EVALUATION OF SUITABLE CHILLED, EXTENDED SEMEN PRESERVATION TIME AND THE EFFECTS OF DIFFERENT ARTIFICIAL INSEMINATION TECHNIQUES ON THE FERTILITY OF INDIGENOUS VENDA GOATS

BY

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DECLARATION

I, Vukosi Monyeleote, the undersigned, student number 11606100, declare that this dissertation for a Master of Science in Animal Science (MScANS) degree at the University of Venda is my original work and has not been submitted for any degree at this or any other university or institution. The dissertation does not contain another person's writing unless specifically acknowledged and referenced accordingly.

Signature…….. Date……

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DEDICATION

This dissertation is dedicated to my family: my parents Mr and Mrs Monyeleote, my siblings: Rhulani, Amukelani, Ntsako, Tebogo, Caswell, Vivian, Tumelo, Nomsa, and Xirhandziwa. My friends: Nhlahla, Wisani, Nsovo and Biggy.

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ABSTRACT

The aims of the study were to evaluate the effects of dilution and chilled storage time on the quality of semen, and of different artificial insemination techniques on fertility in artificially inseminated indigenous Venda does. Fresh semen was collected using an artificial vagina from three Boer bucks aged 4±1.55 years once every four days during July and August 2016. Semen was pooled and samples were divided into two equal parts, which were extended using Biladyl® extender at ratios of 1:5 and 1:10 v/v (semen to extender), before refrigeration for 120 hours at 5 °C. The fresh undiluted semen and freshly extended semen were evaluated in six replicates for sperm motility, live-dead and sperm morphology using the Sperm Class Analyzer (SCA). Extended semen continued to be evaluated at 24 hour intervals for 120 hours. Ninety indigenous Venda does were obtained from different flocks in the Vhembe district and kept intensively in one 10 m x 40 m pen at the University of Venda experimental farm in the goat feedlot. The does were fed and watered *ad libitum*. After acclimatization for 14 days, estrus was synchronized using a controlled internal drug release (CIDR) containing 0.3 g of progesterone. Upon removal of the CIDR, does were injected 10 mg of $PGF_{2\alpha}$ (Lutalyse® dinoprost tromethamine) Sterile Solution. At 24 hours after the removal of the CIDR, the does were injected intramuscularly with 300 international units (IU) of equine chorionic gonadotrophin (eCG). Forty eight hours after the removal of the progesterone, freshly collected and diluted (1:5 ratio ~$150x10^6$ sperm/ml), five day-stored semen were used to inseminate the does using cervical (CAI), trans-cervical (TAI), and laparoscopic artificial (LAI) insemination methods in a complete randomized design (CRD) with a 2 X 3 factorial arrangement of the treatments with 15 replications per treatment. The does were tested for pregnancy after 30 days using ultrasonography. Analyses of variance was performed on the pregnancy, kidding rates and on prolificacy using the GLM procedure of Minitab (Minitab 2013). Significant differences in all motility parameters were observed between the extension ratios and storage time ($P<0.01$). There were significant interactions between the extension ratio and storage time ($P<0.05$) on the sub-population of sperm cells with non-progressive motility (NON-P). Significant ($P<0.01$) interaction was observed between the semen extension ratio and storage time on medium and slow spermatozoa ($P<0.01$). The method of insemination did not ($P>0.05$) affect fertility, though both pregnancy and kidding rates numerically decreased in the order laparoscopic insemination (LAI)≥ trans-cervical insemination (TAI)≥ cervical insemination (CAI). Overall, 71% kidding rate was achieved.

Keywords: Artificial insemination, extended semen, fresh semen, pregnancy rate, kidding rate.

TABLE OF CONTENTS

CONTENTS **PAGE**

LIST OF ABBEVATIONS

AI	: Artificial insemination
ALH	: Amplitude of lateral head displacement
ANOVA	: Analysis of variance
BCF	: Beat cross frequency
°C	: Degrees Celsius
CAI	: Cervical artificial insemination
CASA	: Computer aided sperm analysis
CEAAR	: Centre of Excellence in Animal Assisted Reproduction
CIDR	: Controlled internal drug release
cm	: Centimeter
CSS	: Certified Semen Services
eCG	: Equine chorionic gonadotrophin
ER	: Extension ratio
FGA	: Fluorogestone acetate
g	: Grams
IU	: International units
LAI	: Laparoscopic artificial insemination
LIN	: Linearity
MAP	: Medroxyprogesterone acetate
MGA	: Progesterone acetate
µg	: micrograms
ml	: Milliliter
PBS	: Phosphate-buffered saline
ROS	: Reactive oxygen species
SCA	: Sperm class analyzer
STR	: Straightness
TAI	: Trans-cervical artificial insemination
VAP	: Average path velocity
VCL	: Curvilinear velocity
VSL	: Straight line velocity

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Goats serve a crucial role in both commercial and subsistent farming systems in South Africa. In the commercial sector, goats are mainly kept for meat production, for both local sales and for export, while to subsistent farmers, goats are of greater importance as a source of animal protein and whole communities depend on their flocks of goats, as well as source of milk for poor families, who cannot afford to keep cattle (Casey and Van Niekerk, 1988). About 75 per cent of the goats in the world are in developing countries, kept by small families and are used for the production of fiber, skin, meat and milk (Alan, 1992). Goats contribute to the health and nutrition of several million people in developing countries, especially those on the poverty line mainly in villages and rural areas. According to Devendra and Burns (1983), raising goats provides a crucial supply of animal proteins of high biological value and lipid-soluble vitamins, which are of specific significance for the most vulnerable groups such as pregnant and nursing mothers as well as young children.

According to Evans and Maxwell (1987) artificial insemination (AI) has contributed significantly to animal genetic improvement worldwide. The early development of AI in sheep on a major scale began in Russia (Milovanov, 1938). Initially the most crucial problem to resolve was to find a method to store semen long enough for shipment and use in the field. The first major improvement in the AI procedure that was initiated in the United States was the development of an egg yolk-phosphate semen extender (Phillips and Lardy, 1940). Sperm survival at 5 °C permitted the use of the semen for longer periods, as much as up to 3 days. Citrate dispersed the fat globules in egg yolk, making sperm visible for microscopic examination (Foote, 2001).

Artificial insemination plays an important role in the breeding of goats, particularly in intensive systems of production, whereby it is used to synchronize reproduction, and to facilitate the dissemination of improved genotypes to improve the production of meat, milk and fiber. The use of AI allows the control of reproduction in a specific goat population in order to allow kidding at a precise season of the year. Artificial insemination allows rapid and widespread diffusion of improved genotypes and the exchange of genotypes without transmitting diseases. This

technique allows the transport of semen when the risk of disease prevents buck movement. Synchronization of estrus is an important management tool that has been used as an aid for AI and to reduce seasonal effects in the reproduction of dairy goats (Freitas *et al.,* 1997). It is extremely important to identify the onset of heat to improve control mating systems and for AI schemes. Intrauterine insemination with frozen-thawed semen using laparoscopy makes it possible to deposit the semen directly into the uterus. Baril *et al.* (1993) reported that the difference in the pregnancy rate could be due to the differences in the time of occurrence of estrus and the set time of AI.

1.2 Problem statement

In rural areas, farmers usually have a small number of goats and also have limited access to a high quality sire or even a sire at all. Farmers need to shift from subsistence farming to commercial production by increasing the size and quality of their flocks. This can be achieved through AI. However, most AI has been carried out using frozen semen. Keeping frozen semen requires good quality equipment, materials and techniques. Materials and techniques for frozen semen are more expensive than for chilled extended semen. Therefore, it is necessary to improve AI using semen that can be extended and kept viable for long periods. Trans-cervical AI (TAI) is considered a potential, cost effective, safer and much more convenient alternative to laparoscopic AI (LAI). In South Africa, though LAI is widely used in sheep, the method is rarely used in goats. Goat farmers stand to benefit if cost effective, safer and more convenient protocols for AI application using extended semen are validated.

1.3 Justification

Many of the artificial insemination techniques are mostly used in sheep and cows, especially dairy cows. A cheaper technique with a high pregnancy rate, would be very useful for goat farmers who have a passion to use AI to control reproduction and to improve the genetic value of their flocks. Communal goat farmers cannot readily practice AI due to the fact that the procedures are complicated and the transport and storage of the semen are expensive. The use of chilled extended semen can be helpful to goat breeders who cannot afford frozen semen or the laparoscopic method of applying it.

1.4 Objectives

1.4.1 Broad objective

The broad objective of the study was to evaluate the suitable preservation time and the effects of different artificial insemination techniques on conception rate in indigenous goats.

1.4.2 Specific objectives

i. To determine the suitable preservation time for Boer goat semen in Biladyl® extender
ii. To identify an extension ratio fit to maintain the viability of Boer goat semen in Biladyl® extender for effective application of artificial insemination.
iii. To determine the effect of different artificial insemination techniques (CAI, TAI and LAI) on conception rate.
iv. To determine the effect of different artificial insemination techniques (CAI, TAI and LAI) on kidding rate.

1.5 Hypothesis.

I. Storage time does not affect the viability Boer goat semen in Biladyl® extender and stored chilled at 5° C.
II. The Biladyl® extender ratio does not affect the viability of Boer goat semen extended in Biladyl® extender and stored chilled at 5° C.
III. The method of AI (CAI, TAI and LAI) does not affect the pregnancy rate of indigenous Venda goats
IV. The method of AI (CAI, TAI and LAI) does not affect the kidding rate of indigenous Venda goats.

CHAPTER 2

LITERATURE REVIEW

2.1 General Introduction

According to Lehloenya e*t al.* (2004), indigenous goat breeds in South Africa such as Nguni and Boer goats have moderate productive performance under natural conditions. These breeds are well known for adaptability, hardiness, resistance to diseases as well as good mothering ability. Irrespective of their good characteristics older does may have relatively low fertility due to their age or where AI is applied, possibly because of prior multiple laparoscopic artificial insemination. In South Africa, since the introduction of AI with fresh semen in the 1950s. Many producers use high quality bucks to improve performance of their herd through AI.

Vaginal and cervical AI (CAI) are the most commonly used AI methods. These methods are quite acceptable with fresh semen. Cervical insemination is the preferred option in both ewes and does. Cervical insemination using fresh and chilled semen can achieve conception rates of 40–80% after hormonal control of estrus (Chemineau *et al*., 1991). However, in using frozen/thawed semen, pregnancy rates are too low for commercial application. Evans and Maxwell (1987) reported that use of frozen/thawed semen for cervical insemination yielded lambing rates of 25-40%. This is far below results of 65-75% pregnancy rates when fresh diluted or undiluted semen was used in the same method. However, by using frozen semen, laparoscopic or trans-cervical intrauterine insemination techniques are the only means to achieve acceptable pregnancy rates. Due to the complex anatomy of the cervix in doe, AI is more difficult in does compared to cows. Since the cervix is a physical barrier for the passage of an AI pipette, intrauterine AI with a laparoscope has been used to overcome this problem. However, Laparoscopic AI is the method which is mostly used with frozen semen, to attain conception rates comparable to natural service or use of fresh semen (Maxwell, 1986).

Direct transfer of semen in the lumen of the uterus with laparoscopic technique has resulted in an acceptable pregnancy rate, but it has several disadvantages. These include the costs of owning laparoscopic equipment, costs in the training help for operation, costs associated with drugs for synchronization of ewes, and storing of ram semen. Alternatively, trans-cervical intrauterine insemination techniques have been developed and improved to allow the semen to be deposited deeply into the uterine horns (Buckrell *et al.,* 1994; Sohnrey and Holtz, 2005).

Since goats are seasonal breeders, synchronization plays a major role in allowing AI to be performed during the non-breeding season. Repromap estrus synchronization sponges were introduced in the 1960s which were mainly used in the Karakul industry. Later on the hormonal treatment was developed in goats to induce and synchronize ovulations during or out of the breeding season for AI purposes. The most commonly used treatment consists of a progestagen (i.e. fluorogestone acetate), equine chorionic gonadotrophin (eCG), and prostaglandin analogue (Corteel *et al*., 1988). However, the repeated administration of eCG leads to the production of anti-eCG antibodies, reducing the efficacy of the treatment and decreasing fertility after AI (Roy *et al.,* 1999). Moreover, because eCG is purified from animal tissues, risks associated with the presence of pathogens in commercial eCG extracts remain. The intravaginal devices containing 0.3 g of progesterone (i.e. CIDR-G) were developed for use with the long treatments, even blocking estrus and ovulation in treatments prolonged for approximately 30 days (Wheaton *et al*., 1993). According to previous study, high progesterone concentrations reached by the insertion of a CIDR® seem to induce follicular turnover and the new follicle continues growing to reach ovulation after device withdrawal 5 days later (Menchaca *et al*., 2007). The progesterone released from CIDR® mimic the effects of the doe's natural progesterone by preventing estrus from occurring as long as they are present in the body. Once removed, the doe comes into estrus in 1 to 2 days.

Due to its biochemistry, goat semen preservation is difficult. The storage of semen, particularly in frozen state, causes ultrastructural, biochemical and functional damage to the spermatozoa resulting in a reduction of motility, viability, impaired transport and fertility (Leboeuf *et al*., 2000). At chilling temperature, the fertilizing ability of spermatozoa reduces with time (Shamsuddin *et al*., 2000) and the motility and morphology of spermatozoa deteriorate after two days (Alam *et al*., 2005). Many researchers have encountered the same problem, but the fertility of fresh semen is generally higher than the one for frozen semen. Refrigeration of goat semen is particularly important because of its relatively small volume compared to other livestock species such as cattle, buffalo and pigs. However, the cooling process seems to reduce semen motility at a rate of 10% to 35% per day of storage (Evans and Maxwell, 1987). In rams, cooling and thawing of semen induces the premature capacitation of the spermatozoa, reducing the fertilizing capacity (Gillan and Maxwell, 1998). Blash *et al*. (2000) found that the freezing-thawing process of goat semen reduced the percentage of live spermatozoa from 86% to 60%, and the integrity of the acrosome from 95% to 89%. Due to variety of high quality goat breeds, recently, goat semen preservation invite the interest of local scientist. It became necessary to determine the ability and

the performance of these bucks. Their good performance and abilities can lead them to become potential semen donors for AI using extended semen in chilled conditions.

2.2 Goat Semen and its collection

Semen is a natural fluid produced by the male reproductive system. It includes secretions from as the testicles, the prostate gland, and the seminal vesicles. A major problem constraint to the delivery of AI in goats is the lack of generally accepted optimum semen preservation protocol.

2.2.1 Methods of collecting semen

Methods of goat semen collection include the use of artificial vagina and electro-ejaculation. Electro-ejaculation is a useful procedure to obtain semen from a large number of bucks in the field and can be applied in many species. Artificial vagina is mostly used in domestic animals due to the fact that it is a technique that requires a preliminary training period (Wulster-Radcliffe *et al.,* 2001). The electro- ejaculator is the method of collecting semen that involves an alternative when males are not trained to artificial vagina or for wild species. By this method ejaculation of semen is brought about inserting a probe or electrode in sire's rectum and stimulating nerves of the reproductive system by gradually increasing voltage in rhythmic fashion with a rheostat for a short period.

Each of these semen collecting techniques are mostly used in goats and they have their own advantages and disadvantages. The collection of semen using artificial vagina is considered humane compared with that done through an electro-ejaculator, because it is almost similar to natural service and is the most hygienic technique. Practically the whole ejaculate is collected in uncontaminated and natural stage. A sterile condition of the apparatus ensures disease control. Jiménez-Rabadán *et al.* (2012) observed higher sperm concentration and lower abnormal sperm when using artificial vagina compared to the use of an electro-ejaculator.

However, the temperature of the artificial vagina is more important and should always be checked before making the collection. Too high a temperature may cause injury to the penis and the bull may refuse to serve in future, whereas at lower temperature, there may not be complete ejaculation and the whole ejaculate may be contaminated with urine. The electro-ejaculator can also be an alternative for semen collection from animals that reject the artificial vagina or animals with leg injuries which cannot mate. Successful use requires skill, experience, patience and the knowledge of individual requirement of the stimulation.

2.2.2 Goat semen extension

Semen extenders are liquid diluents which are added to semen to preserve its fertilizing ability or as products that can be mixed with semen to prevent it from deteriorating while it is being held or shipped for eventual use. Protecting the semen using suitable extenders is critical to the success of AI in goat reproduction. Semen extension also increases the number of fertilizations per ejaculate. The extender allows the semen to be stored and to be transported to the females.

The critical components of a good semen extender include simple carbohydrates, such as glucose and or fructose, are added as sources of energy for the sperm. Commercial extenders include skim milk, glycerol, lactose, egg yolk glycerol, egg-yolk citrate, hydroxymethyl, aminoethane, citric acid, Illini Variable Temperature (IVT) extender, Cornell University Extender (CUE), Tris-coconut milk and coconut milk-citrate etc., each with a different preservation power and fertilization rate. Some of these components are found in expensive extenders and may sometimes not be readily available locally (Preciado *et al.,* 2011).

Egg yolk is a common component of semen cryopreservation extenders for domestic animals. It has been shown to have a beneficial effect on sperm cryopreservation as a protector of the plasma membrane and acrosome against temperature related injury, in association with others components (Purdy., 2006). Goats are apparently unique among domestic livestock species in secreting semen in that they produce specific lipase enzymes that interact with egg yolk lipids or skim milk triglycerides to generate products that are toxic to sperm cells (Sias *et al.,* 2005).

2.2.2 Chilled goat semen storage

Significant temperature changes are considered detrimental to semen quality (Lafalci *et al*., 2002). The process of liquid storage, like in cryopreservation may advance maturation of sperm membranes, increasing the proportion of capacitated and acrosome reacted cells with reduced viability (Cseh *et al*., 2012). Liquid goat semen destined for use within 12 h should be stored at 4 °C (Lafalci *et al*., 2002). However, lately, it was indicated that storage of ram semen at 5 °C maintained acceptable motility and viability (O'Hara et al., 2010). Baliarti *et al*. (2012) observed best cold survival motility and viability of ram spermatozoa at 5 °C storage. However, Oghenesode and Udeh (2011) reported that liquid goat semen stored in sodium citrate under refrigerator condition maintained the highest viability of the sperm cells and diluted liquid goat semen stored in the refrigerator should not be kept beyond 24 h before insemination in order to

obtain a good conception rate. The percentage of motile spermatozoa in buck semen stored in liquid form for 72 h, progressively declined overtime irrespective of whether storage occurred at 4 or 18 °C (Peterson *et al*., 2007). Gradual depletion of nutrients such as potassium sodium and plasma protein which are required for the high metabolic demands of sperm transport through the female genital tract may results in drastic decline in the sperm motility of stored liquid semen (Sias *et al.,* 2005).

Using native phosphocaseinate and betalactoglubin (NPPC-BL) and milk as semen extenders, fertility rate of 70% was found after four hours of in vitro storage, but beyond four hours a dramatic decrease in kidding rate was obtained reaching an average of 40% at 76 hours of storage (Leboeuf *et al.,* 2004). However, Mehmod *et al.* (2011) reported a pregnancy rate of 44.4% in does inseminated with skimmed milk extender and 12.5% using Tris-citric acid buffer during low breeding season. Furthermore, Fukui *et al.,* (2008) established that kidding rates were 64.5 and 56.7% for sperm cryopreserved in egg yolk and commercial soybean lecithin extenders, respectively.

2.3 Synchronization of estrus in goats

The synchronization or induction of estrus is helpful in controlling estrus cycle and inducing of estrus in a period out of breeding season (Danko, 2007). Heat synchronization plays a crucial role in artificial insemination and reproduction management. Estrus synchronization can be achieved using different hormones which have direct physiological effect on the female reproductive system. In South Africa MAP, CIDR and FGA sponges are commercially available for estrous synchronization which are efficient in controlling estrous and ovulation in small stock (Gordon, 1997). The estrus or heat phase in goats lasts 12-48 hours with an average length of 36 hours. Successful fertilization during the induced estrous period depends on the time of the artificial insemination.

Out of the season, synchronization protocols are based on controlled internal drug release (CIDR). Intravaginal polyurethane sponges impregnated with progesterone (P4), or their synthetic analogues (progestogens) mainly medroxyprogesterone, melengestrol and fluorogestone acetate forms, plus equine chorionic gonadotropin (eCG) and prostaglandin F2 alfa (PGF2α) or even estrogenic pharmacologic active substances are also used for synchronization (Simoes, 2015). The CIDR-G provides a convenient means to deliver exogenous progesterone to sheep and goats and offers an alternative to the progestogen sponge for reproductive management (Jonathan *et*

al., 1993). In goats, CIDR have been substituted successfully for sponges for estrous synchronization, superovulation, artificial insemination and embryo transfer. However, during non-breeding season 50% estrus behavior rate and 62% pregnancy rate were achieved after a 6-days short-term progestogen (20 mg of fluorgestone acetate) priming followed by male effect treatment (in substitution to eCG) in Serrana goats. (Simoes *et al,* 2008).

2.4 Methods of heat detection in goats

Estrus can be detected through signs of estrus such as a clear mucus discharge from the vulva, vocal sound and being active when they are in heat. These methods are applicable to goats. Timely heat detection is critical to efficient AI. Estrus detection is achieved using vasectomized rams (Alan, 1992) and natural breeding can be avoided when these rams are fitted with marking harnesses with crayons.

2.5 Artificial insemination in goats

2.5.1 Timing of artificial insemination

Standing estrus plays an important role in timing of artificial insemination in goats. The estrus synchronization leads to simultaneously expression of estrus and ovulation in large number of animals, followed by expulsion of fertile ovum and AI in optimal time guarantee successful fertilization (Holtz *et al.,* 2005). Gonadotropin-releasing hormone (GnRH) application during the estrus synchronization has more precise ovulation and improves the success of AI in fixed time AI in does (Pierson *et al.*, 2003). However, it is important to inseminate near the end of standing estrus. Another prerequisite for successful conception after using frozen semen is enough fertile spermatozoa to be deposited into the cervix immediately before the ovulation (Stanimir *et al.,* 2016). Due to the fact that the ovum has a shorter life span than the sperm, it is better to have the sperm waiting for the ovum rather than the ovum waiting for the sperm (Rodning *et al.,* 2012).

2.5.2 Methods of artificial insemination

2.5.2.1 Cervical artificial insemination

Cervical insemination is an easy method to perform since it only requires an insemination pipette (Devendra and Burns., 1983). Basic equipment is required which consists of a speculum with a built in light source and a pipette connected to a one milliliter (1 ml) syringe. The most commonly used pipettes for cervical insemination are plastic disposable pipettes. Straight pipettes can be used in goats for easier penetration of the cervix.

In France, a 65% average pregnancy rate in goats was reported with cervical insemination using vaginal speculum for detection of the cervical opening (Leboeuf *et al*., 2008). Moore *et al. (*1987) reported a kidding rate of less than 30% with the use of one time cervical insemination using frozen-thawed semen in progesterone synchronized oestrus. However, Karatzas *et al.* (1997) reported a 65.5% of kidding rate using fresh semen compared to 53.4% kidding rate using frozen thawed semen. In does that were inseminated twice with fresh and frozen thawed semen, fertility of 70.4 and 59.1%, respectively; were reported (Karatzas *et al.,* 1997). Pinto *et al.* (2014) similar kidding rates of 24.1% did not differ between animals inseminated with semen cooled for 24 and 48 hours kept at a temperature of 5 ˚C. Waide *et al*. (1977) reported 63.3%, 79.4% and 77.7% for semen stored 1 to 30 days, 31-102 days and 210-1022 days, respectively after deep cervical insemination using forceps with non-washed semen stored for 3 years in sodium citrate diluent. However, Fougner (1979) reported 63.4% kidding rate after intrauterine inseminations through the cervix with washed spermatozoa stored for 1–3 years in tris–fructose–citrate–yolk medium.

2.5.2.2 Trans-cervical artificial insemination

The cervix is the most caudal portion of the uterus. Its constricted lumen is surrounded by a thick musculo-connective tissue wall. Trans-cervical AI requires specially designed inseminating equipment and manipulation using forceps. Greater success rate is achieved by depositing semen at the bifurcation of uterine body than at other locations (Seguin, 1986). However, there are concerns about the potential trauma involved in the procedure (More, 1984). There is limited research on the comparative efficacy of trans-cervical insemination. Trans-cervical insemination using thawed semen achieved 71% kidding rate (Sohnrey and Holtz, 2005). A pregnancy rate of 66.7% was achieved in the West African Dwarf Does (Ajibade and Leigh, 2010). Ultrasound pregnancy examination conducted four weeks after insemination revealed that trans-cervical inseminated synchronized does achieve a pregnancy rate of 74% compared to 56% for laparoscopically inseminated does (Sohnrey and Holtz, 2005).

2.5.2.3 Laparoscopic artificial insemination

Before laparoscopic surgery, open surgery was the common method performed through a very long incision which required nursing the animal for 7 days post-operation and 2 to 6 weeks for complete return to its normal conditions (Bonev *et al*., 2005). Nowadays, many of the

abdominal and pelvic surgery are done by laparoscopy. Laparoscopic AI can be performed through the injection of semen into the uterus. It is advantageous to inject semen into the uterus, thus eliminating the barrier of the cervix penetration by spermatozoa. According to Fennessy and Mackintosh (1988) this artificial insemination technique is 10 to 20% higher than intra cervical insemination in terms of pregnancy rates.

Dickson *et al*. (2001) obtained a fertility rate of 59.5% in milking goats (French Alpine and Saanen) which had received intrauterine insemination with frozen semen. However, according to the studies done by Ritar *et al. (*1990) insemination with 10 million spermatozoa has not differed from the insemination with 20 million spermatozoa, with fertility rate of 61.5% and 63.7% respectively. The pregnancy rate obtained using laparoscopic insemination with frozen thawed semen in cashmere does was 64.5% when does were synchronized by CIDR and 62.7 % when estrus was induced using progestagen sponge (Ritar *et al*., 1990).

2.7 Pregnancy detection in goats

Precise and early pregnancy diagnosis is considered to improve the efficiency of production in dairy goat herds, by discriminating between pregnant and non-pregnant animals better management control is made possible in that nutrition can be administered adequate to the status of the doe (Fernando *et al.,* 2004). Clinical methods and hormonal assays are available to diagnose pregnancy in goats at around 3 weeks after breeding. The simplest method involves observation for signs of estrus at approximately 21 days post-breeding. Serum or milk progesterone (P4) levels have been widely used to detect pregnancy in goats at 21–22 days post-breeding (Corteel *et al.,* 1982). Real-time ultrasound scanning provides an efficient tool for early diagnosis of pregnancy in goats. (Haibel, 1990).A variety of examination methods have evolved over the years. Ultrasonography, hormone assay, and radiography have emerged as the most useful methods utilized today. However, the method of choice to diagnose pregnancy in goats will depend upon the availability of equipment, number of days post-breeding if known, and the desired accuracy (Fernando *et al.,* 2004).

2.8 Summary of the literature review

Indigenous goat breeds in South Africa such as the Nguni and Boer goats have moderate productive performance under extensive management. These breeds are well known for their adaptability, hardiness, resistance to diseases as well as good mothering ability. However, there is insufficient description of indigenous goats, which has led to poor understanding of their

potential for reproduction and growth (Gwaze *et al.,* 2009). Most AI has been carried out with fresh and frozen semen. However there is no information of AI in Venda indigenous goats. Many extenders have been used to preserve goat semen, but these extenders can be classified into two major groups. Some extenders have been used to freeze semen, while others are used to preserve semen in a liquid state. However, during storage, the extender should provide the environment that inhibits the formation of harmful reactive species (Orok *et al*., 2010). Presently, egg yolk is the most common ingredient of most semen preservation extenders to protect spermatozoa from damaging during the freezing–thawing process (Forouzanfar *et al.,* 2010). However, the problem about the extenders containing egg yolk in goat semen has been attributed to egg yolk coagulating enzyme (EYCE) which can be harmful to the sperm cells (Leboeuf *et al*., 2000). The main factor limiting more widespread use of frozen semen in caprine reproduction is the reduction of sperm viability during freezing processes (Batista *et al*., 2009). The success of AI is based on the ability to efficiently collect, evaluate and cryopreserve semen from quality bucks for use in inseminating does over generations (Ngoula *et al*., 2012). In order to achieve the economic success of AI, there is need for extension of the semen. Extended buck semen can be used frozen-thawed, chilled or fresh but when fresh semen is used, it offers better fertility and conception rate (Langford *et al*., 1979, Hackett and Wolynetz, 1981).

CHAPTER 3

EFFECTS OF EXTENSION RATIO AND PRESERVATION TIME OF BOER GOAT SEMEN EXTENDED USING BILADYL® AND STORED AT 5 °C.

3.1 Introduction

Few studies have evaluated the efficacy of Biladyl® extender in preserving chilled indigenous South African goat semen. However, medium-term preservation of chilled goat semen is critical for the application of artificial insemination in the unavailability of liquid nitrogen storage in rural areas. The objective of this study was to determine the suitable preservation time and extension ratio fit to maintain Boer goat semen in Biladyl® extender for the purpose of artificial insemination.

3.2 Materials and methods

3.2.1 Experimental site

The study was conducted at the School of Agriculture Experimental Farm and all semen samples were analyzed in the Biotechnology Laboratory of the Centre of Excellence in Animal Assisted Reproduction (CEAAR), University of Venda, Thohoyandou, in the Limpopo Province of South Africa. The daily temperatures at Thohoyandou vary from about 25˚C to 40˚C in summer and between approximately 12˚C and 26˚C in winter. Rainfall is highly seasonal with 95% occurring between October and March. The average rainfall is about 800 mm but varies (Mzezewa *et al*., 2010).

3.2.2 Animals and management

Three adult Boer goat bucks of age 4 ± 1.55 years provided the semen used for this study. The bucks belong to the School of Agriculture Experimental Farm where they are kept in open pens in natural light and were fed daily with 1.8 to 2 kg goat concentrate, and water was provided *ad-libitum.*

Figure 1: Boer goat bucks

3.2.3 Experimental design and procedures

The effect of semen extension ratio and storage time were evaluated in a 2 (extension ratio) x 6 (time of storage) factorial experiment replicated six times. The experimental design and treatments are summarized in table 1.

Table 1: Experimental design and treatments

Collection	Semen type	Extender	Extension ratio	Temperature and storage	Timing of sampling evaluation	Replications
Artificial Vagina	Fresh	Biladyl®	1:5	37ºC prior to evaluation, no storage	0 hours	6
	Extended	Biladyl®	1:5	37ºC prior to evaluation, 120 hours at 5 ºC	0 hours post dilution, every 24 hours to 120 hours	6
	Extended	Biladyl®	1:10	37ºC prior to evaluation, 120 hours at 5 ºC	0 hours post dilution, every 24 hours to 120 hours	6

3.2.4 Preperation of Biladyl® extender

Biladyl® was used as the extender which was prepared according to the specification of the Certified Semen Services (CSS manual). The Biladyl® extender was supplied in three fractions A, B and AB. Fractions A and B are crystalline nutrient and buffer liquid concentrates. Fraction AB is very fine white powdery cocktail of antibiotics. The extender constituted according to the manufacturer's instructions, with proportionate component reduction to the quantities required for each stage of the study in order to avoid wastage due to prolonged cooling during refrigerated storage which could destabilize the media. Table 2 shows the constituents of Biladyl® extender.

Table 2: Constituents of Biladyl® extender

Fraction A Biladyl® (49 g)	**Fraction B Biladyl® (250 g)**	**Fraction AB Biladyl® (250 g)**
Double distilled water (24.9 g)	Double Distilled Water (140 g)	Gentamycin (300 g)
Citric acid (6.9 g)	Citric Acid (6.9 g)	Lincomycin (180 mg)
Fructose (5 g)	Fructose (5 g)	Spectinomycin (360 mg)
TRIS (12.1 g)	TRIS (12.1 g)	Tylocin (60 mg)
	Glycerol (86 g)	

3.2.4.1 Preparation of fraction A of the Biladyl® extender

The extender was prepared a day before semen collection and kept in the refrigerator at 5 °C.

Materials required:

- Biladyl fraction A
- Ultra-pure water
- Fresh egg yolk
- Sterile graduated cylinder or flask
- Sterile filter paper
- Sterile filter funnels

Step 1: A measured 8.1 ml of fraction A was poured into a graduated flask and mixed with 56.3 ml of ultra-pure water to make a combined volume of 64.4 ml.

Step 2: Fresh laid chicken (W-98) eggs were collected from the University of Venda poultry house. The egg shells were sterilized by wiping them thoroughly with 70% ethanol. The eggs were opened carefully and the egg yolks were separated completely from the albumen. The entire egg yolk was put on a clean filter paper and rolled on a paper until it was completely free from albumen. A 20 ml syringe and an 18 G needle were used to aspirate the egg yolk from inside its membrane.

Step 3: After measuring 16.7 ml of egg yolk, stock solution A was added to the egg yolk and was mixed with a sterile glass rod after which 2 ml of antibiotics cocktail AB was added to the solution. The final stock solution was kept in sterile 50 ml Cellstar tubes sealed with parafilim "M"® (American National Can, Chicago, U.S.A.) to avoid taint and stored at 5 °C. Two hours before semen collection, the Biladyl® Fraction A was warmed in the water bath, (Model WBS-450B) to 37 °C before use.

3.2.4.2 Preparation of fraction B of the Biladyl® extender

Exactly 41.7 ml of Biladyl® fraction B was reconstituted from 25 ml ultra-pure water gently mixed into 16.7 ml fresh egg yolk in a separate beaker. The homogenous mixture was aspirated in the 20 ml syringe and was filtered using a sterile syringe filter and cooled to 5 °C. The final stock solution was kept in sterile 50 ml CellStar tubes, sealed with Parafilm "M"® (American National Can, Chicago, U. S. A.) and stored at 5 °C in the refrigerator.

3.2.5 Semen collection and pre-extension evaluation

Three healthy bucks were used for semen collection to ensure maximum quality and quantity of semen. Semen samples from three bucks were collected using an artificial vagina (AV) once in 4 days to allow sexual rest. Three bucks were trained for a period of 4 weeks in the presence of a doe in heat before the onset of the study (Bopape *et al.,* 2015). The AV used resembles a car radiator hose and was about 15 cm in length. The AV had an inner latex liner containing water at a temperature of 45 °C placed between the liner and the hose. The warm water simulates the vagina of a doe. A latex rubber collection cone was placed at the one end of the AV and a pre-warmed collection tube (15 ml Cellstar) was placed on the end of the cone. An assistant technician held the goat doe in heat by the horns and the male was allowed to mount. The penis of the buck was directed into the AV and semen was ejaculated into the test tube. The test tube containing the ejaculate was protected from direct sunlight and cold temperature. Immediately after collection, the color of the goat semen was evaluated. The semen volume from each buck was directly measured from the tube. The test tube with the semen sample was placed

in the vacuum flask at 37 °C and was taken to the laboratory. Upon arrival at the biotechnology laboratory, the pH of each sample was measured using Meter Toledo (AG Analytical, Sonnenbergstrasse 74, Schwerzenbach) pH meter and the concentrations measured using a Spectrophotometer and a Sperm Class Analyzer® (SCA), version 5.4, (Microptic S.L., Barcelona, Spain) and recorded in millions per milliliter. The semen samples were pooled together in a pre-warmed and sterile tube (15 ml Cellstar tube) to eliminate individual difference.

3.2.6 Semen extension and storage

Empty 15 ml Cellstar tubes were pre-warmed in the water bath at 37 °C. Semen samples were diluted at extended at ratio 1: 5 v / v (semen to extender); and 1: 10 v / v. 1 ml of semen from the pooled semen was slowly introduced into pre-warmed 2 µl of Biladyl® fraction A (containing antibiotics cocktail AB) and the mixture was homogenized by gentle shaking and left in the water bath for 10 minutes for the temperature to equilibrate with the water bath temperature of 37 °C. Pre-warmed 3 ml of 37 °C Biladyl® fraction B was then very slowly added into the equilibrated mixture. The extended semen was then divided into two pre-warmed 15 ml Cellstar tubes for storage. The semen samples in the test tubes were placed into a 250 ml beaker with distilled water at 25 °C and placed in a refrigerator at 5 °C to allow slow cooling and avoid cold shock.

3.2.7 Evaluation of semen

3.2.7.1 Sperm motility

The automated computer aided sperm analysis system (CASA) for semen analysis was used to provide the total motility (progressive motility and non-progressive motility) analysis of the spermatozoa using a Sperm Class Analyzer® 5.0 (SCA) (Microscopic, S.L, Barcelona, Spain). A 2 µl of diluted semen was dropped into a 2 µl deep chamber of eight-chamber Leja slide pre-warmed to 37 °C. Spermatozoa were evaluated at 10 x (Nikon, China) magnifications in three different areas on the slide of the same semen sample. The progressive motile and non-motile spermatozoa percentage were automatically calculated. The CASA was used to measure motility parameters such as rapid, medium, slow, static movement. The percentage of sperm motility was assessed subjectively using the phase contrast microscope on same fields used for CASA. All data was recorded.

3.2.7.2 Sperm morphology

Sperm morphology was determined by staining with Spermac® (Stain Enterprises, P.O. Box 152, Wellington 7654, South Africa). Spermac stain consists of stain A (red stain), stain B (pale green), stain C (dark green) and Fix (fixative). For staining, sperm smears were first prepared at room temperature (24 °C) by pipetting a 15µL drop of semen onto a clear end of a slide. The edge of the second slide was then placed on the drop at an angle of 20° on a horizontal plane and then pushed forward to smear across the slide. The sperm smears were thereafter allowed to air dry, for about 5 minutes on a warm plate at 37 °C before staining. The dried smears were placed horizontally on absorbent paper inside a staining tray and about 1 ml of Spermac fixative was then poured on the dried smear using a plastic disposable pipette. After 5 minutes, the slides were placed vertically on one end on absorbent paper to drain off excess fixative. The slides were then washed by dipping seven times slowly (about 1 second a dip) into distilled water. Excess water was drained off by touching the end of the slide onto absorbent paper. About 1 ml of stain A was then poured onto the slide for 2 minutes. The slides were washed for the second time in fresh distilled water to remove the excess of stain A. Stain B was introduced the same way as stain A, and was similarly washed by dipping seven times into fresh distilled water. The third stain, stain C was introduced for another 2 minutes and washed as above. The slides were then left in an upright position (about 70° angle) to let fluid drain until air dry. Thereafter, the stained slides were placed on the CASA microscope stage. A drop of immersion oil was poured on the stained slide and covered with a cover slip before evaluation of the spermatozoa morphology. The CASA morphology program was used at 100X magnification to count 200 spermatozoa per stained slide. The results were recorded.

3.2.7.3 Sperm viability

Live normal and dead sperm were determined using eosin-nigrosin staining (Onderstepoort Faculty of Veterinary Sciences Pharmacy, South Africa). Six micro-liters of the semen sample and 20 µL of eosin stain was 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 nigrosin stain was then poured onto the mixture on the slide and mixed using the same pipette tip. The edge of a second slide also pre-warmed to 37 °C was thereafter place on the mixture at an angle of 20° from the horizontal plane and pushed forward to smear the cocktail across the slide. Immediately after smearing, the smeared slide was place on a hot Buehler® slide warmer (Buehler Ltd., 42 Waukegan road, lake Bluff, Illions, USA) at 120 °C to allow drying. The slide was left to cool down before evaluation. A drop of immersion oil was poured on the stained slide and covered with a cover slip before

evaluation. The CASA vitality program was then used at 60X magnification (Olympus Corporation BX 51FT, Tokyo, Japan) to count the number of live and dead spermatozoa. Under the microscope live spermatozoa heads appeared white, while dead spermatozoa heads appeared pinkish in color. A total number of 200 spermatozoa were counted per stained slide and the results were recorded.

3.2.8 Statistical analysis

Data were subjected to analysis of variance for a 2 x 6 factorial experiment in a completely randomized design with six replications using the GLM procedure of Minitab (Minitab, 2013). Semen quality data were analyzed based on the model:

$$\boldsymbol{Yijk} = \boldsymbol{\mu} + \boldsymbol{\alpha i} + \boldsymbol{\beta ij} + \varepsilon \boldsymbol{ijk}$$

Where;

Y_{ijk} is the observation.

μ is the overall mean.

α_i is the effect of the i^{th} extender, where, (i = 1…2).

β_j is the effect of j^{th} storage time, where, (I = 1....6).

$\alpha\beta_{ik}$ is the interaction of the i^{th} extender and j^{th} storage time.

ε^{ijl} is the residual error.

Means were separated using the Tukey's test at the P<0.05 level of significance

3.3 Results

Table 3 presents the semen parameters of the South African Boer bucks used in the study.

Table 3: Initial characteristics of Boer goat buck semen

Parameters	**Values**
Semen color	Creamy
Volume (ml)	1.73 ± 0.40
pH	7.10 ± 0.7
Motility (%)	98 ± 5.8
Concentration (x 10^6/ml)	610.5 ± 123.0

Table 4 shows the motility characteristics of Boer goat spermatozoa extended in Biladyl® at two different ratios during storage over 120 hours at 5 °C.

Table 4: Motility of extended semen with Biladyl® using two different extension ratios evaluated after 0, 24, 48, 72, 96 and 120 hours storage at 5 °C.

Treatments	Storage Time (Hrs)	TM (%)	NON-P (%)	PROG (%)	RAP (%)	MED (%)	SLOW (%)	STA (%)
ER1	0	98^{a}	3^{f}	96^{a}	95^{a}	4^{f}	0.4^{c}	0.5^{d}
	24	97^{ab}	16^{ef}	80^{ab}	79^{ab}	12^{ef}	5^{bc}	3^{d}
	48	93^{ab}	24^{de}	69^{bc}	68^{bc}	20^{bcd}	6^{bc}	6^{cd}
	72	90^{abc}	27^{de}	62^{cd}	60^{bcd}	23^{bc}	8^{bc}	9^{bcd}
	96	83^{abcd}	29^{cde}	54^{cde}	52^{cd}	23^{bc}	12^{b}	16^{abcd}
	120	80^{abcd}	31^{bcd}	49^{def}	47^{de}	25^{abc}	12^{b}	19^{abcd}
ER2	0	92^{abcd}	26^{de}	58^{cde}	57^{cd}	18^{bcde}	8^{bc}	14^{abcd}
	24	85^{abcd}	50^{a}	42^{efg}	29^{ef}	33^{a}	29^{a}	7^{cd}
	48	81^{abcd}	48^{a}	33^{fgh}	22^{f}	26^{ab}	32^{a}	18^{abcd}
	72	72^{bcd}	43^{ab}	29^{gh}	21^{f}	20^{bcd}	30^{a}	28^{abc}
	96	67^{cd}	43^{ab}	24^{h}	15^{f}	17^{bc}	34^{a}	32^{ab}
	120	62^{d}	42^{abc}	19^{h}	14^{f}	13^{de}	34^{a}	37^{a}
SEM		2.8	2.8	3.41	4	1.78	1.74	5
Extension ratio means								
ER1		91^{a}	22^{b}	69^{a}	67^{a}	18^{b}	8^{b}	9^{b}
ER2		77^{b}	42^{a}	35^{b}	27^{b}	21^{a}	28^{a}	23^{a}
SEM		2.1	1.14	1.4	1.64	0.72	0.72	2.07
Storage time means								
	0	94^{a}	15^{b}	76^{a}	76^{a}	12^{b}	4^{c}	8^{c}
	24	92^{a}	33^{a}	62^{b}	56^{b}	23^{a}	17^{b}	5^{c}
	48	87^{ab}	36^{a}	51^{c}	45^{bc}	24^{a}	19^{ab}	12^{bc}
	72	81^{abc}	36^{a}	46^{cd}	41^{cd}	22^{a}	20^{ab}	19^{abc}
	96	75^{bc}	36^{a}	39^{de}	34^{cd}	20^{a}	23^{a}	24^{ab}
	120	72^{c}	37^{a}	35^{e}	31^{d}	19^{a}	24^{a}	28^{a}
SEM		3.6	1.98	2.41	2.84	1.26	1.23	3.58
P Values								
ER		**	**	**	**	**	**	**
ST		**	**	**	**	**	**	**
ER X ST		ns	*	ns	ns	**	**	ns

For each set of means, different superscripts (a, b, c, d, e, f, g, and h) within the same column indicates significant differences, SEM = Standard Error of Means. ER = extension ratio, ER1 extension ratio (1:5), ER2 extension ratio (1:10), ER X ST = interaction between extension ratio and storage time, TM = total motility of spermatozoa, Bi = Biladyl®, NON-P = sub-population of sperm cells with non-progressive motility, and PROG = sub-population of sperm cells with progressive motility, RAP = sub-population of rapid moving sperm cells, MEDIUM = sub-population of spermatozoa with medium velocity, SLOW = sub-population of slow moving spermatozoa, STA = sub-population of static spermatozoa, ns= Not significant, *= significant (P<0.05), **= Highly significant (P<0.01).

Both the extension ratio and the storage time significantly (P<0.01) influenced all the motility parameters, with significant interaction between the extension ratio and storage time on NON-P (P<0.05) and on MED and SLOW (P<0.01). While the storage time did not (P>0.05) affect the TM at the ER1, there was significant (P<0.05) decrease after 72 hours on the ER2. Compared to dilution at the ER1, semen dilution to the ER2 increased the NON-P, SLOW and STA, but reduced the TM, PROG, RAP and MED (P<0.05). Longer storage reduced TM, PROG and RAP and increased the NOP-P, SLOW, MED and STA (P<0.05).

Table 5 shows the effects of the extension ratio and storage time on the proportion of live spermatozoa in extended semen after 120 hours storage at 5 °C

Table 5: Effects of the extension ratio and storage time on the percentage live spermatozoa after storage for 120 hours at 5 °C

	Storage Time (Hour)					
Extension ratio means	0	24	48	72	96	120
ER 1	100	91.0[a]	90.0	83.3	78.6	69.0
ER 2	96.5	81.0[b]	80.0	77.6[ab]	75.6	68.9
SEM	5.41	4.63	3.27	2.67	2.21	1.89
P values						
ER	ns	**	ns	*	ns	ns
ER X ST	ns	ns	ns	ns	ns	ns

Different superscripts (a, b, and c) within the same column indicates significant differences E = extender, T = ST = storage time, ER1 = Extension ratio (1:5), ER2 = Extension ratio (1:10), SEM = Standard Error of Means, ns= Not significant, *= significant (P<0.05), **=highly significant (P<0.01).

Both extension ratios maintained the viability of spermatozoa for 120 hours, though the percentage of the live spermatozoa decreased gradually as the storage time increases. There was no significant difference (P> 0.05) in the percentages of live spermatozoa between the ER1 and ER2 at 0, 48, 96 and 120 hours of storage, with significant difference only for spermatozoa in the semen stored for 24 hours (P<0.01) and 72 hours (P<0.05). There was no interaction between the storage time and extension ratio (P>0.05) for all of the motility parameters.

Table 6 shows the effect of extension ratio and storage time on spermatozoa morphology

Table 6: Abnormal morphology rates of extended semen in Biladyl® extender after 120 hours of storage at 5 °C.

Treatments	Storage Time (Hrs)	Cut tails (%)	Coiled tails (%)	Cut heads (%)
ER1	0	4.2^{e}	24.6^{f}	15.2^{ef}
	24	6.6^{de}	33.8^{ef}	10.2^{f}
	48	5.6^{de}	56.2^{cd}	16.8^{ef}
	72	8.2^{cde}	66.6^{bc}	24.2^{cde}
	96	9.2^{bcd}	75.2^{ab}	20.6^{def}
	120	8.8^{bcde}	86.4^{a}	27.8^{cd}
ER2	0	5.6^{de}	32.4^{ef}	16^{ef}
	24	10^{bcd}	30^{f}	28.2^{cd}
	48	10^{bcd}	36.6^{ef}	34.8^{c}
	72	11.8^{abc}	46.2^{de}	46.6^{b}
	96	13.2^{ab}	90.4^{a}	53.2^{ab}
	120	15.4^{a}	88.6^{a}	62.4^{a}
SEM		0.98	3.28	2.19
Extension ratio means				
ER1		7.1^{b}	57.1^{a}	19.1^{b}
ER2		11.0^{a}	54.0^{a}	40.2^{a}
SEM		0.40	1.33	0.89
Storage time means				
	0	4.9^{a}	28.5^{a}	15.6^{a}
	24	8.3^{ab}	31.9^{a}	19.2^{b}
	48	7.8^{abc}	46.4^{b}	25.8^{b}
	72	10^{bc}	56.4^{c}	35.4^{c}
	96	11.2^{cd}	82.8^{d}	36.9^{d}
	120	12.1^{d}	87.5^{d}	45.1^{d}
SEM		0.70	2.32	1.55
P Values				
Extender		**	ns	**
ST		**	**	**
ER X ST		ns	**	**

For each set of means, different superscripts (a, b, c, d, e, f, g, and h) within the same column indicate significant differences ($P < 0.05$); SEM = Standard Error of Means. ER 1= Extension ratio

1 (1:5), ER 2= extension ratio 2 (1:10), ST= storage time, ER X ST = interaction between extension ratio and storage time, ns= Not significant, *= significant (P<0.05), **= highly significant (P<0.01).

The highest proportion of abnormal spermatozoa were in the coiled tails in both extension ratios, which were not (P > 0.05) affected by the extension ratio. Cut tail spermatozoa were the least in both extenders, and differed significantly (P<0.05) between the extension ratios. The proportion of cut head spermatozoa were different (P<0.05) between the two extension ratios. Storage time affected all the morphological abnormalities (P<0.01). There was interaction between storage time and extension ratio on coiled and cut head morphological abnormalities (P<0.05).

3.4 Discussion

The color of the semen was creamy, which is considered normal (Steyn, 2010). The semen pH compared favorably with values of 7.5 ± 0.2 and 7.2 ± 0.4 obtained by Mashaba (2010) and Bopape *et al.* (2015), respectively. Buck semen pH usually ranges from 7.0 to 7.8 (Prins, 1999). The sperm concentration was comparable to values of 681.7 ± 74.6 × 10^6 /mL after collection by the AV technique (Orihuela *et al.* (2009).

Sperm viability indicated by at least 55% of motile spermatozoa is considered the minimum to be used for effective AI (Flocerfida, 2014). Motility of spermatozoa diluted at the ratio of 1:5 was 91% after 120 hours of storage, compared to 71% for the 1:10 dilution ratio. Therefore, the test on the optimum storage time indicated that Boer buck semen can be stored in a liquid form using Biladyl® extension ratios of 1:5 and 1:10 (semen to extender) beyond 120 hours. However, spermatozoa survived better at the ER1 throughout the storage. These results are similar to the results obtained by Quareshi *et al.* (2013) who obtained a total motility 72.33% in dairy goats after 192 hours of storage. In this study, the lower dilution ration (ER1) showed higher TM, PROG, and RAP of spermatozoa than the lower ratio (ER2) throughout the storage. The different sperm viability as indicated by different total motility after 120 hours indicated that the higher dilution ratio was inferior in preserving Boer goat spermatozoa compared to lower dilution ratio. These observations were in agreement with findings in Angora goats by Daskin *et al.* (2011), whereby the survival of goat spermatozoa at 1:4 dilution rate (spermatozoa / ml) was superior to a 1:10 dilution rate using Bioxcell® extender.

With regard to storage time, there was no significant difference observed in TM of spermatozoa kept for 0 and 24 hours. However, spermatozoa kept for 0 hours and 24 hours had a higher percentage motility than those kept for 48 hours, 72 hours, 96 hours and 120 hours. The percentage of both PROG and RAP spermatozoa kept in both extension ratios are highest at 0 hours (fresh extended semen) of storage and it is gradually reduced with time until after 120 hours of storage. This result is intimately in line with the observation of Chang-He *et al*. (2016) that spermatozoa motility declined with increasing storage time. Furthermore, this result agrees with the observation of Peterson *et al*. (2007) that the percentage of motile spermatozoa in buck semen stored in liquid form for 72 hours, progressively declined overtime irrespective of whether storage occurred at 4 or 18ºC. In this study sperm viability decreased with time at both extension ratios. The results of this study might be assigned to the effect of refrigeration temperature of 5 °C to the spermatozoa that lose its viability due to cold shock, which can be explained as a rapid change of temperature that the spermatozoa undergo. As the storage is prolonged, increased

concentration of toxic metabolic products such as ROS (Reactive oxygen species) likely affected spermatozoa viability (Abulizi *et al.,* 2012).

Given the greater inclusion of egg yolk in the extender, the more rapid decline in the spermatozoa viability could be due to greater concentration of egg yolk coagulating enzymes in the seminal plasma, which are known to be toxic to spermatozoa (Santiago-Mereno *et al*., 2006). The Biladyl® extender used in this study contained 20% of egg yolk, which likely similarly limited sperm survival particularly at the lower extension ratios. The highest number of normal spermatozoa were at the ER 1, which was similar to findings by Hudson *et al.* (2016) who reported higher percentage of live and morphological normal spermatozoa in less dilute semen irrespective of extender and storage time. However, a study conducted by Bamba and Cran (1988), found that increasing the dilution rate increased the proportion of abnormal acrosomes of spermatozoa. Flocerfida *et al.* (2014) suggested that the poor adaptation of the spermatozoa when stored in chilled conditions may increase the number of semen abnormalities.

3.5 Conclusion

Both extension ratios used in the experiment were able to maintain the spermatozoa motile for 120 hours at 5 °C, though the percentage of live spermatozoa decreased gradually as the storage time increased. Throughout the 120 hour storage, spermatozoa survived better at the lower dilution rate.

CHAPTER 4

EFFECTS OF DIFFERENT ARTIFICIAL INSEMINATION TECHNIQUES ON THE PREGNANCY AND KIDDING RATE IN INDIGENOUS VENDA DOES.

4.1 Introduction

Artificial insemination (AI) is the most widely used technique with the most important contribution to the genetic improvement of goat worldwide (Evans and Maxwell, 1987). Artificial insemination plays an important role in the reproductive management and genetic improvement of indigenous goats, particularly in intensive systems of production, whereby it is used to implement synchronized reproduction. Artificial insemination success is valued by the pregnancy rate, where the higher the pregnancy rate after insemination, the more economically feasible is the technique (Agriculture & Fisheries, 2012). The main objective of semen extension is to maximize the number of females that can be inseminated with a single ejaculate, and the benefits of using extended semen is that high pregnancy rates are obtained using lower number of spermatozoa. However, to achieve this goal, well planned synchronization plays a very crucial role, and better synchronous ovulations reduce costs by the number of insemination needed to optimize fertility (Maria-Teresa *et al*., 2016).

Motility is a phenomenon responsible for transport of spermatozoa from the site of deposition to the site of fertilization, and for penetrating the zona pellucida during fertilization. Lavara *et al*. (2005) reported that a characteristic feature and prerequisite for the fertilizing capacity of spermatozoa is their motility. Hence, it is very crucial considering the importance of motility and motion characters exhibited by sperm in determining the fertility via computer-assisted semen analysis (CASA), (Mukul and Sarvajeet, 2016). In this current study, based on the results from the first experiment, semen that was diluted in the dilution ratio of 1:5 was used for all insemination due to the fact that it was superior compared to dilution ratio of 1:10. Again from the results of the current study, ER 1 showed a better consistency in TM, PROG and RAP from sperm motility parameters (Table 6). Furthermore, some of the semen samples for ER 2 were discarded due to total mortality before 120 hours. The objective of the study was to determine the effects of different artificial insemination techniques on the pregnancy and kidding rate in indigenous Venda does.

4.2 Materials and methods

The bucks used in the study, their management, the methods of semen collection from the bucks and the semen processing and evaluation were as described in chapter 3.

4.2.1 Doe management

A total number of ninety (n=90) indigenous Venda does that weighed between 30 to 85 kg and aged between 1.5 to 4 years of age were borrowed from four different farmers in Vhembe District and they were transported to the University of Venda experimental farm. Seventy (n=70) Indigenous Venda goats were collected from two different farmers of at least 2 km apart in Masisi village kept under an extensive farming system. The other 20 Indigenous Venda does, collected from Mavambe and Makhuvha villages were kept under extensive farming system as well. All does were subjected to pregnancy detection by real-time ultrasonography with a rectal 7.5-MHz linear-array transducer, as described by Padilla-Rivas *et al*. (2005) to avoid using pregnant does in the experiment. Only does that were found not pregnant were selected and transported to the experimental farm. Two weeks before the goats were carried to the experimental farm, they were vaccinated with 2 ml of Multivax P [MSD Animal Health Intervet South Africa (Pty) Ltd.] which was injected under skin and dosed with Prodose Orange (2ml/10kg). The does were vaccinated in order to avoid transmission of different diseases from one place to another. At the arrival, all does were injected 10 mg of $PGF_{2\alpha}$ (Lutalyse dinoprost tromethamine) Sterile Solution (5 mg/ml) [Pfizer Laboratories (Pty) Ltd Reg.no. 1954/00078/07] to ensure that all the does were not pregnant before the start of the experiments.

All does were kept intensively at the University of Venda experimental farm in the goat feedlot. Does were subjected to water at *ad libtum* and kept in one big pen (10 m x 40 m) that had enough space for four feeding troughs, two water troughs as well as enough space for free movement. The pen was provided with roofing to provide the shade to protect the goats from the sun and also act as a refuge during extreme weather conditions such as rain. All goat does were fed with lucern supplied by local farmers and sheep pellets produced by Driehoek Voere and supplied by NTK (Pty) Ltd. During the adaptation period of two weeks before the start of the experiment, goats that had diarrhoea were injected intramuscularly with 300 mg of Duplocillin® Aqueous Suspension (150mg/ml) [MSD Animal Health Intervet S.A. (Pty) Ltd.

Figure 2: Indigenous Venda goats in their holding pen at University of Venda.

4.3 Experimental Design

The effects of insemination method were evaluated in 2 (fresh, extended semen) X 3 (method of insemination) factional experiment

Table 7: Experimental design, treatments and replications

SEMEN TYPE	**AI METHODS**		
	CAI	**TAI**	**LAI**
FRESH	15 does	15 does	15 does
EXTENDED	15 does	15 does	15 does

4.4 Estrous synchronization

The 90 does were synchronized using CIDR (Eazi-Breed CIDR, Pfizer Animal Health, Auckland, New Zealand) that contains 0.3 g progesterone. All does were restrained and the area of the vulva was cleaned thoroughly with water. A CIDR is a T-shaped nylon (Figure 3) insert molded with a silicon rubber skin containing the progesterone. The wings of the CIDR were folded

together towards the front for intra vaginal insertion using the applicator. After insertion of the CIDR, the wings of the CIDR returned to their original T-shape and apply pressure to the vaginal walls and therefore, holding the CIDR in place. The blue nylon string (tail) of CIDR was cut to prevent the does from pulling their own or another doe's CIDR from the vagina. The CIDRs were gently removed after 7 days. At the removal of the CIDR, does were injected 10 mg of $PGF_{2\alpha}$ (Lutalyse® dinoprost tromethamine) sterile solution. At 24 hours after removal of the CIDR, does were injected with 300 IU of equine chorionic gonadotrophin (Folligon®, 1000IU) [MSD Animal Health. INTERVET.SA. (Pty) Ltd] to synchronize the follicular development and ovulation.

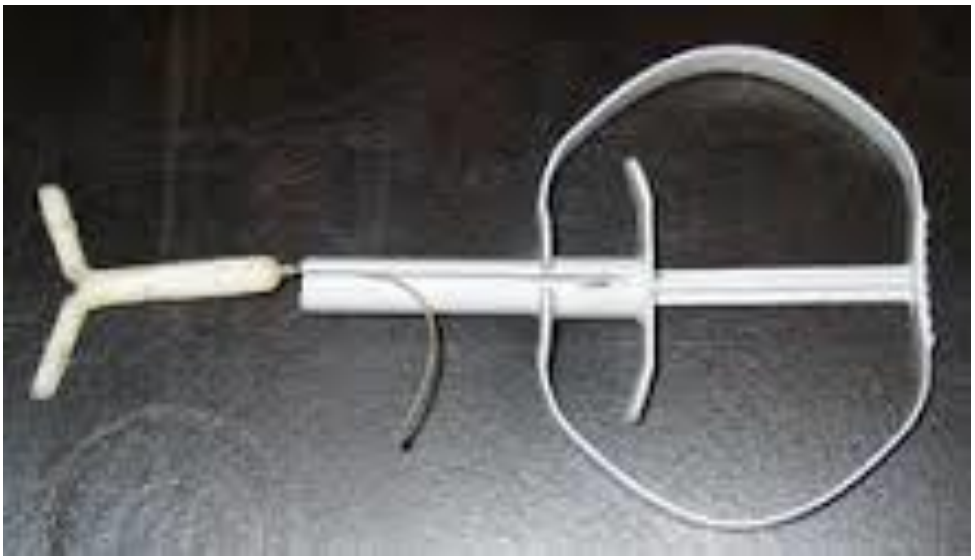

Figure 3: CIDR and applicator.

4.5 Insemination of the does

All does were tagged using ear tags for identification. The 90 does were inseminated according to the experimental design described in Table 7 above. Semen from three different bucks were pooled after collection and diluted with Biladyl® extender to 1:5 (v:v) and sperm concentration of ~$150x10^6$ sperm/ml. The pooled semen was divided into two semen samples. At 48 hours after the removal of the CIDR, a sample of fresh semen was used to inseminate 15 does each by CAI, TAI and LAI. The second sample was kept in the refrigerator at 5 °C for 5 days, after which it was used for insemination. All inseminations were carried out in the metabolic house at the University of Venda.

4.5.1 Cervical insemination

The does were placed in the cage and restrained by putting the elevated hindquarters over a rail with the head pointing downwards. The vulva was cleaned thoroughly using water and wiped with dry paper towels. A clean vaginal speculum approximately 12 cm long and lubricated with clean sunflower oil for better penetration was introduced through the cleaned vulva into the vagina. By applying a twisting motion and a slight pressure, the speculum was inserted. Once the speculum was in place, the laparoscopic light was turned on and the cervix was visualized. An 8 G catheter was attached to the sterile 3 ml syringe and the plunger was removed to allow a stylet to pass through the catheter to the tip. The urinary catheter was stiffened by this stylet and the catheter was inserted into the posterior opening of the cervix. Once the catheter was in place the stylet was removed. Exactly 0.2 ml of semen was aspirated from the test tube containing semen in the water bath at 37°C using a 1 ml syringe. The 0.2 ml of semen was deposited into the 3 ml syringe attached to the catheter. The empty 1 ml syringe was removed and the 3 ml plunger was inserted. Semen was deposited into the cervix by slowly pressing down the plunger of the syringe. After the insemination the catheter and speculum were withdrawn carefully.

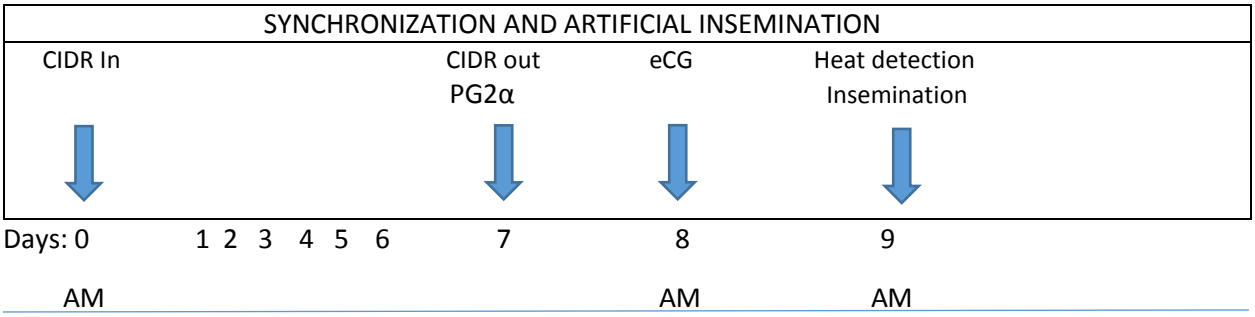

Figure 4: Experimental procedure

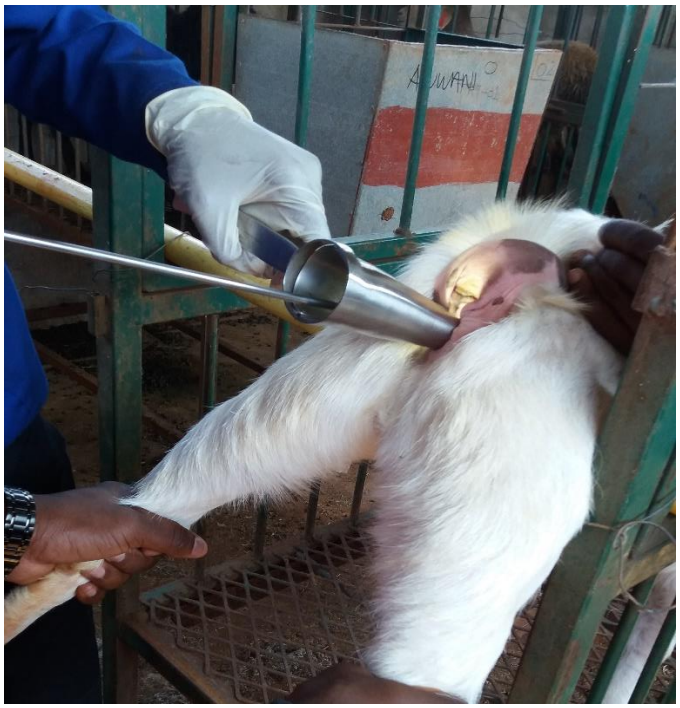

Figure 5: Cervical artificial insemination.

4.5.2 Trans-cervical Insemination

All does were restrained and placed in a crate as described before. The vulva was cleaned thoroughly using water and wiped with dry paper towels. A clean vaginal speculum approximately 12 cm long and lubricated with clean sunflower oil for easier penetration, was introduced through the cleaned vulva into the vagina. By applying a twisting motion and slight pressure, the speculum was inserted. The vaginal vault was cleared of any mucus plug discharge by slowly twisting the speculum and the cervical os identified as a purplish protrusion into the vaginal vault. Using light from the laparoscope, the cervix was grasped at 11 o'clock and 5 o'clock using a ponzi tenaculum forceps leaving the cervical os unobstructed for passage of an 8 G urinary catheter. In a synchronized maneuver, cervical rings were aligned and trans-versed using a pulling and twisting motion of the forceps through 90 degrees anticlockwise. The catheter was attached to the sterile 3 ml syringe and the plunger was removed to allow a stylet to pass through the catheter to the tip. Forward passage of a urinary catheter was made possible by stiffening of the catheter with a stylet and it was introduced as far as possible into or through the cervix. The stylet was removed after passage through the cervix and the 1 ml loaded syringe containing 0.2 ml semen was deposited into the 3 ml syringe barrel. Slowly the syringe plunger was placed back and pushed

forward until the deposition of semen was achieved in the uterine body. All the equipment were withdrawn and cleaned.

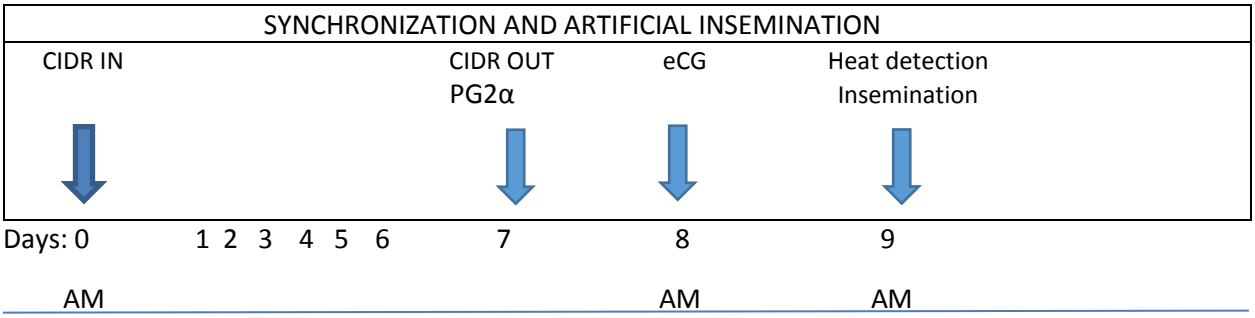

Figure 6: Experimental procedure

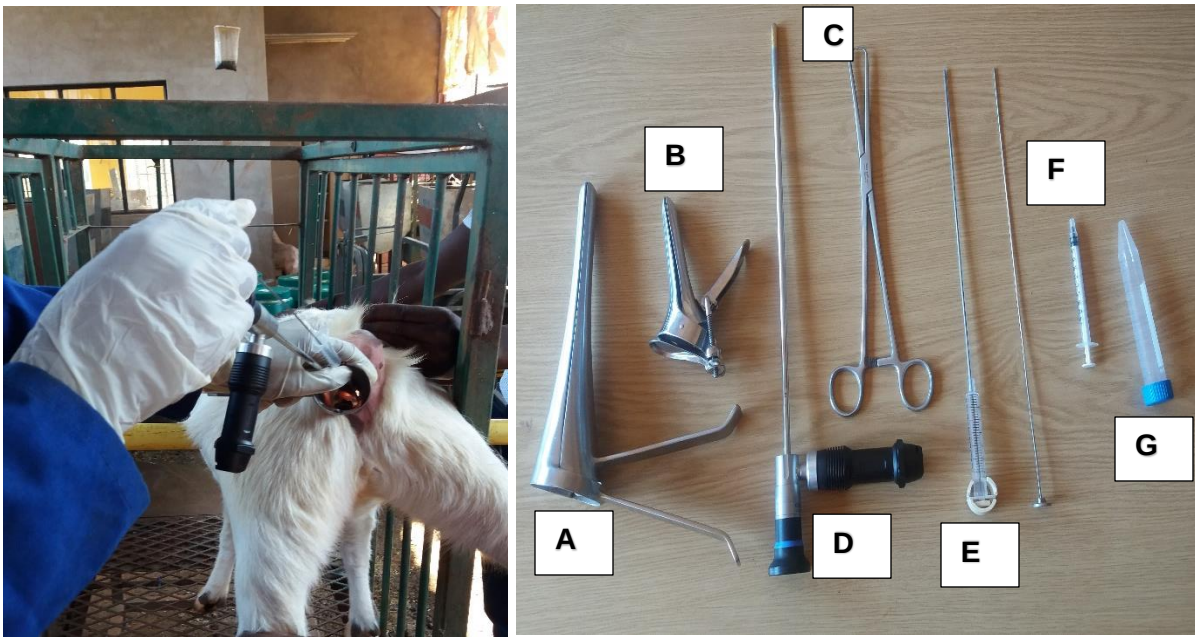

Figure 7: TAI and equipment. (A) Duckbill vaginal speculum for large doe, (B) Duckbill vaginal speculum for small doe, (C) Ponzi tenaculum forceps, (D) Laparoscope with light source, (E) AI lineup of urinary catheter connected to 3 ml syringe stiffened by stylet, (F) Sterile 1 ml syringe, (G) Graduated 10 ml semen storing plastic test tube

4.5.3 Laparoscopic Insemination

In this method of AI, semen was deposited directly into the lumen of the uterus through the wall of uterine horns with the aid of a laparoscope. Before laparoscopic insemination was

performed, the does were restricted from feed and water for 24 hours to reduce the content of the rumen and the bladder. Each doe was restrained on the cradle in dorsal recumbency and tilted at 45^{o} with hindquarters raised above the ground and the head pointing down. All does were injected intravenously with 5 mg of Xylazine [Intervet SA (Pty) Ltd Cnr.Zurich] to reduce stress and to calm down the doe. The abdomen was prepared by applying water and soap on the skin. A pair of scissors and razor with a blade were used to shave the hair to allow a clean and sterile surface to do the laparoscopy. Alcohol was applied on the skin for disinfection. All goats were injected 60 mg of local anaesthetic (Lignocaine) [Bayer (Pty) Ltd. Animal Health Division, Wrench] under the skin and into the muscle on both sides of the midline of the abdomen where the incision will take place. These positions are about 8 cm in front of the udder and about 3 cm on both sides of the midline. A scalpel with blade (15 c/s) was used to make a small incision through the skin of the abdomen for a trocar and cannula to pass through into the abdominal cavity. A 7 mm trocar and the cannula with a valve and latex tubing was then passed through the small incision into the abdominal cavity. The valve was opened and air was blown through the latex tube to slightly inflate the abdomen. The valve was closed after the abdomen was inflated with air. The trocar was removed and the laparoscope with a light source and fitted with a camera was inserted through the cannula. The second trocar and cannula were inserted at the other side of midline after inflation of the abdominal cavity with air. After visualizing the uterus, the trocar was removed and a spring-loaded forceps was inserted to manipulate the uterus horns in a favorable position, and the forceps was removed. The aspic with a 5 to 7 mm needle was slowly inserted into the transcap and locked. Through the assistance of the technician, 0.2 ml of semen was aspirated through the needle of the aspic into the transcap. The metal guide was used to cover the transcap and the aspic with its needle. The loaded transcap covered in the metal guide was inserted through the second cannula into the abdominal cavity. The needle of the aspic was exposed at the front end of the metal guide by pushing the transcap deeper into the metal guide. The needle was inserted into the uterine lumen at a 90 degrees angle to the uterine wall. The plunger on the transcap was rolled upwards to push out the semen with the aspic plunger from the aspic. Half of semen was injected into the lumen of each uterine horn. After injecting the semen the needle was pulled into the metal guide and removed from the cannula. The laparoscope with its light source was safely removed. After insemination the cannulas were safely removed. The air inside the abdominal was allowed to pass out through the small incisions. Vetafil suture was used to close the two small incisions and were treated with topical antibiotics wound spray (Supona Aerosol) [Pfizer Laboratories (Pty) Ltd Sandton, South Africa]. Does were moved to a recovery area and left undisturbed for about 1 to 2 hours to recover from the xylazine sedation.

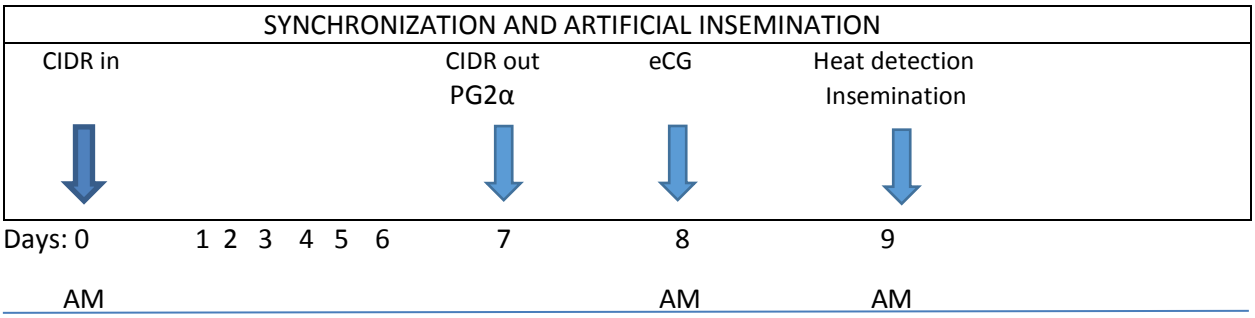

Figure 8: Experimental procedure LAI

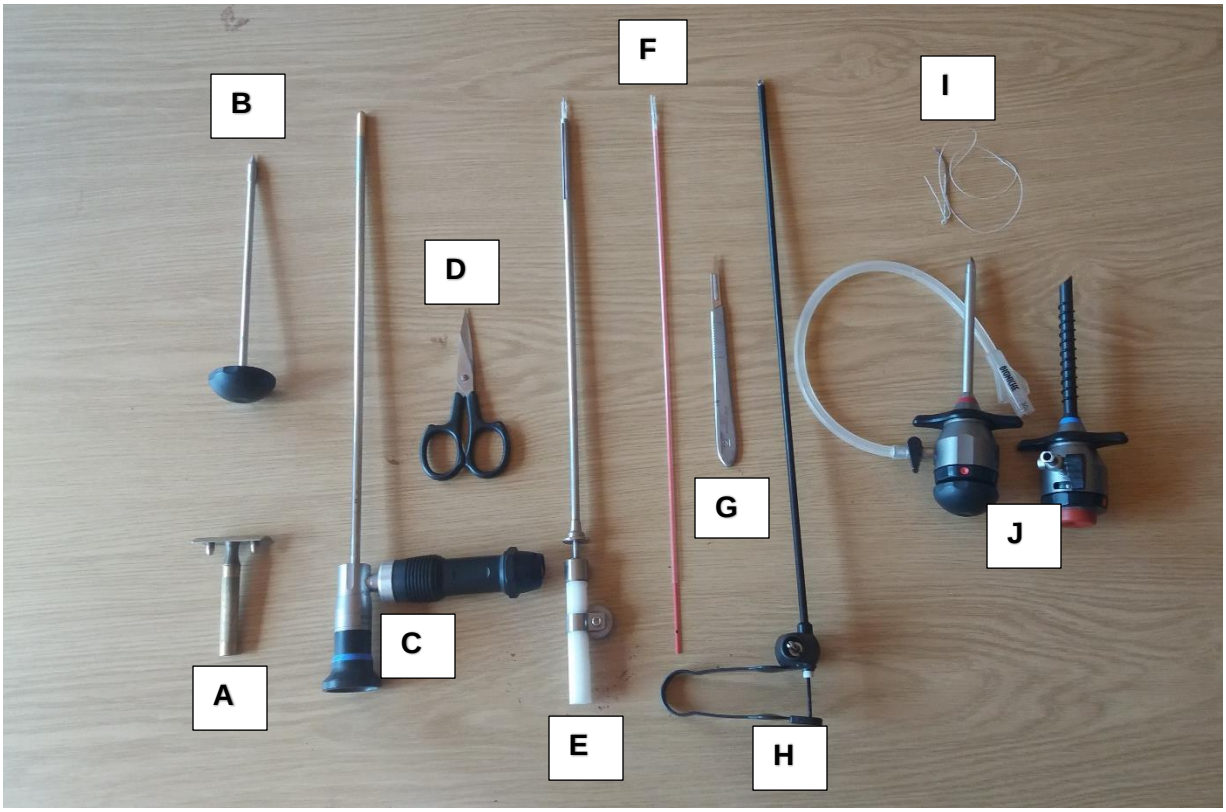

Figure 9: Equipment used for LAI.

Razor (A), Trocar (B), Laparoscope its light source (C), Scissor (D), Transcap fitted with aspic inside the metal guide (E), Aspic with fitted needle and plunger for transcap (F), A scalpel with a blade (G), Spring-loaded forceps (H), Needle with vetafil (I), Cannula with valve and latex tube (J).

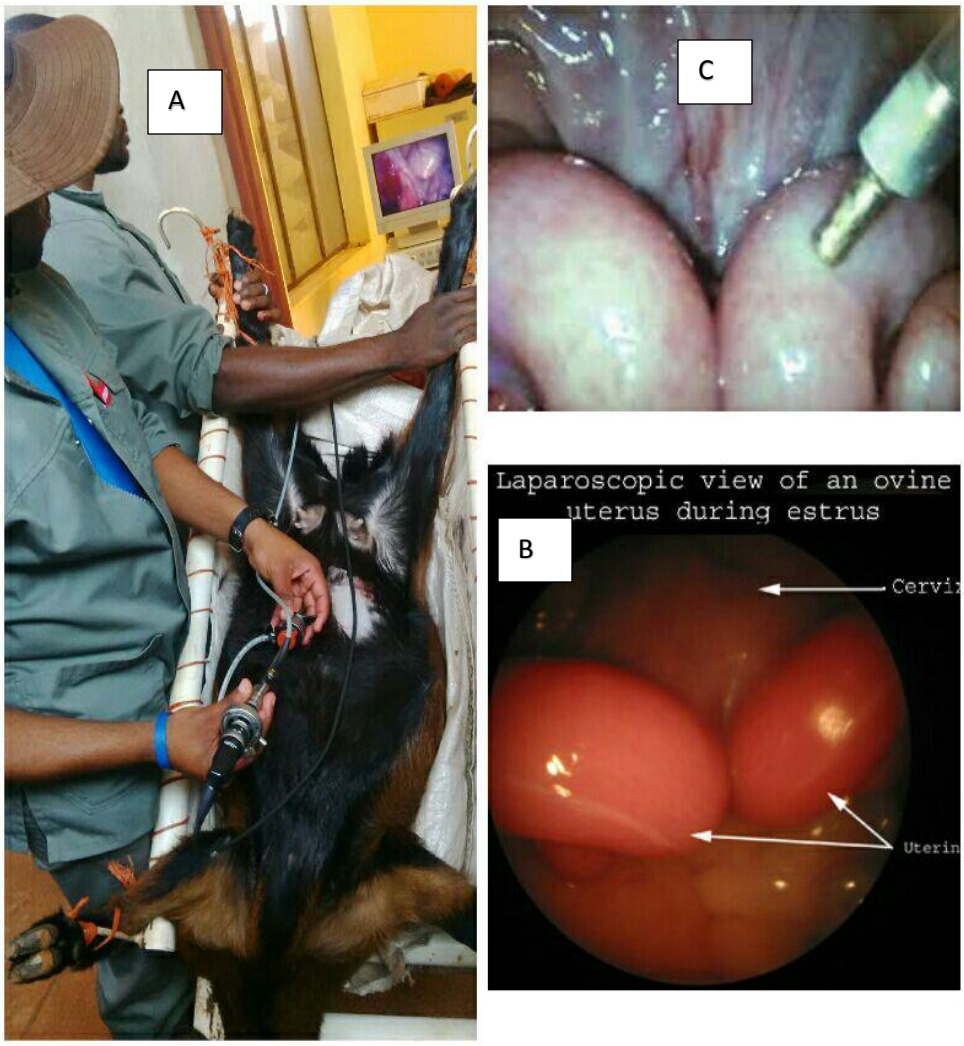

Figure 10: Laparoscopic insemination. (A) Shows a doe restrained on the cradle in dorsal recumbency and tilted at 45° with hindquarters raised above the ground and the head pointing down. The uterus is visualized on the monitor screen. (B) Visualizing the uterus on the screen. (C) The aspic of the transcap fitted with aseptic needle covered in the sheath inserted through the second cannula into the abdominal cavity. The needle was inserted into the lumen of each uterine horn at a 90 degrees angle to the uterine wall.

4.6 Pregnancy diagnosis and kidding

Six weeks after insemination, the does were subjected to pregnancy detection by real-time ultrasonography with a rectal 7.5-MHz linear-array transducer (Figure 11), as described by Padilla-Rivas *et al*. (2005). At parturition, kidding rate, and litter size were recorded. Differences between the number of does pregnant and litter size were tested for significance by χ^2 test (Steel &Torrie, 1960). Litter size was defined as the number of kid(s) born by each goat doe and considered at three levels: single, twin and triplet births. Prolificacy was calculated as the percentage of number of kids born on total number of does delivered according to the following equation. Prolificacy (%) = (No. of kids born/No. of does delivered) x 100.

Figure 11: Ultrasound pregnancy diagnosis.

4.7 Statistical analysis

Pregnancy and kidding rates were compared using the Chi-square test ($\chi 2$). The data were analyzed based on the model:

$$\boldsymbol{Yijk} = \boldsymbol{\mu} + \boldsymbol{\alpha i} + \boldsymbol{\beta ij} + \varepsilon \boldsymbol{ijk}$$

Where;

Y_{ijk} is the observation.

μ is the overall mean.

α_i is the effect of the i^{th} insemination method, where, (i = 1…3).

β_j is the effect of j^{th} type of semen, where, (I = 1....2).

$\alpha\beta_{ik}$ is the interaction of the i^{th} AI method and j^{th} type of semen.

$\mathcal{E}^{ijl}$ is the residual.

Statistical significance was denoted as $P < 0.05$.

4.8 Results

The pregnancy, kidding litter size rate and prolificacy following two types of Boer goat semen after using three different artificial insemination techniques are summarized in Table 8.

Overall 74%, 71% and 141.92 of pregnancy, kidding rates and prolificacy, respectively, were achieved with no significant ($p>0.05$) treatment effects and no interaction. However, numerically, the pregnancy rates were in the order LAI (83%)> TAI (73%)> CAI (66.5%) and kidding rates were LAI (80%)>TAI (76)>CAI (60), whereas prolificacy was in the order; CAI (164.71%), TAI (147.62%) and LAI (126.09) with the higher fertility on the fresh semen regardless of the insemination technique.

The figure below shows the image of a pregnant Venda doe

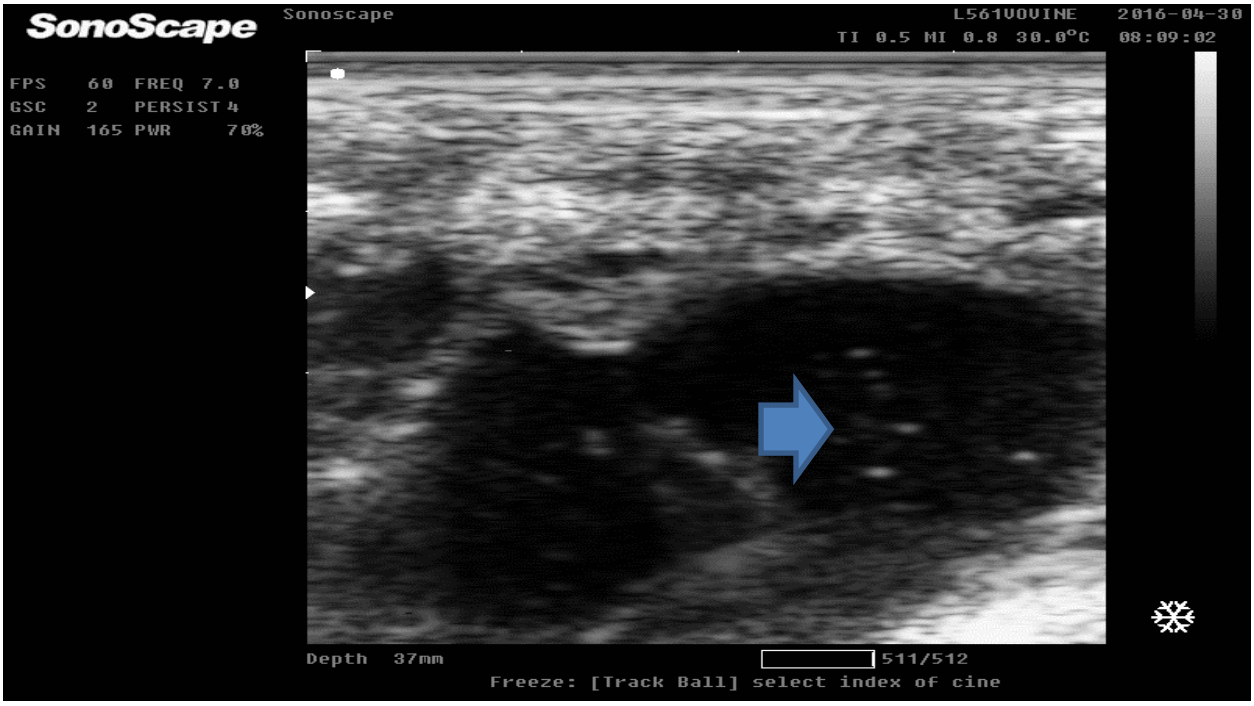

Figure 12: Trans-rectal ultrasound image of a pregnant Venda doe on day 30 after artificial insemination; Note the fetal heart in the area pointed by the arrow.

Table 8: Effects of semen extension and insemination method on goat fertility

	Semen type	Twinning rate	N	CAI (%)	TAI (%)	LAI (%)
Pregnancy rate	Fresh		15	80	80	93
	Extended		15	53	66	73
	Overall		30	66.5	73	83
SEM				0.07	0.09	0.10
P values						
Semen type				ns	ns	ns
AI technique				ns	ns	ns
Semen type X AI technique				ns	ns	ns
Kidding rate	Fresh		15	66	80	86
	Extended		15	53	66	73
	Overall		30	60	73	80
SEM				0.08	0.09	0.08
P values						
Semen type				ns	ns	ns
AI technique				ns	ns	ns
Semen type X AI technique				ns	ns	ns
Prolificacy	Fresh					
		Single		3	5	10
		Twin		3	5	3
		Triple		3	1	0
		Total kids		18	16	16
		Prolificacy		200	145.45	123.07
	Extended					
		Single		6	6	9
		Twin		2	3	2
		Triple		0	1	0
		Total kids		10	15	13
		Prolificacy		125	150	118.18
P values						
Semen type				ns	ns	ns
AI technique				ns	ns	ns
Semen type X AI technique				ns	ns	ns

For each set of means, different superscripts (a and b) within the same column indicates significant differences (P<0.05), SEM= standard error of means, n= number of entries, CAI= cervical insemination, TAI= trans-cervical insemination, LAI= laparoscopic insemination, % percentage of pregnancy rate, ns= no significant.

4.9 Discussion

The use of frozen semen requires expensive equipment to store the semen, as well as a reliable supply of liquid nitrogen. Chilling semen to reduce sperm metabolism and the use of a suitable extender can preserve sperm viability over a number of days. The importance of extending semen is to provide an adequate volume of inseminate with a sufficient number of motile spermatozoa to give a high fertilization rate without wasting spermatozoa. Protecting the semen using suitable extenders is an important ingredient in the success of AI in goat reproduction. In this study, comparative pregnancy rates between fresh and chilled extended semen supported the application of TAI, but not CAI, as a substitute for laparoscopic insemination with extended semen. The lower pregnancy rate with the CAI technique when using extended semen could be due to failure to achieve full penetration of the uterus since the semen was deposited right into the posterior opening of the cervix. Results from the study were similar to findings by Chang-He Liu *et al*. (2016), who achieved pregnancy rates of 79.3% with fresh semen and 65.7% with semen stored in n-mZAP for 12 days. The timing of semen collection from the bucks could partially explain the relatively high fertility recorded in this study. Previous studies suggested semen collected during the breeding season was of superior quality compared to semen collected during the non-breeding season (Bastia *at al*., 2009).

The results of this study indicated that pregnancy rates greater than 80% can be achieved using any of the three AI techniques when using fresh semen. The most commonly used AI technique is cervical insemination (Evans, 1998), whereby semen is deposited into the cervix. Though uterine contractions provide motion to propel sperm towards the fertilization site (Tami, 1994), the longer distance covered by sperm in relation to the site of fertilization is critical to successful fertilization. Cervical insemination is therefore most satisfactory when used with fresh semen. In does and ewes, Chemineau *et al*. (1991) reported pregnancy rates of 40-80% with fresh semen administered via cervical insemination, which they argued should be preferred due to its technical simplicity. Cervical insemination is an easy method to perform since it only requires an insemination pipette (Devendra and Burns., 1983) and speculum which are both affordable. The CAI technique can be most beneficial to the small scale farmers who cannot afford to use TAI and LAI techniques which require skilled technicians and more capital. Paulenz *et al*. (2003) supported the use of cervical insemination with extended chilled semen produced at AI stations. On the other hand, Trans-cervical AI places the semen anterior to the cervix, directly into the posterior part of the lumen of the uterine body. Thus, semen is closer to the fertilization site than in CAI and therefore has a better chance of fertilization. With the LAI, high pregnancy rates were

achieved regardless of semen extension and chilled extension. The superiority of LAI was attributed to the advantageous placement of the semen directly into the lumen of doe's uterine horns (Rodriguez et al., 1990). In does similarly synchronized in estrus using a CIDR device, Tami (1994) and Justin (2008) similarly reported highest pregnancy rates with the application of LAI.

In the present study, an overall 71% kidding rate was achieved, with no significant differences between extended semen and fresh semen. However, in similar pattern to the pregnancy rate, the kidding rate was highest for the LAI, followed by TAI and by the CAI method. Higher kidding rates were recorded from fresh semen compared to extended semen, regardless of the technique used. This difference could be due to the changes that occur during storage which include a reduction in motility, morphologic integrity, and fertility of spermatozoa. These changes are accompanied by a decline in the survival of spermatozoa in the female reproductive tract and may be associated with an increase in embryonic loss (Ohara *et al*., 2010). The kidding percentage of the Venda does were similar to the 70% reported using frozen and chilled semen in the Majorera breed (Bastita-Arteaga *et al*., 2011). At 60% kidding rate, it can be argued that all three techniques are reliable enough to be used commercially in the conditions of the study.

In the current study, the numerical disparity between the pregnancy and kidding rates suggested some of the does lost their pregnancy due to embryo and foetal losses. Embryo and foetal losses can be caused by exposure to stress during the handling of does before and during insemination (Haney Samir *et al.,* 2016). In this study, 4 % percent of the goats lost their pregnancies. However, it is not possible to fully explain the causes of such losses. The goats were subjected to different management post fertilization since they were returned to the owners a month after pregnancy diagnosis with ultrasound. Variable factors included the change in feeds and the overall environment, and transport stress in the early stages of pregnancy. However, supplementary pellets were provided to help the transition of the goats through a uniform nutritional regime after returning to extensive management conditions in their respective original farms. According to Hussain et al. (1996) low energy intake is directly associated with the occurrence of abortion in goats. Under extensive conditions and with native goats, abortion has been associated mainly with malnutrition (Bhattacharyya et al., 1977; Unanian and Feliciano-Silva, 1984).

The present study recorded a prolificacy rate of 145 %, with no statistical differences between the type of semen and among the insemination methods. The most frequent litter size was singles (62.9%), followed by twins (29.0%), and triplets (8.06%). The average litter size of 1.45 was lower compared to breeds such as the Nubian, Pygmy, American Alpine, French Alpine,

Saanen and the Toggenburg, which have average litter sizes of 2.0, 1.9, 1.9, 1.7, 1.7 and 1.6, respectively (Amoah *et al*., 1996). The findings on 37.06% multiple births of which 29% of kids were twins, were much lower than the previously reported by Hassan *et al* (2007) and Haldar *et al.* (2014) who reported 56.3% and 61.70% twinning rates, respectively.

4.10 Conclusion

Overall, numerically, but not statistically different pregnancy and kidding rates were in the order LAI >TAI> CAI insemination, with the fresh semen giving numerically but not significantly higher rates compared to extended semen. In contrast, prolificacy was numerically highest with TAI. The numerical, but not statistical differences in the treatment effects on pregnancy, kidding rates, and in prolificacy suggested more goat numbers might be required to detect effects in the fertility test.

CHAPTER 5

GENERAL DICUSSIONS AND CONCLUSIONS

5.1 Overall discussion

One objective of the study was to identify the threshold preservation time for the chilled storage of extended goat semen. The study showed that extended chilled goat semen could be stored up to 120 hours. However, in short to medium-term storage, a gradual decline in sperm motility was correlated with a decrease in spermatozoa motility and an increase with the number of morphological abnormalities of the spermatozoa.

A second objective of the study was to identify an extension ratio fit to maintain chilled extended semen for the purpose of artificial insemination for 5 days. More rapid decline in spermatozoa motility and a higher number of abnormal spermatozoa were apparent at the high semen dilution rate. Similar effects were observed by Rowida *et al*. (2016), who attributed the effect to the lower concentration of seminal plasma at a higher dilution rate. The stabilizing capacity of the extender likely declines with the dilution rate and therefore limit spermatozoa protection.

A third objective of the study was to evaluate the effect of semen extension in relation to the insemination method on goat fertility. Semen diluted at the ER1 (1:5) which achieved higher score of motility, viability and normal morphological abnormalities compared to semen diluted at the ER2 (1:10) was used in fertility test in comparison with fresh semen. Higher fertility was recorded from fresh semen when compared to extended semen, regardless of the technique used. The difference was attributed to the fact that fresh semen likely supplied the critical threshold in motile spermatozoa and enough viable spermatozoa required at the fertilization sites (Masoudi *et al*., 2016). Thus, in addition to the dilution effect, chilling caused substantial ultrastructural, biochemical and functional damage to the spermatozoa resulting in a reduction of motility, viability, impaired transport and fertility (Leboeuf *et al*., 2000). Consequently, due to closer placement of spermatozoa to the fertilization site, the LAI method achieved higher fertility rates compared to other two techniques of AI in both fresh and extended semen, though with the least prolificacy probably due to higher stress levels at the time of insemination which negatively influences ovulation rate.

5.2 Conclusion

Based on the findings of this study, the following conclusions were made.

i. Though extended chilled goat semen could be stored and remain viable up to 120 hours, there was a gradual decline in sperm motility, which was correlated with a decrease in spermatozoa motility and an increase with the number of morphological abnormalities of the spermatozoa. Therefore, the storage conditions are critically important.
ii. The semen extension ratio affected sperm liveability, morphology and motility, with the semen extended at the 1:5 maintaining better sperm viability compared to dilution at the 1:10 ratio
iii. While the highest pregnancy and kidding rates are likely achieved by using laparoscopic followed by trans-cervical and by cervical insemination methods, prolificacy may be reduced by LAI
iv. The high fertility achieved in this study showed that Venda goats have potential for better reproductive efficiency with improved managerial practices such as well-planned oestrus synchronization and AI.

5.3 Recommendations

The goal of the present study was to assist producers to avoid inbreeding while improving the genetic value of Venda goats in the communal farmers around Vhembe District though application of AI. Many goat farmers are not able to adopt artificial insemination technology due to the fact that transport and storage of frozen semen for laparoscopic artificial insemination is difficult and fairly expensive. The materials and techniques required for AI using frozen semen are more expensive than for chilled extended semen.

Against this background, and based on the findings of the study, for the successful application of AI in Venda goats, the following recommendations were made;

i. Semen extension is recommended at the extension ratio1 ratio [ER1 (1:5)]
ii. Any of the three AI techniques can be used for satisfactory goat fertility, depending on the available skills and financial resources. However, cervical insemination should be recommend given the lower cost and skills required.
iii. Further research using a larger goat population is necessary to detect statistical treatment effects on pregnancy, kidding rates, and in prolificacy.

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APPENDICES

APPENDIX 1: INDEMNITY AGREEMENT FORM

INDEMNITY AGREEMENT

AGREEMENT OF INDEMNIFICATION BETWEEN CEAAR, UNIVERSITY OF VENDA AND THE FARMER.

SECTION A

CEAAR REPRESENTATIVE

Surname & Initials: ……

Student Number: ……

SECTION B

FARM DETAILS

Surname & Initials: ……

Contact Number: ……

Email Address: ……

Residential Address: ……

……

……

SECTION C

LIVESTOCK DETAILS

Number ……

Species..……..……..

Breed……..

Gender: (Male): …… (Female): ……

Last period of vaccination:……..……

Period at which livestock will be at the CEAAR Center:

From: ……..…… Until: ……..……..

SECTION D

CEAAR UNDERTAKES THE FOLLOWING:

1. Vaccination of livestock animals upon arrival at the University of Venda Experimental Farm;

2. Dosing for intestinal parasites upon arrival;

3. Good care, maintenance, feeding, access to clean water and environment;

4. Security of the animals;

5. Care of animal health and veterinary care;

6. Animals will be handled in humane way regardless of the experiment;

7. Return them to the owner after experimental period.

8. Should any loss of animals occur due to unforeseen and uncontrollable environmental effects and diseases, CEAAR may not be held responsible.

9. Should CEAAR fail to provide safety and security for animals or forsake and neglect its responsibilities, then, in such case, CEAAR shall compensate farmers for the loss of animals not with money but with live animals.

10. This agreement shall be unlimited as to amount or duration, and it shall be binding to the benefits of both parties.

I ……..…… Accept the conditions stipulated on this indemnity agreement as binding and subjected to no changes of the agreement after the

animals have been introduced at the University of Venda experimental farm (CEAAR) on the following date ……

…… ……

Name of a Farmer **Date**

…… ……

Signature **Date**

…… ……

Name of CEAAR Representative **Date**

…… ……

Signature **Date**

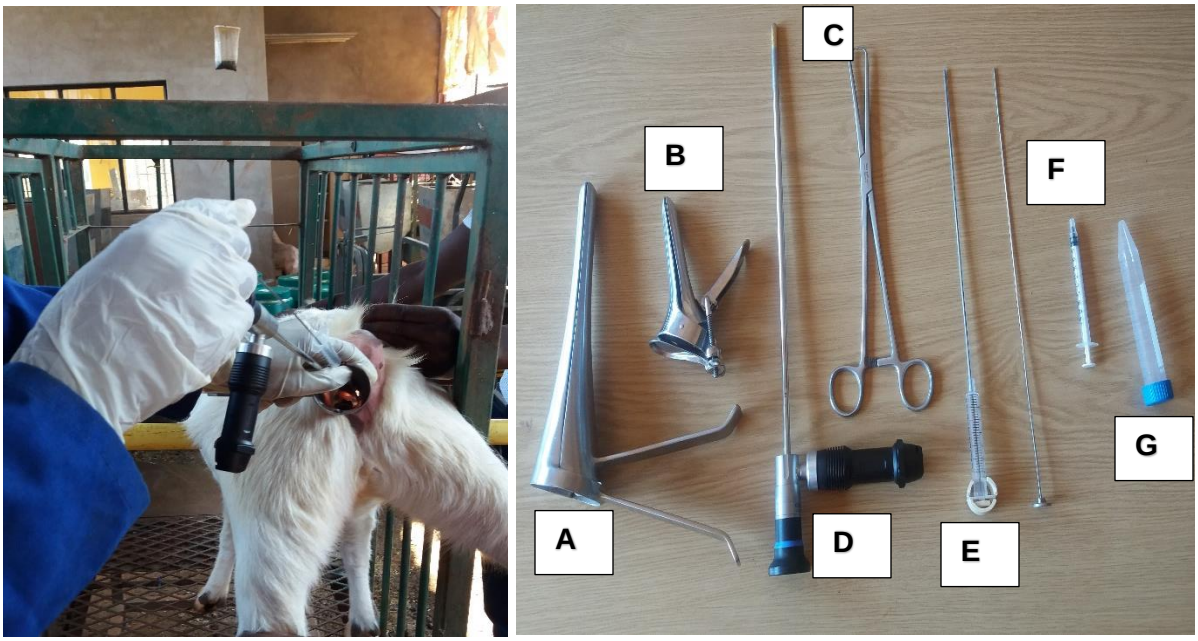
…… ……

Name of Supervisor **Date**

…… ……

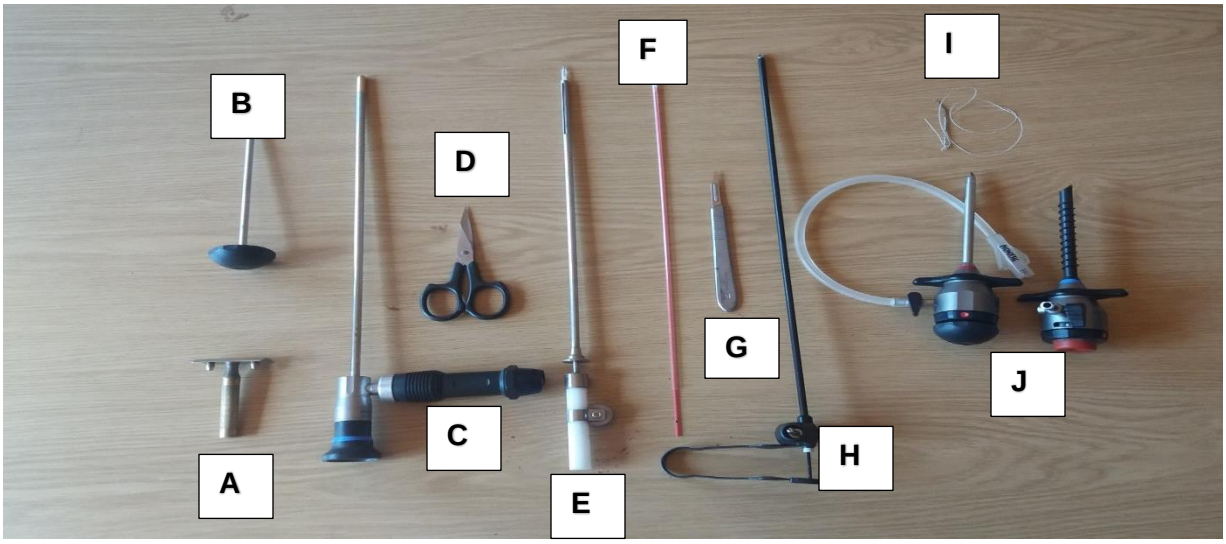
Signature **Date**

APPENDIX 2: Equipment used during CAI and TAI



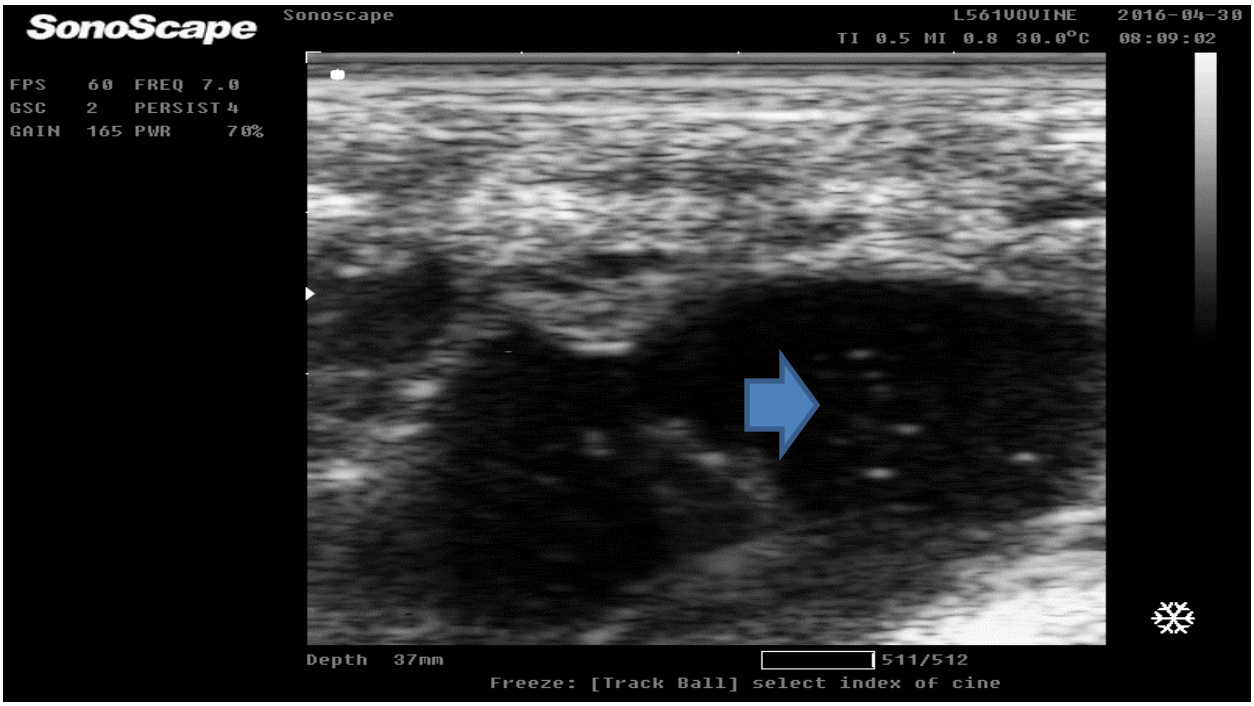
TAI and equipment.(A) Duckbill vaginal speculum for large doe, (B) Duckbill vaginal speculum for small doe, (C) Pozzi tenaculum forceps, (D) Laparoscopic light source, (E) AI lineup of urinary catheter connected to 3 ml syringe stiffened by stylet, (F) Sterile 1 ml syringe, (G) Graduated 10 ml semen storing plastic test tube

APPENDIX 3: Equipment used for LAI.



Razor (A), Trocar (B), Laparoscope (C), Scissor (D), Transcap fitted with aspic inside the metal guide (E), Aspic with fitted needle and plunger for transcap (F), A scalpel with a blade (G), Spring-loaded forceps (H), Needle with vetafil (I), Cannula with valve and latex tube (J).

APPENDIX 3: Trans-rectal ultrasound image of pregnant indigenous Venda doe.



Pregnant indigenous Venda goat on day 30 after artificial insemination; Note the fetal heart with the area pointed by arrow.

APPENDIX 4: Boer buck goats used in the study

APPENDIX 5: Indigenous Venda does

APPENDIX 6: Kids born after AI