammary gland, resulting in a milk let-down. Hafez (1993) reports that ovarian oxytocin is involved in luteal function by acting on the endometrium to induce prostaglandin $F_{2\alpha}$ release, which has a luteolytic action (regression of the corpus luteum). The glycoprotein human chorionic gonadotropin (hCG) consists of α-and β-subunits with a molecular weight of 40.000 daltons. The α-subunit has 92 amino acids and 2 carbohydrate chains. The α-subunit of hCG is like the α-subunits of human, porcine, ovine, and bovine LH. The β-subunit has 145 amino acids and 5 carbohydrate chains (Hafez, 1993). Human chronic gonadotropin, synthesized by the syncytiotrophoblastic cells of the placenta of primates, is found in both the blood and urine. It is detected in the urine 8 days after conception by sensitive radio immunoassays (Hafez, 1993).

Because hCG appears early in human pregnancy, detection of hCG in the urine is the basis of immunologic human pregnancy tests. In addition, the LH-like action of hCG has made it the hormone for treating cystic ovaries in cattle. The hCG treatment of a cow with cystic ovaries usually requires 5,000 to 10,000 IU of hCG, after which the cystic follicle will either ovulate and forms CL or more often, luteinizes. In either case, the luteal structure produces progesterone, and CL is functional for 20 days and regresses normally, allowing the cows to cycle 21 days after treatment. She is expected to breed almost as successfully as a non-cystic cow at that time. Certain malignancies, such as chorioepithelioma and hydatiform moles in women, are associated with high levels of urinary gonadotropins (Hafez, 1993).

1.3. The Diffusion of Innovation Theory

According to Kaminski (2011), diffusion of innovation refers to the process of adopting a new idea, product, practice, philosophy, and so on. Kaminski (2011) cited Rogers (1957), who stated that there are core elements of the Diffusion of innovation theory: innovation,

communication, time, and social system. The Diffusion of innovation theory involves innovation, a new idea or practice, such as semen collection, extension and artificial insemination technology for goats; it involves communication means, which are the channels of sharing information about the invention, such as farmer workshops. It includes time, the period the adoption spreads, and the social system, which capes the community, such as rural farmers.

According to Rogers (1957), Diffusion of innovations has key attributes that influence the adoption of a new idea. He believed that relative advantage is the first crucial aspect in influencing the adoption of an innovative product. Relative advantage is viewed as the perceived benefits of the innovation over current practices, such as higher kidding rates, which may increase productivity and food security. Secondly, compatibility involves understanding the alignment of existing values, experiences, cultures, and norms. For example, does artificial insemination fit with traditional livestock farming methods? Thirdly, complexity, is the simplicity of understanding and using the product or technology. For example, in this case, how accessible and straightforward AI procedures are for farmers to sue. In his ideas, Rogers explained that Triability is one aspect that may influence the adoption of AI. Triability is the ability to test innovation on a limited scale, in this case, do a pilot project for a few goats. Lastly, he mentioned observability, as the visibility of the results or outcome to others, such as healthier and more productive goats in the community, which may influence the community to adopt this new innovative idea after being convinced by the results.

1.3.1. Rogers distinguishes five categories of adopters of innovation.

- **Innovators** are the vision holders, risk-takers, and the first to try new ideas. They are the pioneers in the community, the ones who first experimented with AI for goats. Often well-educated and always willing to take risks, they inspire others with their pioneering spirit.
- **Early adopters:** These individuals want to try new technologies and establish societal utility. They are the opinion leaders who adopt the innovation early and share their positive experiences, influencing others to follow suit. Their influence is a powerful force in the Diffusion of innovations.
- **Early majority:** These individuals pave the way for innovation within mainstream society. They are part of the general population, and their deliberate assessment of the innovation's success before committing is of utmost importance in the Diffusion of innovations.

- **Late majority:** People who follow the early majority into adopting innovation as part of their daily life and are also part of the general population. They are more sceptical and adopt AI only when it becomes a norm in the community.
- **Laggards:** People lag the general population in adopting innovative products and new ideas. They may adopt only when it becomes unavoidable due to economic or social pressure.

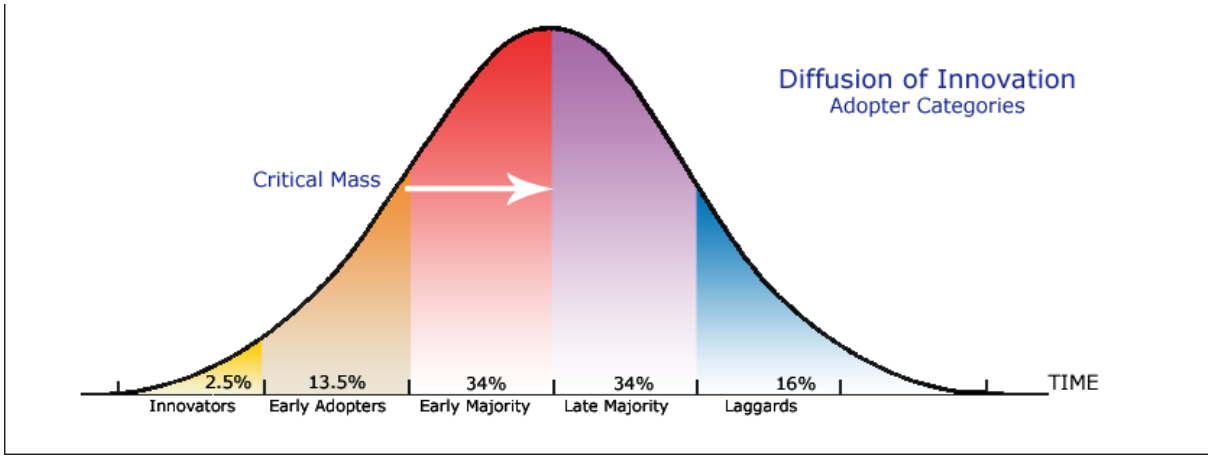


Figure 1.1: **Five categories of innovation adopters**

In conclusion, Diffusion of innovation is a relevant theory that can guide the study or ideas around Artificial insemination for goats in rural areas. This theory seeks to understand how new ideas, technology, or inventions can be communicated in the community, thus taking care of community participation and social and cultural practices. Thus, it discusses five categories of adopters and core elements of Diffusion of the innovation as explained above**.** This theory described the spread effect in the hierarchy of development. It is believed that development in specialized designated areas (poles) will spread benefits to peripheral districts. The theory assumes that growth does not appear everywhere at the same time, but it manifests itself in “points” or “poles” of growth with variable intensity and spreads through different channels with variable terminal effects on the whole of the economy (Perroux, 1950). In this case, this study cannot apply AI in the whole province of Limpopo at the sometime. Thus, the beneficial “spread effects” from growth poles would eventually induce development in the remaining peripheral areas. Also, its geographical base will bring about structural change in other places.

Reference

Aboagla, E.M. and Terada, T., (2004). Effects of egg yolk during the freezing step preservation on the viability of goat spermatozoa. *Theriogenology* 62, 1160 – 1172.

Agarwal, A., Makker, K. and Sharma, R., (2008). Clinical relevance of oxidative stress in male factor infertility: an update. *Anim. J. Reprod. Immunol.* 59, 2 – 11.

Aires, V.A., Hinsch, K.D., Mueller-Schloesser, R., Bogner, K., Mueller-Scloedder, S., and Hinsch, E., (2003). In Vitro and In Vivo comparison of egg yolk-based soyabean lecithin-based extenders for cryopreservation of bovine semen. *Theriogenology* 6, 79-269.

Ajao, A.O., Benyi, K., and Barry, D.M., (2015) Evaluation of the effectiveness of different extenders of goat bucks' semen under refrigerated condition. Masters. Dissertation, University of Venda, South Africa.

Alberts, B., Johnson, A., and Lewis. J., (2002). *Molecular Biology of the Cell* (4th Ed.). New York: Garland Science.

Althouse, G., (2007). Artificial insemination. *Comparative reproductive biology*. (1st Ed). Iowa: Black well publishing.

Amann, R.P., (1981). A critical review of methods for evaluation of spermatogenesis from seminal characteristics. *J. Androl.* 2, 37 – 58.

ANON. (2012). Cornell introductory biology, individualized instruction. BIOG 1105 – 1106.

ARC. (2014). Mobile lab does cow a service. SARAH WILD: SCI-TECH.

Aurich, J.E., Schonherr, U., Hoppe, H., and Aurich, C., (1997). Effects of antioxidants on.

Ball, P.J.H. and Poters, A.R. (2004). Reproduction in cattle. 3rd ed. Blackwell Publishing, Ltd. ISBN 978-1-4051-1545-2.

Barry, D.M., and Godke, R.A., (2001). The Boer goat the potential for cross breeding. Department of animal science, Louisiana University, Boton Ronge, Louisiana. 708008. www.boer goats.com/clean/articles/godke.

Boonsorn, T., Kongbuntad, W., Narkkong, N., and Aengwanich, W., (2010). Effects of catechin addition to extender on sperm quality and lipid peroxidation in Boer semen. *J. Agric. Environ. Sci.* 7 (5), 283 – 288.

Buhr, M.M., (1990). Preservation of Boer sperm alters membrane molecular dynamics. Reproduction in domestic animals. 31(1), 147-152. http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0531.1995.tb00018

Bungum, M., Bungum, L. and Giwercman, A., (2011). Sperm chromatin structure assay catechin additio to extender on sperm quality and lipid peroxidation in Boer semen. *J. Agric. Environ. Sci.* 7, 283 – 288.

Bungum, M., Bungum, L., and Giwercman, A., (2011). Sperm chromatin structure assay (SCSA): A tool in diagnosis and treatment of infertility. *Asian J. Androl*. 13, 69 – 75

Colas, G., (1975). Effect of initial freezing temperature, addition of glycerol and dilution on the survival and fertilizing ability of deep-frozen ram semen. *J. Reprod. Fertil.* 42, 277 – 285.

Colas, G., Dauzier, I., Courot, M., Ortavant, R., Signoret, J.P., (1968). Résultants obtenus au cours de l'étude de quelques facteurs importants de l'insémination artificielle ovine. *Ann. Zootech*. (Paris) 17, 47 – 57.

Dara, O.B., Baloyi, J.J. and Barry, D.M., (2015). Comparison of progesterone$PGF_{2\alpha}$ and novel Synchronization GnRH based synchronization protocols in Boer and Venda indigenous goats of South Africa: Vhembe district. Masters. Dissertation, University of Venda. South Africa.

Dearing, J.W. and Cox, J.G. 2018. Diffusion of innovations theory, principles, and practice. *Health affairs*, *37*(2): 183-190.

De Cock N., D'Haese M., Vink, N., van Rooyen C.J., Staelens L., Schonfeldt H.C. and D'Haese, L., (2013). Food security in rural areas of Limpopo province, South Africa. *Food Security.* 5, 269-282.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S., Courboi, C., (1999). Livestock to 2020 – The Next Food Revolution. Food, Agriculture, and the Environment Discussion Paper 28. IFPRI, Washington D.C.

DeSilva, M., & Reeves, J.J., (1985). Ovulation blockage by intrafollicular injection of indomethacin in the cow. *J. Anim. Sci*. 75, 547.

FAO. (2005). Agricultural data. http://faostat.fao.org.

Freeman, H.A., Kaitibie, H.A., Moyo, S., and Perry, P.D., (2008). Livestock, livelihoods and vulnerability in Lesotho, Malawi and Zambia: Designing livestock interventions for emergency situations in Southern Africa. Nairobi, Kenya. Research Report 8. International Livestock Research institution.

Gadea, J., (2003). Semen extenders used in the insemination of swine. *Span. J. Agric.* 1 (3) 17-2

Gil, J., Lundeheim, N., Soderquist, L., Rodriguez-Martinez, H., (2003a). Influence of extended, temperature and addition of glycerol on post-thaw sperm parameters in ram semen. *Theriogenology* 59, 1241 – 1255.

Gil, J., Rodriguez-Irazoqui, M., Lundeheim, N., Soderquist, L., and Rodriguez-Martinez, H. Goat. (2003b) *Nature* (London), 1, 179 – 318.

Gravance, C.G., Garner, D.L., Pitt, C., Vishwanath, R., Sax-Gravance, S.K., and Casey,

Green, J., and Senge, P. 2011. Diffusion of Innovations Theory.

P.J., (1999). Replicate and technician variation associated with computer aided bull sperm head morphometry analysis (ASMA). *Int. J. Androl.* 22, 77 – 82.

Hafez, E.S.E., (1993). Hormones, growth factors, and reproduction. *Reproduction in farm animals.* (6th Ed), 59-93. Lea and Fibiger. Philadelphia.

Hammersted, R.H., Graham, J.K. and Nolan, J.P., (1990). Cryopreservation of mammalian sperm: What we ask them to survive. *J. Androl*. 11, 73 – 88.

Hansen, G.B.B., Morris, I.D., Ersboll, A.K., Greve, T. and Christensen, P., (2005). DNA integrity in sexed bull sperm accessed by neutral Comet assay and sperm chromatin structure assay. *Theriogenology* 63, 1789 – 1802.

Harrison, R.A.P., Dott, H.M., and Foster, G.C., (1978). Effect of ionic strength, serum albumin and macro-molecules on the maintenance of motility and the surface of mammalian spermatozoa in a simple medium. *J. Reprod. Fertil.* 52, 65 – 73.

Harrison, R.A.P., Dott, H.M., and Foster, G.C., (1982). Bovine serum albumin, sperm motility and the dilution effect. *J. Exp. Zool.* 222, 81 – 88.

Heise, A., (2011). Artificial insemination in veterinary science. Faculty of veterinary science of Pretoria South Africa. 2, 18-32. http://cdn.intechopen.com/pdfs/28665/InTech-Artificial_insemination_in_veterinary_science.pdf

Hiipakka, R.A., and Hammerstedt, R.H., (1978). Deoxyglucose transport and phosphorylation by bovine sperm. *Biol. Reprod.* 19, 368 – 379.

Hinsch, E., (2003). *In vitro and in vivo* comparison of egg yolk-based and soya lecithin-based extenders for cryopreservation of bovine semen. *Theriogenology* 60, 269 – 279.

Hlomendlin, H., (2016). South African commercial farmers key to African food security. Farmer's weekly.16050. www.famersweekly.co.za.

Januškauskas, A., and Žilinskas, H., (2002). Bull semen evaluation post-thaw and relation of semen characteristics to bull's fertility. *Vet. Zoot.* 17, 39.

Johnson, L.A., Weitze, K.F., Fiser, P., and Maxwell, W.M.C., (2000). Storage of Boer semen. 62, 143-172. www.elsevier.comrlocateranireprosci.

Kaminski, J. 2011. Diffusion of innovation theory. *Canadian Journal of Nursing Informatics*, *6*(2): 1-6.

Kathiravan, P., Kalatharan, J., Karthikeya, G., Rengarajan K., and Kadirvel, G., (2011). Objective Sperm Motion Analysis to Assess Dairy Bull Fertility Using Computer-Aided System: a review. *Reprod. Dom. Anim.* 46, 165 – 172.

Kebede, B., (1992). Estimation of additive and non-additive genetic effects for growth, milk yield and reproduction traits of crossbred cattle in the wet and dry environment in Ethiopia. PhD Dissertation, New York. Cornell University, Ithaca.

Koster, M., (2004). Goats in South Africa: A significant role player. University of Pretoria.

Kriel, G., (2016). Laparoscopic artificial insemination in sheep. Farmers weekly 16032. www.famersweekly.co.za.

Lamb, G.C, & DiLorenzo, N., (2014). Current and future reproductive technologies and world food production. Eds. New York.

Leboeuf, B., Restall, B., and Salamon, S., (2000). Production and storage of goat semen

for artificial insemination. *Anim. Reprod. Sci.* 62, 113 – 141.

Lewis, L.L., (1911). The viability of reproductive cells. *Agric. Exp.* Stillwater. Oklahoma Agriculture and Mechanical College.

Lopez-Fernandez, C., Crespo, F., Arroyo, F., Fernandez, J.L., Arana, P., Johnston, S.D. and Gosalvez, J., (2007). Dynamics of sperm DNA fragmentation in domestic animals II. The stallion. *Theriogenology* 68 (9), 1240 – 1250.

Maarse, L., (2010). Livestock: A smart solution for food and farming. Farming Matters, 26 (2), 7–12.

Mahanjane, A.M. and Cronje, P.B., (2000). Factors affecting goat production in communal farming system in the Eastern Cape region of South Africa. 30, 149-155. DOI: https://doi.org/10.4314/sajas.v30i2.3864

Malmgren, L. (1997). Assessing the quality of raw semen: a review. *Theriogenology* 48 (4), 523– 530.

Maxwell, W., & Salamon, S., (1993). Liquid storage of ram semen: a review. *Reprod. Fertil. Dev.* 5 (6), 613 – 638

McCracken, J.A, Schramm, W., Okulicz, W.C., (1980) Hormone receptor control of pulsatile secretion of PGF2P from ovine uterus during luteolysis and its abrogation in early pregnancy. Anim Peprod Sci, 7 (1), 31-55

Miki, K., (2007). Energy metabolism and sperm function. *Soci. Reprod. Fertil. Suppl.* 65 (3), 309 – 325.

Mortimer, S.T. (2000). CASA-practical aspects. *J. Androl.* 21 (4), 515 – 524.

Munyai, F.H., (2012). An evaluation of socio-economic and biophysical aspects of small-scale livestock systems based on a case study from Limpopo province: Muduluni village. PhD. Thesis, University of the Free State.

Murdock, W.J. and Dunn, T.G., (1983). Luteal function after ovulation blockage by intra follicular injection of indomethacin in ewes. J. Reprod. Fert. 3 (1) 69,671.

National Agricultural Marketing Council, (2005). Report on the investigation into the potential for the South African goat's industry. https://www.namc.co.za

Nedambale, T.L. and Nengovhela, B., (2011). Upgrading the smallholder beef cattle farming sector in Limpopo and Eastern Cape Province. *Science Technology*. 31 (2), 64-65.

Nethengwe, L.D., Francis, J., and Barry, D.M., (2016). The effect of three ovulation inducing drugs on pregnancy rates of rural cattle after administration of synchronized artificial insemination in Vhembe district. Masters. Dissertation, University of Venda, South Africa.

Nunes, J.F., Corteel, J.M., Combarnous, Y., and Baril, G., (1982). Study of the involvement of seminal plasma constituents in the seasonal variations of goat spermatozoa motility. Dairy Goat Journal. 13 (2), 17

Orok, E.E., Essien, A., Akpet, S.O., Ibom, L.A. and Etop, S.C., (2010). Mean spermconcentration and percent motility of extended porcine semen as affected byantibiotics from selected sources and storage time. *Int. J. Curr. Res*. 10 (3), 001-006.

Peacock, C.A., (2004). Partway out of poverty. *Small Ruminant. Res.* 60, 179-186. DOI: 10.1016/j.smallrumres.2005.06.011

Pellicer-Rubio, M.T. & Combarnous, Y., (1998). Deterioration of goat spermatozoa in skimmed milk-based extenders as a result of oleic acid released by the bulbourethral lipase BUSgp60. *J. Reprod. Fertil.* 112 (4), 95 – 105.

Peng, W and Berry, E.M., (2019). The concept of food security. Elsevier, 2 1-7. DIO: https://doi.org/10.1016/B978-0-08-100596-5.22314-7

Perroux, F. (1950). Economic space: theory and applications. Quarterly Journal of Economics 64: 90-97.

Phillips, L., (2016). The problem within the goat's industry in South Africa. Farmer's weekly16030. Farmers weekly co.za.

Phillips, P.H., (1939). Preservation of bull semen. Phosphorylation by bovine sperm. *Biol. Reprod.* 19 (4), 368 – 379.

Ramsay, K.A., Hlatshayo, M., and Mamabolo, M.J., (2007). The national livestock development strategy. Department of Agriculture. South Africa.

Ramukhithi, F.V., Nedambale, T.L., Sutherland, B., and Lehhloenya, K.L., (2011). Cryopreservation of South African unimproved indigenous and Boer goat semen. *Afr. J. Bio. 10, 17898-17902.*

Rasby, R.J. and Funston. R.N. (2010). Synchronization of oestrous in beef cattle. Institute of Agriculture and Natural Resources. University of Nebraska-lincoln Extension.

Raseona, A.M., Ajao, O.A., Nethengwe, L.D., Madzhie, L.R., Nedambale, L.T and Barry, D.M., (2017). Viability of bull semen extended with commercial semen extender and two culture media stored at 24 °C. S, AFr. J. *Anim. Sci.* 47 (1), 50-55.

Red Meat Status Report (2005). The livestock development strategy (LDS) and its potential impact on the red meat marketing and development in South Africa. *Reprod. Dom. Anim. Suppl.* 9 (1), 81 – 93.

Rodning, S.P., Elmore, M.F., Edmondson, M.A., Gard, J.A and Lovelady, A.S., (2012). Oestrous Synchronization and Artificial Insemination Programs for Beef Cattle. Alabama Cooperative Extension.

Roets, M., (2004). From folklore to feasibility commercialization of South African's indigenous goats. South Africa. PhD. Thesis. University of Pretoria. South Africa.

Roy, A., (1957). Egg-yolk coagulating enzyme in the semen and Cowper's gland of the goat. London. *Nature*, 179 (1), 179 – 318.

Saacke, R.G., (1998). AI fertility: Are we getting the job done? In, Proceedings of the 17th technical conference in artificial Insemination and reproduction national association in animal breeders, Columbia, MO, 6 –1

Sakkas, D., Mariethoz, E., Manicardi, G., Bizzaro, D., and Bianchi, P.G., (1999). Origin of DNA damage in ejaculated human spermatozoa. *U. Rev. Reprod*. 4 (1), 31 – 37

Salamon, S. & Maxwell, W.M.C., (2000). Storage of ram semen. *Anim. Reprod. Sci.* 62 (3), 77 – 111.

Salisbury, G.W., and Van Dermark, N.L., (1961a). Diluents and extension of semen. *Physiology of Reproduction and Artificial Insemination of Cattle* (*Eds*.). Freeman and San Francisco.

Salverson. R. (2012). Synchronization protocol for beef cows. Applied reproductive strategies in beef cattle. *Proc. West. Sec. Amer. Soc*. *Anim. Sci*. 7 (2), 56:409-412.

Soderquist, L., Jansson, L., Larsson, K., and Einarsson, S. (1991). Sperm morphology and fertility in AI bulls. *J. Vet. Medi*. 38 (5), 534 – 43.

Steyn, J. (2016). Why South Africa's goat farmers must get down to business. Farmers weekly 16046. Farmersweekly co.za.

Steyn, J.J., (2012). Application of artificial insemination on commercial sheep and goat production, 9 (2), 1-13

Steyn, O.P.J., (1982). General flock management in mutton sheep. College of agriculture. Glen farming in South Africa. Department of agriculture and fisheries. https://books.google.co.za.

Taminaude Agricultural University. (2008). Artificial Insemination. tnau agritech portal, animal husbandry. www.agritech.tnau.ac.in/ta/animalusbandry/animhuscattleAI.

Tavirimirwa, B., Mwembe, R., Ngulube, B., Banana, N.Y.D., Nyamushamba, G.B., Ncube S. and Nkomboni, D., (2013). Communal cattle production in Zimbabwe. Livestock Research for Rural Development. 25 (12).

Toro, E., Fernandez, S., Colomar, A., Casanovas, A., Alvarez, J.G., Lopez- Teijon, M., and Velilla, E., (2009). Processing of semen can result in increased sperm DNA fragmentation *Fertil. Steril.* 92 (10), 2109 – 2112.

Upreti, G.C., Hall, E.L., Koppens, D., Oliver, J.E., and Vishwanath, R., (1999). Studies on the measurement of phospholipaseA2 (PL A2) and PL A2 inhibitor activities in ram semen. *Anim. Reprod. Sci.* 56 (2), 107 – 121.

Varner, D.D., & Johnson, L., (2007). From a Sperm's Eye View - Revisting Our Perception of this Intriguing Cell. 53th Annual Convention of the American Association of Equine Practitioners. 53 (10), 104 – 177.

Waberski, D., S. Meding, G. Dirksaen, K. F. Weitze, C. Lewiding and R. Hahn. 1994. Fertility of long term-stored boar semen: influence of extender (Androhep and Kiev), storage time and plasma droplets in the semen. *Anim. Reprod. Sci*. 36:145-151.

Waberski, D., Weitze, K.F., Rath, D., and Sallmann, H.P. (1989). Effect of bovine serum albumin and zwitterionic buffers on stored liquid Boer semen. Zuchthygiene. *Anim. Reprod. Sci*. 24 (3): 128-133.

Waters-Bayer, A., & Bayer, W., (1992). The role of livestock in the rural economy. Commission on nomadic people. Nomadic People. 3 (1): 3-18.

Willet, E.L., & Salisbury, G.W. (1942). The effect of various dilutors, cooling rate, temperature of storage, and some other factors on the liveability of spermatozoa in stored samples of bull semen. *Mem. Univ. Agric. Exp. Stn.* 9 (4) 249-251.

Woodruff, L. (2012). Fertility Clinic Testing for Sperm DNA Fragmentation. *Theriogenology* 18 (12); 1838-50

Yacoub, A.N., Gauly, M., Sohnrey, B., and Holtz, W., (2011). Fixed-time deep uterine insemination in $PGF_{2\alpha}$-synchronized goats; *Theriogenology* 76 (9):1730-1735.

CHAPTER 3: SOCIOECONOMIC DYNAMICS ASSOCIATED WITH ESTABLISHING CENTERS FOR DELIVERY OF ARTIFICIAL INSEMINATION SERVICE TO COMMUNAL GOAT FARMERS IN THE VHEMBE DISTRICT

3.1. Abstract

The study aimed to determine the socioeconomic dynamics associated with establishing assisted reproduction centres designed and equipped to facilitate effective artificial insemination (AI) service to improve South African indigenous goat production in the Vhembe district. A total of 140 communal goat farmers were selected from villages to participate in this study. In-depth one-on-one interviews and focus group discussions were conducted to collect qualitative data. Quantitative data was gathered through questionnaires. Cluster sampling was used to select villages within random wards, from which one hundred and forty communal goat farmers participated in this study. Qualitative data was analysed using Thematic Content Analysis (TCA), whereby themes were generated for participants verbatim. Computer software IBM-Statistical Package for Social Sciences (SPSS) Version 25 was used to analyse quantitative data. Respondents indicated that the main reason behind goat production is to supplement insufficient household income, which suggested goat production generates significant cash household income. Respondents valued goats as a trading currency. The gender distribution was slightly skewed towards females (55%). Both male (43.6%) and female (31.6%) farmers with tertiary education strongly agreed that livestock production contributed towards generating income, followed by 29.8% female and 25.6% males with secondary education and 21.1% illiterate females. The largest percentage of farmers (85.7%) indicated cash income as the main reason for keeping goats, while the remaining 14.3% keep goats for household foodstuff (meat and milk). About 66.4% of the communal farmers practised extensive goat farming, while the remaining 33.6% did semi-extensive farming. Most (89.3 %) of farmers primarily accepted AI, premise on the Boer goats as a breed previewed to be valuable to combat inbreeding. Seventy per cent of the respondents showed interest in pen feeding and separating does from bucks during oestrous synchronization and AI, while the remaining 30% could not afford to pen feed and separate their animals, though receptive to being part of the AI program. It is then concluded that research and well-equipped animal assisted reproductive technology centres can address the technical constraints to implementing AI and advance the socio-economic development of communal goat farmers for sustainable rural development and poverty alleviation in the Vhembe district.

Keywords: Artificial insemination; communal goats farming; inbreeding; livelihood.

3.2. Introduction

The world human population is rapidly growing and predicted to reach at least 9.6 billion by 2050 (Omontese, 2018). The rapid population growth will exert massive pressure on food resources. Goats could provide an excellent food source for the growing world population (IFAD, 2016). Goats produce meat (chevon), milk and skin (Abd-Allah *et al.*, 2019). In many rural communities, goats serve as a store of economic value against poverty and are used for cultural purposes (FAO, 2009). Goat farming requires less start-up capital than larger ruminants and can be easily raised on limited land resources (Sibisi, 1981). Many development organizations in African rural areas encourage the rearing of goats to improve the income and nutrition status of resource-poor people (Randolph *et al*., 2007). South Africa has about 6.6 million goats (De Villiers *et al*., 2009). Sixty-three per cent of these are indigenous and owned by rural communal farmers (DAFF, 2013).

Goats are well known for their tolerance to harsh tropical environments but generally exhibit signs of inbreeding resulting from decades of uncontrolled breeding (Kulaksiz & Daskin, 2012). Indications of inbreeding include petit body size, slow average daily weight gain, decreased fertility, and loss of natural resistance to diseases and parasites (Raseona *et al*., 2017). The communal farming system does, and bucks run together throughout the year. Typically, one or two bucks stay in a flock of 10-50 does for up to five or more years (Webb *et al*., 2010), which increases inbreeding. Currently, no interventions are designed to improve reproductive management and goat genetics in communal goat farming systems.

Although considerable research has been conducted and published on the reproductive aspects of indigenous breeds of goats in the tropical areas of Africa (Webb & Mamabolo, 2004), including South Africa (Dara *et al*. 2015), most of the research was performed under controlled conditions, and at research stations (Nethengwe *et al*., 2016). Consequently, results may not directly apply to the communal production systems across South Africa (Kifaro *et al.,* 2007). To date, no attempt has been made to establish animal assisted reproduction centres designed to curb goat inbreeding, from which efficient oestrous synchronization and AI protocols could be administered. Moreover, assisted animal reproduction centres could facilitate effective goats AI service, transfer skills to communal farmers, and enhance sustainable goat reproduction performance to a better socio-economic development in Vhembe district. Meanwhile, these farmers are receptive to being part of AI programmes (Nethengwe *et al.,* 2016).

In goats, artificial insemination using fresh or frozen semen has been used commercially in several countries for many years (Heise, 2011). Although AI has long been established in developed countries in cattle, sheep, and goat reproductive management, it has not been fully transferred into native goat breeds reared in extensive grazing farming systems. Similarly, in South Africa, particularly in the communal farming system, AI in goats has rarely been performed in ecologically marginal rural areas. The commonly cited reasons include access to AI technology, the requisite skills, availability of liquid nitrogen, land/camps to separate does from bucks throughout oestrous synchronization and the perception that AI is suited only to commercial farming (Raseona *et al.,* 2017). However, there might be other constraining factors which may be unique to the study area. The objective of this study was, therefore, to determine the socioeconomic dynamics and goat management practices critical to the establishment of successful assisted reproduction centres designed and equipped to facilitate effective AI services to improve communal South African indigenous goat production in Vhembe district.

3.3. Methodology

3.3. 1. Ethics

This study was carried out under the recommendations, care, and use of animals under the University of Venda Animal Ethics Committee guidelines. Ethical clearance was obtained from the University of Venda Ethics Committee with a project registration number (SARDF/17/IRD/08/2908) for permission to conduct this study.

3.3. 1. Site of the study

This study was conducted in Rabali (ward 34), Tshanzhe (ward 4), Tsidzi (ward 10) and Mabayeni (ward 35) villages of Makhado, Thulamela, Musina, and Collins Chabane local municipalities, respectively. These four local municipalities are in the Vhembe district of Limpopo province of South Africa. The province is in northern South Africa, sharing borders with Botswana, Mozambique, and Zimbabwe. The area of the district is 25597 square kilometres, and the population of the province is 1402779 (StatsSA, 2015). Rural farmers rely heavily on communal goat production to meet their socio-economic needs.

3.3.2. Experimental procedure

Topical participatory rural appraisal was used to identify farmers' goat production, production management, and goat production challenges. Topical participatory rural appraisal focuses on a particular topic (goat farming) unlike exploratory participatory rural appraisal, which explores different fields, such as agriculture, mining, and others within the study area. The appraisal was used to describe the reproductive management practices and the interventions required to improve communal goat reproduction and genetics to develop sound management, which is key to the success of goat production in the target areas. The current goat production systems were investigated. This included communal goat farmers' challenges in goat reproduction and production management. Cluster random sampling was used to select the ward and villages within wards in the Vhembe district. Cluster random sampling is the sampling method where different groups within a population are used as a sample. This differs from stratified sampling in that an entire group or cluster is used as a sampling unit rather than a randomly selected group member. 140 communal goat farmers were selected from villages to participate in this study.

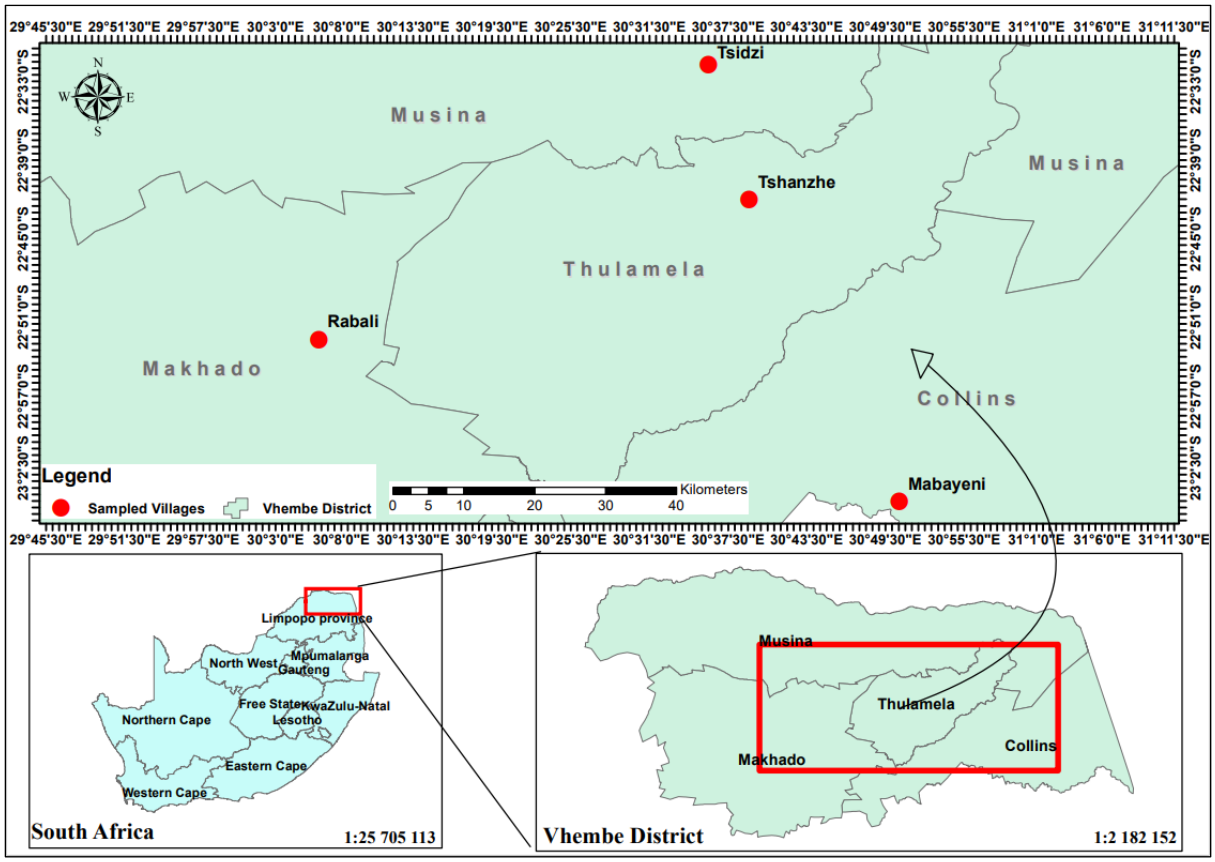


Figure 3.1: Map of the four targeted areas of the study in Vhembe district municipality

3.3.3. Data collection

In-depth one on one interviews and focus group discussions were conducted using participatory rural appraisal (PRA) to collect both qualitative and quantitative data through administering structured questionnaires (Appendix 1). The questionnaire was pre-tested on 10 household heads who were not part of the study participants to ensure that the questions were well understood. Data collection commenced on 08 April 2019 using face-to-face interview procedures to ensure that group discussions did not affect individual thoughts and expressions. The focus group discussions were a follow up on face-to-face interviews, to confirm some of the answers provided by the respondents. Face-to-face interviews were done using structured and open-ended questionnaires to Vhembe household heads. Smallholder farmers (18 years and above) in the target area were interviewed in their homes or farms. Questions that seemed difficult for the respondents were clarified using farmers' local languages with the help of the local extension officers from the Limpopo Department of Agriculture. A focus group interview (containing 10 households per group) was conducted in the four municipalities. This was replicated three times in each centre, totalling 30 households per municipality and 120 in the study.

3.3.4. Data analysis

The main techniques of analysis were descriptive statistics. Computer software IBM-Statistical package for the Social Sciences (SPSS) version 23 was utilized for the analysis and used to compute descriptive statistics and cross-tabulations. The technique was used to determine and compare household demographics, income groups and challenges, goat flock size, and production systems. Thematic Content Analysis (TCA) was used, whereby themes were generated for participants verbatim.

3.4. Results

3.4.1. Household and socio-demographics

Table 3.1 outlines the findings on the household socio-demographic characteristics. The communal goat farmers' gender distribution was skewed towards females (55%), the majority (66.4%) of whom raised their goats under an extensive farming system, with the remaining 33.6% on a semi-extensive farming system. About 29% of the respondents had formal employment, followed by 26% old-age pension grant beneficiaries, 17% informally paid employment and 16% volunteers, while 10% are self-employed. The income status of the respondents in this study was evaluated to determine the communal goat farmers' main sources of income apart from goat production. The age of the respondents ranged from 18 to 70 years (Figure 3.1). The largest (21%) age bracket of the participants in communal goat farming was between 41 and 60 years. Those between the age of 18 and 25 years constituted the least participants. The old-age group reported that youths were ashamed of farming and herding goats.

3.4.2. Farmer perceptions on household cash income generation from goat production in relation to level of education and gender

Table 3.2 shows the findings on the level of awareness of goat production and understanding of production management about the level of farmer education and gender. Both male (43.6%) and female (31.6%) farmers with tertiary education strongly agreed that livestock production has contributed towards generating income, followed by 29.8% female and 25.6% male with secondary education and 21.1% illiterate females. In addition, 60% of female respondents possessing secondary education agreed that goat farming is one of their most important sources of income, followed by 46.7% male with tertiary qualifications and 40% males with secondary education. The largest percentage (85.7%) of farmers indicated income as the main reason for keeping goats, with the remaining 14.3% stating household food.

3.4.3. Farmers response to goat production

Table 3.3 shows farmers responses to goat production. The majority of the respondents (92.9%) reported that their does kid for the first time at about 17 to 19 months of age. The highest kidding rate (65.7%) is in autumn, followed by spring (34.3%). About 95% of the

respondents reported seldom weaning kids, and only 5% weaned at 4 months of age. More than half (65.7%) of the respondents seldom vaccinate their goats, and the remaining 34.3% buy vaccine from the reputable agricultural stores and vaccinate their animals against lamb dysentery, pulpy kidney, tetanus, blackleg, clostridial metritis (malignant oedema of the uterus), blood-gut, infections caused by Clostridium novyi type B, tick-borne gall-sickness (anaplasmosis), heart-water, pneumonia, foot rot, joint-ill and navel-ill. Most (65.7%) farmers indicated they hardly experience diseases in their flocks.

3.4.4. Goats flock size distribution per household

Table 3.4 shows goat flock size distribution. Goat flocks were generally small in the target areas of the study, with a tad variability between households (Table 3.4). About a quarter (28.6%) of the households owned less than 10 goats, and 22.8% owned between 11 and less than 30 goats. The remaining 48.6% owned more than 30 goats per household.

3.4.5. Farmer perceptions on feeding and reproductive management

Table 3.5 shows participant responses towards feeding and separating goats during oestrous synchronization and introduction of AI. The commitment and enthusiasm of the communal goat farmers in Vhembe district municipality; separating goat does from bucks during oestrous synchronization and AI is represented in Table 3.5. Seventy percent of the respondents showed interest in the proposal to pen feed and separate goats for oestrous synchronization and AI. Other 30% of the respondents reported that they could not afford to pen feed and separate their animals, though they were receptive to be part of the AI programme. Table 3.5 shows a summary of farmer perceptions in relation to the adoption of AI during the introduction of artificial insemination in Vhembe district using highly improved semen of a Boer goat buck. Most of the respondents (72.1%) strongly agreed to the introduction of improved genetics through AI using the Boer buck semen, followed by 17.1% farmers who agreed to use AI with Boer buck semen and 10.8% of the farmers who were against the Boer goat breed. Most respondents (89.2%) primarily accepted AI on the premise that the Boer goat breed could be valuable in combating the prevailing inbreeding. Few (10.8%) farmers refused the Boer goat breed, as they are anxious that Boer goat semen could cause dystocia in their animals.

Socio-demographic results of goat farmers in the Vhembe district are tabulated in Table 3.1 below.

Table 3.1: Socio-demographics of 140 interviewed goat framers in the Vhembe district

Characteristics	**N**	**Proportion (%)**
Gender		
Male	62	44.3
Female	78	55.7
Total	**140**	**100.0**
Income group		
Formal paid employment	41	29.3
Volunteers	23	16.4
Self-employment	15	10.7
Informal paid employment	24	17.2
Old-age support grant	37	26.4
Total	**140**	**100.0**
Farming system		
Extensive farming system	53	66.4
Semi-extensive farming system	47	33.6
Total	**140**	**100.0**

The age distribution of the participants is presented in Figure 3.2 below.

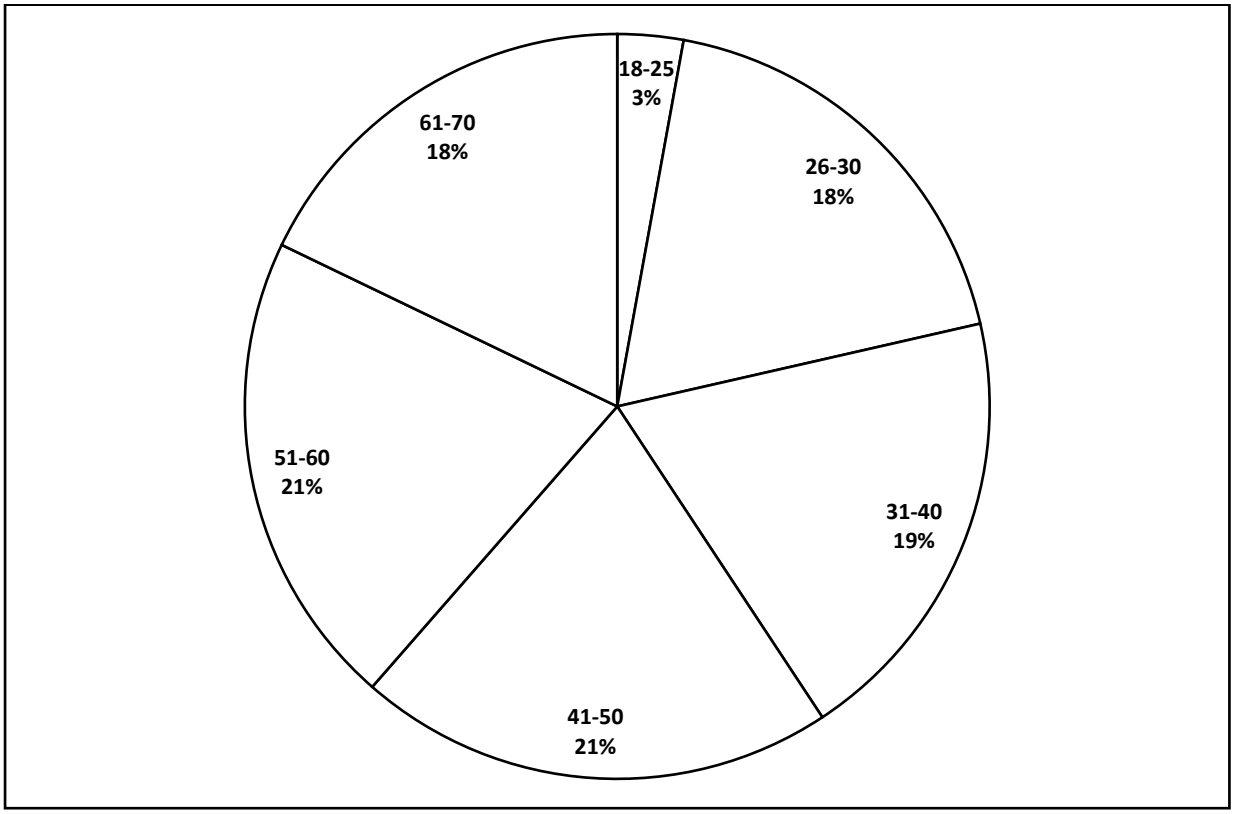


Figure 3.1: The age (18 to 70 years) distribution of the participants.

Farmers' perceptions of the household cash income generation from goat production in relation to education and gender are shown in Table 3.2 below.

Table 3.2. Level of education and gender influence on farmer perceptions towards household cash income generation from goat production

Farmer perceptions on household cash income generation from goat production in relation to level of education and gender								
		Strongly agreed		**Agreed**		**Disagreed**		
Gender	**Education**	N	%	N	%	N	%	Total
Male	Illiterate	7	17.9	0	0.0	1	12.5	8
	Primary education	5	12.8	2	13.3	0	0.0	7
	Secondary education	10	25.6	6	40.0	5	62.5	21
	Tertiary education	17	43.6	7	46.7	2	25.0	26
	Total	39	100.0	15	100.0	8	100.0	62
Female	Illiterate	12	21.1	1	10.0	4	36.4	17
	Primary education	10	17.5	1	10.0	4	36.4	15
	Secondary education	17	29.8	6	60.0	2	18.2	25
	Tertiary education	18	31.6	2	20.0	1	9.0	21
	Total	57	100.0	10	100.0	11	100.0	78

Reproduction performance results following communal goat production management are tabulated in Table 3.3 below.

Table 3.3. Responses toward goat production management

Goat production	N	%
Reason for keeping goats		
Cash income	120	85.7
Foodstuff	20	14.3
Age at first, kidding.		
Months	130	92.9
Kidding interval		
< 240 days	55	39.3
>365 days	62	44.3
Others	23	16.4
Kidding season		
Autumn	92	65.7
Spring	48	34.3
Weaning		
Yes	7	5.0
No	133	95.0
Others	0.0	0.0
Vaccination		
Yes	48	34.3
No	92	65.7
Performance records		
Yes	35	25.0
No	105	75.0

Goat flock size results in four local municipalities are presented in table 3.4 below.

Table 3.4. Number of goats per flock in Vhembe district

Municipality	Goats flock size						Total
	5-10		11-30		Over 30		
	N	%	N	%	N	%	
Thulamela	9	22,5	9	28.1	17	25.0	35
Makhado	12	30.0	6	18.7	17	25.0	35
Collins Chabane	9	22.5	10	31.2	15	22.0	34
Musina	10	25.0	7	21.8	19	27.9	36
Total	**40**	**28.6**	**32**	**22.8**	**68**	**48.6**	**140**

Communal goat farmers' responses to feeding and separating goats during oestrous synchronization and artificial insemination are tabulated in 3.5 below.

Table 3.5. Responses towards feeding and separation of goats during oestrous synchronization and introduction of artificial insemination in rural areas.

Farmers' response to feeding and separation of goats	**N**	**%**
Yes	98	70.0
No	42	30.0
Farmers response towards the introduction of AI	**N**	**%**
Strongly agreed	101	72.1
Agreed	24	17.1
Disagreed	15	10.8

3.5. Discussion

Anteneh *et al*. (2004) observed that the male household head takes all decisions related to the production and sale of cattle and goats. Female members will, however, be consulted before selling any livestock. Conversely, this study showed that women are more involved in goat production than men. Similar to this study, Aldosari (2018) reported that women are more involved in extensive and semi-extensive goat farming systems. These results therefore confirm the trend across Africa where women dominate agricultural activities. Thagwana (2009) noted that socio-economic factors were critical in influencing women to participate in agriculture. For example, this may be because farming has been regarded as one of the domesticated chores designated for women in communal lands (Doss, 2011). Men tend to take-up employment for financial sustenance of their families, which in most cases takes them out of their provinces to work in mines or other money earning businesses (Mboma *et al*., 2018). Women therefore engage in agricultural activities to alleviate poverty by supplementing their husbands' inadequate incomes (Gordon & Craig, 2001). The International Fund for Agricultural Development (2004) testified that many women in sub-Saharan Africa (SSA) countries had been associated with small livestock roles, particularly sheep and goats, for many years. Hulela (2010) revealed that women in Botswana played a significant role towards food security and income generation in both domestic and national development through managing goat production in rural communities.

The income status of the respondents in this study was evaluated to determine the communal goat farmers' main sources of income, apart from goat production. As in this study, the main reason behind goat production was to supplement insufficient household income. This reveals that goat production generates cash income in their households by selling goats and their products. In addition, respondents conveyed that they valued goats as their currency to satisfy household needs. Some respondents indicated that they have managed to pay for their children's education with the revenue from communal goat farming. This agrees with Abd-Allah *et al*. (2019), who reported that the largest percentage of farmers (89%) indicated that cash income from the sale of goats was one of the important reasons for keeping the goats. Visser & van Marle-Koster (2017) testified that goats are often seen as a form of 'fluid capital, as they can easily be sold to cover immediate expenses such as school fees or fodder purchases for the rest of the herd. In contrast, some respondents rear animals for household food (Omontese, 2018). They also conveyed that goats provide humans with meat, milk, and skin, particularly in rural areas. In support of this notion few respondents (14.3%) are keeping goats as the source of household diet because red meat is expensive to buy, and its price keeps increasing. The findings were consistent with those by El Aich & Waterhouse (1999),

who argued that food products from goats are a delicacy in many parts of the world. About 85.75% of respondents emphasise that goat production is vital in socioeconomics, reducing poverty and enriching the poor communal farmer's living standards. Furthermore, farmers alluded that goats contribute to the food security, economic and employment to both formally (43.6%) and informally (17.9%) paid employees in Vhembe district. These findings agree with Aldosari (2018), who reported that goat management is extremely important and valuable for farming communities, particularly those whose livelihood partially or depends on livestock production. De Cock *et al*. (2013) also noted that livestock production is a major contributor to household food production and security in rural areas, and there is a vast potential for increased production efficacy and economic activity. Several challenges such as petit body size, decreased fertility and long kidding interval could be overcome for communal goat farmers to develop from being small scale goat farmers for household food security to viable commercial small-scale goat producers (VIsser & Van Marle-Koster, 2017).

The rural poor who cannot afford to maintain a cow find goats as the best alternative source of supplementary cash income (85.7%) and foodstuff (14.3%). This is supported by Nedambale & Nengovhela (2011), who indicated that it is much easier for small farmers with little/no land to farm goats than cattle because 10 goats could be kept in the space of one cattle. The same observation was made by Visser & van Marle-Koster (2017), who reported that more goats can be kept and cared for on a piece of land that can support only one large bovine. This study reveals that respondents (66.4%) tend to practice extensive farming because 30% of respondents seldom supplement their goats. Hence, those who practice semi-extensive farming (33.6%) often leave their goats to fend for themselves and are occasionally supplemented with household food leftover/wastes or cultivated field leftovers. These findings echo the observation made by Webb *et al*. (2010), who reported that in communal goat production systems, the focus is generally on free ranging goats, grazing around the village, old-cultivated fields, or areas of regrowth or harvest. Goats are called 'nonselective browsers because of their desire to choose from a large variety of vegetative types to graze from (Erasmus, 2018). Consequently, this grazing behaviour enables them to survive harsher climates and more marginal grazing conditions than sheep or cattle in communal areas. Kumar (2007) supported this, by reporting that goats are particularly useful in the semiarid, arid, and mountainous regions, where they can sustain sparse vegetation and extreme climatic conditions. Further, wherever irrigation facilities are poor, one can generally find large areas of common property or wasteland on which the small ruminants of rural resource-constrained households can survive (Visser & van Marle-Koster, 2017). A major part of their fodder requirement is met through waste and other common property lands. It has

been argued that these rural households have often developed highly efficient agricultural and livelihood systems that make the most rational and conservative use of their scarce resources (Barbier, 1989).

Afande *et al*. (2015) indicated that young people prefer employment in non-farm sectors. Negative attitudes towards agriculture have been associated with drudgery, low returns and lack of prestige compared to white collar jobs and awareness of the disparities between rural and urban life. In contrast, this study does not align with Aldosari (2018), who affirmed that both male and female youth were not ashamed of participating in goat farming. The eldest group of respondents (18%) reported that children under 18 are responsible for herding goats after school within their families. Meanwhile, some of these children are even hired/paid by neighbours or other families to herd goats, particularly by families without having a male child (Awan *et al.,* 2011). Moreover, children from families without goats, end-up been attracted by money that their mates receive from herding goats and end-up doing the same chore. However, this money is important to these children and their families (Afenyadu, 2008).

Respondents testified that they use this money in many ways, such as pocket money or allowance at school, paying school fees and buying school uniforms for the children. Likewise, goats are targeted towards food security and income generation initiatives as they are easy to keep and manage particularly by women and children within the poor community. According to Byaruhanga *et al.* (2015), the involvement of children in various aspects of goat production underlines the importance of targeting them in improving goat production programs intended to improve household nutrition and income.

The findings of this study indicate that goat production remains one of the most important components in literate (43.6%) and illiterate (17.9%) households' livelihood strategies in the Vhembe district. Munyai *et al.* (2012) reported that apart from the fact that education is one of the important variables which enhance the farmers' ability to acquire, process and use agriculturally related information, it is widely recognised as a vehicle for empowerment, economic growth, and general improvements in welfare. Stats SA (2022) revealed that youth remain vulnerable in the labour market. However, 62.5% of males with secondary education and 36% of females with primary education disagreed that goat farming contributed to income generation. The reasons behind the controversy or disagreement include how clients negotiate for lower or reduced goat prices; in some cases, customers refuse to buy due to the

small body frame of the goats. Communal goat farmers need to fully understand the industry so that they are not taken advantage of by unscrupulous traders who would underpay them for their goats. Erasmus (2018) reported that the South African smallholder sector mainly keeps multi-purpose and uncharacterised veld-type goats with small body frames and low meat yield. In addition, ruminants are associated with low growth rates and mature body weights as goats, but in this sector, goats have not been genetically improved for quality meat yield. Comparable findings were reported by Visser & Van Marle-Koster (2017), that goats kept in smallholder and communal systems are usually hardy and adept at foraging in degraded environments but tend to have low productivity and poor meat quality.

According to Webb & Mamabolo (2004), animal production is a South African tradition. In the present study, respondents generally depended on livestock production for income (85.7%) generation or food (14.3%) supply. While crop production is often associated with livestock production. In this study, the majority of respondents (92.9%) reported that indigenous does in communal farming systems kid for the first time at about 17 to 19 months of age, which is similar to West African dwarf goats in Chad, but it takes longer than Togo (15 months), Sahel (13 months) and Maradi goats (14 months) (Wilson *et al.,* 1989) and west African dwarf goats in Nigeria (Ikwaegbu *et al.,* 1995). The first kidding age for communal goats in Vhembe district is shorter than the Rwandan goats (21 months; Wilson *et al.,* 1989). Consequently, these does reach puberty at a later stage, approximately 8 to 12 months of age. Conversely, Webb & Mamabolo (2004) reported that the indigenous goats in Mpumalanga are early breeders, reaching puberty at about 6 to 7 months of age. Although, this could be influenced by the farming systems practiced within that area. This study shows that, mating is anarchic, hence it occurs immediately after the does reach puberty. Additionally, Bucks and does run together during the day and corralled together at night, influencing random mating and resulting in inbreeding within the flock. In this study, most farmers (44.3%) reported an inter-kidding period of 365 days, followed by 39.3% of 240 days, and 16.4% of other farmers were unaware of their inter-kidding period from the first to the next kid. The results of this study indicate that most of the communal does in Vhembe district only kid once per annum. However, these does are well known for fecundity under extensive communal farming conditions of Vhembe district (Monyeteti *et al*., 2017). Moreover, these have the potential to kid with twins, and even triplets, only if communal farmers use acceptable animal-assisted reproduction technology, such as AI, to increase the quality and quantity of the kids they produce. The results of this study agree with the findings of Mann (2007), who reported the inter-kidding period of 365 days in an extensive communal goat system and the inter-kidding period of 240 days in the intensive goat farming system. Barry & Godke (2001) also reported a similar inter-kidding period of 234 days

in South African Boer goats. This finding differed from the kidding interval of 238 days for the South African indigenous does in rural communal farming systems of Mpumalanga (Webb & Mamabolo, 2004).

The findings indicate that the highest kidding rates are attained in autumn, followed by spring. This is because the daylight length generally stimulates the sexual activity of does. When the daylight length is long, the sexual activity of does is low, and the opposite is true for the short daylight length. This was supported by Van der Westhuizen (1979) and Greyling (1990) that shortening daylight length generally stimulates the does, with a peak in sexual activity during the months of April and May in the southern hemisphere. The period of least sexual activity is usually from October to January. Webb & Mamabolo (2004) reported similar findings for the South African indigenous does in Mpumalanga, where the highest kidding rates were attained in autumn (96%), followed by spring (93%), winter (63%) and summer (0%).

Sebei *et al.* (2004) observed that kids on communal grazing are naturally weaned at about 6 months of age (180 days), which is longer than the findings of this study (4 months of age). This means a doe will kid once a year, as the average gestation period is 154 days. Research has shown that, by the time the kid is 8 weeks old, it can digest grass just like an adult goat (Goat Farming, 2022). This means that after 8 weeks, a kid relies less on their mother's milk for food and depends more on available pasture feed (Mann, 2007). Kids can be weaned successfully when they are 8 weeks old, and the weaning mass should be 15 to 20 kg (Steyn, 1982; Belanger-Naud, 2020; Vickery *et al.,* 2022). In this study, respondents reported that from the first four weeks after kidding, they tether the suckling kids in the corral to allow the milking does and the rest of the flock to go out and graze/browse during the day but corralled together with the suckling kids at night. This is done to protect the suckling kids from predators. Goats are generally associated with small body frames and are easily hunted and killed (prey) by wild predators, such as feral dogs and baboons, in most mountainous rural areas of the Vhembe district. Baboons can survive in arid environments, such as grasslands and deserts, because they can subsist on grass alone for a long time, just like sheep and goats. Moreover, baboons are known to prey on sheep and goats from human homesteads. The baboons find it difficult to prey on mature goats, but young goats are threatened (Smuts, 2008).

The results of this study revealed that more than half of the respondents seldom vaccinate their goats. They hardly experience diseases in their flocks, which is similar to the findings reported by Webb *et al*. (2010), who stated that indigenous goats appear to be resistant to diseases such as blue tongue, pulpy kidney and gall sickness and are less susceptible to internal parasites compared to sheep. This means these animals still possess strong

characteristics (traits) of local breeds regarding their adaptive capacities to relatively poor nutrition and superior ability to survive tick-borne diseases compared with commercial goat types (Erasmus, 2018). It was noted that 75% of rural farmers seldom keep performance records of their flock. This highlights poor management practices from the rural communal goat farmers in the Vhembe district.

Goat flock sizes are generally small in communal areas because many kids die due to inclement weather resulting from poor management strategies and housing at kidding time (Devendra, 1992). In most circumstances, poor people keep goats in small flocks and may run with other animals, such as sheep or cattle, and they have been neglected politically and scientifically for decades (Wilson, 1988). Similar to this study, Oni *et al*. (2003) reported that it can be calculated that among the households with agricultural sources of livelihood in Sekhukhune district of Limpopo province, only 9% do not have any goats, 31% have 1-5 goats, 28% have 6-10 goats, 16% have 11-20 goats and 16% have more than 20 goats. Meanwhile, increasing the goat population is hindered by the lack or the high price of breeding bucks that can be used to improve communal goat stocks (Soriano *et al.,* 2021).

Goats like dry weather, so farmers would ensure they have a dry place when it rains. They built goat houses with a wall and let them give birth in clean, warm, and dry places (Visser & Van Marle-Koster 2017). The size of the flocks differed across the targeted areas; most of these flocks are non-descript, crossbred and indigenous goats. However, these does have the potential to reproduce and increase the number of offspring from a highly superior buck when AI is employed. Nethengwe *et al.* (2016) reported comparable findings where livestock owners in rural areas of Vhembe district encountered serious challenges with separating cows from bulls for synchronization programmes. In addition, these people are farming in communal lands, where they cannot control and isolate bulls from cows due to a lack of farming infrastructure, such as fences to separate their grazing land into different camps. Moreover, implementing such a programme is challenging because of limitations such as the availability of land, liquid nitrogen, and the cost of semen tanks. However, establishing animal assisted reproduction centres could be a valuable strategy that enables the exchange of desired indigenous genotypes within rural communities. This would promote the resource-constrained farmer's access to superior exotic genotypes through AI. Improved goat production will likely significantly enhance the livelihoods of poor communal farmers.

The same observations were made by Ebanyat *et al*. (2010), who stated that crossbreeding between indigenous goats and exotic breeds is prone to positive effects on goat productivity. Arrebola *et al*. (2013) revealed that no reproductive technology has contributed to such a

degree to the genetic improvement of livestock as has AI. This has been successfully achieved through AI in many African countries, and recently, at a research station, Boer goats and Venda indigenous goats were combined with AI and a 78.6% conception rate was achieved (Dara *et al*., 2015). This agrees with Soriano *et al*. (2021), who reported that goat AI sub-centres in the Philippines were established in seven different municipalities, and a 60.29% conception rate was observed. Kifaro *et al*. (2007) also obtained a 55% conception rate in dairy goats in Tanzania. These results are in close agreement with those obtained by Motlomelo *et al*. (2002), who alluded that the 60 % conception rate range was achieved following oestrous synchronization protocols and AI in goats.

Although AI is a technique that has already been used for years in goats, especially in countries such as France, Italy, and Spain, it has not been fully transferred into rustic goat breeds reared in extensive grazing farming systems (Arrebola *et al*., 2013). This was supported by Jiabi *et al*. (2004), who reported that AI in small ruminants, using fresh and frozen semen, has been used commercially in several countries for many years. However, in Africa, particularly in Tanzania, AI in goats has rarely been performed (Kifaro *et al.,* 2007). AI can spread elite genetic material in each population (Gordon, 1997; Romano *et al.*, 1996). The Diffusion of Innovation Theory assumes that growth does not appear everywhere at the same time, but it manifests itself in “points” or “poles” of growth with variable intensity and spreads through different channels with variable terminal effects on the whole of the economy (Perroux, 1950). In this case, this study cannot apply AI in the whole province of Limpopo at the sometime. Thus, the beneficial “spread effects” from growth poles would eventually induce development in the remaining peripheral areas. Also, its geographical base will bring about structural change in other places (Rogers, 1957).

3.6. Conclusion and recommendation

The communal goat flocks in the Vhembe district municipality are generally small, with variability between households. Communal goat farmers raised their goats under an extensive farming system. Hence, mating is anarchic, as it occurs randomly when does reaches puberty. Most farmers strongly agreed that goat production has contributed to generating household income. Moreover, most (72.1%) farmers accepted using AI in goats. Acceptance of AI was on the premise that the Boer goat breed could be valuable in combating the prevailing inbreeding. Therefore, it is fundamental to develop innovative tools and measures like animal assisted reproduction centers designed to facilitate controlled animal breeding to ensure the reproductive improvement of communal goat farmers. Research and well-equipped animal-assisted reproductive technology centers can address the technical constraints to implementing AI and advance communal goat farmers' socio-economic development for sustainable rural development and poverty alleviation in the Vhembe district. Therefore, it is recommended that cooperation and support on AI are needed from rural-based Universities, Colleges, and Government institutions to ease the livelihood of the communal goat farmers. Additionally, artificial insemination with fresh semen diluted from cheaper and easily available quality semen extenders to both communal and commercial goat farmers could enhance livestock production performance and increase small goat flock size, supplementing the communal farmers' insufficient household income in Vhembe district.

3.7. Reference

Abd-Allah, S., Mohamed, M.I., Abd-Elrahman, H.H., and EL-Kady, R.I., (2016). Assessment of some productive performance of Boer goats and their crosses with Egyptian Baladi goats. *Int J ChemTech Res.* 9,259–265.

Abd-Allah, S., Salama, R., Mohamed, M.I., Mabrouk, M., El-Kady, R.I., Kadry, A.I., Ahmed, S.M., (2015). A comparative study on reproductive and productive performance of Boer and Baladi goats raised under similar environmental conditions in Egypt. *Int J ChemTech Res* 8, 225–236.

Abd-Allah, S., Mohamed, M.I., Shoukry, M.M., Salman, F.M and Abd- El Rahma, H.H., (2019). Assessment of the traditional goat production systems in rural areas of the Nile Delta in Egypt. Bulletin of the National Research Centre. DOI: https://doi.org/10.1186/s42269-019-0153-3. 43-114.

Abd-Allah, S., (2014). Application of some crossbreeding and feeding programs to improve the productive performance in Baladi goats. Unpublished Ph.D. Thesis, Fac. of Agric., Al-Azhar Univ. Egypt. http://thesis.mandumah.com/Record/ 277060.

Afande, F., Maina, W., and Maina, M., (2015). Youth engagement in agriculture in Kenya, *Journal of culture, society, and development*, 7: 4-20.

Afenyadu, D. 2008. Children in Cattle Herding: A Baseline Study of the North and South Tongu Districts. Commissioned by the International Needs Network Ghana, unpublished paper.

Aldosari, F.O., (2018). Gender participation in sheep and goat farming in Najran, Southern Saudi Arabia. *Saudi. J Biol Sci.* 25 (1) 144-148.

Anteneh, N.T., Mekala, D.G., Mnisi, P.E., Mukisira, C., Muthui, M., Murungweni, C.S.O. (2004). Goats' production and livelihood systems in Sekhukhune District of the Limpopo province, South Africa. Working Document Series 118

Arrebola, F. Gonzalez, O, Torres, R and ABECIA, J. (2013). Artificial insemination in Payoya goats: Factors affecting fertility. *Anim Prod Sci*. (54) 3. http://dx.doi.org/10.1071/AN13138

Awan, M.S., Waqas, M., and Aslam, M.A., (2011). Why do Parents Make their Children Work? Evidence from Multiple Indicator Cluster Survey. Int. J. Acad. Res. 3, 545-549.

Barry, D.M., and Godke, R.A., (2001). The Boer goat the potential for cross breeding. Department of animal science, Louisiana University, Boton Ronge, Louisiana. 708008.

Byaruhanga, C., Oluka, J., Olinga, S., (2015). Socio-economic Aspects of Goat Production in a Rural Agro-pastoral System of Uganda. *Univers. J. of Agric. Res.* 3, 203-210.

DAFF. (2013). A profile of the South African goat market value chain. Retrieved on 30 June 2014 from www.nda.gov.za.

Dara, O.B., Baloyi, J.J. and Barry, D.M., (2015). Comparison of progesterone$PGF_{2\alpha}$ and novel Synchronization GnRH based synchronization protocols in Boer and Venda indigenous goats of South Africa: Vhembe district. Masters. Dissertation, University of Venda. South Africa.

De Cock, N., D'Haese, M., Vink, N., van Rooyen, C.J., Staelens, L., Schönfeldt, H.C. and D'Haese, L., (2013). Food security in rural areas of Limpopo province, South Africa. *Food Security.* **5**, 269-282.

De Villiers, J.F., Gcumisa, S.T., Gumede, S.A., Thusi, S.P., Dugmore, T.J., Cole, M., Du Toit, J.F., Vatta, A.F., and Stevens, C., (2009). Estimation of live body weight from the heart girth measurement in KwaZulu-Natal goats. *Anim. Husb. Rural Dev*. 2, 1-8.

Department of statistics South Africa. (2019). www.statssa.gov.za

Doss, C., (2011). The roll of woman in agriculture. The food and agriculture organisation of the united nation. 11-02. www, FAO.org/economics/esa

Dossa, L.H., Wollny, C., and Gauly, M., (2007). Smallholders' perceptions of goat farming in southern Benin and opportunities for improvement, *Trop. Anim. Health Prod*. 39, 49-57.

Ebanyat, P., de Ridder, N., de Jager, A., Delve, R.J., Bekunda, M.A., and Giller, K.E., (2010). Drivers of land use change and household determinants of sustainability in smallholder farming systems of Eastern Uganda, Population and Environment. 31 (6) 474-506.

El Aich, A., Waterhouse, A., Small ruminants in environment conservation. *Small Rumin. Res*. 34:271-287.

Erasmus, D., (2018) Goat breeding: the challenges faced by smallholders. Farmers weekly.

Fahad, O.A., (2018). Gender participation in sheep and goat farming in Najran, Southern Saudi Arabia. *Saudi J. BioL. Sci*. 25, 144-148.

FAO, 2009.ftp://ftp.fao.org/docrep/fao/012/i1034e/i1034e02.pdf.

Gordon, A., and Craig, C., (2001). Rural non-farm activities and poverty alleviation in sub-Saharan Africa (NRI Policy Series 14). Natural Resources Institute Policy Series. Natural Resources Institute, Chatham Maritime, Kent. ISBN 0-85954-533-4

Gordon, L.R., (1997). Controlled Reproduction in Sheep and Goats. CAB International, UK.

Greyling, J.P.C., (1990). Sexual activity of the Boer goat doe. Boer Goat News 9:58.

Hassen, A.S., Tesfaye, Y., (2014). Sheep and goat production objectives in pastoral and agro-pastoral production systems in Chifra district of Afar, Ethiopia, *Trop. Anim Health Prod*. 46, 1467-1474.

Heise, A. (2011). Artificial insemination in veterinary science. Faculty of veterinary science of Pretoria South Africa. In Tech. 2, 18-32. (ISBN 978-953-51-0031-7). DOI: 10.5772/20120

Homann, S., van Rooyen, A., Moyo, T., and Nengomasha Z., (2007). Goat production and marketing: Baseline information for semi-arid Zimbabwe. Bulawayo, Zimbabwe: International Crops Research Institute for the Semi-Arid Tropics. 84 pp.

Hulela, K., (2010). The Role of Women in Sheep and Goats Production in Sub-Saharan Africa. *Int. J. Sci. Educ*, 3, 177-187. http://www.ijsre.com.

IFAD. (2016). https://www.ifad.org/documents/10180/bcb2e5dd-ad6b-4a5d-a23b-6031d5f99303.

Ikwuegbu, O.A, Njwe, R.M., and Tarawali, G., (1995). On -farm reproductive performance of the West African Dwarf goat at Ganawari in the sub-humid zone of Nigeria. *Trop. Agric.* 73, 49-55.

Jiabi, P., Zegao, D. and Taiyong, C., (2004). Extension of Artificial insemination in Boer goats. www.igagoatworld.org/publicationlproceeding/abst ractl4.pdfsite visited on 27/512005.

Kifaro, G.C., Eik, L.O., Mthenga, LA., Mushi, D.E., Safari, J., Kassuku, A.A., Kimbita E.N., Maeda-Mchang'u A.D., Kanuya, N.L., Muhikambele, V.R.M., Ndemanisho, E., and Ulvund, M.J., (2007). The Potential use of Artificial Insemination in sustainable Breeding of Dairy Goats in Developing countries: A Case Study of Norwegian Dairy Goats in Tanzania. Sokoine University of Agriculture. *J. Agric.Sc*. 19-24.

Kulaksiz, R, and Daskin, A. (2012). Reproductive performance of primiparous and multiparous Saanen goats after laparoscopic intrauterine insemination: a field study. Turk. *J. Vet. Anim.* 36, 201-204.

Mann, P. (2007). Timeline production of sheep and goats. Department of Animal Science. Thohoyandou. University of Venda. South Africa.

Mboma, Z.M., Greer, G., and Lorenz., L.M., (2018). To be honest, women do everything': understanding roles of men and women in net care and repair in Southern Tanzania. *Malar J* 17, 459. DOI: https://doi.org/10.1186/s12936-018-2608-7

Motlomelo, K.C., Greyling, J.P.c. and Schwalbach, L.M.J., (2002).synchronization of oestrous in goats: the use of different progestagen treatments. *Small Rumin. Res*. 45: 45-49.

Munyai, F.R., Swanepoel, F., Ndlovu, L., and Wilke, P., (2012). An evaluation of socio-economic and biophysical aspects of small-scale livestock systems based on a case study from Limpopo Province: Muduluni village. *Doctor of Philosophy Thesis*. University of Free State, South Africa.

Nedambale, T.L., and Nengovhela, B., (2011). Upgrading the smallholder beef cattle farming sector in Limpopo and Eastern Cape Province. *Sci. Technol*. **31**, 64-65.

Nethengwe, L.D., Francis, J., and Barry, D.M., (2016). The effect of three ovulation inducing drugs on pregnancy rates of rural cattle after administration of synchronized artificial insemination in Vhembe district. *Masters. Dissertation*, University of Venda, South Africa.

Omontese, B.O., (2018). Oestrous synchronization and artificial insemination in goats. DOI; 10.5772. Intechopen. 74236

Oni, S., Nesamvuni, A.E., Odhiambo, J.J.O., and Dagada, M.C., (2003). Agriculture as a cornerstone of the economy of Limpopo Province. Agricultural policies and institutions in

agricultural investment, financing, and credit in the Limpopo Province. Department of Agricultural Economics and Extension, South Africa. 1(12).

Randolph, T.F., Schelling, E., Grace, D., Nicholson, C.F., Leroy, J.L., Cole, D.C., Demment, M.W., Omore, A., Zinsstag, J., Ruel, M., (2007). Role of livestock in human nutrition and health for poverty reduction in developing countries. *J Anim Sci*. 85:2788–2800.

Raseona, A.M., Ajao, O.A., Nethengwe, L.D., Madzhie, L.R., Nedambale, L.T and Barry, D.M., (2017). Viability of bull semen extended with commercial semen extender and two culture media stored at 24 °C. S, AFr. *J. Anim. Sci.* **47**, 50-55.

Romano, J.E., Rodas, E., Ferreira, A., Lago, I., Benech, A., (1996). Effects of progestagen, PMSG and artificial insemination time on fertility and prolificacy in Corriedale ewes. *Small Rumin. Res*. 23: 157-162.

Salman, H., and Abd-EI Rahman, H., (2019). Assessment of the traditional goat production systems in rural areas of the Nile Delta in Egypt. Bulletin of the National Research Centre. 43:114

Sebei, P.J., McCrindle, C.M.E and Webb, E.C., (2004). An economic analysis of communal goat production. Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria. 0038-2809 *J S.Afr. Vet. Ass*. 75, 19–23.

Sibisi, H., (1981). Keen farmers on Swazi nation land: economic and analysis section. Ministry of Agriculture and Cooperatives, Mbabane.

Smuts, B., (2008). Predators on livestock farm. Land foundation projects.

Soriano, A.P., Celestino, E.F. and Uprpiano, E.M.V., (2021). Application of artificial insemination as a breeding method for dairy goats herd built-up in Region 3, Philippines. ISSN: 2507-9638.

Statistical Package for Social Sciences for IBM. Version 24. (2015). Available from: https://www.ibm.com/br-pt/analytics/spss-statistics-software

Steyn, O.P.J., (1982). General flock management in mutton sheep. College of agriculture. Glen farming in South Africa.

Thagwana, S.T., (2009). Trends in women's participation in agriculture at Tshiombo irrigation scheme, Limpopo province. Master dissertation. Institute for Poverty, Land and Agrarian Studies. University of Western Cape. South Africa.

Van der Westhuizen, J.M., (1979). The oestrous response and changes in plasma progesterone concentration of Angora and Boer goat does following injection of a GnRh analogue. S. Afr. *J. Anim. Sci*. 9:17-19.

Van Rooyen, A.F., Freeman, A., Moyo, S. and Rohrbach, D., (2007) Livestock development in Southern Africa: Future Research and Investment Priorities. International Crops Research Institute for the Semiarid Tropics, Bulawayo, Zimbabwe. Unpublished.

Visser, C and van Marle-Koster, E., (2017). Challenges to the development of a rural goat industry in South Africa. Department of Animal and Wildlife Sciences, University of Pretoria.

Webb, E.C, and Mamabolo, M.J., (2004). Production and reproduction characteristics of South African indigenous goats in communal farming systems. *J. Anim. Sci.* 34, 236-239.

Webb, E.C., Mamabolo, M.J., Du Preez, E.R., and Morris, S.D., (2010). Reproductive status of goats in communal systems in South Africa. Department of Animal and Wildlife Sciences, University of Pretoria. South Africa.

Wilson, R.T., Murayi, T.H. and Rocha, A., (1989). Indigenous African small ruminants' strain with potentially high reproductive performance. *Small Rumin. Res.* 2, 107-117.

CHAPTER 4: EFFECTS ON BOER BUCK SPERM QUALITY OF 7-DAY STORAGE AT 5°C IN BOVINE AMNIOTIC FLUID-TRIS EXTENDER

4.1. Abstract

The study sought to evaluate the motility, velocity, morphology properties and viability of Boer goat sperm stored at 5°C for 168 h. Semen was diluted in Tris extender-based egg yolk (TEY) as a control, Tris extender-based bovine amniotic fluid of 10, 13 and 18 cm head-tail length fetus (60, 70 and 80 days pregnancy, respectively), denoted (BAF10, BAF13 and BAF18, respectively). A total of 48 ejaculates were collected from four Boer goat bucks twice a week for 6 weeks. Only uncontaminated ejaculates containing spermatozoa with >80% progressive motility rate and concentration >1.0 x 10^9 spermatozoa/ml were used. Ejaculated spermatozoa samples were pooled together before dilution at a ratio of 1:4 with TEY, TBAF10, TBAF13 and TBAF18. Diluted spermatozoa samples were then stored at 5°C for 168 h. Total motility (TM %) showed no significant different (P>0.05) on all the extenders from 0-96 h of the storage time. However, a significant difference was highly (P<0.001) observed from 120-168 h, whereby TBAF10, TBAF13 and TBAF18 prolonged the viability of spermatozoa far better than TEY as a control. The highest (P<0.001) spermatozoa motility was observed with TBAF18 at 0h, and the lowest was with TEY at 168 h. The spermatozoa PM, RAP, VCL, VSL, VAP, STR, AT, CT, BT, and LS differed (P<0.05) from 0-168 h of the storage time between extenders. Linearity and WOB showed no significant difference (P>0.05) for all the extenders. while, the storage time showed differences (P<0.05). Interaction between spermatozoa and storage time was observed for STR and AT % (P>0.05). The study concluded that tris extender-based bovine amniotic fluid (TBAF) contains the necessary nutrients and growth factors needed for semen dilution for storage at 5°C for more than a week (7 days), cheaper and easily manageable to both commercial and communal farmers.

Keywords: Bovine amniotic fluid, Morphology, Motility, Tris-egg yolk

4.2. Introduction

In the communal areas in South Africa, 86% of the land is classified as exclusively grazing land, leaving just 14 per cent for crop production (Rootman *et al*., 2015). The communal areas house 50% of the country's livestock population (Stroebel *et al*., 2011; Mafukata, 2014; 2015). Goat farming can potentially drive the socio-economic development activities in these communities, primarily because of its easy adaptability and as a food source for needy household farmers (Azeredo *et al*., 2001). However, production is hampered by poor breeding, among other factors. Different strategies are proposed to improve livestock production in communal areas. These include strengthening reproductive management and genetic improvement through assisted reproduction technology (ART), such as semen preservation for artificial insemination (AI) (Ferreira *et al*., 2014). AI facilitates the infusion of improved livestock genetics into rural livestock production systems (Lebbie & Kagwini, 1996). For effective AI, efficient semen preservation is critical for effectively improving the goat breeding program (Salamon & Maxwell, 2000). Semen contains spermatozoa suspended in the fluid secretions of the three male accessory sex glands (Frandson *et al*., 2009). The fluid portion, the seminal plasma, which is the transport medium, contains a variety of substances that include various electrolytes, fructose, citric acid and sorbitol. Fructose serves as the main source of energy for spermatozoa. Semen is evaluated as part of the protocol to evaluate the fertility of a male animal. The parameters vital for fertility are sperm concentration, motility, velocity, and morphology (Naz *et al*., 2018). Sperm motility is considered the most important (Januskauskas & Zilinskas, 2002). Sperm evaluation is achieved by computer-aided sperm analysis (CASA), a system in which several semen parameters can be analysed from digital images of the spermatozoa (Kathiravan *et al*., 2011).

After collection, semen ejaculates are diluted in media called semen extenders. Extenders are designed to provide spermatozoa with nutrients, energy, and antimicrobials, which protect cells against damage from cold shock and changes in pH and osmotic pressure. Semen is either extended for fresh use within several hours post-collection or can be frozen to be used after many years (Gadea, 2003). Extended semen should have a minimum shelf-life of 2 to 4 days of chilled storage to provide its transportation for AI in distant locations (Salisbury & Van Dermark, 1961). The primary objective in extending semen is to achieve maximal inseminations and conception with a single ejaculate. Preservation of bovine semen has been practised worldwide for many reasons. The main benefit of using fresh semen is that high conception rates are achieved with a relatively lower number of sperm cells (Anzar *et al*., 2003). Due to the freezing and thawing damage, a higher sperm concentration is required for frozen-thawed, compared to fresh semen (Holt, 2000). Freezing and thawing reduce sperm

motility and fertilising capacity, with the disadvantage of the high cost of liquid nitrogen and storage (Nethengwe, 2015). Different extenders have been used to preserve goat semen. These include commercial and non-commercial animal-source lipoprotein-based extenders or plant-source lipoprotein-based extenders (Kasimanickam *et al*., 2011). The animal source is mostly egg yolk, and the plant source is soybean lecithin. Whole or skimmed milk is also used to preserve semen (Rahman, 2014). The decline in spermatozoa viability and fertility related to cryopreservation motivated conducting studies to address the preservation of semen in refrigerated form for the buffalo species (Almeida *et al*., 2022). Therefore, this study aimed to evaluate the effects on Boer buck sperm quality of 7-day storage at 5°c in the bovine amniotic fluid-tris extender.

4.3. Materials and methodology

4.3.1. Ethics

This care and use of animals followed procedures approved by the University of Venda Animal Ethics Committee, project registration number (SARDF/17/IRD/08/2908).

4.3.2. Study area

The study was conducted at the School of Agriculture in the Centre of Excellent in Animal Assisted Reproduction biotechnology laboratory, University of Venda in Thohoyandou, Limpopo province of South Africa. Daily temperature at Thohoyandou differs from 22°C to 40°C in summer and between 12°C and 26°C in winter (Novela *et al*., 2020). Rainfall is highly seasonal, with 95% occurring between October and March. The average annual rainfall is 55.48mm but differs yearly (Weather and Climate, 2024). Vhembe region extends northwards to the Limpopo River, forming the boundary between South Africa and Zimbabwe (Rix *et al*., 1989).

4.3.3. Animals and management

Semen was collected from four healthy (1–2-year-old) Boer bucks managed at the University of Venda experimental goat feedlot. Each buck was fed 1.8 to 2 kg Driehoek pellets daily, and water was provided *ad libitum*.

4.3.4. Source and preparation of bovine amniotic fluid

Bovine uteri containing fetuses 10-20 cm from head-tail base length (60-90 days pregnancy) were collected from mixed beef cattle of unknown origin from a local commercial abattoir. The uteri were collected immediately upon slaughter using a cooled polystyrene box at 5-7 °C and transported within 2 h to the University of Venda Animal Science Laboratory. The uterine wall was then dissected open using a sterile surgical blade, and the amniotic fluid was aspirated with a sterile rubber less 50 ml syringe (Normo-jet Zentrisch, Henke Sass Wolf, Germany) and an 18-gauge needle. The amniotic fluid was heat inactivated at 56°C for 30 minutes using a water bath and then stored at -20°C in the screw cap 15 ml plastic vials until needed (Barry, 2005).

4.3.5. Preparation of egg yolk

Chicken eggs were randomly collected from the commercial flock at the University of Venda Experimental Farm a day before the extenders were prepared. All the eggs were washed in distilled water and wiped with a disinfected paper towel. This was done to ensure that no microorganisms could contaminate the extenders. The eggshell was wrecked by slightly tapping the egg against the side of an egg divider. Egg yolks were obtained using a sterile egg diver and then transferred to a clean filter paper to absorb and remove the remaining albumin. A sterile needle was then used to carefully puncture the yolk membrane and aspirate it into a 20 ml syringe. Therefore, the egg yolk was poured into plastic vials (Cellstar, Greiner Bio-One).

4.3.6. Preparation of extenders

The formulation of the experimental extenders is presented in Table 4.1. To prepare the extenders, 100 ml of distilled water was added to a borosilicate glass beaker, in which the different ingredients of Tris-based extenders were split into 15 ml CellStar tubes of Egg yolk, BAF10-60, BAF13-70, and BAF18-80. The extenders were stored at 5°C in the refrigerator and used for a maximum of 8 days after preparation. The extender pH was measured using a pH meter (Metter-Toledo AG, Analytical, Sonnenbergstrasse 74, Schwerzenbach) and adjusted to 6.8 using acetic acid and sodium hydroxide.

The composition of fresh semen Tris extender-based egg-yolk and Tris extender-based bovine amniotic fluid 10,13, and 18 are tabulated in Table 4.1 below.

Table 4.1. Composition of fresh semen extenders

Extender Ingredients	TEY	TBAF10-60	TBAF13-70	TBAF18-80
Tris (g)	2.422	2.422	2.422	2.422
Citric acid (g)	1.36	1.36	1.36	1.36
Monohydrate glucose (g)	1	1	1	1
Antibiotic (g)	0.1	0.1	0.1	0.1
Egg yolk (% v/v)	20	_	_	_
BAF10-60 (% v/v)	_	20	_	_
BAF13-70 (% v/v)	_	_	20	
BAF18-80 (% v/v)	_	_	_	20
Total volume (ml)	100	100	100	100

TEY: Tris extender-based egg-yolk TBAF10-60: TBAF13-70; TBAF18-80; Tris extender-based bovine amniotic fluid of 10,13,18 cm head-tail base length fetus (60, 70, and 80 days pregnancy, respectively)

4.3.7. Semen collection, processing, and experimental design

Semen was collected from four Boer goat bucks with proven fertility using an electro-ejaculator pulsator IV, auto: ajustTM, 15 A. 125 V (lane manufacturing INC Denver Colorado, USA). A total of 48 ejaculates were collected from the bucks (twice per week per buck) over 6 weeks. The electro-ejaculation was performed in the morning with the bucks restrained in the pen in a standing position. Just before semen samples were collected, the preputial hair of each buck was clipped, and the orifice was thoroughly washed, rinsed and dried. The sheath was washed with phosphate buffer saline (PBS) to avoid contamination of the ejaculates.

Immediately after collection, the volume of each semen sample was measured. Low semen volume is associated with low sperm concentration (Ajao, 2015). The colour of each semen sample was appraised visually and recorded. The samples were placed into labelled, pre-warmed 15 ml Cellstar tubes and transported to the biotechnology laboratory in a vacuum flask with warm water (37°C). The pH of each sample was measured using a Metler Toledo (AG Analytical, Sonnenbergstrasse 74, Schwerzenbach) pH meter. The sperm concentrations were determined using sperm class analyzer® (SCA), version 5.3 (Microptic S.L Barcelona, Spain) and the concentration was recorded in millilitres. Ejaculates containing spermatozoa with >80% progressive motility rate and concentrations >1.0 x 10^9 spermatozoa/ml were used. Daily ejaculates for the four bucks were pooled to avoid individual animal effects and diluted at a ratio of 1:4 (v/v) with TEY, TBAF10-60, TBAF13-70, and TBAF18-80. The semen samples were stored at 5^{o}C for 168 hours. Semen was evaluated for spermatozoa motility, viability, and morphology defects in a randomised design.

4.3.8. Spermatozoa motility evaluation

The spermatozoa motility parameters: total motility, progressive motility, rapid cells, curvilinear velocity, straight-line velocity, average path velocity, linearity, straightness, and wobble were evaluated using the CASA system (Sperm Class Analyzer® 5.3, Microptic, Barcelona, Spain) using the motility program at phase contrast (Ph1) and 10x magnification at 0, 24, 48, 72, 96, 120, 144 and 168 h of storage time. Three micro-litres of semen from the four extended samples were placed inside one chamber of the eight chambered Leja® slides (Leja Products B.V, Nieuw-Vennep, Netherlands) on the warm glass stage of the CASA microscope at 37°C for evaluation. Five fields were captured for each analysis, and the software automatically calculated the motility parameters of spermatozoa samples.

4.3.9. Spermatozoa morphology defects

Spermatozoa morphology defect was determined by staining the spermatozoa with spermac® (Stain Enterprise, Wellington, South Africa). Thin smears were prepared by pipetting a 15 µl drop of extended spermatozoa at one end of a clean microscope slide, seared across, and allowed to dry. The dried smears were stained with spermac® stains following the manufacturer's recommendations. A drop of immersion oil was placed onto the dried, stained slide and covered with a coverslip before evaluation using the CASA microscope at 60x magnification. The percentage of spermatozoa with morphology defects was determined by counting 2000 spermatozoa per stained slide (Raseona *et al*., 2017).

4.3.10. Spermatozoa viability test

Spermatozoa were stained with a nigrosin-eosin stain. Two microscope slides were pre-warmed at 37°C then 6 µl of the extended sample and 20 µl of eosin stain were poured on the end of the slide and mixed using a pipette tip on a warm glass stage at 37°C. A drop of 20 µl of nigrosin stain was then poured onto the mixture on the slide and mixed using the same pipette tip. The edges of the second slide were also pre-warmed at 37°C. Thereafter, placed on the mixture at an angle of 20° from the horizontal plane and pushed forward to make a thin smear on the side. The smeared slide was immediately placed on a hot Buehler® slid warmer (Buehler LTD, Lake Bluff, Illinos, USA) at 120°C to allow drying. The dried, stained slides were placed at room temperature on the CASA microscope stage. A drop of immersion oil was placed on the stained slide and covered with a coverslip before evaluation. The CASA vitality program was used at 60x magnification to count the number of live and dead spermatozoa. 200 spermatozoa were counted per stained slide, and the results were recorded (Soltan *et al*., 2017).

4.3.11. Statistical analysis

The data were subjected to mixed-model analysis of variance (ANOVA) for a 4 x 8 factorial arrangement in a randomized design using the GLM procedures of Minitab software version 17 (Minitab, 2014).

$$Y_{ilK} = \mu + E_i + T_j + (ET_{ij}) + \varepsilon_{ijk}$$

Where Y_{ilK} is the observation – TM, PM, RAP, VCL, VSL, VAP, LIN, STR, WOB, AH, AT, BT, LS.

μ = overall mean common to all observations.

E_i = effect of spermatozoa extender, i =1, 2, 3 and 4; and

T_j = effect of storage time, j = 1, 2, 3, 4, 5, 6, 7 and 8

ET_{ij} = interaction between the j^{th} spermatozoa extender and j^{th} storage time.

ε_{ijk} = residual error

When significant differences were observed, treatment means were compared using Tukey's post hoc test (Steel Torrie, 1981), with the significance level set at $P < 0.05$.

4.4. Results

The results of sperm motility are tabulated in Table 4.2 below.

Table 4.2 Motility mean values of extended semen with TEY, TBAF10, TBAF13 and TBAF18 and stored at 5 °C for 168 hours

Extender	ST (h)	TM %	PM %	RAP %
TEY	0	99.8^{a}	96.0^{a}	96.0^{a}
	24	99.3^{a}	83.2^{abcd}	79.5^{abc}
	48	97.2^{a}	71.3^{abcdef}	64.7^{abcdefgh}
	72	83.0^{a}	52.8^{ef}	45.7d^{efgh}
	96	82.2^{a}	45.2^{fg}	35.7^{hij}
	120	42.5^{b}	19.5^{gh}	9.7^{ijk}
	144	24.5^{b}	6.0^{h}	2.2^{k}
	168	17.7^{b}	6.2^{h}	2.7^{jk}
TBAF10	0	99.5^{a}	92.8^{abc}	92.0^{ab}
	24	98..5^{a}	83.5^{abcd}	81.0^{abc}
	48	96.5^{a}	74.8^{abcd}	70.8^{abcdef}
	72	96.5^{a}	75.0^{abcd}	68.0^{abcdefgh}
	96	94.7^{a}	70.7^{abcdef}	60.8^{bcdefgh}
	120	94.5^{a}	65.8^{abcdef}	56.8^{cdefgh}
	144	92.5^{a}	65.7^{cdef}	55.7^{cdefgh}
	168	89.0^{a}	60.5^{def}	49.5^{cdefgh}
TBAF13	0	99.7^{a}	94.2^{ab}	93.8^{ab}
	24	97.5^{a}	81.5^{abcd}	77.8^{abcd}
	48	97.2^{a}	75.2^{abcde}	67.2^{abcdefgh}
	72	94.2^{a}	69.0^{abcdef}	60.8^{bcdefgh}
	96	94.2^{a}	63.8^{def}	52.2^{cdefgh}
	120	90.0^{a}	63.5^{def}	52.2^{cdefgh}
	144	89.3^{a}	56.7^{def}	48.5^{cdefgh}
	168	87.2^{a}	56.5^{def}	40.7^{ghi}
TBAF18	0	100^{a}	95.7^{a}	95.3^{a}
	24	99.0^{a}	81.3^{abcd}	76.5^{abcde}
	48	98.2^{a}	80.0^{abcde}	74.0^{abcdef}
	72	96.2^{a}	75.3^{abcde}	69.7^{abcdefgh}
	96	93.2^{a}	66.5^{bcdef}	58.0^{cdefgh}
	120	92.3^{a}	65.7^{cdef}	54.5^{cdefgh}
	144	84.5^{a}	57. ^{bcdef}	43.8^{egh}
	168	84.3^{a}	52.0.^{ef}	37.0^{efghi}
SEM		1.49	1.59	1.88
	E	***	***	***
	ST	***	***	***
	E X ST	***	***	***

Mean values with different letters in the same column differ significantly (P < 0.05); SEM = standard error of the mean; ST = storage time; TM = total motility; PM = progressive motility; RAP = rapid cells; TEY = tris egg yolk extender; TBAF1 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with (70 days) 9 cm length fetus from head to tail; TBAF2 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with an (80 days) 11 cm length fetus from head to tail; TBAF3 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with (90 days) 16 cm length fetus from head to tail.

The results of velocity are tabulated in Table 4.3 below.

Table 4.3 Velocity mean values of extended semen with TEY, TBAF10, TBAF13 and TBAF18 and stored at 5 °C for 168 hours

Extender	ST (h)	VCL %	VSL %	VAP %	LIN %	STR %	WOB %
TEY	0	91.0^{a}	24.6^{ab}	47.0^{ab}	49.7	67.8^{ab}	72.6
	24	58.3^{cde}	18.0^{bcde}	32.0^{cd}	37.4	60.8^{abcdef}	62.8
	48	45.0^{cdef}	15.5^{cdef}	26.8^{cde}	35.3	59.4^{abcdef}	58.0
	72	44.6^{cdef}	11.5^{efg}	19.6^{efgh}	33.5	58.2^{abcdef}	57.1
	96	38.4^{cdef}	11.8^{defg}	19.5^{efgh}	32.5	56.7^{abcdef}	56.0
	120	21.8^{ghi}	10.3^{fg}	15.5^{fgh}	32.4	56.1^{abcdef}	55.5
	144	18.7^{hi}	7.2^{g}	11.8^{h}	30.9	54.6^{abcdef}	55.0
	168	18.4^{hi}	6.0^{g}	10.5^{gh}	27.0	52.1^{ef}	51.7
TBAF10	0	84.1^{ab}	26.5^{a}	47.2^{ab}	43.1	67.5^{abc}	61.1
	24	65.7^{bc}	20.8^{abc}	36.5^{bc}	40.4	66.0^{abcd}	61.0
	48	53.6^{cdef}	18.7^{bc}	31.0^{cd}	36.7	62.9^{abcdef}	58.5
	72	50.0^{cdef}	18.3^{bcde}	29.0^{cde}	35.8	62.7^{abcedf}	58.1
	96	45.0^{cdef}	18.2^{bcde}	27.7^{cde}	35.4	61.0^{abcdef}	58.0
	120	43.6^{defg}	17.1^{cdef}	26.9^{cde}	34.8	60.2^{abcdef}	57.8
	144	43.4^{defg}	16.3^{cdef}	25.6^{cdef}	32.0	57.1^{abcdef}	56.0
	168	42.0^{def}	16.0^{cdef}	25.6^{cdef}	31.4	50.7^{f}	55.8
TBAF13	0	93.5^{a}	26.9^{a}	50.5^{a}	45.4	69.9^{a}	64.4
	24	63.9^{bcd}	21.0^{abc}	35.8^{c}	44.4	69.6^{a}	63.3
	48	50.1^{cdef}	18.7^{bc}	28.5^{cde}	37.3	62.5^{abcdef}	59.1
	72	46.1^{cdef}	17.9^{cdef}	26.5^{cde}	36.2	62.3^{abcdef}	57.8
	96	43.4^{defg}	16.0^{cdef}	26.0^{cdef}	34.8	61.0^{abcdef}	57.1
	120	41.3^{efg}	15.7^{cdef}	23.6d^{ef}	34.4	60.7^{abcdef}	56.9
	144	41.2^{efg}	14.7^{cdef}	23.5d^{ef}	33.8	59.0^{abcdef}	56.6
	168	36.9^{efghi}	14.6^{cdef}	23.0^{def}	28.8	53.3^{def}	53.9
TBAF18	0	92.6^{a}	27.2^{a}	50.4^{a}	43.7	68.4^{ab}	63.4
	24	54.3^{cdef}	18.6^{bcd}	30.9^{cd}	42.7	68.0^{ab}	62.7
	48	54.3^{cdef}	18.6^{bcd}	29.9^{cde}	38.5	64.9^{abcde}	60.0
	72	51.1^{cdef}	17.1^{cdef}	29.7^{cde}	38.3	63.0^{abcdef}	59.3
	96	43.6^{defg}	16.4^{cdef}	27.5^{cde}	36.3	62.5^{abcdef}	58.0
	120	42.9^{defg}	15.8^{cdef}	25.6^{cdef}	34.5	59.9^{abcdef}	87.3
	144	37.8^{efghi}	15.6^{cdef}	23.4^{def}	32.1	57.4^{abcdef}	55.7
	168	35.1^{fghi}	15.0^{cdef}	22.4^{defg}	29.4	53.9^{cdef}	54.4
SEM		1.24	0.39	0.62	0.79	0.78	0.65
	E	***	***	***	**NS**	**	**NS**
	ST	***	***	***	***	***	***
	E X ST	**	**	**	*	**NS**	**

Mean values with different letters in the same column differ significantly (P < 0.05); SEM = standard error of the mean; ST = storage time; VCL = curvilinear velocity; VSL = straight-line velocity; VAP = average path velocity; LIN = linearity; STR = straightness; WOB = wobble TEY = tris egg yolk extender; TBAF1 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with (70 days) 9 cm length fetus from head to tail; TBAF2 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with an (80 days) 11 cm length fetus from head to tail; TBAF3 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with (90 days) 16 cm length fetus from head to tail.

The results of sperm morphology are tabulated in Table 4.4 below.

Table 4.4 Morphology mean values of extended semen with TEY, TBAF10, TBAF13 and TBAF18 and stored at 5 °C for 168 hours

Extender	ST (h)	AH %	AT %	BT %	LS %
TEY	0	0.4^{f}	0.4^{f}	0.3^{d}	99.9^{a}
	24	5.2^{abcdefghi}	4.0^{abcdef}	4.2^{bcd}	90.7^{abcd}
	48	6.17^{abcdefghi}	5.0^{abcdef}	4.8^{bcd}	67.8^{efgh}
	72	8.3^{abcdefghi}	8.7^{abcedf}	10.5^{abcd}	66.2^{fgh}
	96	10.3^{abcdefg}	9.8^{abcde}	10.5^{abcd}	50.3^{h}
	120	12.0^{abcde}	10.2^{abcde}	10.8^{abcd}	27.7^{i}
	144	13.8^{ab}	11.3^{abcd}	14.2^{ab}	5.3^{j}
	168	14.3^{a}	13.3^{a}	18.2^{a}	2.8^{j}
TBAF10	0	1.8^{ghi}	1.2^{ef}	2.3^{cd}	99.7^{a}
	24	2.0^{efghi}	2.3^{def}	2.5^{cd}	98.3^{ab}
	48	3.2^{cdefghi}	3.5^{bcdef}	2.8^{cd}	92.2^{abcd}
	72	4.2^{bcdefghi}	6.0^{abcdef}	5.7^{bcd}	88.8^{abcde}
	96	6.3^{abcdefgh}i	6.3^{abcdef}	5.8^{bcd}	74.5^{cdefg}
	120	7.8^{abcdefghi}	7.0^{abcdef}	7.0^{bcd}	68.3^{efgh}
	144	8.3^{abcdefghi}	9.2^{abcdef}	8.2^{abcd}	65.^{8fgh}
	168	9.5^{abcdefghi}	10.8^{abcd}	10.0^{abcd}	63.0^{gh}
TBAF13	0	0.3^{i}	2.5^{cdef}	3.5^{cd}	100^{a}
	24	1.0^{hi}	2.5^{cdef}	3.7^{bcd}	96.7^{ab}
	48	2.2^{fghi}	5.2^{abcdef}	6.2^{bcd}	93.0
	72	3.3^{cdefghi}	6.3^{abcdef}	6.3^{bcd}	84.0^{abcdefg}
	96	5.5^{abcdefghi}	8.7^{abcedf}	9.3^{abcd}	77.8^{bcdefg}
	120	10.8^{abcdefg}	9.8^{abcde}	9.2^{abcd}	74.8^{cdefg}
	144	11.8^{abcdef}	9.8^{abcde}	9.8^{abcd}	71.8^{defh}
	168	12.6^{abc}	10.2^{abcde}	14.2^{ab}	71.7^{defgh}
TBAF18	0	0.1^{i}	7.0^{abcdef}	6.5^{bcd}	99.7^{a}
	24	0.7^{i}	7.3^{abcdef}	6.7^{bcd}	98.3^{ab}
	48	2.8^{decefghi}	8.5^{abcdef}	8.3^{abcd}	96.8^{ab}
	72	4.3^{bcdefghi}	9.2^{abcdef}	9.0^{abcd}	94.5^{abc}
	96	5.5^{abcdefghi}	10.^{2abcde}	9.7^{abcd}	86.0^{abcdef}
	120	5.0^{abcdefghi}	11.8^{abc}	10.0^{abcd}	84.0^{abcdefg}
	144	10.7^{abcdefgh}	12.2^{ab}	10.7^{abc}d	78.0^{bcdefg}
	168	12.5^{abcd}	12.8^{ab}	10.8^{abcd}	71.3^{defgh}
SEM		0.55	0.53	0.60	0.19
	E	**	***	***	***
	ST	**	***	***	***
	E X ST	**NS**	**	*	***

Mean values with different letters in the same column differ significantly (P < 0.05); SEM = standard error of the mean; AH = absent head; AT = absent tail; BT = bent tail; LS = live sperm; TEY = tris egg yolk extender; TBAF1 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with (70 days) 9 cm length fetus from head to tail; TBAF2 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with an (80 days) 11 cm length fetus from head to tail; TBAF3 = bovine amniotic fluid collected 0 h from a slaughtered trimester pregnant cow with (90 days) 16 cm length fetus from head to tail.

Boer goat semen diluted in Tris extender-based bovine amniotic fluid and tris extender-based egg yolk and stored at 5°C in the portable refrigerator for 168 h, were evaluated for total spermatozoa motility, progressive motility, rapid cells, curvilinear velocity, average path velocity, straight-line velocity, linearity percentage, straightness, wobble, absent head, absent tail, bent tail and live sperm. The spermatozoa samples were analyzed after every 24 h until 168 h, and the results are presented in Table 4.2 (motility mean value for TEY, TBAF10, TBAF13 and TBAF18), Table 4.3 (velocity mean value for TEY, TBAF10, TBAF13 and TBAF18) and Table 4.4 (morphology value for TEY, TBAF10, TBAF13 and TBAF18).

Storage time and semen extenders used influenced spermatozoa motility parameters ($P<0.01$) as shown in Table 4.2. Total motility was similar ($P>0.05$) on all the extenders from 0-96 h of the storage. Total motility was highest in sperm diluted with TBAF18 at 0 h, while progressive motility showed the lowest value in TEY at 96 h. However, there was an interaction between the extender and storage time. TBAF13 and TBAF10 offered approximately equal sperm total motility percentages (99.7 % and 99.5 %), but the progressive motility was higher in TEY. However, a difference ($P<0.001$) was observed from 120-168 h, whereby TBAF10, TBAF13 and TBAF18 showed consistency in spermatozoa motility far better than TEY as a control. At 168 h, the highest sperm motility percentage was observed with TBAF10 (84.3 %), and the lowest value was in TEY (17.7 %).

As seen in Table 4.3, sperm velocity parameters (VCL, VSL, VAP and STR) showed significant differences ($P<0.01$) from all the extenders, with an interaction observed between the extender and storage. Curvilinear velocity, VSL and VAP were lowest for sperm diluted in TEY, while STR diluted in TEY at 0 h demonstrated higher sperm velocity percentage (67.8 %) than (67.5 %) of sperm velocity diluted in TBAF10, but the difference was not significant ($P<0.05$). Linearity and WOB showed no significant difference ($P>0.01$) for all the extenders (Table 4.3). Sperm morphology parameters showed significantly different $P<0.001$ from 0-168 h storage time from all the extenders. Concerning spermatozoa morphology defects, shown in Table 4.4, TEY extender gave better results on sperm morphology compared to all the BAF, although the differences were not significant. However, sperm morphology parameters of these three TBAF extenders had the highest ($P<0.05$) live sperms and normal sperm cells compared to the TEY extender.

4.5. Discussion

Boer goat semen diluted in Tris bovine amniotic fluid and stored at 5°C in the portable refrigerator for 168 h showed consistency in total motility for more than 168 h of the storage time. These results suggest that BAF10, 13 and 18 could be an alternative extender to TEY and many more commercial extenders that keep semen viable for 72 h of storage. Moreover, BAF extenders could be a solution for communal livestock farmers who cannot buy fresh semen from reliable suppliers in the city due to the expensive equipment for handling semen, transport costs and liquid nitrogen and tank in case of frozen semen. The results of this study are in agreement with Tirpak *et al*. (2015), who reported that Semen should be extended so that the initial spermatozoa concentration is reduced to a viable number. Motility is one of the vital parameters associated with fertility and is an expression of sperm viability and integrity (Naz *et al*., 2018). Computer-Aided Sperm Analysis (CASA) provides comprehensive and precise information on sperm motion attributes (Kathiravan *et al*., 2008).

In this study, storage time and extender used influenced spermatozoa motility $P<0.01$, as shown in Table 4.2. Tris with heat-inactivated first trimester bovine amniotic fluid extender (TBAF3) stored at 5°C for 168 h recorded the highest sperm motility with less damage to sperm cells. These results are in the same range with previously reported lowest spermatozoa total motility at 5°C for 72 hours on the South African Boer goats breed (85%, Ajao, 2015; 72.6%, Raseona *et al*., 2017; 80%, Manyeleote, 2017), South African indigenous goats (83.1%, Ramukhithi *et al*., 2011; 86.3%, Bopape *et al*., 2015), Saanen goats (62.5%, Nur *et al*., 2005), and the Tankwa goats (70.2%, Ramukhithi, 2016; 78.2%, Ngcauzele, 2018). Like the current study, heat-inactivated first-trimester bovine amniotic fluid (BAF) with 10% added fetal bovine serum supported the development of pre-compacted morula stage in sheep, goat, and cow embryos to the hatched blastocyst stage better than the standard embryo culture medium such as tissue culture medium (TCM)-199 (Barry, 2005). Raseona *et al*. (2017) also reported that for the first time, bull semen was extended in Ham's F10 and TCM-199 culture media and survived for three days. Contrary to the results from this study, Raseona and Barry (2018) observed that spermatozoa motility and viability of cauda epididymal spermatozoa diluted with Ham's F10 supplemented with 30% BAF significantly decreased after 24 h of storage. Moreover, the spermatozoa source is less important (Martinez *et al*., 2008). Tris extender-based egg yolk (TEY) (control) showed more damage to spermatozoa total motility (17.7%), progressive motility (6.0%) and rapid motility (2.2%) when compared to TBAF10, TBAF13 and TBAF18. These results disagree with the observation of Forouzanfar *et al.* (2010) and Soltanpour & Moghaddam (2014), who stated that a diluent of citrate egg yolk had better sperm protection ability than milk-based extenders after eight days of liquid storage at 4 °C,

according to sperm motility and sperm viability. Barry (2005) reported that the earlier the stage of pregnancy, the more concentrated the constituents (growth factors and cytokines) present in amniotic fluid that are beneficial for embryonic development *ex vivo.* Bovine amniotic fluid from a fetus of 80 days of pregnancy supported 46% of grade 1 pre-compacted morula stage embryos to develop to the hatched blastocyst stage. Likewise, this figure increased to 69% when BAF from a 60-day pregnancy was used as a medium inside an incubator. The total sperm motility findings in the current study revealed no significant difference in total motility between TBAF10, TBAF13 and TBAF18. However, the highest motility rate (100%) was recorded from a TBAF18 from a fetus at 90 days of pregnancy, which does not agree with the study in embryos by Barry (2005). Nevertheless, at 40 days of pregnancy, the volume of amniotic fluid present is very small and not feasible to collect.

The result from the current study is in accord with Afroz *et al*. (2008), who reported that commercial extender Triladyl® gave consistently better results in sperm motility rates throughout the storage time at 5°C of semen. Raseona *et al*. (2017) also found consistency in buck spermatozoa motility rates (91.5%) when using Triladly at 5°C for 72 h of storage time. However, these findings were not numerically higher than the result recorded with TBAF13 (94.2%) at 5°C for 72 h, but higher than the control (83.0%) in this study at 72h. This influence could be because BAF contains the necessary nutrients and growth factors for semen dilution (Barry 2005). Sadeghi (2020) reported that sperm motility and viability gradually decreased over time for various reasons such as nutrient depletion in the extenders, which is like the results of this study. This was supported by Salvador *et al*. (2016), who reported that sperm motility declined as time increased. Reduced sperm motility is the main variation as storage time increases (Raseona *et al.,* 2017). Moreover, this result agrees with the observation of Peterson *et al.* (2007) that the number of motile spermatozoa in buck semen chilled for 72 hours progressively dropped over time regardless of whether storage occurred at 4 or 18°C. Findings from this study indicated that total sperm motility parameters diluted with Tris-egg yolk decreased rapidly with the increase in storage time compared to three TBAFs, which slightly decreased as storage time increased. It is assumed that reduced motility and kinematics of spermatozoa in egg yolk-based extenders might be attributed to delayed energy production mechanism at the cellular level due to the presence of high-density lipoproteins (HDL) and other granular substances of egg yolk, which was not observed from the other treatments (Pace & Graham, 1974; Amirat *et al*., 2005). Crespilho *et al*. (2014) reported that the decrease could be due to the production of reactive oxygen species (ROS) that limit spermatozoa motility and fertility of refrigeration sperms. This species occurs as a normal consequence of spermatozoa metabolism and results in an irreversible decline in the quality of cooled spermatozoa (Coian *et al*., 2010). Even though a decrease in temperature causes a

considerable reduction in spermatozoa metabolism, decreasing the rate of fructolysis and oxygen consumption (Blackshaw *et al*., 1956), spermatozoa quality decreases throughout the refrigeration period regardless of the extenders, dilution rate or storage conditions (O'Hara *et al*., 2010). Equally, these findings agree with the results of this study. In this study, both semen extenders and storage time increased spermatozoa VCL, VSL, VAP, STR AT CT, BT, and LS ($P<0.01$) without affecting the LIN and WOB ($P<0.05$). A previous study showed that the semen samples with higher values of VCL, VSL and VAP parameters provided higher rates of pregnancy (Donnelly *et al*., 1998). Additionally, each of these velocities describes a different aspect of the progression of the spermatozoon, along with other parameters such as LIN, STR and WOB (Maksimovic *et al*., 2018). Dunson *et al*. (1999) observed that VCL is constantly the highest among the three velocity parameters, which is parallel to the results of this study. The Boer goat sperm velocity for VCL, VSL, and VAP diluted with TEY at 5°C for 24 hours of the storage time were 45.0µm/s, 15.5µm/s, 26.8µm/s, respectively, which were lower to the values recorded in TBAF10 and TBAF13 except for TBAF18 on VCL (54.3µm/s) and VAP (30.9µm/s) in the current study.

Salamon and Maxwell (2000) reported that sperm morphology refers to the size, shape, and appearance of the sperm, which includes the head, neck, middle piece, plasma membrane and tail. When the sperm cell is abnormal, it could decrease fertility and make it more difficult to fertilize the ovum (Ball & Potters, 2004). Sperm could be misshaped based on the head size, extra head, and no head or tail. Other sperm defects include bent tail, coiled tail, and cut/stump tail. The results of the current study exposed that the largest number (18.2%) of abnormally shaped spermatozoa was recorded in bent tails with TEY extender at 168 h of the storage time. The lowest (0.1%) spermatozoa morphological abnormalities were recorded in the absent head with TBAF13 and TBAF18 at 0 h, followed by TBAF10 in the absent tail at 0 h, respectively. Contrary, these findings are not in agreement with Rammutla (2018), who reported that the highest number (17.7 %) of absent heads at 0 h and the lowest number (1.0 %) was recorded with bent tail at 120 h of the storage time. However, Medeiros *et al.* (2002) stated that every male, fertile or infertile, has different percentages of abnormally shaped spermatozoa. Conversely, these defects might have been caused during the semen smearing and staining process. Many factors could lead to abnormally shaped sperm, including increased testicular temperature, exposure to toxic chemicals, infection, and genetic traits (Barbas and Mascarenhas, 2009). There was a highly significant difference in the live sperm cells from all the extenders. The highest percentage of live sperm cells was observed from TBAF13 (71.7%), whilst the lowest percentage was on TEY (2.8%) as a control at 168 h of the storage time. The reduction in the percentage of live spermatozoa continued to decrease with time, but it is lowest in TEY after 96 h of the storage time. However, the study findings are

similar to Ajao (2015), who stated that the 20% egg yolk content of the Biladyl® and Triladly® maintained Boer goat semen livability and morphology at 5°C after 72 hours. This finding did not align with Raseona *et al*. (2017), who reported the highest semen viability obtained by Triladyl (41%). Trimester bovine amniotic fluid in this study maintained Boer goat semen livability far better than commercial extender Triladyl and TEY as control. Semen extender and long storage time reduced STR and AT% ($P>0.05$). Highly significant improvement was recorded in spermatozoa motility diluted with TBAF18, TBAF13 and TBAF10 compared to TEY extender stored at 5°C for 168 h of the storage time. However, there were no significant differences ($P>0.05$) recorded in spermatozoa motility from all the extenders from 0-96 h of the storage time, and the total motility was above 80% from all the extenders.

4.6. Conclusion

This study shows that tris extender-based bovine amniotic fluid (TBAF) contains the nutrients and growth factors needed for semen dilution compared to tris extender-based egg yolk as a control. In addition, TBAF could be stored at five degrees Celsius for more than a week (7 days), and the sperm total motility will be more than 80% after 168 h of the storage time. The TBAF is cheaper and easily available from the local commercial abattoir and could be used as an alternative to commercial extenders. Therefore, this can accelerate the use of AI in commercial and communal goat farming, expanding the income and nutrition status of needy households.

4.7. References

Afroz, S., Islam, R., Khandoker, M.A.M.Y. and Akter, Q.S., (2008). Cryopreservation of Black Bengal buck semen: effects of diluents and freezing on sperm motility and morphology. *Anim. Sci. J*. 79, 550-553.

Ajao, A.O., Benyi, K., and Barry, D.M., (2015) Evaluation of the effectiveness of different extenders of goat bucks semen under refrigerated condition. Masters. Dissertation, University of Venda, South Africa.

Amirat, L., Anton, M., Tainturier, D., Chatagnon, G. R., Battut, I., & Courtens, J. L., (2005). Modifications of bull spermatozoa induced by three extenders: Biociphos, low density lipoprotein and Triladyl, before, during and after freezing and thawing. *Reproduction*. 129, 535–543.

Anzhar, M., Faroog, U., Mirza, M.A., Shahab, M., and Ahmad, N., (2003). Factors affecting the efficiency of artificial insemination in cattle and buffalo in Punjab. Pak. *Vet. J.* 23, 106-113.

Azeredo, G. A., Esper, C. R., and Resende, K. T., (2001). Evaluation of plasma membrane integrity of frozen thawed goat spermatozoa with or without seminal plasma. *Small Rumin. Res*. 41, 257-263.

Ball, P.J. and Peters, A.R., (2004). Reproduction in cattle. (3rd edition). John Wiley & Sons.

Barbas, J.P. and Mascarenhas, E. R. D., (2009). Cryopreservation of domestic animal sperm cell. Cell Tissue Bank. 10, 49-62. (ISBN: 9781405115452). DOI:10.1002/9780470751091.

Christensen, P., Stenvang, J.P., Z., Korsgaard, I.R., Pedersen, K.M., Jensen, J., and Lehn-Jensen, H., (2000). Objective parameters for estimation of the fertility of bull semen, in procceding of the 14th International Congress in Animal Reproduction. Stockholm. *J Androl*. 26, 98-106.

Dunson, D.B., Weinberg, C.R., Perreault, S.D., and Chapin, R.E., (1999). Summarizing the motion of self-propelled cells: applications to sperm motility. *Int, J. Biom*, 55, 537–543. DOI: 10.1111/j.0006-341X.1999.00537.

Ferreira, V.S., Mello, M.R.B., Fonseca, C.E.M., Dias, A.C.F., Jéssica Machado Cardoso, J.M.C, Silva, R., Pereira, M.J., (2014). Effect of seminal plasma and egg yolk concentration on freezability of goat semen. *R. Bras. Zootec*. 43, 513-518.

Forouzanfar, M., Sharafi, M. and Hosseini, M.S., Ostadhosseini, S., Hajian, M., Hosseini, L., Abedi, P., Nili, N., Rahmani, R.H., Nasr-Esfahani, H.M., (2010). In vitro comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of ram semen. *Theriogenology*. 73, 4, 480-487.

Frandson. R.D., Wilke, W.L., and Fails, A.D., (2009). Anatomy and physiology of farm animals, 7th ed. Wiley-Blavkwell. Ames, Iowa.

Gadea, J. 2003. Semen extenders used in the insemination of swine. *Spain J. Agric*. 1, 17-27.

Holt, W.V., (2000). Basic aspects of frozen storage of semen. *Anim. Reprod. Sci*. 62, 3-22.

Januskauskas, A., and Zilinskas, H., (2002). Bull semen evaluation post-thaw and relation of semen characteristics to bull's fertility. *Vet. Zoot*. 17, 19.

Kalonji, M.P.V., (2017). Evaluation of Nguni bull semen extended in Tris Egg yolk extender, soyabean milk and coconut water based extenders and stored at different temperatures, Master's Dissertation University of Venda, Thohoyandou.

Kathiravan, P., Kalatharan, J., Edwin, M., and Veerapandian, C., (2008). Computer automated analysis of crossbred bull spermatozoa and its relationship with in vitro fertility in zona-free hamster oocytes. *Anim Reprod Sci*. 104, 9–17. DOI10.1016/j.anireprosci.

Kathiravan, P., Kalatharan, J., Karthikeya, G., Rengaraian, K., and Kadirvel, G., (2011). Objective Sperm Mortion Analysis to Assess Dairy Bull Fertility Using Computer-Aided System: a review. Repro Domest Anim. 46, 165-172. DOI. 10.1111/j.1439-0531.2010.01603.

Lebbie, S.H.B., Kagwine, E., (1996). Small Ruminant Research and Development in Africa. In, Proceeding of the Third Biennial Conference of the African Small Ruminant Research Network, UICC, Kampala, Unganda, 5-9 December 1994. ILRI Nairobi, Kenya, 326.

Mafukata, M.A., (2014). Predicting the factors affecting farm productivity amongst communal cattle farmers in Vhembe District, Limpopo Province. *Int. J. Agric. Sci*. 4, 12.

Mafukata, M.A., (2015). Factors having the most significance on the choice and selection of marketing channels amongst communal cattle farmers in Vhembe District, Limpopo Province. *J. Hum. Ecol*. 19, 77-87.

Maksimovic, N., Milovanovic, A., Barna T., Caro Petrovic, V., Pantelic V., Lazarevic, M., Stojanov., I., (2018). Short-term liquid storage of ram semen in various extenders. S, Afri. J. Anim Sci. 48, 718-723.

Manyeleote, V., (2017). Evaluation of suitable chilled, extended semen preservation time and the effect of different artificial insemination on the fertility of indigenous goats. Master's Dissertation, University of Venda, Thohoyandou. South Arica.

Martinez, A.F., Martinez-Psator, F., Alvarez, M., Fernandez-Santos, M.R., Esteso, M.C De Paz, P., (2008). Sperm parameters on Iberian red deer: electro-ejacution and post-mortem collection. *Theriogenology* 70, 216-26.

Medeiros, C. M. O., Forell, F., Oliveria, A.T.D., and Rodrigues, J. L., (2002). Current status of semen cryopreservation: why isn't it better?. *Theriogenology*. 57: 327-44.

Naz, S., Umair, M., 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. *J. Androl*. 10, 1-6.

Nethengwe, L.D., Francis, J., and Barry, D.M., (2016). The effect of three ovulation inducing drugs on pregnancy rates of rural cattle after administration of synchronized artificial insemination in Vhembe district. Masters Dissertation, University of Venda, South Africa.

Ngcauzele, A., (2018). Seasonal differences in semen characteristics and sperm functionality in Tankwa goats. Master's Dissertation. University of the Western Cape, Bellville. South Africa.

Nur, Z., Dogan, I., Gunay, U., and Soylu, M.K., (2005). Relationships between Sperm Membrane Integrity and Other Semen Quality Characteristics of the Semen of Saanen Bucks. Bulletin of Veterinary Institute in Pulawy, 49, 183-187.

Pace, M. M., and Graham, E. F., (1974). Components in egg yolk which protect bovine spermatozoa during freezing. *J. Anim. Sci*, 39, 1144–1149.

Peterson, K., Kappen, M.A.P.M., Ursem, P.J.F., Nothling, J.O., Colenbrander, B., Gadella., and BM., (2007). Microscopic and flow cytometric semen assessment of Dutch AI-bucks: Effect of semen processing procedures and their correlation to fertility. *Theriogenology*. 67, 863-871.

Rammutla, T.L., (2018). Effect of different disaccharides as energy supplements in Tris-Egg yolk semen extender on the quality of cryopreserved Boer goat spermatozoa. Master's Dissertation, University of Venda, Thohoyandou. South Africa.

Ramukhithi, F.V., (2011). Characterization and Cryopreservation of South African Unimproved Indigenous and Boer Buck Semen. Master's Dissertation, Tshwane University of Technology, Pretoria. South Africa.

Ramukhithi, F.V., (2016). Prediction of fertility in South African indigenous goats using semen and phenotypic parameters. Doctoral Dissertation, Tshwane University of Technology, Pretoria. South Africa

Raseona, A.M., and Barry D.M., (2018). Extension of fresh bull semen in Ham;s F10 stored at different storage conditions for 72 h. *J. Vet. Adv*. 17, 133-138.

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. S. *Afr. J. Anim sci*. 47, 49-55.

Sadeghi, S., Del Gallego, R., García-Colomer B., Ernesto A. Gómez, J., Jesus L., Gosalvez, J., Lopez-Fernandez., C., and Miguel A., (2020). Effect of Sperm Concentration and Storage Temperature on Goat Spermatozoa during Liquid Storage. *Biology*. 9, 1-13

Salamon, S and Maxwell, W., (2000). Storage of ram semen. *Anim. Reprod. Sci*. 62. (1), 77-111.

Salisbury, G.W., and Van Demark, N.L., (1961). Significance of semen quality. In, physiology of reproduction and artificial insemination in cattle 1st ed. Freeman, W.H., and San Francisco, C.O. 359-379.

Salvador, I., Yaniz, J., Viudes-de-Castro, M.P., Gomez, E.A., Silvestre, M.A., (2006). Effect of solid storage on caprine semen conservation at 5 ◦C. *Theriogenology*. 66, 974–981.

Soltanpour, F. and Moghaddam G., 2014. Effect of diluents on storage of ram semen. JAAS 2, 6, 179-183. Vaić, D., 2011. 'Optidyl' is a concentrated extender for freezing of bulls semen doses. In: Proceedings of XXV Conference of Agronomist, Veterinarians and Technologists. Belgrade, Serbia, 101-104

Stroebel, A., Swanepoel, F.J.C. & Pell, A.N., 2011. Sustainable smallholder livestock systems – a case study of Limpopo Province, South Africa. *J. Livest. Sci*. 139, 186-190.

Tripak, F., Slanina, T., Ofukany, M., Lukac, N., Massinyi, P., (2015). Effect of taurine on bovine spermatozoa motility parameters following cryopreservation. Slovak *J. Anim. Sci*., 48, 49-56.

Watson, P. F. 1995. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reproduction, Fertility and Development 7, 871-891. DOI: 10.1071/RD9950871

Almeida, J., Brito, M., Neves, B.P., Becerra, V.A.B., Auler, P.A., Haddad, J.P., Baruselli, P.S., Henry, M., (2022). Use of cooled buffalo semen as a strategy to increase conception rates in fixed-time artificial insemination programs during unfavorable reproductive periods. Arquivo Brasileiro de Medicina Veterinária e Zootecnia (online).73, 560-570.

Kasimanickam, R., Kasimanickam, V., Tibracy, A. & Pelzer, K., (2011). Effect of semen extenders on sperm parameters of ram semen during liquid storage at 4°C. *Small Rumin. Res*. 99, 208-13.

Rahman, S., (2014). Preservation of indigenous ram semen with skim milk diluent. MSc thesis, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

CHAPTER 5: EFFECT OF BOVINE AMNIOTIC FLUID-TRIS EXTENDER ON THE BOER GOAT SEMEN CRYOPRESERVATION

5.1. Abstract

This study investigated incorporating heat-inactivated bovine amniotic fluid instead of the traditional egg yolk in goat semen extender. The semen samples were collected from four healthy Boer goat bucks, aged 1–2 years, using an electro-ejaculator. Collected semen was divided into 4 parts; the first was diluted with Tris–egg yolk extender (TEY) as control, while the others were diluted with Tris extender based bovine amniotic fluid of 10, 13 and 18 cm head-tail length fetus (60, 70 and 80 days pregnancy, respectively), denoted (BAF10, BAF13 and BAF18, respectively). The semen samples were loaded into 0.25 ml France straws, sealed, and cooled at 5 °C for 4 h to equilibrate. After equilibration, the straws were frozen in liquid nitrogen (LN_2) vapour, 4 cm above the surface of LN_2, for 10 minutes. The straw was immersed into LN_2 and kept at -196 °C for 7 days. After thawing at 37 °C for 60 seconds, the spermatozoa were transferred into a 37 °C pre-warmed sterile 5 mi tube and subsequently evaluated for spermatozoa motility, viability, and morphology defects in a randomised design. The results revealed that there was a significant difference between the TEY, TBAF10, TBAF13 and TBAF18 observed from all the evaluated spermatozoa morphology (AH, AT, and BT) and TM ($P<0.05$), except for PM and RAP cells. However, PM was highest in sperm diluted with TBAF18 (58.8%), while RAP cells showed the lowest value in TEY (38.0%) during frozen semen (FR). Additionally, 50% of sperm velocity parameters (VSL, VAP and STR) and Sperm viability (LS) showed no significant difference from all the extenders ($P>0.05$). After freezing, TBAF10 and TBAF13 presented equal sperm velocity percentages (37.2%), but the VCL was higher with semen diluted in TBAF18 (43.1%). Cryopreservation of refrigeration (RF) and frozen semen affected the motility of spermatozoa regardless of the extender used. The highest spermatozoa motility percentage was observed with TBAF18 (99.3%) even after the freezing effect (90.3%), and the lowest value was in TBAF10 (85.0%) after freezing. Therefore, the present study concluded that semen cryopreserved in TBAF18 prolonged the viability of spermatozoa when compared with TEY as a control.

Keywords: Bovine amniotic fluid, Boer bucks, Cryopreservation, Extender, Tris-egg yolk

5.2. Introduction

Semen cryopreservation is a technique to preserve and minimise detrimental effects on sperm cells kinematics, plasma membrane, acrosome, and DNA integrity (Celeghini *et al*., 2008). Semen could be used successfully for many years after cryopreservation (Kalonji, 2017). Formerly, goat semen was very challenging to freeze due to the high concentration of enzyme reactions interacting with the common components of cryopreservation media (Purdy 2006). Recently, several successful attempts have been made to freeze goat semen using various protocols (Morrell *et al.,* 2022). Goat semen preservation is a complex process that involves many factors to obtain good results (Ngoula *et al*., 2014). Therefore, an extender should not only increase the volume of semen but also be capable of prolonging the life span of spermatozoa without compromising productivity (Karim *et al.,* 2018). The most used extender for liquid or frozen semen has egg york as a basic ingredient (Makkawi *et al*., 2002). Egg yolk provides an excellent fortification for goat spermatozoa in fighting against cold shock and lipid-phase transformation (Aboagla, 2004). To conduct semen cryopreservation, selecting a suitable and economical diluter is essential to test the efficacy of the common component of the freezing diluents (Roca *et al*., 2000). Egg yolk is added to buck semen extender as a source of energy that stretches the sperm cells with lecithin, protein, and lipoproteins (Semenova, 1987). Egg yolk is a major constituent of extenders used for storage and cryopreservation of semen of domestic animals, including bull, ram, goat and pig. The main advantage of an egg yolk extender is the fraction of low-density lipoprotein, which protects the sperm phospholipids during cryopreservation (Amirat *et al*., 2005). However, wide variations in egg yolk constituents make assessing the beneficial effect difficult (Gil *et al*., 2003). Moreover, the fertilising capacity of spermatozoa is negatively affected by the risk of microbial contamination associated with egg yolk (Aires *et al*., 2003). It has also been reported that the fat globules of egg yolk make the evaluation of sperm difficult (Singh *et al*., 2012). Contrarily, several studies have stored goat semen at different temperatures using various diluents such as sodium citrate-yolk, sodium citrate-fructose-yolk, coconut water, and milk with or without egg yolk (Lebouef *et al*.,2000). Amiri (1997) reported that Tris-fructose-egg-yolk (TFEY) and glucose-citrate-egg-yolk (GCEY) are better diluents than skim milk diluents. Previous studies revealed that including soybean milk in semen extender improved post-thawed sperm characteristics far better than egg yolk extender (Papa *et al.,* 2011; Singh *et al.,* 2012; Sorongbe *et al.,* 2019). Bovine amniotic fluid (BAF) is rich in different important semen extender constituents such as protein, fatty acid, glucose, cholesterol, and vitamins, which are essential components of semen extender (Ning *et al.,* 2005; Cambell and Farrel, 2007; Zhang *et al.,* 2009; USDA, 2018). It seems rational to compare the cryo-survival of spermatozoa, considering characters with different diluters in preserving buck semen. Therefore, the

development of a suitable and affordable extender for preserving buck semen is essential for the adoption of AI in goats and to improve the livelihood of communal farmers. The objective of this study was, therefore, to evaluate the effect of bovine amniotic fluid extender on the Boer goat semen cryopreservation.

5.3. Material and methods

5.3.1. Ethics

This study was carried out under the recommendations, care, and use of animals through the University of Venda Animal Ethics Committee guidelines. Ethical clearance was obtained from the University of Venda Research Ethics Committee with a project registration number (SARDF/17/IRD/08/2908) for permission to conduct this study.

5.3.2. Study area

The study was conducted at the School of Agriculture in the Centre of Excellent in Animal Assisted Reproduction biotechnology laboratory, University of Venda in Thohoyandou, Limpopo province of South Africa. In Thohoyandou, the summers are long, warm, humid, and partly cloudy, and the winters are short, cool, dry, and clear (Osidele, 2016). Over the course of the year, the temperature typically varies from *9°C* to *29°C* and is rarely below *7°C* or above *35°C* (Mzezewa *et al*., 2010).

5.3.4. Animals and management

Semen was collected from four healthy (1–2-year-old) Boer bucks managed at the University of Venda experimental goat feedlot. Each buck was fed 1.8 to 2 kg concentrate peiiets and water was provided *ad-libitum*.

5.2.4. Source and preparation of bovine amniotic fluid

Bovine uteri containing foetuses 10-20 cm from head-tail base length (60-90 days pregnancy) were collected from mixed beef cattle of unknown origin from a local commercial abattoir. The uteri were collected immediately upon slaughter using a cooled polystyrene box at 5-7 °C and transported within 2 h to the University of Venda Animal Science Laboratory. The uterine wall

was then dissected open using a sterile surgical blade, and the amniotic fluid was aspirated with a sterile rubber less 50 ml syringe (Normo-jet Zentrisch, Henke Sass Wolf, Germany) and an 18-gauge needle. The amniotic fluid was heat inactivated at 56°C for 30 minutes using a water bath and then stored at 5°C in the screw cap 15 ml plastic vials until needed (Barry, 2005).

5.3.5. Preparation of egg yolk

Chicken eggs were randomly collected fresh from the commercial flock (white leghorn) a day before the extenders were prepared. Chickens were kept under an open-sided caged house at the University of Venda Experimental Farm. Feed (commercial laying mess) and water were provided *ad libitum*. All the eggs were washed in distilled water and wiped with a disinfected paper towel with 70% alcohol. This was done to ensure that no microorganisms could contaminate the extenders. The eggshell was wrecked by slightly tapping the egg against the side of an egg divider. Egg yolks were obtained using a sterile egg diver and then transferred to a clean filter paper to absorb and remove the remaining albumin. A sterile needle was then used to carefully puncture the yolk membrane and aspirate it into a 20 ml syringe. Therefore, the egg yolk was poured into a plastic vial (Cellstar, Greiner Bio-One) (Foot, 1960).

5.3.6. Preparation of extenders

The formulation of the experimental extenders is presented in Table 5.1. To prepare the extenders, 100 ml of distilled water was added to a borosilicate glass beaker, in which the different ingredients of Tris-based extenders were split into 15 ml CellStar tubes of Egg yolk, BAF10-60, BAF13-70, and BAF18-80. The extenders were stored at 5°C in the refrigerator and used for a maximum of 8 days after preparation. The extender pH was measured using a pH meter (Metter-Toledo AG, Analytical, Sonnenbergstrasse 74, Schwerzenbach) and adjusted to 6.8 using acetic acid and sodium hydroxide.

The composition of frozen semen, Tris extender-based egg yolk, and Tris extender-based bovine amniotic fluid 10,13 and 18 are tabulated in Table 5.1 below.

Table 5.1. Composition of frozen semen extenders

Extender Ingredients	TEY	TBAF10-60	TBAF13-70	TBAF18-80
Tris (g)	2.422	2.422	2.422	2.422
Citric acid (g)	1.36	1.36	1.36	1.36
Monohydrate glucose (g)	1	1	1	1
Antibiotic (g)	0.1	0.1	0.1	0.1
Glycerol (ml)	14	14	14	14
Egg yolk (% v/v)	20	_	_	_
BAF10-60 (% v/v)	_	20	_	_
BAF13-70 (% v/v)	_	_	20	
BAF18-80 (% v/v)	_	_	_	20
Total volume (ml)	**100**	**100**	**100**	**100**

TEY: Tris extender-based egg-yolk TBAF10-60: TBAF13-70; TBAF18-80; Tris extender-based bovine amniotic fluid of 10,13,18 cm head-tail base length foetus (60, 70, and 80 days pregnancy, respectively).

5.3.7. Semen collection, processing, and experimental design

Semen was collected from four Boer goat bucks with proven fertility using an electro-ejaculator pulsator IV, Auto: ajustTM, 15 A. 125 V (lane manufacturing INC Denver Colorado, USA). A total of 48 ejaculates were collected from the bucks (twice per week per buck) over 6 weeks. The electro-ejaculation was performed in the morning with the bucks restrained in the pen in a standing position. Just before semen samples were collected, the preputial hair of each buck was clipped, and the orifice was thoroughly washed, rinsed and dried. The sheath was washed with phosphate buffer saline (PBS) to avoid contamination of the ejaculates.

Immediately after collection, the volume of each semen sample was measured. Low semen volume is associated with low sperm concentration (Ajao, 2015). The colour of each semen sample was appraised visually and recorded. The samples were placed into labelled, pre-warmed 15 ml Cellstar tubes and transported to the biotechnology laboratory in a vacuum flask with warm water (37°C). The pH of each sample was measured using a Metler Toledo (AG Analytical, Sonnenbergstrasse 74, Schwerzenbach) pH meter. The sperm concentrations were determined using sperm class analyzer® (SCA), version 5.3 (Microptic S.L Barcelona, Spain) and the concentration was recorded in millilitres. Ejaculates containing spermatozoa with >80% progressive motility rate and concentrations >1.0 x 10^9 spermatozoa/ml were used. Daily ejaculates for the four bucks were pooled to avoid individual animal effects and diluted at a ratio of 1:4 (v/v) with TEY, TBAF10-60, TBAF13-70, and TBAF18-80. The semen samples were loaded into 0.25 ml France straws, sealed and cooled at 5 °C for 4 h to equilibrate. After equilibration, the straws were frozen in liquid nitrogen (LN_2) vapour, 4 cm above the surface of LN_2, for 10 minutes. The straw was immersed into LN_2 and kept at -196 °C for 7 days. After thawing at 37 °C for 60 seconds, the spermatozoa were transferred into a 37 °C pre-warmed sterile 5 mi tube and subsequently evaluated for spermatozoa motility, viability, and morphology defects in a randomised design.

5.3.8. Spermatozoa motility evaluation

The spermatozoa motility parameters were evaluated using the CASA system (Sperm Class Analyzer® 5.3, Microptic, Barcelona, Spain) using the motility program at phase contrast (Ph1) and 10x magnification. For each of the samples, 3 µl was placed inside one chamber of the eight chambers of Leja® slides (Leja Products B.V, Nieuw-Vennep, Netherlands) on the warm glass stage of the CASA microscope at 37°C for evaluation. Five fields were captured for each analysis, and the software automatically calculated the motility parameters of spermatozoa samples.

5.3.9. Spermatozoa morphology defects

Spermatozoa morphology defect was determined by staining the spermatozoa with spermac® (Stain Enterprise, Wellington, South Africa). Thin smears were prepared by pipetting a 15 µl drop of extended spermatozoa at one end of a clean microscope slide, seared across, and allowed to dry. The dried smears were stained with spermac® stains following the manufacturer's recommendations. A drop of immersion oil was placed onto the dried, stained slide and covered with a coverslip before evaluation using the CASA microscope at 60x magnification. The percentage of spermatozoa with morphology defects was determined by counting 2000 spermatozoa per stained slide (Raseona *et al*., 2017).

5.3.10. Spermatozoa viability test

Spermatozoa were stained with the nigrosine stain previously described by Soltan *et al.* (2017). After staining, a drop of immersion oil was placed on the stained slide for evaluation using the CASA viability program at 60x magnification. A total of 200 spermatozoa were counted per stained side.

5.3.11. Statistical analysis

The data were subjected to mixed-model analysis of variance (ANOVA) for a 4 x 2 factorial arrangement in a randomized design using the GLM procedures of Minitab software version 17 (Minitab, 2014).

$$Y_{ilK} = \mu + E_i + T_j + (ET_{ij}) + \varepsilon_{ijk}$$

Where Y_{ilK} is the observation – TM, PM, RAP, VCL, VSL, VAP, LIN, STR, WOB, AH, AT, BT, LS;

μ = overall mean common to all observations;

E_i = effect of spermatozoa extender, i =1, 2, 3 and 4; and

T_j = effect of storage time, j = 1 and 2

ET_{ij} = interaction between the j^{th} spermatozoa extender and j^{th} storage time.

ε_{ijk} = residual error

When significant differences were observed, treatment means were compared using Tukey's post hoc test (Steel Torrie, 1981), with the significance level set at $P < 0.05$.

5.4. Results

Boer goat semen samples were diluted with TEY, TBAF10, TBAF13, and TBAF18 extenders and were stored at 5 °C for 4 h to equilibrate and cryopreserved in LN_2 at -196 °C for 7 days. The results of the analyses are presented in Table 6.1 below.

Table 5.2 Spermatozoa motility, morphology and viability mean values of TEY, TBAF10, TBAF13 and TBAF18 extenders evaluated before and after thawing.

Extender (E)	SM	TM%	PM%	RAP%	VCL%	VSL%	VAP%	LIN%	STR%	WOB%	AH%	AT%	BT%	LS%
TEY	RF	98.0^{a}	87.3^{a}	85.2^{a}	63.5^{bc}	20.6^{ab}	36.1a^{b}	33.1^{bcd}	57.1^{abc}	57.5^{cde}	0.1^{b}	0.2^{b}	0.2^{c}	95^{a}
	FR	88.8^{bc}	49.7^{c}	38,0^{b}	34,3^{d}	15.3^{bc}	23.6^{c}	44.8^{a}	65.2^{a}	68.7^{a}	0.1^{b}	0.5^{b}	0.5^{c}	52^{b}
BAF10	RF	98.2^{a}	93.7^{a}	93.0^{a}	85.2^{a}	26.1^{a}	46.6^{a}	30.3^{cd}	50.4^{c}	54.6^{de}	8.5^{a}	7.7^{a}	8.7^{ab}	96^{a}
	FR	85.0^{c}	48.3^{c}	40.2^{b}	37.2^{d}	14.9^{bc}	23.1^{c}	39.7^{ab}	64.4^{ab}	62.2^{bc}	10.8^{a}	9.8^{a}	15. ^{a}	49^{b}
BAF13	RF	98.8^{a}	77.0^{ab}	89.2^{a}	77.8^{ab}	23.5^{a}	43.0^{a}	30.4^{cd}	54.6^{abc}	55.4^{de}	6.7^{ab}	5.7^{b}	10.^{ab}	97^{a}
	FR	88.5^{bc}	50.0^{c}	43.5^{b}	37.2^{d}	14.2^{c}	23.7^{c}	36.0^{bc}	60.8^{abc}	59.1^{bcd}	10.5^{a}	7.7^{a}	12.^{ab}	47^{b}
BAF18	RF	99.3^{a}	93.0^{a}	92.3^{a}	83.3^{ab}	23.4^{a}	44.9^{a}	28.4^{d}	53.4^{bc}	53.1^{e}	10.2^{a}	4.8^{ab}	6.5^{bc}	97^{a}
	FR	90.3^{a}	58.8^{bc}	50.2^{b}	43.1^{cd}	15.3^{bc}	25.5^{bc}	38.1^{ab}	59.6^{abc}	63.8^{ab}	12.8^{a}	7.8^{a}	10.^{ab}	49^{b}
SEM		0.71	3.17	1.27	2.80	0.78	1.46	0.95	1.49	0.73	0.92	0.75	1.03	1.19
	E	*	NS	NS	**	NS	NS	**	NS	***	***	***	***	NS
	SM	***	***	***	***	***	***	***	***	***	**	**	**	***
	E ×SM	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS

Mean values with different letters in the same column differ significantly (P < 0.05); SEM = standard error of the mean; SM = storage method; RF = refrigeration; FR = frozen; TM = total motility; PM = progressive motility; RAP = rapid cells; VCL = curvilinear velocity; VSL = straight-line velocity; VAP = average path velocity; LIN = linearity; STR = straightness; WOB = wobble; AH = absent head; AT = absent tail; BT = bent tail; LS = live sperm; TEY = tris egg yolk extender; TBAF10-60: TBAF13-70; TBAF18-80; Tris extender based bovine amniotic fluid of 10,13,18 cm head-tail base length foetus (60, 70, and 80 days pregnancy.

Table 5.1. Shows the summarised results of sperm motility, morphology and viability percentage characteristics of semen exposed to TEY, TBAF10, TBAF13, and TBAF18 extenders during refrigeration (RF) and frozen (FR) storage methods. Semen was evaluated for total spermatozoa motility (TM), progressive motility (PM), rapid cells (RAP), curvilinear velocity (CVL), straight-line velocity (VSL), average path velocity (VAP), linearity percentage (LIN), straightness (STR), wobble (WOB), absent head (AH), absent tail (AT), bent tail (BT) and live sperm (LS). The spermatozoa were analysed at two different times namely immediate dilution and post-thawing. The total spermatozoa motility rate for RF varied between 98.0%, 98.2%, 98.8% and 99.3%, while the FR rate varied between 85.0%, 88.5%, 88.8% and 90.3% average from all the extenders. Furthermore, a significant difference among the TEY, TBAF10, TBAF13 and TBAF18 was observed from all the evaluated spermatozoa morphology (AH, AT and BT) and TM ($P<0.05$), except for PM and RAP cells. However, PM was highest in sperm diluted with TBAF18 (58.8%), while RAP cells showed the lowest value in TEY (38.0%) during FR semen. Additionally, 50% of sperm velocity parameters (VSL, VAP and STR) and Sperm viability (LS) showed no significant difference from all the extenders ($P>0.05$). After freezing, TBAF10 and TBAF13 presented equal sperm velocity percentages (37.2%), but the VCL was higher with semen diluted with TBAF18 (43.1%). Cryopreservation of RF and FR affected the motility of spermatozoa regardless of the extender used. The highest spermatozoa motility percentage was observed with TBAF18 (99.3%) even after the freezing effect (90.3%), and the lowest value was in TBAF10 (85.0%) after freezing. No significant difference ($P>0.05$) was observed between TEY, TBAF10 and TBAF13 during RF. Subsequently, this was also observed with TBAF13 (88.5%) and TEY (88.8%) spermatozoa motility percentages evaluated with frozen semen.

5.5. Discussion

Semen cryopreservation requires good-quality diluents, which could maintain spermatozoa motility, morphology and viability parameters and curb temperature changes during the cooling and freezing phase (Nisfimawardah *et al.*, 2023). The present study confirmed that cryopreservation of Boer goat semen with bovine amnionic fluid-Tris extender before and after freezing could preserve spermatozoa motility, morphology, and viability. However, all refrigeration semen diluted with bovine amniotic fluid-Tris extender had a higher spermatozoa motility rate than Tris egg yolk extender as a control, but after freezing, the highest value in spermatozoa viability was recorded with a control. Furthermore, total spermatozoa motility and live sperm were significantly decreased after freezing and thawing. Similar findings for a decrease in motility due to freezing and thawing compared with refrigeration semen have been reported in Boer goats (Rammutla, 2018), Saanen goats (Nisfimawardah *et al*., 2023), Florida goats (Dorado *et al*., 2010) and Beetal goats (Ahmad *et al*., 2014).

The reduction of spermatozoa motility and viability in this study could be due to what was reported by Shah (2020) that semen cryopreservation could damage and decrease sperm quality due to temperature changes, formation of ice crystals and changes in electrolyte concentration due to storage of fresh semen in a frozen state. According to Tekin & Daskin (2019), these damages could produce reactive oxygen species (ROS), lack of energy, ionic imbalance, change in cell volume, hyperosmolarity and protein denaturation. More than 50% of mammalian sperm are usually injured by cryopreservation (Watson 2000). Similarly, the motility rate decreased significantly after freezing and thawing in bull (Kalonji, 2017) and buffalo sperm (*Rasul et al*., 2000). To prevent this cryodamage in goats, a semen diluent is required to maintain the spermatozoa's quality and alter the freezing rate during cryopreservation (Ugur *et al*., 2019). Adding egg yolk into the freezing extender increases the proportion of motile sperm (Aboagla & Terada 2004). This was also supported by Aboagla (2004), who stated that egg yolk provides excellent protection for goat spermatozoa in fighting against cold shock and lipid-phase alteration. However, the post-thawing results obtained in TEY (88.8%) were higher than TBAF10 and TBAF13, but the TEY value was lower than those observed with TBAF18 during FR. However, the results observed in TBAF13 and TBAF10 were greater than those reported by Raseona, (2022) and Kakati *et al*. (2019). Motility is the movement of spermatozoa that moves forward with time. Motility is important in determining sperm viability and ability to fertilise an oocyte (Setiyono *et al.,* 2020). In the current study, the highest post-thawing spermatozoa percentage was observed in TBAF18, and the lowest was in TBAF10. Additionally, TEY was higher than TBAF10 and TBAF13. Similar to the present study, Kalonji (2017) also found that cryopreservation of post-thawing spermatozoa diluted

with TEY maintained total spermatozoa motility rate and the percentage of viable cells when compared to soya bean-milk-based extender (SOBM) and Coconut water-based extender (COWE) in the frozen semen. Celeghini *et al*. (2008) also reoported that more effective post-thawing spermatozoa TM, AH, AT BT and LS values were connected with the use of Botu Bov® containing 20% egg yolk compared to Bio cell® medium constituted of soy lecithin. However, the findings of the previous study were lower than the results observed with FR semen diluted in TBAF18 in the present study. Additionally, there was a significant difference $P<0.05$ observed in the spermatozoa velocity after freezing, verifying that the TEY extender presented the highest values for LIN and WOB. Therefore, the highest value for VCL was observed with semen diluted with TBAF18. This could probably occur due to the difference in composition and components present in each of the extenders used, causing the osmolarity and viscosity of the medium to contribute to a variation in sperm velocity (Almeida & Resende, 2023). These findings were consistent with those by Celegnini (2005), who testified that the density of the medium can directly influence sperm velocity.

The extenders used, and storage method findings obtained in the present study revealed that colling and post-thawing were efficient in preserving spermatozoa viability. Furthermore, the spermatozoa viability rate before and after thawing was similar to all the extenders, which agreed with the study in stallions reported by Monteiro *et al*. (2011). TBAF10 and TBAF18 offered approximately equal sperm viability rates, but the sperm viability was higher in TEY, and the lowest value was observed in TBAF13 with no significant differences between the treatments. Hence, it agrees with the study conducted by Chaveiro *et al*. (2015). The viability of semen drops during the process of cryopreservation, which is due to the storage of liquid semen in a frozen state (Shah *et al*., 2022). This decrease in viability is caused by damage to the cell membrane, which is a complex composition including two layers of phospholipids, proteins, glycoproteins, and carbohydrates (Peris-Frau *et al*., 2020). This is caused by a decrease in temperature during the cold storage process, which causes the lateral movement of phospholipids to change between the liquid and frozen phases leading to the membrane being stiff and brittle (Card *et al*., 2013).

5.6. Conclusion

From the results achieved, it can be concluded that there was a better preservation of the semen samples kept under frozen phase with TBAF18 extender at -196 °C for 7 days, providing greater protection against spermatozoa injuries and defects when compared to TEY extender. Thus, trimester heat-inactivated bovine amniotic fluid has the potential to maintain buck sperm quality for many years after cryopreservation, and it could be an alternative to egg yolk extender.

5.7. References

Aboagla, E.M.E and Terada, T., (2004). Effects of egg yolk during the freezing step of cryopreservation on the viability of goat spermatozoa. *Theriogenology*, 62, 1160-1172. DOI: https://www.doi.org/10.1016/j.theriogenology.2004.01.013

Ahmad, M., Nasrullah, R., Riaz, H., Sattar, A. and Ahmad, N., (2014) Changes in motility, morphology, plasma membrane and acrosome integrity during stages of cryopreservation of buck sperm. Journal of the South African Veterinary Association. 85, 4. DOI: 10.4102/jsava. v85i1.972

Amiri, Y., (1997). Effects of collection frequency, ejaculate number and diluents on the survival and morphology of buck spermatozoa. MS thesis, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Bangladesh.

Campbell, M. K and Farrel, S. O., (2007): Lipids and proteins are associated with biological membranes. (In Portuguese). Campbell MK, Farrel SO (Eds.), Bioquímica (5th ed.), Thomson Learning, São Paulo), (Chapter 8).

Card, C.J. Anderson E.J. Zamberlan, S. Krieger, K.E. Kaproth, M. and Sartini, B.L., (2013). Cryopreserved bovine spermatozoal transcript profile as revealed by high-throughput ribonucleic acid sequencing. *Biol Reprod*. 88, 49. DOI: https://www.doi.org/10.1095/biolreprod.112.103788

Celeghine, E.C.C., (2005). Efeitos da criopreservaçao do semen bovino sobre a membranas plasmática, acrossomal e mitocondrial e estrutura da cromatina dos espermatozoides utilizando sondas fluorescentes. Tese (Doutorado) - Faculdade de Medicina Veterinaria e Zootecnia, Universidade de Sao Paulo, Sao Paulo, 186.

Celeghini, E.C., De Arruda, R.P., De Andrade, A.F., Nascimento, J., Raphael, C.F., Rodrigues, P.H., (2008). Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. *Anim Repro Sci*. DOI: 104(2-4). 119-131.

Dorado, J., Munoz-Serrano, A. and Hidalgo, M., (2010). The effect of cryopreservation on goat semen characteristics related to sperm freezability. *Anim Reprod Sci.* 121, 115–123. http://dx.doi.org/10.1016/j.anireprosci.2010.04.182

El-Battawy, K.A., (2019). Preservation of goat semen at 5°C with emphasis on its freezability and the impact of melatonin. Int. *J. Vet. Sci. Res.* 5, 035-038. DOI: http://dx.doi.org/10.17352/ijvsr.000039

Kakati, U., Sinha, S., Deka, B.C., Biswas, R.K, Baruti, M., Nahardeka, N., and Borah, R.S., (2019). Effect of soybean lecithin-based extender and OviXcell on quality of frozen semen in Beetal, Sirohi and Assam Hill goat. *Int. J. Livest Res,* 9, 95-109. DOI: https://www.doi.org/10.5455/ijlr.20108509033715

Kalonji, M.P.V., (2017). Evaluation of Nguni bull semen extended in Tris Egg yolk extender,

soyabean milk and coconut water-based extenders and stored at different temperatures, Master Dissertation University of Venda, Thohoyandou. South Africa.

Karim, MF, Khandoker M.A.M.Y and Husain S.S., (2018). Fact findings about the frozen buck semen characters freezing with Egg Yolk Citrate and TRIS extender and productivity of Black Bengal does as the potential genetic resource in Bangladesh. *Res. Agric. Livest*. *Fish*. 5, 341-350.

Lebouef, B., Restall, B., and Salamon, S., (2000). Production and storage of goat semen for artifi cial insemination. *Anim Reprod Sci.* 62, 113-141.

Makkawi, A., Elrahman, A and Threlfall W.R., (2002). Influence of steer and heifer blood serum in egg yolk extender on non-cryogenic preservation of bovine semen. Proceedings of the 3rd Scientific Conference of Agricultural Sciences, Assiut, Soudan, pp: 139-151.

Morrell, J.M, Malaluang, P, Ntallaris, T, Johannisson, A., (2022). Practical Method for Freezing Buck Semen. *Animals*. 12, 352. DOI: https:// doi.org/10.3390/ani12030352.

Ngoula, F., Tarla, D.N., Kenfack, A., Bayemi, P.H.D., Bessong, O.W., Tadondjou, C.D., Kamtchouing, P and Tchoumboue, J., (2014). Effect of Egg Yolk Concentration in Semen Extender, pH Adjustment of Extender and Semen Cooling Methods on Bovine Semen Characteristics. *Glob. Vet.* 12, 292-298. DOI: 10.5829/idosi.gv.2014.12.03.8236

Nisfimawardah, L. Firmawati, A. Ihsan, MN, Susilawati T., and Wahjuningsih S. (2023). Semen Cryopreservation Quality and Sperm Kinematics of Saanen Goats using Different Diluents. *World Vet. J*. 13, 300-309. DOI: https://dx.doi.org/10.54203/scil.2023.wvj3.

Osidele, O., (2016). An Analysis of Patterns and Trends of Road Traffic Injuries and Fatalities in Vhembe District, Limpopo Province, South Africa. Thohoyandou: University of Venda.

Papa, F.O. Felicio, G. B. Melo-O na, C. M. Alvarenga, M. A. De Vita, B. Trinque, C. Puoli-Filhob, J. N. P. Dell'Aqu, J. A., (2011). Replacing egg yolk with soyabean lecithin in the cryopreservation of stallion semen. *Anim Reprod Sci.* 129: 73– 77.

Peris-Frau, P. Soler, A.J. Iniesta-Cuerda, M. Martin-Maestro, A. Sánchez-Ajofrin, I. Medina-Chávez, D.A. Fernández-Santos, M.R. Garcia-Alvarez, O. Maroto-Morales, A. Monotoro, V., (2020). Sperm cryodamage in ruminants: Understanding the molecular changes induced by the cryopreservation process to optimize sperm quality. *Int J. Mol. Sci*, 21, 2781. DOI: https://www.doi.org/10.3390/ijms21082781

Purdy, P.H. (2006). A review on goat sperm cryopreservation, *Small Ruminant Res*. 63, 215–225.

Rammutla, T.L., (2018). Effect of different disaccharides as energy supplements in Tris-egg yolk semen extender on the quality of cryopreserved Boer goat spermatozoa. Master Dissertation University of Venda, Thohoyandou. South Africa.

Roca, J, Martinez, S, Vazquez, J.M, Lucas, X, Parrilla, I. 2000. Viability and fertility of rabbit spermatozoa diluted in tris-buffer extenders and stored at 15°C. *Anim Reprod Sci.* 64, 103-

112.

Semenova, V.A., (1987). The use of egg yolk lipoproteins of low molecular weight in diluents for frozen semen of ram. *Anim. Breed*. 55: 4476.

Setiyono, A., Setiadi, M.A., Kaiin, E.M., and Karja, N.W.K., (2020). Movement pattern of bull sperm following incubation in fertilization media supplemented with heparin and/or caffeine. *Jurnal Veteriner,* 21, 458-469. DOI: https://www.doi.org/10.19087/jveteriner.2020.21.3.458

Shah, S.A.H., Haider, M.S., Ahmed, H., Fayyaz, M.H., and Andrabi, S.M.H., (2022). Validation of double freezing protocol of beetal buck (Capra hircus) spermatozoa. *Reprod Domest Anim*. 57, 1615-1622. DOI: https://www.doi.org/10.1111/rda.14238

Singh, A.K., Singh, V.K., Narwade, B.M., Mohanty, T.K., Atreja, S.K., (2012): Comparative quality assessment of buffalo (Bubalus bubalis) semen chilled (5°C) in egg yolk- and soya milk-based extenders. *Reprod Domest Anim.* 47, 596–600.

Sorongbe, T.A., Daramola, J.O., Onagbesan, O.M, Adebayo, A.V., Adekunle, E.O., (2019). Soybean milk extender improves sperm functional and oxidative stress parameters of goat sperm during slow and rapid freezing. *Agricultura Tropica ET Subtropica*, 52, 89-96. DOI: https://doi.org/10.2478/ats-2019-0010

Tekin, K and Daşkin, A., (2019). Effect of polyvinyl alcohol on survival and function of angora buck spermatozoa following cryopreservation. *Cryobiology,* 89: 60-67. DOI: https://www.doi.org/10.1016/j.cryobiol.2019.05.004

USDA. (2018): Organic whole ground tiger nuts. Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 24. Washington, DC:US Department of Agriculture.

Zhang, S.S., Hu, J.H., Li, Q.W., Jiang, Z.I., Zhang, X., Xiao-Ying, Z., (2009). The Cryoprotective effect of soybean lecithin on Boer spermatozoa quality. *Afr. J. Biotechnol.* 18, 6476–6480.

CHAPTER 6: EFFICACY OF USING CHILLED EXTENDED SEMEN AS AN ALTERNATIVE TO FROZEN SEMEN IN AN ARTIFICIAL INSEMINATION PROGRAMME FOR COMMUNALLY MANAGED INDIGENOUS GOAT DOES IN VHEMBE DISTRICT

6.1. Abstract

This study established four animal assisted reproduction centres to facilitate controlled animal breeding, efficient oestrous synchronization, ovulation, and artificial insemination (AI) protocols that accelerate reproductive performance in rural South African indigenous goats of the Vhembe district. The objective of this study was to establish animal-assisted reproduction centres designed to compare the efficacy of using fresh extended semen as an alternative to frozen semen and evaluate the efficacy of three ovulation inducing drugs on the doe's fertility measured as pregnancy rates at the time of AI programme in Vhembe district. One hundred and twenty-eight does were kept in animal-assisted reproduction centers in four local municipalities in the Vhembe district. Does were synchronized using a (CIRD) containing 0.3 g of progesterone on day 0 of synchronization. On day 7 in the morning, CIRD were removed from the vagina and injected 10 mg of $PGF_{2\alpha}$. During day 8 in the morning, does was injected intramuscularly with 300 IU of (eCG). Oestrous was detected by visual observations by experienced personnel with a teaser buck walking around the does pen with adequate visibility and cajoling but of limited physical contact. All does were then trans-cervical inseminated with fresh/ frozen semen diluted in BAF10, BAF13, BAFF18 and TEY 48 h after the removal of the CIRD. Therefore, all does were alternately assigned to one of the three ovulation inducing drugs and a control immediately after AI: (1) 300 IU hCG (Chorulon) injection under the tail into the vein, n = 32; (2) 10 mg dinoprost $PGF_{2\alpha}$ hormone (Lutalyze) intramuscular injection, n = 32; (3) 25 IU oxytocin hormone (Fentocin) intramuscular injection, n = 32 and (4) 5 ml sterile isotonic saline intramuscular injection as a control, n = 32. Pregnancy diagnosis was performed 42 days post-insemination by transrectal ultrasonography. Oxytocin, $PGF_{2\alpha}$ and hCG significantly improved doe's pregnancy rates in Vhembe district. A significant effect ($P>0.001$) was observed in fresh semen diluted in BAF18 (93.8%), with the highest pregnancy rate percentage. However, numerically, the highest fertility rate was in fresh semen diluted with BAF18 (93.8 %), while the BAF10 (81.3 %) showed a lower fertility rate when compared to the pregnancy rate observed with frozen semen diluted with BAF10 (87.5 %). Oestrous ovulation inducing drugs with frozen semen diluted in BAF13, BAF18 and TEY offered approximately equal pregnancy rate (68.8 %, 62.5% & 62.5 %), but the highest percentage was in BAF13. Therefore, there were acceptable beneficial effects of oxytocin, hCG and $PGF_{2\alpha}$ at the time of AI, and control adversely affected the pregnancy rate in communal goats in this study.

Keywords: Human chorionic gonadotropin, Oxytocin, Prostaglandin F2α, Pregnancy rate

6.2. Introduction

Commercialization of goat farming has evolved greatly in the 20th century with particular emphasis towards improving genetics, reproductive efficiency, and kid survival rates (Teleb & Ashmawy, 2007). Raising the goats is crucial in both commercial and subsistent farming systems in South Africa. In the commercial sector, goats are mainly kept for meat and milk production, for both local sales and for export, while to subsistent farmers, goats are of greater importance as a source of animal protein, milk and manure, and most needy communities depend on these goat flocks (van Marle-Koster *et al.* 2004). However, goat farming has been discouraged in other countries, such as Pakistan and Zambia, due to their influence on environmental degradation and destabilisation of ecology balances (Ngambi *et al.,* 2012). Contrary, goat farming is an important component of the livestock sector in the Vhembe district, and most of the goat farmer are small, marginal, and landless farmers (Ajao, 2015). There are several important constraints in the communal goat farming system. These include a shortage of good quality breeding bucks, indiscriminate mating using non-descript and genetically inferior bucks, which result in inbreeding problems, and loss of valuable goat germplasm (Gokuldas *et al*., 2023). Addressing these challenges through capacity building, improved infrastructure, better veterinary services, and market linkages can further enhance the growth and sustainability of goat farming in rural areas (Kochewad *et al.,* 2023). However, it is not feasible to buy an expensive breeding buck of R 20 000.00 for a small communal farmer who own a small flock size of goats, but the adoption of animal assisted reproduction (ART), such as artificial insemination (AI) and oestrous synchronization, has proven to be a very effective reproductive technology that selectively increases genetic gain through increased selection pressure on males (Nethengwe, 2015). Artificial insemination is one of the most important, practical, and valuable livestock technologies used for genetic improvement in farm animals for many years. Artificial insemination, the act of mechanical and unnatural depositing of semen into the female reproductive tract with the goal of achieving conception, was the first ART to be practised. As the most frequently used ART procedure today, AI has had and continues to tremendously impact the various animal breeding industries (Athouse, 2007). AI is a way to achieve reproductive efficiency and production in production animals. In Holstein cattle, for example, AI supported selection for the milk production trait and within 40 years milk production has nearly doubled (Heise, 2011). The oestrous cycle of females can be manipulated to establish efficient insemination programs. With oestrous synchronization programs, large groups of females can be inseminated simultaneously. This has the advantage of concentrating work on a specific day during breeding and will ultimately simplify communal goat flock management before and after the offspring are born nearly all simultaneously. Parturition observation, vaccination programs, and castration of kids are just

a few examples of improved flock management practices through the group effect achieved through oestrous synchronization of does. Another reason for AI is to ensure the effectiveness of the use of semen. An increased number of offspring from a superior buck could be produced when AI is employed in the communal goat farming system and manifold increases the flock size. For example, a buck's ejaculate could be sufficient to inseminate 15 to 20 does when split into doses instead of impregnating one doe. Bull ejaculates can also be split into up to 200 or more fresh AI doses. Does could be inseminated with either chilled or frozen semen. In general, shipping chilled or frozen semen nationally and internationally involves fewer health risks and welfare implications than transporting live animals. Thus, AI could provide a unique opportunity for faster genetic gain in communal goat farming (Foote, 2002). The objective of this study was to establish animal-assisted reproduction centres designed to compare the efficacy of using fresh extended semen as an alternative to frozen semen and evaluate the efficacy of three ovulation inducing drugs (Oxytocin, hCG and $PGF_{2\alpha}$) on the doe's fertility measured as pregnancy rates at the time of AI programme in Vhembe district.

6.5. Material and Methods

6.3.1. Ethics

This study was carried out under the recommendations, care, and use of animals through the University of Venda Animal Ethics Committee guidelines. Ethical clearance was obtained from the University of Venda Research Ethics Committee with a project registration number (SARDF/17/IRD/08/2908) for permission to conduct this study.

6.3.2. Study area

This study was conducted in Rabali (ward 34), Tshanzhe (ward 4), Tsidzi (ward 10) and Mabayeni (ward 35) villages of Makhado, Thulamela, Musina, and Collins Chabane local municipalities, respectively. These four local municipalities are in the Vhembe district of Limpopo province of South Africa. The province is in northern South Africa, sharing borders with Botswana, Mozambique, and Zimbabwe. The area of the district is 25597 square kilometres, and the population of the province is 1402779 (StatsSA, 2016). Rural farmers rely heavily on communal goat production to meet their socio-economic needs.

6.3.3. Buck management

Semen was collected from four healthy (1–2-year-old) Boer bucks managed at the University of Venda experimental goat feedlot. Each buck was fed 1.8 to 2 kg concentrate pellets and water was provided *ad-libitum*.

6.3.4. Does management

One hundred and twenty-eight (n=128) South African indigenous goat does that weighed between 25 and 80 kilograms and aged between 1.5 to 4 years of age (dentition) were used in this trial. All does belong to communal farmers participated in chapter 4. Thirty-two does kept under tropical rangeland sweet and mixed veld condition were collected from Rabali, Tshanzhe, Tsidzi and Mabayeni and surrounding villages of Makhado, Thulamela, Musina, and Collins Chabane local municipalities from different farmers. Indigenous does collected were of unknown fertility, and there is no current literature citation on the fertility of this breed of goats in Vhembe district. Does were subjected to pregnancy diagnosis by foetal ballottement and real-time ultrasonography with a rectal 7.5-MHz linear-array transducer, as described by Padilla-River *et al.* (2005), to avoid using pregnant does in the trial. Two-weeks before the trial, does who were not pregnant were vaccinated using Multiclos®+P, dosed with

an antihelminthic Prodose® orange, supplemented with Multimin® mineral injections and transported to the animal assisted reproduction centre in each of the four local municipalities. All does were kept intensively at one of the four village animal assisted reproduction centres. Animal-assisted reproduction centres were established to pool the resources needed for oestrous synchronization and AI services in the Vhembe district. These centres were established after communal goat farmers expressed interest in pen feeding and separating does from bucks during oestrous synchronization and AI program. Similarly, animal-assisted reproduction centres were established and designed to overcome goat production challenges faced by communal farming system, such as poor infrastructures, limited land access and a lack of veterinary services. These factors were prevented by the establishment of animal-assisted reproduction centres designed to facilitate controlled goat breeding, from which efficient oestrous synchronization and AI protocol was administered in a space of two weeks. Thereafter, does were released into their day-to-day communal grazing land until 42 days of the pregnancy diagnosis. Does were fed compressed Driehoek® ewe pellet and water was provided. Goat does were conditioned through a dietary pre-adaption period of two weeks during which lucerne hay, grass hay and dry pellets were fed. Does developing diarrhoea or bloat had their diet switched to grass hay and withdrawn from trial diet of compressed pellets and lucerne hay.

6.3.5. Oestrous synchronization and calendarized events

Does were synchronized using a controlled internal drug release (CIRD) containing 0.3 g of progestero (Eazi-Breed^{TM}, Zoetis SA) intravaginal devices inserted on day 0. The CIRD was removed after 7 days with 10 mg prostaglandin$F_{2\alpha}$ (Lutalyset^{TM}, dinoprost 5 mg/ml, Zoetis SA) injected intramuscularly. After 24 hours of the removal of the CIRD, does were injected with 300 IU of equine choronic gonadotrophin (folligon®, 1000IU/ie, MSD Animal Health, INTERVET. SA) (PTY) Ltd.

6.3.6. Oestrus detection

All goat does (n = 128) were tagged using an ear tag applicator for identification. Oestrous was detected by visual observations by experienced personnel daily for 4 days after treatment withdrawal for onset of standing heat in the morning from 7am-10am and in the afternoon from 3pm-5pm using a teaser buck walking around the does pen with adequate visibility, cajoling but of limited physical contact. Oestrus detection in goats is never fairly accomplished in the absence of the buck since the highly regarded definitive sign of oestrous is receptivity and standing of goat doe whilst being mounted by the buck (Rahman *et al.,* 2008). Sixty-four does were inseminated using fresh semen diluted in BAF10, BAF13, BAF18 and TEY (control),

while frozen semen diluted with the same extenders was used to inseminate another sixty-four does remaining.

6.3.7. Semen collection and processing for AI

The buck semen in this chapter, their management, the methods of collection and semen processing and evaluation were described in chapter 4 and 5.

6.3.9. Fresh and frozen semen storage

After semen processing and evaluation, the fresh semen samples were placed into 15 ml Cellstar tubes and stored at 5ºC inside the National luna portable refrigerator. The National Luna portable refrigerator was plugged into a cigarette lighter hole in the vehicle to keep the refrigerator temperature constant at 5ºC. While frozen semen was stored inside the liquid nitrogen (LN_2) tank full of canisters that contained semen straw.

6.3.8. Preparation of automatic semen apparitor

An electric kettle was used to heat water to the boiling point of 100°C at the University of Venda experimental farm. Then, the water was poured into the thermos vacuum flask and taken to the animal assisted reproduction centre. At the AI centre, boiled water from the thermos flask was poured into the thawing apparatus and then mixed with cold water to drop the water temperature from boiling into warm water. The water temperature was measured at 37 °C by a thermometer in the thawing apparatus. The thawing apparatus was plugged into a cigarette lighter hole in a car to keep the water temperature constant at 35 °C. The water temperature was monitored every 15 minutes before thawing another straw from the National luna portable refrigerator or the LN_2 tank.

6.3.9 Thawing of frozen semen

The LN_2 full of canisters that contained semen straws was placed closed to the car with the plugged-in thawing apparatus. The selected canister with the record label of a specific buck's semen was lifted to the neck of the LN_2 tank. A single semen straw was removed within 5 seconds and then placed into the thawing apparatus in warm water at a temperature of 37 °C for a period of 30 seconds. The semen straw was then removed from the thawing apparatus and dried off with a paper towel to prevent water from coming into contact with the semen. A 0.5 ml semen straw was then cut off by a clean scissor through the middle of the end sticking out, and semen was deposited into the pre-warmed 3 ml syringe attached to the catheter.

6.3.10. Trans-cervical AI

The does were placed in a crate and appropriately placed on a bar to allow the technician to pass a 12 cm duckbill speculum into the vagina. The vaginal vault was cleared of any mucus plug discharge, and the cervical os was identified as a purplish bulge into the vaginal vault. Using laparoscopic light, the cervix was grasped at 11 o'clock and 5 o'clock using a pozzi tenaculum forceps, leaving the cervix os open for passage of the urinary catheter. In a synchronized tactics, cervical rings were aligned and trans-versed using pull and twist motion of the forceps 90 degrees anticlockwise and forward passage of a urinary catheter stiffened by a stylet. The stylet was removed after the passage of the cervix, and a 1 ml loaded syringe of 0.5 ml semen from the thawing apparatus was deposited into the 3 ml syringe tub. Slowly, the syringe plunger was pushed until deposits were achieved in the uterine body. The urinary catheter was slowly pulled out whilst releasing semen by the plunger in a double tactic technique to allow for intrauterine and intra-cervical insemination with fresh and frozen semen.

6.3.11. Ovulation Inducing Drugs

All does were alternately assigned to one of the three ovulation inducing drugs and a control immediately after insemination with fresh/frozen semen diluted in BAF10, BAF13, BAFF18 and TEY: (1) 300 IU hCG (Chorulon) injection under the tail into the vein, n = 32; (2) 10 mg dinoprost $PGF_{2\alpha}$ hormone (Lutalyze) intramuscular injection, n = 32; (3) 25 IU oxytocin hormone (Fentocin) intramuscular injection, n = 32 and (4) 5 ml sterile isotonic saline intramuscular injection as a control, n = 32.

6.3.12. Pregnancy diagnosis

All inseminated does were examined for pregnancy diagnosis 42-day post-insemination using real-time ultrasonography with a rectal 7.5-MHz linear-array transducer, as described by Padilla-River et al (2005).

6.3.13. Statistical analysis

The data were subjected to mixed-model analysis of variance (ANOVA) for a 2 x 4 x 4 factorial arrangement in a randomized design using the GLM procedures of Minitab software version 17 (Minitab, 2014).

$Yijk = \mu + S_i + E_j + D_k + (SD)_{ij} + \varepsilon_{ijk}$

Yijk = data obtained from the K^{th} does on the j^{th} extenders, j^{th} ovulation inducing drugs and inseminated with the i^{th} semen.

μ = overall mean

S_i = effect of the i^{th} semen

E_j = effect of the extenders

D_k = effect of the j^{th} ovulation inducing drugs

(SD) is interaction of the i^{th} semen and j^{th} ovulation inducing drugs.

ε_{ijk} = error

When significant differences were observed, treatment means were compared using Tukey's post hoc test (Steel Torrie, 1981), with the significance level set at $P < 0.05$.

6.3. Results

The effect of four oestrous ovulation-inducing drugs and four different semen extenders on the pregnancy rates of communal goats after synchronization of oestrous and artificial insemination with fresh and frozen Boer goat semen are presented in Table 6.1.

Table 6.1: The effect of semen extenders and four oestrous ovulation-inducing drugs after AI with fresh and frozen semen.

Semen Type	Extender	Hormones	Pregnancy rate %
Fresh semen	TEY	Saline	50
		Oxytocin	100
		hCG	50
		PGF	100
		Overall	75
	BAF10	Saline	50
		Oxytocin	100
		hCG	100
		PGF	75
		Overall	81.3
	BAF13	Saline	75
		Oxytocin	100
		hCG	100
		PGF	75
		Overall	87.5
	BAF18	Saline	75
		Oxytocin	100
		hCG	100
		PGF	100
		Overall	93.8
Fresh semen Overall			84.4
Frozen semen	TEY	Saline	50
		Oxytocin	75
		hCG	50
		PGF	75
		Overall	62.5
	BAF10	Saline	50
		Oxytocin	100
		hCG	100
		PGF	100
		Overall	87.5
	BAF13	Saline	25
		Oxytocin	75
		hCG	100
		PGF	75
		Overall	68.8
	BAF18	Saline	50
		Oxytocin	100
		hCG	50
		PGF	50
		Overall	62.5
Frozen semen overall			70.3
SEM			**3.71**
Significance			
Semen			**Ns**
Extender			**Ns**
Hormone			****
Semen × Extender			**Ns**
Semen × Hormone			**Ns**
Extender × Hormone			**Ns**
Semen × Extender × Hormone			**Ns**

SEM- Standard error of the mean

*Significant at P<0.05; P>0.05- not significant (ns)

**Significant at P<0.001

Table 6.1 shows the effect of four oestrous ovulation-inducing drugs (hormones) with four different semen extenders along with AI with fresh and frozen semen. Overall, the pregnancy rate was achieved with no significant difference (P>0.05) observed between fresh (84.4 %) and frozen semen (70.3 %). Hormones showed a significant (P<0.05) effect that might have caused some improvement in the pregnancy rate. A significant effect was highly (P>0.001) observed in fresh semen diluted in BAF18 with the highest fertility on fresh semen regardless of the oestrous ovulation drugs, as observed with the control (75 %). However, numerically, the highest fertility rate was in fresh semen diluted with BAF18 (93.8 %), while the oestrous ovulation drugs in fresh semen diluted with BAF10 (81.3 %) showed lower fertility rate when compared to pregnancy rate observed with frozen semen diluted with BAF10 (87.5 %). Oestrous ovulation inducing drugs with frozen semen diluted in BAF13, BAF18 and TEY offered approximately equal pregnancy rate (68.8 %, 62.5% & 62.5 %), but the highest percentage was in BAF13.

6.4. Discussion

Establishing animal assisted reproduction centers to facilitate controlled animal breeding, efficient oestrous synchronization and artificial insemination (AI) protocols accelerated reproductive performance in rural South African indigenous goats of the Vhembe district. Many efforts have been made to increase goat fertility, including oestrous synchronization and timed artificial insemination protocols (Gumen *et al.,* 2011). Therefore, this study was aimed to improve fertility in tropical communal goat by using the pharmacological synchronization protocol and three oestrous ovulation-inducing drugs. However, these hormones induced synchronization and ovulation following artificial insemination using fresh/frozen Boer goat semen in rural South African indigenous goat does. On average, the result of this study shows that the highest pregnancy rate was observed in fresh semen (84.4 %) when compared to frozen-thawed semen (70.3 %) following artificial insemination. However, there was no significant difference observed amongst the two-semen type. Similar to this study, Monyeleote (2017) reported that a pregnancy rate greater than 80 % could be achieved using any of the three (intracervical, trans-cervical and laparoscopic insemination) AI techniques when using fresh semen. Interestingly, 84.4 % pregnancy rate obtained in this study, and it is close to the 85 % reported by (Fonseca *et al*., (2005) and lower than the 93.4 % reported by Duricic *et al*., (2012). Additionally, frozen semen documented a 70.3 % pregnancy rate, which was approximately equal to the 70 % reported by Agossou & Koluman (2018) and higher than the 53.1 % reported by Kharche *et al.* (2013) in Jamunapari goat with frozen semen. However, the result of this study did not align with Naraini *et al.* (2021), who observed the highest

pregnancy rate in natural mating compared to AI (73 % vs 21 %) with frozen semen. The administration of ovulation-inducing drugs probably influenced this discrepancy. The results of the present study showed that amongst the three-oestrous ovulation-inducing drugs and the control group, the highest pregnancy rate was achieved from the oxytocin treatment group. Similar to this study, administering oxytocin following AI increased the pregnancy rate in lactating dairy cows (Palomares *et al*., 2010). This could be due to changes in the uterine contractility, cervix dilation and possibly increases the acceleration of the sperm transport in the reproductive tract in goats (Yildiz, 2005; Xiaoyu *et al*., (2021). Previous studies have shown that exogenously administering oxytocin in amounts less than 400 IU increases uterine contractility (Sayre and Lewis, 1996, 1997; Stellflug *et al*., 2001). This shows that the exogenous use of oxytocin could lead to more contractions of the uterus, allowing the semen to move further. Like in the current study, oxytocin treatment significantly influenced the pregnancy rate after trans-cervical AI with fresh and frozen semen. The administration of hCG injection following AI also improved pregnancy rate in rural South African indigenous goat. In several previous studies, GnRH improved pregnancy rate (Nakao *et al*., 1983; Stevenson *et al*., 1990; Kaim *et al*., 2003; Lopez-Gatius *et al*., 2006). This study does not align with the observation made by Gumen *et al*. (2011), who concluded that the administration of hCG at the time of AI does not increase pregnancy rates in lactating dairy cows. Human choronic gonadotropin has an LH action in cows. It could stimulate follicle development towards maturation, induce ovulation, bring luteinisation of the granulosa cells, maintain the corpus luteum's functional life, and increase progesterone secretion from luteinised cells. Human choronic gonadotrophin also augment the action of follicle stimulation hormone (FSH) on ovarian growth (Broers, 1995). The results of this study indicated that there was a reasonably higher pregnancy rate achieved in $PGF_{2\alpha}$ treatment group with fresh semen diluted with in BAF18 and TEY as a control. These results are similar to the 67.3 % pregnancy rate reported by Nethengwe, (2017), but not in line with Gumen, (2011) who reported that $PGF_{2\alpha}$ at the time of AI following spontaneous oestrous did not have any beneficial effect. Lopez-Gatius *et al*. (2004) also showed that $PGF_{2\alpha}$ administered at the time of fixed timed insemination did not affect conception rate in cows with acceptable reproductive performance. The use of additional oxytocin hormones in reproductive methods began in the 1970s in Europe (Duzinski *et al*., 2013). In recent studies, attempts have been made to achieve more successful pregnancies by adding oxytocin to semen rather than administering oxytocin exogenously to females (Langendijk *et al*., 2003; Okazaki *et al*., 2014; Kandemir *et al*., 2017; Manjarin, *et al*., 2019; Lu *et al*., 2020; Kandemir, 2023). Viudes-de-Castro *et al*. (2009) reported that oxytocin possibly exerted its influence by stimulating prostaglandin production.

6.5. Conclusion

Animal assisted reproduction centres designed to facilitate controlled animal breeding, efficient oestrous synchronization and artificial insemination (AI) protocols significantly improved the reproductive performance in rural South African indigenous goat of Vhembe district. The administration of hCG, $PGF_{2\alpha}$ and oxytocin at the time of AI with fresh and frozen semen diluted in BAF10, BAF13 and BAF18 increased the pregnancy rate in this study. The Oxytocin treatment group induced ovulation and manifold increased the changes in uterine contractility and cervix dilation and possibly accelerated sperm transport in the reproductive tract in communal goats. Thus, there were acceptable beneficial effects of oxytocin, hCG and $PGF_{2\alpha}$ at the time of AI in this study.

6.7. References

Agossou, D.J and Koluman, N., (2018). The effects of natural mating and artificial insemination using cryopreserved buck semen on reproductive performance in Alpine goats. *Anim Breed*. 61, 459–46. https://doi.org/10.5194/aab-61-459-2018

Ajao, O.A.A., (2015). Evaluation of the effectiveness of different exten6ders of goat buck semen under refrigerated conditions. Master's Dissertation, University of Venda, Thohoyandou. South Africa.

Palamares R.A., Gutierrez, J.C., Portillo, G., Boscan, J.C, Montero, M., Lopez, Y, Maxwell, H.S., Carson, R.L and Soto, E. (2010). Oxytocin treatment immediately after calving does not reduce the incidence of retained fetal membranes or improve reproductive performance in crossbred Zebu cows. *Theriogenology*. 74, 1414-1419. DOI: https://doi.org/10.1016/j.theriogenology.2010.06.013

Althouse, G., (2007). Artificial insemination. *Comparative reproductive biology*. (1st Ed). Iowa: Blackwell publishing, USA.

Amarantidis, I., Karagiannidis, A., Saratsis, P.h., Brikas, P., (2004). Efficiency of methods used for oestrous synchronization in indigenous Greek goats. *Small Rumin. Res.* 52, 247252.

Duricic, D. J., Grizelj, T., Dobrani ´ c, I., Harapin, S., Vince, P., ´ Kocila, I., Folnozi , C,M., Lipar, G., Greguri, C,G., and Samardzija, M., (2012). Reproductive Performance of Boer Goats in a Moderate Climate Zone, *Arch.Vet*, 82, 351–358.

Duzinski, K., Knecht, D., and Gajewczyk, P., (2013). Effect of oxytocin treatment on the reproductive performance of sows after artificial insemination with liquid semen, *Turkish J. Vet. Anim. Sci.*, 37, 575–581, https://doi.org/10.3906/vet-1211-35, 2013.

Fatel, A., Rubioa-Pellicer, M.T., Leboeuf, B., (2010). Reproductive cycle of goats. *Anim. Reprod. Sci*, 124:211-219.

Fonseca, J.F., Torres, C.A.A., Costa, E. P., Maffili, V.V., Carvalho, G.R., Alves, N.G., and Rubert, M.A., (2005). Progesterone profile and reproductive performance of oestrous-induced Alpine goats given hCG five days after breeding, *Anim. Reprod*. 2, 54–595.

Gemen, A., Keskin, A., Yilmazbas-Mecitoglu, G., Karamya, E., Cevik., S, and Balici, F., (2011). Effects of GnRH, PGF2α and oxytocin treatments on conception rate at the time of artificial insemination in lactating dairy cows. *Czech J. Anim. Sci*. 56, 279–283.

Gokuldas, P.P., Ralkumar, S and Narnaware, S., (2013). Artificial insemination technology for sustainable goat production in coastal regions. Extension folder NO.104. ICAR-Central Coastal Agricultural Research Institute.

Heise, A., (2011). Artificial insemination in veterinary science. Faculty of veterinary science of Pretoria South Africa. 2, 18-32. http://cdn.intechopen.com/pdfs/28665/InTech-Artificial_insemination_in_veterinary_science.pdf

Kaim, M., Bloch, A., Wolfenson, D., Brawtal, R., Rosenberg, M., Voet H., Folman, Y. (2003). Effects of GnRH administered to cows at the onset of oestrous on timing of ovulation, endocrine responses, and conception. *Journal of Dairy Science*, 86, 2012–2021.

Kandemir, C., (2023). Effect of oxytocin added into sperm on artificial insemination in sheep. *Anim. Breed*. 66, 61–69 https://doi.org/10.5194/aab-66-61-2023

Kandemir, Ç., Ta¸skin, T., and Ko¸sum., N. (2017) Effect of oxytocin and clitoris massage on the pregnancy rate of Saanen goats, Res. Opin. *Anim. Vet. Sci.* 7, 25–28, 2017.

Kharche, S.D., Jindal, S.K, Priyadarshini, R., Kurmar, S., Goel, A.K., Ramachandra, N and Rout, P.K., (2013). Fertility following frozen semen artificial insemination in Jamunapari goats. *Indian J Amim Sci*. 83:107-1073

Kochewad, S.A., Chavan, S.B, Kumar, N, Tayade, A.S., Dhumal, N., Jadhavar, P and Reddy, K.S., (2023). Self-sustaining goat farming model for livelihood improvement of small and marginal farmers. Technical Bulletin No.40. ICAR-National Institute of Abiotic Stress Management, Baramati, Pune, Maharashtra, India, pp 25.

Langendijk, P., Bouwman, E. G., Schamps, D., Soede, N. M., and Kemp, B., (2003). Effects of different sexual stimuli on oxytocin release, uterine activity, and receptive behaviour in oestrous sow, *Theriogenology,* 59, 849–861, https://doi.org/10.1016/s0093- 691x (02)01157-3, 2003.

Lopez-Gatius F., Santolaria P., Martino A., Deletang F., Rensis F.D., (2006). The effects of GnRH treatment at the time of AI and 12 days later on reproductive performance of high producing dairy cows during the warm season in northeastern Spain. *Theriogenology*, 65, 820–830.

Lopez-Gatius F., Yaniz J.L., Santolaria, P., Murugave,l K., Guijarro, R., Calvo, E., Lopez-Bejar, M., (2004). Reproductive performance of lactating dairy cows treated with cloprostenol at the time of insemination. *Theriogenology*, 62, 677–689.

Lu, X., Liu, Y., Zhang, J., Wu, X., and Li, X., (2020). Oxytocin increases pregnancy rates after fixed time artificial insemination in Kazak ewes, *Reprod. Domest. Anim*. 56, 942–947, https://doi.org/10.1111/rda.13931.

Manjarin, R., Kirkwood, R. N., Ngula, J., Martinez-Pastor, F., Alegre, B., and Domínguez, J. C. (2019) Effect of oxytocin, cloprostenol or buserelin in semen doses on sow fertility, *Animals*. 9, 746. DOI: https://doi.org/10.3390/ani9100746, 2019.

Motlomelo, K.C., Greyling, J.P.C., Schwalbach, L.M.J., (2002).synchronization of oestrous in goats: the use of different progestagen treatments. *Small Rumin. Res.* 45:4549.

Nakao, T., Narita, S., Tanaka, K., Hara, H., Shirakawa, J., Noshiro, H., Saga, N., Tsunoda, N., Kawata, K., (1983). Improvement of first-service pregnancy rate in cows with gonadotropin-releasing hormone analog. *Theriogenolog,* 20, 111–119.

Naraini, D.M., Prastowo, S and Widyas, N., (2021). Reproductive performance comparison

between natural mating and artificial service Jawarandu goat. IOP conference series: Earth and Environmental Science. 637: doi:10.1088/1755-1315/637/1/012028

Nethengwe, L.D., Francis, J., and Barry, D.M., (2016). The effect of three ovulation inducing drugs on pregnancy rates of rural cattle after administration of synchronized artificial insemination in Vhembe district. Masters. Dissertation, University of Venda, South Africa.

Ngambi, J.W., Alabi, O.J., Norris, D., (2012). Role of goats in food security, poverty alleviation and prosperity with special reference to Sub-Saharan Africa: A review, Agricultural Research Communication Centre, *Indie j Anim*. *Res*. 47, 1-9.

Okazaki, T., Ikoma, E., Tinen, T., Akiyoshi, T., Mori, M., and Teshima, H. (2014). Addition of oxytocin to semen extender improves both sperm transport to the oviduct and conception rates in pigs following AI, *Anim. Sci. J.*, 85, 8–14, https://doi.org/10.1111/asj.12089.

Perry, G.A., Perry, B.L., (2009). GnRH treatment at artificial insemination in beef cattle fails to increase plasma progesterone concentrations or pregnancy rates. *Theriogenology*. 71, 775–779.

Sayre, B. L., and Lewis, G. S., (1997). Fertility and ovum fertilization rate after laparoscopic or transcervical intrauterine artificial insemination of oxytocin-treated ewes, *Theriogenology,* 48, 267–275, https://doi.org/10.1016/s0093-691x(97)84074-5

Sayre, B.L. and Lewis., G.S., (1996). Cervical dilation with exogenous oxytocin does not Affects perm movement into the oviducts in ewes, *Theriogenology*, 45, 1523–1533, https://doi.org/10.1016/0093-691X(96)00120-3

Sayre, B.L., Lewis, G.S., (1996). Cervical dilation with exogenous oxytocin does not affect sperm movement into the oviducts in ewes. *Theriogenology*, 45 1523 1533

Stellflug, J.N., Wulster-Radcliffe, M.C., Hensley, E.L., Cowardin, E.A., Seals, R.C, and Lewis, G.S. (2001). Oxytocin-induced cervical dilation and cervical manipulation in sheep: ffects on laparoscopic artificial insemination. *J Anim Sci*, 79 568 573.

Stevenson, J.S., Call, E.P., Scoby, R.K., (1990): Double insemination and gonadotropin-releasing hormone treatment of repeat-breeding dairy cattle. *Journal of Dairy Science,* 73, 1766–1772.

Teleb, D.F and Ashmawawy T.A.M., (2007). Using FGA sponge & GnRH for improving fertility in goats during breeding season. *Egyptian J. of sheep and Goat Science*, Vol 2 (2): 1-14.

van Marle-koster, E., Visser, C., Casey, N.H., (2004). Goats in South Africa: a significant role layer? *South Africa J Amim Sci*, 34: 26-28

Viudes-De-Castro, M. P., Salvador, I., Marco-Jiménez, F., Gómez, E. A., and Silvestre, M. A. (2009). Effect of oxytocin treatment on artificial insemination with frozen-thawed semen in Murciano-Granadina goats, *Reprod. Domest. Anim*. 44, 576– 579, https://doi.org/10.1111/j.1439-0531.2007.00993

Viudes-de-Castro, M.P., Salvador, I., Marco-Jimenez, F., Gomez, E.A and Silvestre, M.A.,

(2009). Effect of oxytocin treatment on artificial insemination with frozen-thawed semen in Murciano-Granadina goats. *Reprod Domest Anim*. 44, 576-9. doi: 10.1111/j.1439-0531.2007

Xiaoyu, L.U., Yiyong, L., Jing, Z., Xinglong, W and Xianglong, L., (2021). Oxytocin increases pregnancy rates after fixed time artificial insemination in Kazak ewes. *Reprod Domest Anim.* (46)1: 82-6. doi: 10.1111/j.1439-0531.2010.01586.x.PMID: 20210882

Yildiz, A., (2005): Effect of oxytocin on conception rate in cows. *Journal of Firat University Health Science,* 19, 75–78

.

CHAPTER 7: OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Discussion

The goal of the research was to address low goat productivity in the Vhembe District and similar communal goat farming systems by introducing animal-assisted reproduction centres to facilitate controlled breeding and artificial insemination. These interventions were designed to enhance genetic improvement and productivity of indigenous goats. A participatory rural appraisal approach was employed for the preparatory situation analysis, an approach which is rooted in people-centred rural development principles (Francis *et al.,* 2010). The situation analysis confirmed the socio-economic importance of goats and showed that most farmers rely on them for cash income through sales, rather than consumption for household food security. The high rate of acceptance of AI (89.3%) suggested potential for broad adoption. However, strong socio-economic barriers, such as the inability of 30% of farmers to afford pen feeding during confinement for controlled breeding, must be addressed to enable broader implementation.

Rural development interventions should comply with established sustainability principles, particularly the three pillars, which are the social, economic, and environmental dimensions (Purvis *et al.,* 2019). Accordingly, in this study, the participatory approach was able to identify the context-specific potential barriers and solutions which are necessary to improve goat reproduction in the target communal farming area, to ensure long-term socio-economic and technical sustainability. Such engagement of stakeholders to co-develop practical innovations was consistent with the consistent with the diffusion of Innovations theory, which informs such a Continuous Improvement and Innovation (CI&I) development approach (Clark *et al.,* 2003), which emphasises an iterative collaborative process in the testing and implementation processes of project implementation, with broad stakeholder partnerships, to adaptively improve innovative interventions.

The study introduced two key technical innovations. The first was the use of Tris-based bovine amniotic fluid (TBAF) semen extenders, which demonstrated the highest efficacy with TBAF18, which preserved sperm motility and viability for up to 168 hours under chilled-storage. This is a practical, low-cost alternative to conventional cryopreservation, especially for farmers who are spread across wide geographic areas from the central service areas. Secondly, a unique ovulation-inducing hormonal protocol for oestrus synchronisation was successfully used to achieve high pregnancy rates (84.4 %) from the 7-day chilled stored semen. However, going forward, CI&I principles demand continuous future monitoring and

feedback, with the necessary technical refinement to address challenges such as the limited veterinary infrastructure, poor farmer access to the AI centres, and socio-economic barriers to adoption.

People-centred rural development emphasizes integrating IKS to respect local traditions and ensure inclusive innovation delivery (Francis *et al.,* 2010). However, the study did not explore the Indigenous Knowledge Systems (IKS), which can play a vital role in ensuring cultural acceptance and optimizing AI delivery, such as heat detection, pregnancy diagnosis, and adapting to local norms. Further, socio-economic analyses and cost-benefit evaluations were outside the scope of the study, which limits the scalability of the interventions. Economic feasibility studies are critical for assessing long-term sustainability, especially in resource-constrained communal farming systems.

7.2. Conclusions

The study demonstrated the technical feasibility and potential benefits of animal-assisted reproduction centres in improving goat productivity through AI in communal farming setting. The results confirmed that Tris-based bovine amniotic fluid extenders, particularly TBAF18, effectively preserved sperm quality under prolonged refrigeration. Ovulation-inducing oestrus synchronisation protocols resulted in acceptable pregnancy rates. These innovations show promise for resource-limited settings by lowering costs and expanding access to controlled breeding.

However, deeper socio-economic and infrastructural challenges remain, which must be addressed to ensure the successful implementation of AI programs. By employing, people-centred rural development principles and leveraging CI&I processes, future interventions can continuously infuse community-specific needs, indigenous knowledge to enhance adoption and sustainability.

7.3. Recommendations

i. **Infrastructure and Capacity Building:** In implementing the innovations, a continuous improvement and innovation (CI&I) approach is recommended which includes partners such as the local government, NGOs, and community-based organizations to co-develop programs that improve the veterinary infrastructure and farmer training in AI and reproductive management. This should include continuous feedback mechanisms to refine interventions based on farmer experiences.

ii. **Integration of indigenous knowledge systems:** Further participatory research is recommended to incorporate local practices and Indigenous Knowledge Systems into goat reproductive management according to people-centred development principles, to ensure cultural relevance, acceptance, and effective delivery of AI technologies.

iii. **Economic feasibility studies:** Detailed cost-benefit analyses to evaluate the long-term financial viability of AI centres and delivery value chains. The analyses should consider farmer affordability, return on investment, and the integration of low-cost innovations such as chilled semen preservation to ensure scalability.

iv. **Scalability and sustainability:** Pilot AI programs in other regions, to validate and refine scalable models, within a similar CI&I framework, to document and learn from these pilots, to iteratively improve the project design, implementation, and adoption.

v. **Long-term monitoring and evaluation:** Further, longitudinal studies to assess the socio-economic and technical impacts of AI programs. Embed CI&I principles by using monitoring data to identify gaps and adaptively manage interventions for improved outcomes.

vi. **Participatory communication strategies:** Develop communication strategies that foster two-way engagement with farmers, ensuring that information on AI practices is accessible and relevant. Use formal documentation as part of the CI&I process to promote iterative learning, shared understanding, and broader dissemination of findings.

7.4. References

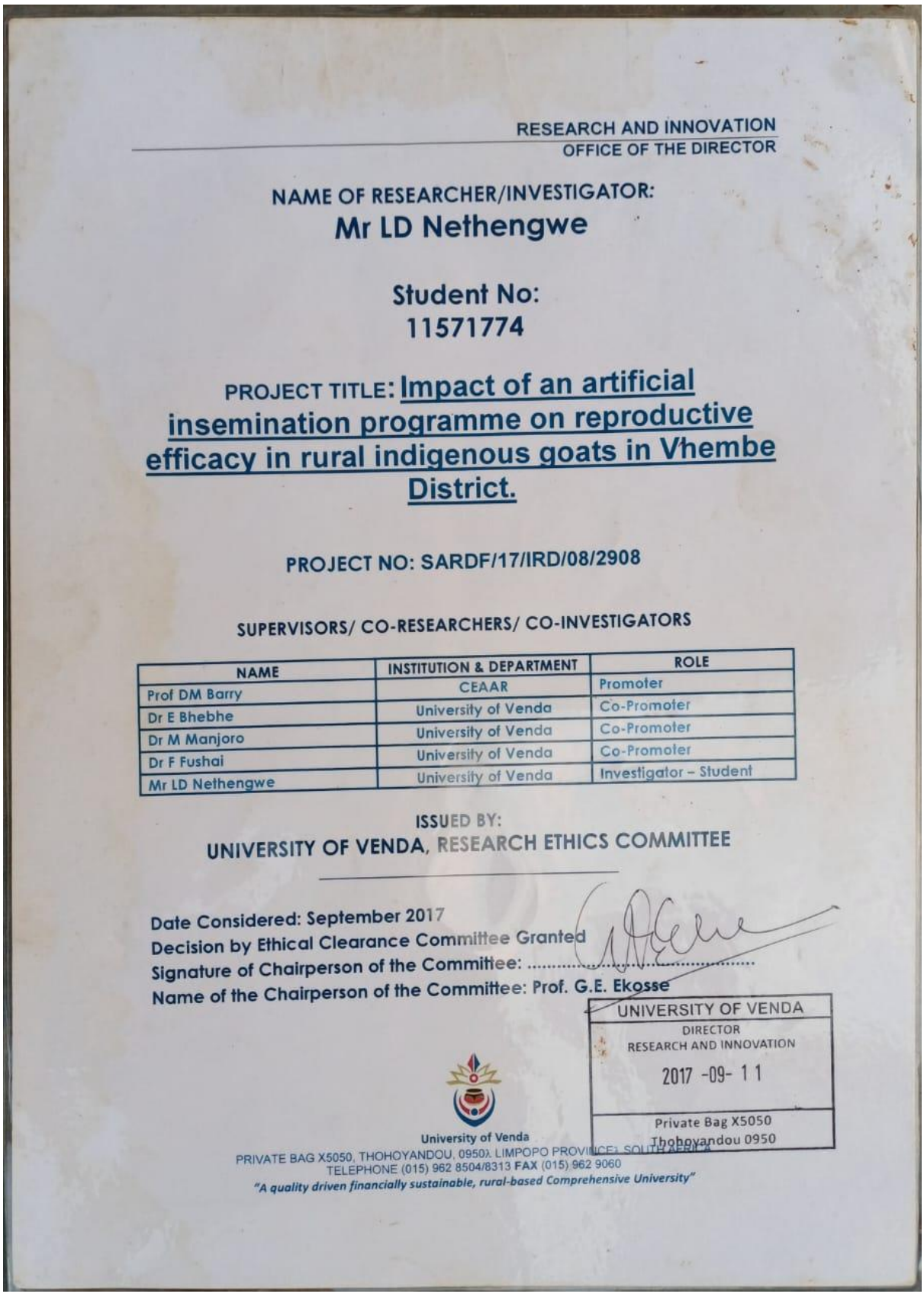
Clark, R., Griffith, G., Madzivhandila, T., Mulholland, C., Nengovhela, N. and Timms, J. (2003). Learning by Writing: Applying Continuous Improvement and Innovation Principles to Project Management by Formal Documentation and Publication. *Australasian Agribusiness Perspectives.* 94: 1-14

Francis, J., Dube, B., Mokganyetji, T. and Chitapa, T. (2010). University-rural community partnership for people-centred development: Experiences from Makhado Municipality, Limpopo Province. *SAJHE. 24(3): 345-373.*

Purvis, B., Mao, Y. and Robinson, D. (2019). Three pillars of sustainability: in search of conceptual origins. *Sustainability Science* 14:681–695.

APPENDICES

Appendix1: Ethical clearance certificate



RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Mr LD Nethengwe

Student No:
11571774

PROJECT TITLE: **Impact of an artificial insemination programme on reproductive efficacy in rural indigenous goats in Vhembe District.**

PROJECT NO: SARDF/17/IRD/08/2908

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof DM Barry	CEAAR	Promoter
Dr E Bhebhe	University of Venda	Co-Promoter
Dr M Manjoro	University of Venda	Co-Promoter
Dr F Fushai	University of Venda	Co-Promoter
Mr LD Nethengwe	University of Venda	Investigator – Student

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE

Date Considered: September 2017
Decision by Ethical Clearance Committee Granted
Signature of Chairperson of the Committee: .....
Name of the Chairperson of the Committee: Prof. G.E. Ekosse

UNIVERSITY OF VENDA
DIRECTOR
RESEARCH AND INNOVATION
2017 -09- 11
Private Bag X5050
Thohoyandou 0950

University of Venda
PRIVATE BAG X5050, THOHOYANDOU, 0950, LIMPOPO PROVINCE, SOUTH AFRICA
TELEPHONE (015) 962 8504/8313 FAX (015) 962 9060
"A quality driven financially sustainable, rural-based Comprehensive University"

Appendix2

QUESTIONNAIRES FOR COMMUNITY MEMBERS

Section A: Personal Data

1. Age categories (please tick or mark an **X**)

   18-25 [ ] 26-30 [ ] 31-40 [ ] 41-50 [ ] 51-60 [ ] 61-70 [ ]

2. Sex:

   Male: [ ] Female: [ ] Others: [ ]

3. Current Marital Status

   Single [ ] Engaged [ ] Married [ ] Divorced [ ] Single Parent [ ]

4. Highest level of education

   ABET [ ] Grade 12 [ ] Degree [ ] Honours [ ] Masters [ ] PhD [ ] None [ ]
   Other [ ]

5. Are you currently employed?

   Yes [ ] No [ ] If **No** Please explain whether you are self-employed.

   ……

   ……

6. Type of farming system practised by the communal farmers

   a) Intensive farming system [ ]

   b) Extensive farming system [ ]

   c) Others [ ]

Section B:

In this section, I would like your comments on the level of awareness and understanding. Mark (√) the relevant box that mostly captures your views, based on the scale: SA for strongly agree, A for agree, D for disagree, SD for strongly disagree, and N for neutral

Part 1: Community/Social Development

		SA	A	D	SD	N
1	Livestock production helped improve the livelihood of communal farmers.					
2	Livestock production has contributed as a source of nutrients for the rural farmer and their communities.					
3	Livestock production has contributed to income generation for the communal goat farmers.					
4	Would you welcome the introduction of improved goat breeds like the Boer goat?					

For **question 1, please Probe**:

……

……

……

……

……

……

For **question 2, please Probe**:

……

……

……

……

……

……

For **question 3, please Probe**:

……

……

……

……

……

……

4. Why do you keep goats?
   a) Source of income [ ]
   b) Traditional reasons [ ]
   c) Food production [ ]
   d) Other [ ]
5. How many goats are in your flock?
   a) 5 to 10 [ ]
   b) 11 to 30 [ ]
   c) 30 and above [ ]

6. What type of breeds are you breeding? ……..……
7. How many males are in your flock? ……..
8. Do you have a breeding season?
   a) Yes [ ]
   b) No [ ]
9. When is your breeding season?
   a) Summer [ ]
   b) Autumn [ ]
   c) Winter [ ]
   d) Spring [ ]
10. Do you keep performance records?
   a) Yes [ ]
   b) No [ ]
11. At which age do your goats start giving birth? ……..
12. What is the kidding-interval
   a)
   b)
   c)
13. What challenges do you encounter during breeding?
   ……
   ……
   ……
   ……
   ……..
   ……
14. What action do you take to prevent or overcome those challenges?
   ……
   ……
   ……
   ……
   ……
   ……
   ……
   ……
15. Can anyone or an organisation assist you in overcoming some of these challenges?

   ……
16. On average, how many kids are born in a year? .....
17. How many kids die in a year? ……
18. Do you wean your kids?
   a) Yes

b) No

19. At what stage do you wean your kids?
20. Do you vaccinate your animals?
    a) Yes
    b) No
21. Do you know anything about animal-assisted reproduction?
    a) Yes
    b) No
22. Do you have a farm?
    a) Yes
    b) No
23. Can you separate bucks from does during oestrous synchronization?
    a) Yes
    b) No
24. Can you feed all the separated does during thesynchronization period?
    a) Yes
    b) No
25. Do you have goats handling facilities in your community?
    a) Yes
    b) No
26. Do you get any advisory services from the government about goat breeding?
    a) Yes
    b) No
    c) If yes, please probe

    .....
    .....
    .....
    .....

27. What is your suggestion about what should be done to improve your goat reproduction and production management in communal farming areas?

    .....
    .....
    .....
    .....
    .....
    .....
    .....
    .....
    .....

Thank you very much

Ethical Clearance Tools

Appendix 3

LETTER OF INFORMATION FOR PARTICIPANTS

Consent to be a research participant.

Dear Participant,

I am Luvhengo D. Nethengwe, completing my PhD in Rural Development at the University of Venda, Department of Animal Science within the School of Agriculture. I am conducting a study investigating the impact of an artificial insemination programme on reproductive efficacy in rural South African indigenous goats in the Vhembe district. I humbly request that you answer the following questions honestly for this research, which will benefit the community and the country. I assure you that the information given will be treated with confidentiality and your details will not be revealed at any time.

Your cooperation in this regard is most appreciated. Participation in this research project is voluntary. You can stop any time you choose to leave. You do not have to answer questions you feel uncomfortable with throughout the study.

_____ Date: ……/ ……. / 2017

Signature of participants

_____ Date: ……. /……. / 2017

Signature of interviewer

Appendix 4

STATEMENT OF CONSENT

I have read the information provided above. I have had the opportunity to ask questions about the study, which were answered satisfactorily. I voluntarily agree to participate in this study.

_____ Date: ……/ ……. / 2017

Signature of participants

_____ Date: ……. /……. / 2017

Signature of interviewer

THANK YOU FOR ACCEPTING TO PARTICIPATE IN THE STUDY

Appendix 5

PERMISSION TO CONDUCT RESEARCH

E26 Riverside Residence
University of Venda
Thohoyandou, 0950
South Africa
25 March 2017

The District/Municipal Manager
Vhembe District Municipality
Thulamela Municipality
Makhado Municipality
Mutale municipality
Collins Chabane municipality
Thohoyandou
0950

Dear Sir/Madam,

REQUEST FOR PERMISSION TO CONDUCT RESEARCH

This letter requests permission to conduct a study entitled: VHEMBE DISTRICT IN LIMPOPO PROVINCE.

I hereby formally request permission to carry out the above study in the Vhembe District area, particularly in Thulamela, Makhado, Mutale, and Collins Chabane Municipalities, for the PhD in Rural Development in the School of Agriculture at the University of Venda.

This study investigates the impact of an artificial insemination programme on reproductive efficacy in rural South African indigenous goats in Vhembe. This will involve questionnaires, interviews, focus group discussions and personal observation. This study is quite significant because the findings will be used for academic qualification and to develop interventions to improve or strengthen the roles of indigenous goats in promoting food security in Vhembe District and the entire country (South Africa).

Kindest regards,
Mr. Luvhengo D. Nethengwe