Effect of dietary selenium supplementation and oestrous synchronisation on reproductive performance of South African indigenous goats

by

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Submitted in partial fulfilment of the requirements for the degree Masters of Science in Agriculture (Animal Science): Production Physiology

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Declaration

I, **Mamodike Ophelia Mashamaite**, declare that this dissertation which I hereby submit for the degree Masters of Science in Agriculture (Animal Science) at the University of Pretoria is my own original work and has not previously been submitted for a degree at this or any other tertiary institution.

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Mamodike Ophelia Mashamaite

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Abstract

Optimum reproduction performance of small ruminants managed under extensive production system can be influenced by the availability of nutrients, especially the mineral content of forages and reproductive management. The present study was conducted to evaluate the effect of Se supplementation and oestrous synchronisation protocols on the reproductive performance of South African indigenous goats during the autumn breeding season. Does (n = 48) were randomly allocated to six treatment groups in a 2x3 factorial design. The factors in the study were Se supplementation (Se supplemented and non-supplemented) and oestrous synchronisation protocols; (progesterone (P4) + male effect, P4 + equine chronic gonadotrophin (eCG) and male effect). The Se supplemented group was dosed with 0.34 mg Se per kg body weight in the form of sodium selenite at 10-days interval. For oestrous synchronisation protocols, all does in the P4 groups were treated with controlled internal drug release (CIDR) for 11 days. At CIDR removal, does in the P4 + eCG group, were intramuscularly injected with 300 IU eCG. While does in the P4 + male effect group, were introduced to a teaser buck wearing an apron. Does in the male effect group did not receive CIDR devices, instead oestrous was naturally synchronised with the aid of teaser buck wearing apron. Reproductive measures taken included, the number of follicles and size of the largest follicle, oestrous response, oestrous onset and duration, gestation length, kidding weight, litter size and pregnancy rate. The results revealed that Se supplementation had no significant effect on the number of follicles, size of the largest follicle, oestrous onset and duration, pregnancy rate, oestrous response, kidding weight, litter size and gestation length. There was no significant difference between oestrous synchronisation treatment groups for their effect on the number of follicles, size of the largest follicle, oestrous response and duration, pregnancy rate, kidding weight and litter size. However, there was significant difference ($P < 0.05$) between the different oestrous synchronisation protocols on the onset of oestrous. The P4 + male effect protocol had resulted in an earlier onset of oestrous compared to P4 + eCG and male effect groups. The interaction between Se and oestrous synchronisation was significant in terms of kidding weight and oestrous response.

It was concluded that Se had no significant influence on the reproductive performance of South African indigenous goats while the addition of the male effect following P4 treatment improved the reproductive performance of South African indigenous goats. Further studies need to be conducted on the use Se supplementation and the addition of the male effect as a reproductive management technique in South Africa indigenous goats.

Keywords: Progesterone, follicles, male effect, selenium, indigenous goats

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List of abbreviations

AD: Average daily gain

AI: Artificial insemination

ANOVA: Analysis of variance

AOB: Accessory olfactory bulb

ART: Assisted reproductive technologies

ATP: Adenosine triphosphate

B-mode: Brightness mode

B-cells: Bone-marrow cells

CIDR: Controlled internal drug release

CL: Corpus luteum

DNA: Deoxyribonucleic acid

eCG: equine chronic gonadotrophin

FAOSTAT**:** Food and Agriculture Organisation Statistic

FGA: Flourogestone acetate

FSH: Follicle stimulating hormone

GLM: General linear model

GnRH: Gonadotrophin releasing hormone

GSH-Px: Glutathione peroxidase

hCG: Human chronic gonadotrophin

HGP-axis: Hypothalamus pirtuary gonadal-axis

IgG: Immunoglobin

IM: Intramuscularly

IU: International Units

IVF: *In Vitro* fertilization

LH: Luteinizing hormone

MOET: Multiple ovulation and embryo transfer

MAP: Medroxyprogesterone

MGA: Melengestrol Acetate

m: metre

mg: milligram

mg/kg: milligram per kilogram

MHz: Mega Hertz

mm: millimetre

NCR: National Council of Research

NMD: Nutritional muscular dystrophy

P4: Progesterone

$PGF2_{\alpha}$: Prostaglandin

PMSG: Pregnant mare serum gonadotrophin

PUFA: Polyunsaturated fatty acids

ROS: Reactive oxygen species

S: Sulphur

Se: Selenium

SEM: Standard error mean

T-cells: Thymus cells

VNO: Vomeronasal olfactory

WB: Whole blood

WMD: White muscle disease

WBC: White blood cells

WB-Se: White Blood-Selenium

Zn: Zinc

Chapter 1
Introduction

1.1 Overview

The demand for food is expected to increase in order to meet the nutritional needs of this rapidly increasing population. Farm animals are recognised as a source of nutrition for this growing population which could address malnutrition and poverty (Gore, 2016). The demand for protein is expected to increase by at least 60 % in 2050 (United Nations, 2015). Compared to other farm animals, goats contribute immensely to the socio-economic and livelihoods in rural areas of African countries (Mamabolo & Webb, 2005; Peacock, 2005). Goat meat plays a significant role in the supply of animal protein in the rural areas of developing countries (Mamabolo & Webb, 2005). Improving the production efficiency of goats in Africa would improve economic status and access to a quality protein source for people in rural areas (Schonfeldt & Hall, 2012).

Majority of goats in developing countries are found in rural areas (Peacock, 2005). In many of these countries, rural communities prefer to keep goats (Rumosa-Gwaze *et al*., 2008). Compared to cattle, goats occupy a small area and are able to utilise a wide variety of poor quality forage (Visser & Van-Marle-Koster, 2016). They are also easier to manage compared to other farm animals, for people with little farming experience (Mamabolo & Webb, 2005). In South Africa, goats are kept mainly as a source of food (meat and milk), as well as for use during traditional and religious ceremonies (Roets & Kirsten, 2005; Aziz, 2010; Qekwana *et al*., 2017). Goats are also kept as a source of income through sales (Rumosa-Gwaze *et al*., 2008).

On the African continent, the largest goat populations are found in Nigeria, Sudan and Kenya (Skapetas & Bampidis, 2016). South Africa is also one of the countries in Africa with a large number of goats, estimated at 6.14 million (FAOSTAT, 2013). Goats in South Africa are one of the less recognized livestock species despite their contribution to the livestock industry (Visser, 2018). Goat contributes to food security, social and economic roles in the lives of people in rural areas around South Africa (Meissner *et al*., 2013). Apart from the established commercial breeds, 63% of South Africa's goats are unimproved indigenous goats found in rural areas (Mohlatlole *et al*., 2015). Compared to other farm animals, indigenous goats make a valuable contribution to the livelihoods of people in rural areas (Lehloenya *et al.*, 2005). However, the industry is still lagging behind with regard to reproduction efficiency and improvement of traits (Mohlatlole *et al*., 2015). Indigenous goats in South Africa are genetically and phenotypically diverse (Mohlatlole *et al.*, 2015) based on their adaptation to different geographical locations.

South African indigenous goats are classified into various ecotypes namely: Nguni type goats, Eastern Cape Xhosa, Northern Cape type goats and Kunene type (Snyman, 2014). Indigenous goats are extremely hardy and can reproduce under extreme environmental conditions. According to Lehloenya *et al*. (2005) the reproductive performance of indigenous goats under normal conditions is not optimum, therefore there is a need to optimize their reproduction efficiency. There are several factors such as genetic factors, nutritional requirements, management strategies etc. that can hinder optimum reproduction efficiency. It is critical to consider these factors when enhancing the reproduction efficiency of unimproved indigenous goats. Therefore, appropriate reproductive management strategies should be implemented to optimize productivity of South African indigenous goats.

Reproduction efficiency is one of the most important production factors that influence animal production (Casey & Webb, 2010). The use of assisted reproductive technologies (ART) such as artificial insemination (AI), oestrous synchronisation and transrectal ultrasonography can be used to optimize the efficiency of reproduction (Rahman *et al*., 2008). For example, oestrous synchronisation is a valuable reproductive management tool used to enhance the efficiency of reproduction, especially in small ruminants (Koyuncu & Alticekic,2010). The benefits of controlling the oestrus cycle by synchronising the occurrence of oestrus allows effective controlled breeding and also improves fertility (Motlomelo *et al*., 2002; Ramukhiti *et al.,* 2012). Nonetheless, different oestrous synchronisation techniques for sheep and goats continue to evolve throughout the years based on their effectiveness, sustainability and ease of application.

The most commonly used oestrous synchronisation techniques include hormonal applications such as: progesterone (P4) treatment in the form of intravaginal devices such as CIDR or its synthetic analogues (MAP and FGA) (Ramukhiti *et al.*, 2012). Progesterone (P4) treatment is often used with co-treatments such as equine chronic gonadotrophin (eCG), male effect, prostaglandin ($PGF2_{\alpha}$) etc. However, the use of P4 treatment has been routinely incorporated with eCG and has been used effectively and regularly in different goat breeds.

Regardless of the success of eCG for inducing ovulation following P4 treatment, the frequent use of eCG as a co-treatment in oestrous synchronisation negatively affects long term fertility (Wildeus, 2000). Therefore, alternative treatments such as the male effect have been sought that have no immunological reactions (Gore, 2016). Several studies have reported the efficiency of the male effect, which is considered a clean, natural and effective method of synchronising oestrus and ovulation (Lopez-Sebastian *et al*., 2014). The male effect is commonly used, with or without P4 treatment as an oestrous synchronisation protocol (Whitley & Jackson, 2004).

Apart from improving oestrous performance, the male effect can be a cost efficient alternative oestrous synchronisation method as it will not require the purchase of synthetic hormones by small

scale farmers with limited financial inputs. Regardless of the method of oestrous synchronisation used to improve reproduction, climatic conditions also play a crucial role in reproduction performance.

The climate of Southern Africa region is getting drier and hotter (Dzama, 2016). Climate change has a detrimental effect on the quantity and quality of forage (Rojas-Downing *et al*., 2017) especially for livestock in rural areas which depend mainly on natural grazing and browsing. Nutritional constraints are a major challenge faced by communal farmers that result in low productivity. One of these is selenium (Se) which is leached out of planted fodder such as lucerne (*alfalfa*) and is also lacking in some parts of South Africa (Van Ryssen, 2001). It has been suggested that lack of Se can lead to reproductive failures in livestock. Reproductive failures linked with Se deficiency include weak or silent oestrus, poor fertilization, cystic ovaries, abortions, still births and retained placentas (Kumar, 2015). Earlier studies on Se have highlighted the possible role of oxidative stress in oocyte maturation, ovulation, fertilization, luteal regression and maintenance (Riley & Behman, 1991).

The accumulation of oxygen free radicals leads to oxidative stress which negatively affect the quality of oocytes and ovulation (Kala *et al*., 2016). In addition, Se is a potent anti-oxidant nutrient which could play a critical role in alleviating reproductive failures caused by oxidative stress in livestock (Smith & Akinbamijo, 2000). It also plays a significant role by destroying toxic oxygen free radicals and maintaining the integrity as well as structure of cells, through the enzyme glutathione peroxidase (GSH-Px). The effect of Se on male fertility and semen parameters in animal species has been extensively studied. However, few studies have explained the effect of Se on doe fertility (Argawal & Allamaneni, 2004; Kala *et al.,* 2016). This has led to a lack of understanding about the role of Se in doe fertility.

1.2 Problem statement

The frequent use of eCG is known to have negative long term consequences on the fertility of animals (Wildeus, 2000). The male effect as an oestrous synchronisation technique has not been studied and it can provide an alternative to eCG in South African indigenous goats where it could have a better impact. Small ruminants have also been found to be highly susceptible to reproductive failures associated with Se deficiency with severity in sheep and goats (Hefnawy & Perez, 2010). The deficiency of anti-oxidant nutrients such as Se has been associated with suppression of oestrus, silent oestrus, poor follicular development with delayed ovulations (Soni, 2015) resulting in poor fertility. It is also not known whether Se deficiency plays a major role in fertility of South African indigenous female goats and this is important because South Africa has regions where Se is deficient.

1.3 Overall objective:

The broad objective of the study was to improve reproductive performance of South African indigenous goats through dietary Se supplementation and identification of suitable oestrous synchronisation protocols.

The specific objectives were to:

- To evaluate the effect of dietary Se supplementation performance of South African indigenous goats.
- To compare the efficiency of three oestrous synchronisation protocols on the reproductive performance of South African indigenous goats.
- To evaluate the interaction of Se and oestrous synchronisation on the reproductive performance of South African indigenous goats.

1.4 Hypotheses:

- **H_1:** Selenium supplementation will improve the reproduction performance of South African indigenous goats.
- **H_1:** Oestrous synchronisation protocols will improve the reproductive performance of South African indigenous goats.
- **H_1:** The interactions of Se supplementation and oestrous synchronisation will have a significant impact on reproductive performance of South African indigenous goats.

Chapter 2

Literature review

2.1 Introduction

Optimum reproductive performance is an important aspect in animal production (Dube, 2015). Reproduction inefficiency is a major factor negatively influencing profitability of farm animals. The efficiency of reproduction is influenced by genetic and phenotypic factors such as genetics, climatic and environmental conditions, management and nutrition etc. that hinder productivity (Greyling, 2010). The use of assisted reproductive technologies (ART's) has enabled farmers to optimize reproduction efficiency in livestock. Therefore, knowledge about factors that can negatively impact the outcome of ART's is essential (Agarwal *et al*., 2006). Oestrous synchronisation is a valuable and key component of several ART's (Baldasserre & Karatzas, 2004). There are different oestrous synchronisation techniques and protocols used in goats varying with some degree of success and limitations (Rahman *et al.*, 2008).

The fertility of does following oestrous synchronisation is dependent on factors such as characteristics of follicles that ovulate and the oocyte that they contain (Evans *et al*., 2004). Research on oestrous synchronisation is important to establish optimal doses as well as agents to apply for optimum synchrony and fertility in different breeds (Hashemi & Safdarian, 2017). It also establishes simple and reliable oestrous synchronisation protocols that can be adopted by farmers (Hashemi & Safdarian, 2017). This chapter reviews literature on the efficiency and limitations of the different oestrous synchronisation protocols that are used in goats.

Nutrition has a profound impact on animal reproduction. Poor nutrition limits productivity of animals. In rural areas, natural grazing and poor quality crop residue are the primary sources of feed for goats (Lanyasunya *et al*., 2005). Due to severe shortage of feed and poor quality vegetation during the dry season, animals are often in poor physiological state resulting in poor reproductive performance (Tolera *et al*., 2000). Extensive production systems may be severely affected by the consequences of climate change because they depend primarily on natural grazing to feed their livestock (Rust, 2013). Unfavourable climatic conditions and food scarcity can cause stress. Stressed and undernourished animals have weak immune system and are highly susceptible to diseases as well as parasite infestation (Talukder *et al*., 2017). Physiological processes such as oocyte development, ovulation, fertilization, embryo development and pregnancy can be influenced by certain nutrients (Robinson *et al*., 2006).

Therefore, cost effective nutrient supplementation strategies to compensate for poor quality vegetation is essential in order to remedy low reproductive performance (Brown *et al.,* 2016) of livestock in extensive production systems.

Nutrients containing anti-oxidants have been established to significantly influence reproduction and immunity of livestock (Smith & Akinbamijo, 2000; Vazquez-Armijo *et al*., 2011). Nutrients such as vitamin E and C, β-carotene as well as Se are essential components of antioxidant enzymes (McDowell *et al*., 2007). However, very few studies have highlighted the relationship between antioxidant nutrients and female fertility (Argwal & Allamaneni, 2004). Of all nutrients with anti-oxidant functions, the trace mineral Se, which is a key component of the selenoenzyme glutathione peroxidase (GSH-Px) has the most notable impact on the reproduction and immune status of livestock.

Selenium is an indispensable trace mineral in the diet of humans and livestock (Zarczynska *et al*., 2013). It plays an important role in the physiological processes such as immune and reproductive functions, thyroid hormone metabolism and antioxidant defence (Hosnedlova *et al*., 2017). Selenium deficiency in livestock is a global challenge, especially in extensive production systems where animals depend primarily on natural grazing as a source of feed (Fordyce, 2007). Selenium deficiency increases the susceptibility of animals to diseases (Lyons *et al*., 2007). The impact of diseases and parasite may be manifested through high morbidity, mortality, abortions, sub-clinical symptoms such as body weight loss and poor reproductive performance resulting in huge economic losses (Dube, 2015; Rumosa-Gwaze *et al*., 2008). Therefore, Se supplementation may be important to sustain reproductive function if it is deficient in the vegetation (Ahsan *et al*., 2014). Nonetheless, the relationship between mineral nutrition especially Se and reproduction is complex and the responses are often inconsistent (Robinson *et al*., 2006). This section will also discuss available literature on the role of oxidative stress on female fertility and the role Se on the reproductive performance of livestock following oestrous synchronisation. As well as the impact of Se deficiency and its role on the immune status of dams and their offspring.

2.2 Oestrous synchronisation techniques

Oestrous synchronisation was developed in the early 1960's as a reproductive management tool. Since then several oestrous synchronisation protocols have been developed (Rahman *et al*., 2008; Dardente *et al*., 2016). Oestrous synchronisation focuses on targeting female animals to come on heat (sexually receptive) simultaneously. This can be achieved by controlling the oestrus cycle using methods such as; manipulation of photoperiod, melatonin treatment, the use of the male effect and hormonal treatments (Whitley & Jackson, 2004; Abecia *et al*., 2012).

The advantages of synchronising oestrus in small ruminants include; increased application of AI, better oestrus detection, it is also a prerequisite for multiple ovulation and embryo transfer (MOET) (Wildeus, 2000; Rahman *et al*., 2008). Oestrous synchronisation shortens the lambing and kidding intervals (Abecia *et al*., 2012; Ali, 2014). It also provides an opportunity to use genetically superior sires (Ali, 2014). Oestrous synchronisation allows mating or AI to be at a pre-determined time (Ramukhiti *et al*., 2012) therefore, making it easier to manage nutrition adjustment based on the physiological state of batched kids (Fatet *et al*., 2011). Any oestrous synchronisation technique that is used should not only result in synchrony but also ensure an optimum level of fertility (Wildeus, 2000). Oestrous synchronisation involves controlling the luteal or follicular phase of the oestrus cycle (Figure 2.1) (Wildeus, 2000). Several studies have confirmed that the window of opportunity for efficient oestrous synchronisation is pronounced during the luteal phase (Rahman *et al*., 2008).

The luteal phase has an extended duration and responds well to manipulation (Rahman *et al*., 2008). According to Rahman *et al.* (2008) there are two strategies that can be used to control the oestrous cycle. The first involves extending the lifespan of the corpus luteum (CL), therefore inhibiting the occurrence of oestrus. This is achieved by administering progesterone (P4) in the form of an intravaginal sponge (progestagen) or CIDR. The synthetic P4 releases the hormone P4, which provides the same physiological function as the CL thereby inhibiting the occurrence of oestrus behaviour. The second strategy which is applied commonly when synchronising oestrus during the natural breeding season, involves shortening the lifespan of the CL. This can be achieved by administering exogenous luteolytic agents such as PGF2α or its synthetic analogues (Abecia *et al*., 2012; Haldar & Ghosh, 2015) thus inducing oestrous behaviour simultaneously.

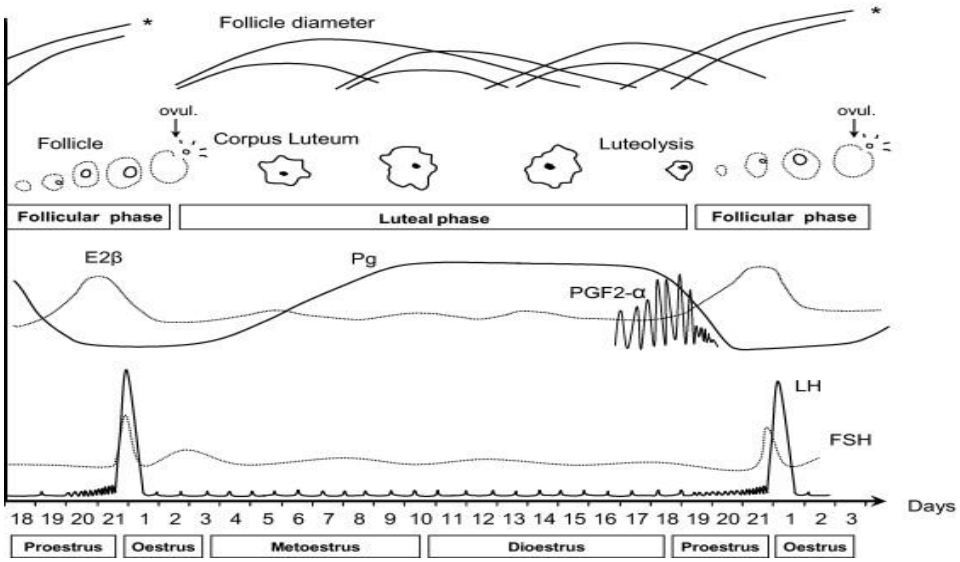

Figure 2.1: Schematic representation of the ovarian cycle and hormonal regulation in goats (Fatet *et al*., 2011).

2.1.1 The use of progesterone treatment during oestrous synchronisation

The use of P4 treatment has been proven to be effective in inducing and synchronizing oestrus in small ruminants in and out of the breeding season. The basic principle for treating animals with P4 is based on that it mimics the physiological function of the CL. Thereby, blocking the occurrence of oestrus and ovulation through the negative feedback mechanism to the pituitary gland (Noakes *et al*., 2009; Simoes, 2015). When the source of P4 is removed, the concentration of P4 decreases while circulating concentration of oestradiol-17β increases and oestrus occur simultaneously (Noakes *et al*., 2009). The efficiency of P4 treatment depends on the breed, duration of treatment (5-16 days) (Greyling & Van der Nest, 2000; Knights & Singh-Knights, 2016), co-treatment and management (Wildeus, 2000). For example, with regard to duration of P4 treatment, Vinoles *et al.* (2001) concluded that 12 days (long-term) P4 treatment resulted in lower pregnancy rate compared to six days (short-term) treatment. Furthermore, the authors observed that the lifespan of the ovulatory follicle and the diameter of the largest follicle was larger for the short-term treatment. Greyling *et al.* (1985) reported that oestrous response using 14 days of progestagen was 100 % and 88 % in 12 days' treatment in synchronisation of oestrus in Boer goats.

Different modes of administering P4 are available and they include; intravaginal devices (CIDR and intravaginal sponges), norgestomet subcutaneous ear implants (Freitas *et al*., 2004) and melengestrol acetate (MGA) that is supplemented in feeds (Abecia *et al*., 2012). However, the most widely used P4 treatment for controlling the oestrus cycle is the use of a CIDR containing 0.3 g natural P4 or intravaginal sponges namely; flourogestone acetate (FGA) containing between 20 and 40 mg P4 per sponge, medroxyprogesterone acetate 60mg P4 per sponge (MAP) (Holtz, 2005; Knights & Singh-Knights, 2016). However, Motlomelo *et al.* (2002) reported that intravaginal sponges can cause drainage of vaginal secretion causing foul smelling discharge on removal. For example, Sonmez *et al.* (2009) investigated the use of intravaginal sponges for oestrous synchronisation in goats, and observed that intravaginal sponges cause oxidative stress due to vaginal discharge at sponge removal.

Recently the CIDR device has been the preferred P4 treatment for oestrous synchronisation in small ruminants. The device is simple to use compared to progestagen sponges (Knights & Singh-Knights, 2016). The CIDR device is reported to have higher retention rate than progestagen sponges (Knights & Singh-Knights, 2016). However, several studies have shown that both CIDR and progestagen are effective in synchronising oestrus (Wheaton *et al*., 1993; Motlomelo *et al*., 2002). Ozyutlu *et al.* (2010) the authors observed no significant difference with regard to oestrous response, onset and duration of oestrus between CIDR and FGA sponges. Similarly, Ashwag and Mohammed-Nour (2015) reported no significant differences in the onset and duration of oestrus, time of luteinizing hormone (LH) surge in does synchronised with either CIDR or FGA.

Zeleke *et al.* (2005), reported that there was no significant difference in the oestrous response, oestrous onset and duration when MAP and FGA sponges during the transition period in Dorper ewes. Dogan *et al.* (2004) observed no significant rates in conception rates when MAP and FGA were used to synchronise oestrus in Saanen does. However, other authors have concluded that there is variation in fertility when CIDR or progestagen sponges are used. For example, Motlomelo *et al.* (2002) argued that the time to onset of oestrus was advanced in South African indigenous goats that received CIDR compared to those that were impregnated with progestagen. Greyling *et al.* (1988) observed that FGA sponges were more effective than MAP sponges based on improved conception and lambing rates of Dorper ewes. Romano (1996) concluded that the onset of oestrus was earlier when Nubian goats were treated with FGA than MAP. The variation in results may be a result of the rate of absorption and metabolization of the P4 devices (Romano, 1996). Oestrous response and fertility vary in their degree of success when CIDR or progestagen are used. Nonetheless, P4 treatment is often used in conjunction with gonadotrophin hormones such as eCG, FSH or hCG (Wildeus, 2000) near the end of P4 treatment.

The use of gonadotrophin hormones during oestrus synchronisation enhance ovulation rates (Rahman *et al*., 2008; Bhardwaj *et al*., 2012) which subsequently determines the occurrence of multiple pregnancies (Gordon, 2017). The ovulation rate is determined by ovarian activity (number and size of follicles). The size of follicles is an essential aspect that shows the competency of the oocyte and subsequently embryo development. Also, the diameter of follicles is linked to increase in oocyte growth and number of oocytes with cumulus cell layers (Mpebe *et al*., 2016). Some studies have tested the use P4 treatment without the addition of gonadotrophins and the ovulation rate was low. To overcome these challenge, gonadotrophin hormones such as eCG are incorporated with P4 treatment to tighten the onset of oestrus and enhance ovulation rate at P4 treatment as part of the protocol (Gore, 2016).

2.2.2 The use of equine chronic gonadotrophin during oestrous synchronisation

According to Wildeus (2000), eCG previously known as PMSG (pregnant mare serum gonadotrophin) is a placental glycoprotein hormone harvested from the serum of pregnant mares. Administration of eCG is the key for inducing ovulation (Simoes, 2015). The administration of eCG as a gonadotrophin is routinely incorporated into oestrous synchronisation protocol preferably out of season to induce ovulation (Wildeus, 2000). It has a gonadotrophic effect similar to FSH that inducing follicular development (Baldasserre & Karatzas, 2004). The administration of eCG enhances follicular development and ovulation rate. Ultimately, improving the quality of CL and ultimately P4 secretion, which positively impacts embryo development and survival (De Renesis & Lopez- Gatius, 2014).

Different doses of eCG have different effects on the induction of ovulation and fertility (De Renesis & Lopez-Gatius, 2014). P4 treatment is often used with eCG dose ranging between 250 and 500 IU in small ruminants (Baldasserre & Karatzas, 2004). The dose of eCG administered is dependent on season, breed, weight, age, previous response and desire for multiple kidding (Regueiroad *et al.*, 1999; Baldasserre & Karatzas, 2004). Different dose levels of eCG, the timing and route of administration have influence on the fertility rate, each varying with some degree of success (Wildeus, 2000; Mohtar *et al*., 2014). For example, Zonturlu *et al*. (2011) observed that different doses (300, 400 and 500 IU) did not affect fertility which is in agreement with Mohtar *et al.* (2014) who reported that different doses of eCG did not affect pregnancy rate in Boer goats.

With regard to the route of administration, some studies have reported that the subcutaneous administration of eCG results in better fertility rates compared to intramuscular administration. For example, Zeleke *et al*. (2005) and Koyuncu & Alticekic (2010) observed higher fertility rates and litter size following subcantenous administration of eCG compared to intramuscular administration. However, Lehloenya and Greyling (2009) reported that subcutaneous route of gonadotrophin administration resulted in lower number of unfertilized ova although both intramuscular and subcutaneous routes were effective. In Boer goats, oestrous response following 300 IU of eCG intramuscularly and subcutaneously was 93.3 and 100% respectively (Greyling & Van-Niekerk, 1990). The same authors also concluded that the use of eCG with progestagen is advantageous irrespective of route of administration. The reason for the variation is not yet clear because intramuscular injection absorbs faster than subcantenous injections.

With regard to timing of administration, Zeleke *et al.* (2005) observed that pregnancy, lambing and fecundity rates were significantly higher in ewes administered 300 IU eCG 24 h prior to sponge removal or at sponge removal compared to that administered 24 h after sponge removal. The oestrous duration was shorter in the control group than in does administered with 300 IU eCG (Greyling & Van Niekerk, 1990). Despite giving acceptable results the repeated use of eCG delayed the timing of oestrus, and as a result it also delayed the pre-ovulatory LH surge which can hinder long-term fertility (Rekik *et al*., 2012). Simoes (2015) and Dardente *et al.* (2016) reported that eCG is capable of developing antibodies when it is administered repeatedly.

A major challenge associated with eCG is prolonged biological life because it promotes the recruitment of antral follicles (Wildeus, 2000), therefore resulting in the increase of the number of ovulated follicles, resulting in a larger number of unovulated follicles (Wildeus, 2000). This has a negative effect on fertility when the treatment is repeated again on the same animal overtime. Furthermore, the use of eCG in some countries i.e. United States is prohibited in animals (Wildeus, 2000). As a result, this has fostered the need for clean and hormone free efficient alternative method such as the male effect to induce ovulation without using synthetic hormones (Lopez-Sebastian *et al*., 2014).

2.2.3 The male effect

Due to concerns associated with the use of hormones to control the oestrus cycle (Delgadillo *et al*., 2009; Hawken & Martin, 2013) the male effect is an efficient natural alternative oestrous synchronisation method. The male effect was first reported in the 1940's in sheep. The introduction of a male to sexually receptive females stimulates the secretion of LH which induces the occurrence of oestrus behaviour and ovulation (Van-Vuuren *et al*., 2004; Gelez & Nys, 2004). The male effect has been used with success to induce and synchronise ovulation in AI programs. The male effect is a non-pharmacological reproductive management technique that is used to synchronise oestrus and induce ovulation in females (Neto *et al*., 2016). The male effect has the advantage of being cost effective (Ungerfield *et al.*, 2004; Delgadillo *et al*., 2009), does not raise concern to consumers about the use of hormones in food-producing animals and is also an alternative to decrease hormonal use for oestrous synchronisation. Therefore, the male effect complies with principles of clean, ethical and sustainable animal production (Delgadillo *et al*., 2009).

The male effect is regarded as a sexual bio-stimulation between males and females (Delgadillo *et al*., 2009). The sight and smell of males stimulate sexual behaviour in females (Booth & Webb, 2010; Delgadillo *et al.*, 2009). The male effect is mediated by pheromones secreted by the males received and processed by the females (Van-Vuuren *et al*., 2004). Neto *et al.* (2016) described pheromones as a chemical signal transported by air and excreted through faeces, urine and skin glands. The pheromones are picked up by the female's accessory vomeronasal (VNO) olfactory system (Booth & Webb, 2010; Lopez-Sebastian *et al*., 2014) which causes behavioural and endocrine changes. Van-Vuuren *et al.* (2004) reported a direct link between the accessory olfactory bulb (AOB) and the hypothalamus. The olfactory signal causes changes in hypothalamic pulse generator which increase gonadotrophin releasing hormone (GnRH) pulses and stimulates secretion of LH (Booth & Webb, 2011; Lopez-Sebastian *et al*., 2014). The male effect increases LH pulse frequency from 0.3 pulses every 3 h, to 2.2 pulses every h, promoting follicular growth (Lopez-Sebastian *et al*., 2014) thus causing preovulatory LH surge and subsequently ovulation. However, the view that the male effect is solely the result of smell has been argued (Van Rooyen, 2013; Delgadillo *et al*., 2009). The behaviour and libido of males can also enhance the response of females to the male effect (Van Rooyen, 2013).

Several factors such as: physiological stage of females, intensity of stimulation, breed, age, season and nutrition influence the response to the male effect (Neto *et al*., 2016). For example, for females to respond to male stimuli, the male used should be sexually mature (Lopez-Sebastian *et al*., 2014; Fatet *et al*., 2011). Sexually inactive males cannot induce male effect in receptive females (Lopez-Sebastian *et al.*, 2014). Chemineau *et al.* (1987) stated that the buck effect is more effective in inducting sexual activity in breeds of low seasonality.

Furthermore, some studies have also reported that, for the females to respond to the male effect they have to be isolated from males (Delgadillo *et al*., 2009). Isolation means the females cannot see, hear and smell males during that period. Other reports show that prior separation time is not necessary for the male effect to be effective (Van Rooyen, 2013; Zaragaza *et al*., 2017). However, it is important to prevent the females from becoming refractory to a particular group of males. This can be ensured by rotating bucks to receptive females (Lopez-Sebastian *et al*., 2014; Van Rooyen, 2013; Fitz-Rodriguez *et al*., 2009). There is limited studies on the precise duration of isolation (Rosa & Bryant, 2002). Proper nutrition also influences the response of females to the male effect (Fatet *et al*., 2011). According to Van Rooyen (2013), the buck effect induce oestrus in does following buck introduction and between 80 and 100% respond to the buck effect provided they are healthy and fed well.

Several studies conducted on the male effect in synchronising oestrus is often used in combination with hormonal treatment. For example, the male effect is often used with P4 treatment (Neto *et al.*, 2016). Recently the use of melatonin with the male effect is gaining recognition. Celi *et al.* (2013) observed greater fecundity and fertility 91.7% in Payoya goats when melatonin implants were used with the male effect. Through the use of P4 treatment and male effect, Gore (2016) observed a high conception rate of 97 % compared to the use of P4 and eCG 72 %. Furthermore, priming P4 prior male introduction induces oestrus at the first ovulation and reduces the time to onset of oestrus (Knight, 1983; Martin & Scaramuzzi, 1983; Freitas *et al*., 2004). Gonzalez-Bulnes *et al.* (2006) observed that goats treated with P4 and introduced to a buck had a higher response compared to those only subjected to male. The absence of P4 priming causes a delay in LH surge which can decrease the conception rate (Martin & Scaramuzzi, 1983). According to Romano *et al.* (2000) immediate exposure of a ram at sponge removal hasten oestrus onset in ewes during the breeding season.

The male effect is often associated and efficient in anoestrus animals (Chemineau *et al*., 2006; Dardente *et al*., 2016;). The use of the male effect in inducing ovulation during the natural breeding season has not been studied extensively (Delgadillo *et al*., 2009). The response to the male effect is assumed to be inhibited by periods of elevated P4 during the luteal phase (Delgadillo *et al*., 2009; Hawken *et al*., 2009). However, the introduction of teaser rams is capable of nullifying the effect of luteal P4 resulting in a more robust response, which also apply in goats (Van Rooyen, 2013). For example, Hawken *et al.* (2007) observed that ram introduction to cyclic ewes stimulates an increase in LH pulsatic secretion. There is evidence suggesting that males can still have a significant effect on the hypothalamus pituitary gonadal axis (HPG-axis) (Delgadillo *et al*., 2009; Van Rooyen, 2013). Previous studies have reported that P4 treatment prior male introduction, suppresses short cycle but does not delay ovulation, instead it blocks $PGF2_{\alpha}$ synthesis thus preventing luteolysis (Chemineau *et al*., 2006).

Few studies have indicated that the male effect can be used to synchronise and induce oestrus as well as ovulation without incorporating hormones in cyclic females (Restall, 1988; Van-Rooyen, 2013). The information on the effect of male exposure to cycling females during the breeding season is scarce (Valencia *et al*., 2010). When the male effect is used to induce ovulation in females that are not treated with P4, in most cases the ovulation following male introduction is not accompanied by oestrus behaviour (Alvarez *et al*., 2003; Freitas *et al*., 2004). The second ovulation five to seven days later is accompanied by oestrus behaviour because the ovulatory follicle has matured to good quality CL and normal P4 secretion (Restall, 1988). In AI programs, the second oestrus five to seven days following buck introduction is a target for AI (Restall, 1988; Freitas *et al*., 2004; Gelez & Nys, 2004). The first ovulation following buck introduction is usually without oestrus behaviour and is of low fertility, with premature regression of the CL (Lopez-Sebastian *et al*., 2014; Freitas *et al*., 2004). For example, when the male effect was used to synchronise oestrus in the breeding season in AI programs, Restall (1988) reported 80% fertility when does were inseminated from day six to seven following buck introduction. However, when the does were inseminated between the first and fifth day following buck introduction fertility of the does was lower than 52%. In a study by Ott *et al.* (1980) the authors reported that the influence of bucks on does was more intense seven days following buck introduction early in breeding season. Peciller-Rubio *et al.* (2016) concluded that insemination 24 hours after pre-ovulatory LH surge resulted in higher kidding rates from day seven following buck introduction.

The male effect has profound physiological effect in cycling does (Delgadillo *et al*., 2009) but there is limited literature on using the male effect as the sole oestrous synchronisation strategy in AI programs (Neto *et al.*, 2016). The lack of literature prevents the development of reproductive management strategies of the male effect for oestrous synchronisation during the natural breeding season (Chemineau *et al*., 2006; Delgadillo *et al*., 2009). Synchronising and inducing oestrus using the male effect can be more economical compared to exogenous hormone administration especially in extensive production systems. The male effect offers a potentially useful, cost effective, clean and ethical strategy to improve reproductive efficiency of goats (Omontese *et al*., 2016).

2.3 The role of oxidative stress on female reproduction

Oxygen (O_2) is the most abundant element on earth and it is a critical element for all living things (Rizzo *et al*., 2012). Aerobic biological processes require O_2, the metabolism of O_2 represents a principal source of energy (Rizzo *et al*., 2012). During O_2 metabolism, glucose is transformed into ATP (Adenosine triphosphate) which is used by cells for energy. However, some O_2 molecules escape the process and result in the formation of free radicals also referred to as reactive oxygen species (ROS). Free radicals are molecules containing an unpaired electron in their outer orbit, thus making molecules highly reactive and unstable (Sharma & Argwal, 2004; Lykkesfeldt & Svendsen, 2007). Reproductive processes are metabolically active and increased metabolism result in the increased production of ROS (Talukder *et al*., 2017).

Reactive oxygen species have been reported to cause DNA damage, lipid peroxidation and protein damage (Sugino, 2006). The accumulation of free radicals' damage cells and tissues thereby causing oxidative damage (Celi, 2010; Rizzo *et al.*, 2012). Deficiencies of minerals such as Se, Zn or Mn, environmental factors such as high ambient temperature and humidity as well as radiation causes stress therefore contributing to the accumulation of free radicals in cells (McDowell *et al*., 2007; Puppel *et al*., 2015).

Free radicals have both beneficial and harmful effects (Opuwari & Henkel, 2016). At low concentrations free radicals have beneficial effects on cellular response and immune function (Sugino. 2005; Kabel, 2014). For example, they play an important role during apoptosis. Apoptosis is a natural procedure in which the body eliminate old cells (Agarwal & Allamaneni, 2004). In addition, free radicals in general are important for cell signalling and other physiological functions (Sharma & Agarwal, 2004). However, when the production of free radicals exceeds the capacity of anti-oxidants available in the body, animals develop oxidative stress (Spears & Weiss, 2008; Rizzo *et al*., 2012). Oxidative stress negatively affects several physiological functions in the reproductive tract and excessive levels can cause reproductive failures (Celi, 2010). For example, oxidative stress occurs in numerous physiological process from steroidogenesis, oocyte maturation, CL function and luteolysis, fertilization, embryo development and parturition (Sugino, 2006; Konvicna & Kovac, 2015). With regard to assisted reproduction, Agarwal *et al.* (2006) and Agarwal *et al.* (2012) stated that oxidative stress is an essential factor that can hinder the success of ART's in human beings. Several studies have highlighted the negative effect of oxidative stress during *in vitro* fertilization (IVF) in humans (Agarwal *et al*., 2006 & Gupta *et al*., 2009). Oxidative stress can be prevented by treatment with nutrients containing anti-oxidants which neutralize free radicals to less reactive molecules by donating a molecule. Thereby, maintaining the structure and integrity of cells (McDowell *et al.*, 2007). Anti-oxidants are defined as molecules that prevent the accumulation of free radicals.

Anti-oxidants form a natural defense mechanism against free radicals (Cronje *et al*., 2006). Anti-oxidants are classified as enzymatic and non- enzymatic anti-oxidants. Enzymatic anti-oxidants are superoxide dismutase, GSH-Px and catalase which destroy free radicals. The non-enzymatic anti-oxidants include: Vitamin E and C, β-carotene, Se and Zn. (Rizzo *et al*., 2012). Both enzymatic and non-enzymatic are found in follicles and they shield oocytes from oxygen free radicals (Kala *et al.*, 2016). For example, Behl and Pandey (2002) concluded that the anti-oxidant enzyme catalase may have a functional role in goat ovarian follicular development under endocrine regulation. Selenium has anti-oxidant functions and inhibit oxidative damage and enhance the immune system (Diaz-Sanchez *et al*., 2017). However, not much is known about the impact of Se on the immune system and reproductive performance of goats (Hall *et al*., 2012).

Theoretically, nutrients containing anti-oxidants provide a potentially important and cost-effective alternative to diseases related to oxidative stress in animals.

2.4 Selenium

Selenium is an indispensable trace mineral of several physiological functions in livestock and humans (Hefnawy & Perez, 2010). Selenium occur naturally in inorganic forms as selenide, selenite and selenate. Organically, Se is found in amino acids as selenomethionine and selenocysteine. Selenium is an essential component of more than 30 selenoproteins (Kruzhel *et al*., 2014). Selenium was first discovered by a Swedish Chemist J. J. Berzelius in 1817 (Fordyce, 2007). Initially Se was considered a toxic mineral causing severe poisoning in livestock (Neve, 1991). However, in 1957 Schwartz and Flotz observed the importance of Se in animals by using it to prevent deficiency of diseases, which were nutritionally related such as liver necrosis in rats (Neve, 1991).

Selenium is widely distributed although the concentration and availability vary between and within regions (Van Metre & Callan, 2001; Khanal & Knight, 2010). For example, the Se status in plants differ globally due to the Se status which vary based on geographical conditions (Fordyce, 2007). The availability of Se in the soil and vegetation affects overall animal nutrition requirements and health (Saha *et al*., 2016). The Se content in the soil and bioavailability to livestock differs with soil type, soil pH, texture, organic matter and rainfall (Fordyce, 2007; Lyon *et al*., 2007). In South Africa for example, based on geographical distribution map of the Se status of herbivores in South Africa, areas in parts of Gauteng and Mpumalanga province including parts of the Western Cape have variable Se concentrations (Van Ryssen, 2001). The Se status vary from marginally deficient to adequate (Van Ryssen, 2001).

Areas with acidic soil, high rainfall and soil prone to water logging contain low forage Se (33Van-Ryssen, 2001). Lyon *et al.* (2007) described that the reason for Se deficiency in forage is due to the leaching of Se from the top soil. For example, in South Africa these areas include the midland and mountain areas of Kwa-Zulu Natal and Western Cape (Van Ryssen, 2001). In some parts of the Limpopo Province, it was observed that the average Se concentration in the whole blood of sable antelope was 0.062 mg/l suggesting a possible marginal deficiency in the province (Van Ryssen, 2001). Marginal Se deficiency can hinder fertility (silent heat, cystic ovaries and birth of unthrifty kids with poor immunity) (Ziaei, 2015). Van Ryssen (2001) concluded that there is limited data available that indicates that the vegetation in arid parts of South Africa is Se sufficient to meet requirements of grazing animals.

2.4.1. The role of glutathione peroxidase

Selenium has been established to be an essential component of the enzyme glutathione peroxidase (GSH-Px) (Spears & Weiss., 2008; Konvicna & Kovac, 2015). The selenoenzyme GSH-Px is considered the most studied and important enzyme in animal reproduction (Maiorino *et al.,* 2009). GSH-Px catalyse the degradation of lipid peroxidase and hydrogen peroxidase (Sugino, 2006). The GSH-Px activity is directly proportional to Se intake and it is the first enzyme to be affected as a result of Se deficiency. For example, Misurova *et al.* (2009) and Pavalata *et al.* (2012) observed a significant increase in GSH-Px activity following Se-meth supplementation in white shorthair goats and kids compared to the control group. Witchel *et al.* (1996) reported that the serum activity of GSH-Px increased in response to Se supplementation compared to controls within 24 hours of supplementation in Angora goat kids following oral supplementation with sodium selenite. The authors also pointed out that the serum analysis of GSH-Px reflect the short-term Se status, which is in agreement with Van Ryssen (2016). In addition, GSH-Px maintains immunity of animals (Yatoo *et al*., 2013) by acting as a defense against cellular peroxidase damage through scavenging free radicals (Spears & Weiss., 2008). According to Hefnawy and Perez (2010) GSH-Px was the first establish selenoenzyme capable of inhibiting oxidative damage of the cell membrane. Tissue concentration of GSH-Px vary from specie to specie, with the highest concentration found in the cytosol and mitochondrial matrix (NCR, 1983; Neve, 1991).

2.4.2. The effect of selenium deficiency

The deficiency of Se is influenced by the Se content in the vegetation (Saha *et al*., 2016). Ruminants appear to be highly susceptible to Se deficiency with more severity in sheep and goats (Hefnawy & Perez, 2010). Selenium deficiency causes exudative diathesis and dietary necrotic liver degeneration in poultry. In pigs, Se deficiency causes mulberry heart diseases (Zarczynska *et al*., 2013). Kruzhel *et al*. (2014) reported that Se deficiency in cattle may cause diarrhoea in young animals and increase cirrhosis in beef cattle. In dairy cows there has been substantial evidence of Se deficiency in the occurrence of mastitis (Underwood, 1999). In sheep and goats Se deficiency is commonly manifested as white muscle disease (WMD) also known as nutritional muscular dystrophy (NMD) (Hostetler *et al*. 2003; Hefnawy & Perez, 2010). This condition is commonly observed in lambs when they are born (Donald *et al.*, 1994). However, Mehdi *et al*. (2013) stated that kids are more susceptible than lambs. The primary symptoms observed include; walking with a stiff gait, arched back, avoid movement, difficulty swallowing, rapid breath and cardiac arrest (NCR, 1983; Mehdi *et al.*, 2013). According to Haughey (1991) WMD may be prevented by oral administration of five mg of Se as a solution of sodium selenite or sodium selenate.

The manifestation of Se deficiency differs in young and adult animals (Mehdi *et al*., 2013). In adult animals, the sub-clinical symptoms of Se deficiency include; reduced performance, slower weight

gains and poor reproductive performance (McDowell, 2002; Saha *et al*., 2016). Sub-clinical symptoms of Se deficiency are more difficult to recognize and hence they are often ignored. As a result, animals continue to grow and reproduce but at a significantly reduced rate (Larson, 2005). Fertility is reduced before the evidence of clinical symptoms (Larson, 2005). Increased morbidity is also observed in new born animals with compromised immune and endocrine functions (Sevcikova *et al.*, 2011). Adequate Se strengthens the immune system and protect the body against pathological conditions (Courtman *et al*., 2012; Gill & Walker, 2008).

Inadequate supply of nutrients from the dam to the foetus may have detrimental effects on the growth and health of the offspring (Hostetler *et al*., 2003). Newborn animals are born with little immunity against diseases. Maternal immunity plays a crucial role, as a defense mechanism early in life until their immunity is developed. Failure of passive transfer of immunoglobin to offspring contributes to neonatal mortality (Habeeb *et al*., 2013). Several studies have reported that Se level in the foetus is in correlation with maternal Se levels (Sevcikova *et al*., 2011). Selenium is easily transferred across the barrier of the placenta to the foetus during gestation (Stewart *et al*., 2013). Ghany-Hefnawy *et al.* (2007) suggested that it is due to the strong affinity between the dam and foetus relative to Se metabolism in small ruminants. Selenium treatment during pregnancy is critical because it is the period when the demand for nutrients is essential. Selenium improves the overall performance and viability of the offspring (Maiorino *et al*., 2009). McDowell *et al*. (2007) observed greater lambing rates in ewes treated with Se during gestation compared to ewes not supplemented with Se. Munoz *et al.* (2008) concluded that ewes supplemented with Se had heavier lambs, they had faster progression to stand and higher activity of GSH-Px, thus improving the immune status resulting in lower perinatal mortality.

2.4.3. The effect of excess selenium

Selenium has beneficial and detrimental effects depending on the balance (Vazquez-Armijo *et al*., 2011; Smith & Akinbamijo, 2000). Selenium is known to be toxic when taken up in excessive doses and beneficial at low or moderate concentration (Khanal & Knight, 2010; Kabel, 2014). In animals, excess Se causes selenosis resulting in central nervous system paralysis, increased salivation, swallowing disorders, hair loss, abdominal pain and eventually death (Konvicna & Kovac, 2015). An oversupply of Se is not a serious risk resulting in toxicity as previously assumed (NRC, 2005). For example, in a study conducted by Cronje *et al.* (2004) the authors observed high concentration of Se in the livers of ewes supplemented with sodium selenite following a dosage of 10 times more instead of 0.16 to 0.32 mg Se / sheep /day. Although, the amounted doses approached toxic levels the authors observed no toxic symptoms. Similarly, Hall *et al*. (2012) observed that ewes receiving Se in the form of sodium selenate, sodium selenite and Se-yeast above recommended levels displayed clinical signs of toxicity at any time during the study. Selenium tolerance by domestic animals differ with the

chemical form, duration and continuity of intake (McDowell, 2002). Therefore, precaution should be taken when treating animals with Se (Andrieu, 2008; Ziaei, 2015).

2.5 Sources of selenium

Selenium exist as either organic or inorganic form. Organic Se sources are more biologically accessible than inorganic Se sources (NCR, 1983; Hostetler *et al*., 2003). The reason is that inorganic forms must first be converted to hydrogen selenide and then to selenophosphate before they can be utilized in selenoproteins synthesis (Pavlata *et al*., 2011). While organic Se is accessed through absorption system of amino acids (Pavlata *et al*., 2011). Sodium selenite is considered the most economical inorganic source and it is commonly used for Se supplementation (Ahsan *et al*., 2014). Supplements of selenite and selenate have been used with success to correct selenium deficiencies (Hall *et al*., 2012). According to Lyon *et al.* (2007) the inorganic Se is more toxic and a high proportion of the mineral is excreted and not absorbed (Palmieri & Szarek, 2011). However, based on the study by Pavlata *et al.* (2012) the authors observed no significant difference in Se concentration and GSH-Px activity amongst four different forms of inorganic Se sources namely; sodium selenite, lactate-protein complex, Se enriched yeast and Se-proteinate supplemented in the diet of pregnant goats. However, the activity of GSH-Px in the blood of kids were higher only in the Se-enriched yeast. Hence, the use of Se-yeast is gaining more recognition as an efficient Se supplement compared to inorganic Se sources (Lyon *et al*., 2007). For example, the replacement of sodium selenite by organic Se in the form Se-yeast is considered to improve fertility (Lyon *et al*., 2007).

According to Wilde (2006) and Hostetler *et al.* (2003) Se-yeast which is an organic source of Se has been established to have higher retention in tissues, hence it has been recommended by several authors in ensuring adequate Se availability. Furthermore, based on the data over the past 10 years, replacing inorganic Se with organic Se sources, such as Se-yeast has been established as an effective strategy in correcting Se deficiency in the dairy, beef and sheep industry (Lyon *et al*., 2007). Compared to inorganic Se sources Se-yeast is reported to significantly increase Se levels in the blood, increase the activity of GSH-Px and maximize Se concentration in the colostrum as well as milk of dairy cows (Lyon *et al*., 2007). This contradicts Van Metre and Callan (2001) who reported superior absorption and retention of inorganic sources. Hall *et al*. (2012) reported that supranutritional Se-yeast supplementation increased whole-blood Se (WB-Se) and serum Se concentration. However, ewes receiving sodium selenite above recommended levels achieved similar WB-Se concentration as ewes receiving Se-yeast.

2.6. Selenium supplementation strategies

Optimum Se intake can be achieved through supplementation. There are different ways that can be used to treat livestock with Se such as fertilizing soil with Se salts, intraluminal Se pellets or glass

boluses (Hefnawy & Perez, 2010). They serve as an immediate available source of Se, however these products are not available in South Africa (Lukusa & Lehloenya, 2017). For monogastric, feedlot animals and dairy cows fed concentrate the most efficient method of giving supplemental Se is through the use of Se containing minerals incorporated in feeds (McDowell, 2002). In grazing animals, the ideal method of supplementing Se includes; oral drench and Se ruminal pellets (McDowell, 2002). According to Ekermans (1982) injection and oral dosing may provide the best method because of having the advantage of knowing exactly how much each animal received.

In a study by Kafilzadeh *et al.* (2014) when comparing the effect of oral and injectable supplementation of Se and vitamin E on reproductive performance and immune function of diary calves and heifers, the authors concluded that there was lack of significant difference between injection and oral supplementation. Therefore, the authors recommended oral supplementation instead of an injection. In intensive production system animals receive a balanced ration including Se hence, oral supplementation is seldomly used. However, in extensive production oral administration is more practical (Lukusa & Lehloenya, 2017). Oral drench supplementation of one mg Se in the form of sodium selenite to moderately dystrophic lambs was reported to improve the Se status within 24 hours (NCR, 1983). With regard to period of supplementation, Saha *et al.* (2016) recommended the application interval to be every one to three months for small ruminants and every three-four months in critical production stages in beef and dairy cattle. Lukusa and Lehloenya (2017) supplemented Se in the form of sodium selenite at 10 day intervals and obtained acceptable results. A study by Mojapelo *et al.* (2017) showed that Se as sodium selenite was supplemented every three months in Saanen bucks and the author reported acceptable results with regard to reproductive parameters.

2.7. Selenium metabolism and absorption

Selenium is found in all tissues and cells of the body (Konvicna & Kovac, 2015). However, the kidney contains the highest concentration of Se followed by the liver, spleen and pancreas respectively (NCR, 1983; McDowell, 2002). The liver is the primary storage organ and the most sensitive to measure for Se status (Van-Ryssen, 2016; Saha *et al.*, 2016). Selenium is mainly absorbed in the small intestine (Hostetler *et al*., 2003; NRC, 2005) specifically in the duodenum and caecum. From there it is transported in the liver, muscles, tissues and kidney (Hostetler *et al*., 2003). The metabolism of Se is influenced by the chemical form and the dose supplemented (Konvicna & Kovac, 2015). Inorganic and organic Se sources are metabolized differently (Petrera *et al*., 2009). Most of the absorbed Se is accumulated in the liver. From there a large proportion of absorbed Se is transferred into the serum, hence the serum is a major pool of Se in the body (Van Metre & Callan, 2001; Habeeb *et al*., 2012). However, Se concentration can also be measured from whole blood, plasma and the liver (Kruzhel *et al*., 2014). Van Ryssen (2016) noted that serum Se is considered an indicator of short-term Se intake while whole blood and liver reflects long-term Se intake.

Courtman *et al*. (2012) and Van Ryssen (2001) stated that Se is among the few trace minerals where the liver and blood concentrations are fairly correlated with Se intake. The bioavailability of Se is efficiently absorbed in monogastric than ruminants (Van Metre & Callan, 2001; Spears, 2003). The low bioavailability of Se in ruminants is due to reduction in rumen microbes that cause Se insoluble, therefore making it unavailable for further metabolism in ruminants (Spears, 2003; Lyon *et al*., 2007).

2.8. Interactions of selenium with other nutrients

The interaction between some trace minerals can influence the absorption and bioavailability of other minerals (Sandstrom, 2001). Minerals with similar chemical and physical properties can compete for protein transportation (Sandstrom, 2001). For example, Se and sulphur (S) have similar physical and chemical properties (Mehdi & Dufrasne, 2016; Spears, 2003). Several studies have reported that higher dietary S reduces the bioavailability of Se (Spears, 2003; Saha *et al*., 2016). The S analogues of Se compounds seem to have the greatest impact on Se metabolism (NCR, 1983). For example, a study in rats showed that nearly a three-fold increase in urinary excretion of Se after a dose of sodium selenate when rats were given S either parenterally or in the diet (NCR, 1983). In soils with adequate concentration of Se, the presence of other trace minerals such as Calcium (Ca), S, copper (Cu) and arsenic(As) can interfere with its utilization by the plant (Hefnawy & Perez, 2010).

Nonetheless, elements such as Mercury (Hg), Silver (Ag), Cu and Cadmium (Cd) are capable of reducing the toxicity of Se but research on their practical influence is limited (McDowell, 2002). Also, the relationship between Se and vitamin E is well established. Selenium and vitamin E have anti-oxidant function. They protect the membranes from oxidative damage because of this shared function. For example, Koyuncu and Yerlikaya (2007) reported that Se and Vitamin E improved the occurrence of oestrus, fertility and body weight in lambs. Vitamin E acts as the first line of defense against peroxidation, while Se acts as the second line of defense which destroys peroxidase (McDowell *et al.*, 2002). The P4 concentrations in the blood plasma of Zairabi goats were significantly increased in animals supplemented with adequate Se and/or Vitamin E compared to animals receiving no supplementation (Habeeb *et al*., 2012). However, the concentration of P4 in goats supplemented with Se and Vitamin E together increased than the concentration of P4 in goats supplemented with Se or Vitamin E independently.

2.9. The effect of selenium on female fertility

The negative effect of Se deficiency on female fertility in livestock has been fairly studied (Maiorino *et al*., 1999). Selenium deficiency has been implicated as the cause of numerous reproductive failures such as cystic ovaries, low fertility, metritis, abortions, poor uterine involution, suppressed or silent oestrus and retained placentas (Lykkesfeldt & Svendsen, 2007; Kumar, 2015; Hosnedlova *et al*., 2017). Detrimental effects of dietary Se deficiencies were also reported during different stages of the reproductive cycle including onset of oestrus to conception, ovulation rate and offspring performance

(Smith & Akinbamijo, 2000). Selenium also plays an essential role in the activation of thyroid hormones through Se-containing enzymes (Beckett & Arthur, 2005). Selenium is an essential component of type I iodothyronine- 5'- deiodinase, and converts T4 (Thyroxin inactive form) to T3 (active form) (Munoz *et al.*, 2008; Hefnawy & Perez, 2010). Several studies (Rooke *et al*., 2004) have also reported the beneficial effect of Se in the application of ART's. The effect of Se on male reproduction has been well described. For example, the presence of optimum Se in the male reproductive tract is important for spermatogenesis and sperm maturation (Ahsan *et al*., 2014). Selenium is essential for normal spermatozoa development and puberty attainment in Saanen bucks (Mojapelo *et al.*, 2017). Lukusa and Lehloenya (2017) reported that supplementation with sodium selenite improved testicular characteristics and semen quality of Saanen bucks. In the same study, Se supplementation also increased the concentration of GSH-Px, LH and testosterone. The male reproductive system is severely affected by Se deficiency and significantly reduces sperm motility ultimately resulting in poor semen characteristics and infertility (Maiorino *et al.*, 1999). However, the influence of Se on oocytes and reproductive function in females remain scarce (Agarwal *et al*., 2012).

Mineral supplementation prior oestrus synchronisation protocols, improves the number of ovarian follicles and result in the improvement of pregnancy (Abdollahi *et al*., 2015). Habeeb *et al.* (2012) reported that Se treatment before breeding may improve fertility. The use of nutrients containing anti-oxidants can improve conception rates by inhibiting accumulation of oxidative stress during early embryonic development (Yildiz *et al*., 2015). Palmieri and Szarek (2011) also stated that Se treatment in cattle and ewes is associated with increased embryo survival, high foetal mass and reduced incidence of retained placenta. Koyuncu and Yerlikaya (2007) observed 100 %, 96.7 %, 100 and 131.0 % oestrus response, pregnancy, lambing and fecundity rates respectively. The authors concluded that maternal Se supplemented prior gestation seems to correlate positively with improved fertility.

Van-Niekerk *et al*. (1996) argued that supplementing Se post-mating could interfere with embryo development and survival in sheep. The authors concluded that supplementation with Se during the period 18-35 days after fertilization may affect the implantation resulting in embryo losses. Munoz *et al*. (2009) observed no difference in the length of the gestation period, abortion, mortality, litter size and birth weight in the group supplemented with Se and the group not supplemented. Gabryszuk and Klewiec (2002) observed 100 % vs 76 % oestrus response in three-year-old ewes supplemented with Se (Na-selenate 0.1 % injection) and the control respectively. The lambing rates were 68 % vs 100 % in the control and Se supplemented group respectively. The contradicting results of Se supplementation on fertility could be determined by several factors namely; severity of deficiency, dose rate given, conditions of supplementation, the ability of the system to prioritize the enzymatic synthesis (Hefnawy & Perez, 2010).

2.9.1. The role of selenium on reproductive hormones and ovarian activity

Reproductive processes in animals are influenced by hormones. Abnormal secretion of pituitary hormones can also cause reproductive failures. For example, LH is required for maturation and release of oocyte. Poor nutrition reduces the frequency at which pulses of LH are released (McDonald *et al*., 2009). Specific trace minerals can influence the concentration of these hormones which alter events in the reproductive cycle. There is scarce information with regard to the effect of Se on reproductive hormones. Kamada *et al.* (2014) proposed that there is a possibility that Se plays an essential role in P4 production. Based on the results by Kamada and Hodate (1998) the authors reported that Se supplementation contributes to P4 concentration in the CL of cows.

Proper functioning of the ovary is essential to maintain fertility, several studies have reported evidence of the influence of oxidative stress on the ovarian activity (Devine *et al*., 2012). Poor nutrition reduces the number of follicles that emerge and subsequently the number of follicles destined to ovulate (Robinson *et al*., 2006).

Abdollahi *et al.* (2015) stated that when ewes are dosed with minerals prior oestrous synchronisation the number of ovarian follicles might increase. Nutrients containing anti-oxidants stimulate the process of steroidogenesis and stimulate the anterior pituitary gland to secrete gonadotrophin hormones, thus initiating folliculogenesis in the ovaries (El- Shahat & Abdel-Moem., 2011). According to Devine *et al.* (2012) studies have shown that high concentration of the anti-oxidant GSH-Px in the oocytes are necessary for normal fertilization and subsequent pre-implantation embryonic development.

In humans, it has been reported that the exposure of germ cells (spermatozoa & oocytes) to high levels of free radicals and low concentrations of anti-oxidants protections will render cells dysfunctional and thus failing fertilization process (Opuwari & Henkel, 2016). According to Kala *et al.* (2016) ROS species are capable of influencing follicles, oocytes, endometrium and their environment. Although the interaction between ROS and anti-oxidants in oocyte development have been established in humans (Kala *et al*., 2016), these aspects in female animals warrants further research. Current knowledge has shown that oxidative stress has a severe impact on female fertility (Devine *et al*., 2012). Therefore, interventions that serve to counteract the negative influence of oxidative stress on female fertility are important.

2.10. The effect of selenium on the immune system

Selenium is essential for proper functioning of many aspects of the immune system in both animals and human beings (Arthur *et al.*, 2003). Reproductive failures such as abortion, still birth, retained placenta and perinatal mortality are associated with systematic diseases that impairs the immune system, therefore lowering reproduction efficiency (Mukusa-Mugerwa, 2011). The main function of the immune system is to act as a defence mechanism against invasion of pathogens.

Selenium plays an important role in enhancing the immune system and building immunity against pathogens (Hall *et al*., 2009). According to the NCR (1983) treating cattle and sheep with Se improves weight gain through its protection of the immune system. Selenium deficiency suppress the immune system, decrease resistance to bacterial and viral infections, neutrophil and antibody function (Hall *et al*., 2009). The immune system can also be weakened by glucocorticoids causing stress (McDowell *et al*., 2007; Puppel *et al*., 2015). In pigs, cattle and sheep the major glucocorticoid produced is cortisol Puppel *et al*., 2015). Stress induces the accumulation of ROS resulting in oxidative stress (Prasad *et al*., 2016). Weakened immune system will result in reduced animal production efficiency as a result of increased susceptibility to diseases (McDowell *et al.*, 2007). McDowell *et al.* (2007) stated that anti-oxidants are capable of reducing microbe infestation.

Immunity is compromised in Se deficient animals and Se supplementation can compensate for deficiencies (Arthur *et al*., 2003). The levels of Se above the recommended requirements have been observed to enhance the immune system in several species (McDowell, 2002; Hall *et al*., 2012). However, the exact mechanism through which Se interacts with the immune system is still unclear (McDowell, 2002; Zarczynska *et al*., 2013). Different immune cells and their mechanisms of phagocytic functioning are influenced by the deficiency of nutrients containing anti-oxidants.

Phagocytosis is defined as a mechanism whereby animals immunologically destroy invasion of bacteria. Se deficiency causes a significant reduction in the ability of the phagocytic neutrophils to kill bacteria (NCR, 1983). Selenium is reported to enhance the capacity of phagocytic neutrophil (Yatoo *et al*., 2013; Salles *et al*., 2014). Selenium deficiency influences blood levels of immunoglobin (IgG) and T cell functions, which results in a higher prevalence and severity of diseases. Kachuee *et al.* (2014) and Habeeb *et al.* (2012) reported that white blood count (WBC), neutrophil and lymphocytes counts were higher in the kids supplemented with Se in the form of Se-methionine compared to the control from birth until seven days. In the same study, the Se concentration also increased significantly in the colostrum of treated goats.

Immune cells are highly sensitive to oxidative stress because their membranes contain high concentrations of polyunsaturated fatty acids (PUFA) that are vulnerable to peroxidation (Spears & Weiss, 2008). The T and B cell represent the key factors of adaptive immunity. They are highly specialised defender cells in the immune system. Selenium treatment has shown to have beneficial effects in improving T-cell proliferative response. Furthermore, the activity and lifespan of neutrophils, macrophages and lymphocytes are reduced as a results of a decrease in the activity of GSH-Px (Hefnawy & Perez., 2010). The use of Se as an immune system enhancer has been reported to have a positive effect on the immune competence and quality of colostrum. For example, positive correlation between blood Se levels and increased concentrations of IgG in serum and colostrum have been reported in cows (Hefnawy & Perez, 2010). Diaz-Sanchez *et al.* (2017) observed that IgG levels increased in groups treated with Se compared to groups which were not treated with Se.

Chapter 3
Materials and methods

3.1 Ethical clearance

The materials and procedure of this study were approved by the Animal Ethics Committee of the University of Pretoria project no. EC074-17.

3.2 Study site

This study was conducted during the natural breeding season in autumn and ended in summer one month after the kidding season. The experiment was conducted at Mara Research Station in Limpopo Province, South Africa. The site is located at latitude 23^0 05'S and longitude 29^0 25'E at an elevation of 956 m above sea level.

3.3 Does management

Forty-eight South African indigenous does aged between 1-6 years were used in this experiment. All experimental does were kept in an open pen throughout the experimental period. All does were kept isolated from the males for one-month prior oestrous synchronisation protocols. The does were fed lucerne (*Medicago sativa*) *ad libitum* and also had access to clean water throughout the study. The does had no access to fresh growing forage or any other feed throughout the study. Before the onset of the experiment the lucerne sample was analysed for Se concentration. The lucerne had a concentration of 0.003mg/kg.

3.4 Experimental design and treatment groups

A total of (n=48) South African indigenous does were blocked according to body weight, age and parity thereafter allocated randomly into one of the following two groups namely: Se supplementation (n=24) and non-supplemental group (n=24). Each of the two groups were further sub-divided into three oestrous synchronisation protocols; (A) P4 (CIDR) + eCG (equine chronic gonadotrophin), (B) P4 (CIDR) + male effect and (C) male effect. The experimental design was a 2 x 3 factorial design as shown in Table 3.2.

Table 3.2 : Experimental design and number of does per group[1]

Selenium supplemented (24)	Non-supplemented (24)
P4 + eCG (8)	P4 + eCG (8)
P4 + male effect (8)	P4 + male effect (8)
Male effect (8)	Male effect (8)

3.5 Selenium treatment

The treatment does were individually dosed with Se in the form of sodium selenite (ACECHEM, South Africa) at 10-day intervals. Selenium supplementation started a month before the initiation of oestrous synchronisation protocols until 16 days' post mating. The does received a dosage of 0.34 mg Se per kg as adjusted by Lukusa and Lehloenya (2017). An amount of 0.2125 mg sodium selenite powder was weighed inside an oval plastic weighing boat on a weighing balance (Sartorius AG, M-power, Germany). The powder was then dissolved in 1000 ml of distilled water. Each animal received 1.6 ml per kg body weight (0.34 mg Se per kg / 0.2125 mg = 1.6 ml per kg). For example, if a goat weighed 37 kg it received 37 kg x 1.6 ml = 59 ml of the solution at 10-day intervals. The solution was administered orally using a 60 ml dosing syringe.

3.6 Oestrous synchronisation protocols

Does were grouped into three groups based on oestrous synchronisation protocols; (A) P4 + eCG, (B) P4 + male effect and (C) male effect. All does in the P4 group were treated with CIDR-G (Pfizer, New Zealand) containing 0.3 g P4 left intravaginally for 11 days.

3.6.1 Progesterone and male effect

At CIDR removal does in the P4 and male effect were introduced to a new teaser buck wearing an apron for a period of 72 h.

3.6.2 Progesterone and eCG

At CIDR withdrawal, does in the P4 and eCG were injected intramuscularly with 300 IU (2.5 ml) of eCG (Intervet Schering-Plough Animal Health, South Africa).

3.6.3 Male effect

Does in this treatment group did not receive CIDRs. They were naturally synchronised by introducing a teaser buck wearing an apron on the same day that P4 treated does received CIDRs. The teaser buck was left over a period of 13 days as described by Peciller-Rubio *et al*. (2016).

[1] Abbreviations of oestrous synchronisation protocols: P4: Progesterone, eCG: equine chronic gonadotrophin

3.7 Procedure of inserting CIDR devices

The experimental does were restrained by one assistant and another assistant holding the hind-legs to avoid disturbance during CIDR insertion. The external vulva was cleaned with a paper towel. The CIDR device was loaded on the CIDR applicator is such a way that the CIDR is tightly fixed on the applicator and the CIDR string was left hanging from the end of the applicator. Prior insertion, the CIDR device was sterilized with antibiotic cream (RAMSEM, South Africa) that also acted as a lubricant. The loaded CIDR applicator was then inserted at 45^0 angle to the roof of the vaginal passage by pushing it out of the plunger until the CIDR string was left hanging outside of the vagina.

3.8 Breeding of does

Five sexually mature indigenous bucks were used for mating the females following oestrous synchronisation. All does were naturally mated following oestrus observations. The does in the male effect were introduced to a buck to mate 24 h following observation of oestrus behaviour starting from day seven as described by Peciller-Rubio *et al*. (2016). While does in the P4 treatment groups were also naturally mated 48 hours following CIDR withdrawal. The bucks were removed from the does after 17 days (one oestrus cycle) following buck introduction.

3. 9. Data collection

3.9.1 Teasing and oestrous detection

A teaser buck wearing an apron was used for oestrous detection to evaluate the onset and duration of induced oestrus. Oestrous detection was monitored for 72 h at 12 h interval (06:00 and 18:00) in all treatment groups. For the male effect group oestrous detection started from day seven following buck introduction and continued for 72 h. In the P4 treated groups, oestrous detection commenced 12 h following CIDR removal. The occurrence of "standing heat" was considered as the true sign of oestrus in all the treatment groups.

3.9.2 Ultrasound evaluation of follicular response

A real-time B-mode ultrasound scanner (Aloka, 500®-SSD, Tokyo, Japan) equipped with transrectal 7.5 MHz linear array probe (UST-660-7.5 model) was used to evaluate the number of follicles and size of the largest follicle. The animals were restrained in a standing position.

The rectal probe was lubricated with lubricating gel (K-Y Jelly, Johnson & Johnson) before inserting the probe into the rectum. The probe was placed in the rectum with the transducer aligned at the right angle to the abdomen wall. When the urinary bladder was passed and the uterine horns located, the probe was rotated laterally 90^0 clockwise and 180^0 counter-clockwise to observe ovaries and the follicles (Gonzalez-Bulnes *et al*., 2006). During ultrasound scanning, the relevant images of the follicles were frozen to allow measurements to be taken.

The ultrasonographic evaluation of ovarian activity (number of follicles and the size of the largest follicle) was performed on the day of mating before they were mated to the bucks.

3.9.3 Measuring of kidding weight

Each kid weight was taken within 24 hours after kidding. Body weight (kg) was determined with an electronic livestock scale Crane Scale (Cap .300 kg) (TALTEC Ltd, South Africa).

3.10. Reproductive measures evaluated:

The following parameters were assessed;

- Oestrous response: number of does in oestrus/ number of does synchronised x 100.
- Onset of oestrus: the time from CIDR withdrawal and buck introduction to first observations of oestrous behaviour
- Duration of oestrus: hours from when the animals started showing oestrus until last observation of oestrous behaviour
- Pregnancy rate: number of does that gave birth / number of does mated x 100.
- Gestation length: period starting from mating date until kidding.
- Litter size: the number of kids born per doe kidded.
- Kidding weight: average weight of kids at birth

3.11. Statistical analysis

The initial weight, number of follicle size of largest follicle, oestrus duration, oestrus onset, litter size, gestation length and kidding weight were analyzed using the General Linear Model (GLM) procedure of SPSS 11.5 for Windows (2003, SPSS version 11.5, SPSS Inc., Chicago, IL, USA). The statistical model used in the analyses of these parameters:

$Y_{ij} = \mu + S_i + P_j + SP_{ij} + e_{ij}$

Where μ = population mean of the appropriate trait;

S_i = effect of the i^{th} supplementation treatment;

P_j = effect of the j^{th} protocol treatment;

SP_{ij} = effect of the interaction of the i^{th} supplementation and j^{th} protocol treatment;

e_{ij} = random effects

The interactions were not significant in most cases. Significant interaction means were separated by Tukey's test at a 5% significance level using the Statistix 8.0 for Windows (2003, Analytical Software, Tallahassee, FL, USA). The pregnancy status and oestrus response were analyzed by Pearson chi-square test at a 5% significance level using the SPSS 11.5 for Windows (2003, SPSS version 11.5, SPSS Inc., Chicago, IL, USA). The Post Hoc after chi-square was done as described by Beasley and Schumacker (1995).

Chapter 4

Results

4.1 The effect of selenium supplementation and oestrous synchronisation protocols on the reproduction performance of South African indigenous goats

Nine (9) does did not complete the experiment due to poor health. The does were excluded from the experiment at the beginning of synchronisation of oestrus. Out of the (n=39) does remaining, a total of five (5) does had reproductive tract abnormalities (their ovaries could not be detected and therefore their follicles could not be measured and counted) at ultrasound scanning, the does were excluded from the experiment and were also omitted from the analysis. Only (n=4) kids died from birth until weaning. Some of the does dropped the CIDR devices during the experimental period but they were replaced as soon as it was noticed.

The results revealed that there was no interaction ($p > 0.05$) between Se supplementation and oestrous synchronisation protocols on the number of follicles, diameter of the largest follicle, onset of oestrus, oestrous duration, litter size and gestation length. The results revealed that there was no significant difference ($P > 0.05$) regarding the effect of Se supplementation on the number of follicles and diameter of the largest follicle (Table 4.1). There was no significant difference ($p > 0.05$). regarding the effect of oestrous synchronisation protocols on the number of follicles. Although, there was no statistical difference with regard to oestrous synchronisation protocols on the size of the largest follicle. The results showed that P4 + male effect protocol had a larger diameter of the largest follicle compared to P4 + eCG and male effect protocols. There was no significant difference ($P > 0.05$) regarding the effect of Se supplementation and oestrous synchronisation protocols on the gestation length, pregnancy rate and litter size (Table 4.2).

The results also revealed that there was a significant difference ($p < 0.05$) with regard to the effect of oestrous synchronisation protocol on the onset of oestrus (Table 4.3.) The time to onset of oestrus was significantly advanced in the P4 + male effect treatment group compared to the male effect and P4 + eCG. The results revealed an interaction ($P < 0.05$) between Se supplementation and oestrous synchronisation on the kidding weight (Table 4.4) and oestrous response (Table 4.5). The kidding weight in this study was lesser when the protocol P4 + male effect was used in Se supplemented does. However, the kidding weight was greater in does using the same protocol and were not supplemented with Se.

The interaction between Se and oestrous synchronisation on the oestrous response revealed that oestrous response was greater when Se was not supplemented and the protocol P4 + eCG was used, however it was lesser when Se was supplemented and the same protocol was used.

A total of 30 kids were born in this study of which the proportion of single was (n = 20/30) and twins (n = 5/30). There was no triplet born in this study. The twin births were in the P4 + male and male effect protocols none in the P4 + eCG.

Table 4.1: The effect of selenium supplementation and oestrous synchronisation protocols on the number of follicles and size of the largest follicle (Mean ± SEM) of South African indigenous goats

	Number of follicles	**Size of the largest follicle (mm)**
Supplementation		
Selenium supplemented	1.6 ± 0.10	6.4 ± 0.36
Non-supplemented	1.7 ± 0.20	6.9 ± 0.37
p-value	0.791	0.319
Protocols		
P4 + eCG	1.6 ± 0.26	6.9 ± 0.46
P4 + male effect	1.8 ± 0.24	7.2 ± 0.44
Male effect	1.6 ± 0.24	5.8 ± 0.43
p-value	0.852	0.067

P4: progesterone, eCG: equine chronic gonadotrophin,

Table 4.2: The effect of selenium supplementation and oestrous synchronisation protocols on the pregnancy rate, gestation length and litter size (Mean ± SEM) of South African indigenous goats.

	Gestation length (days)	**Pregnancy rate (%)**	**Litter size**
Supplementation			
Selenium supplemented	156.8 ±2.11	63.2	1.2 ± 0.11
Non-supplemented	160.2 ±1.91	63.2	1.0 ± 0.12
p-value	0.246	0.631	0.312
Protocols			
P4 + eCG	156.0 ± 2.87	60.0	1.0 ± 0.16
P4 + male effect	156.1 ± 2.14	76.9	1.2 ± 0.12
Male effect	163.3 ± 2.34	53.3	1.2 ± 0.13
p-value	0.072	0.422	0.416

P4: progesterone, eCG: equine chronic gonadotrophin

Table 4.3: The effect of selenium supplementation and oestrous synchronisation protocols on oestrous onset, duration of oestrus and oestrous response (Mean ± SEM) of South African indigenous goats

	Oestrous onset (h)	Oestrous duration (h)	Oestrous response (%)
Supplementation			
Selenium supplemented	23.6 ± 2.69	33.0 ± 4.01	50.6
Non-supplemented	23.0 ± 2.56	37.4 ± 4.26	51.4
p-value	0.869	0.466	0.923
Protocols			
P4 + eCG	30.5 ± 3.56 [a]	41.6 ± 5.70	55.0
P4 + male effect	17.5 ± 2.50 [b]	39.5 ± 4.34	57.8
Male effect	22.0 ± 3.41[ab]	24.6 ± 5.24	37.8
p-value	0.026	0.060	0.101

Values with superscript in the same column are significantly different ($p < 0.05$), h: hours. P4: Progesterone, eCG: equine chronic gonadotrophin

Table 4.4: The interaction effect between selenium supplementation and oestrous synchronisation on the kidding weight of South African indigenous goats

		Selenium Supplementation		
Parameter	**Protocols**	**Non-supplemented**	**Selenium supplemented**	**P-value**
Kids birth weight (kg)	P4 + eCG	2.4 ± 0.20[aA]	2.2 ± 0.21 [aA]	**0.023**
	P4 + male effect	2.6 ± 0.17[aA]	1.8 ± 0.17[aB]	
	Male effect	2.1 ± 0.19[aA]	2.3 ± 0.19 [aA]	

Within a column means followed by different small letter superscript significantly differ ($p < 0.05$) whereas within a row, means followed by different capital letter superscript significantly differ ($p < 0.05$); kg: kilogram, eCG: equine chronic gonadotrophin, P4: Progesterone

Table 4.5: The interaction effect between selenium supplementation and oestrous synchronisation on the oestrous response of South African indigenous goats.

		Selenium Supplementation		
Parameter	**Protocols**	**Non-supplemented**	**Selenium supplemented**	**P-value**
Oestrous response (%)	P4 + eCG	86.7^{aA}	36.0^{bB}	**0.006**
	P4 + male effect	50.0^{aA}	64.7^{aA}	
	Male effect	32.0^{aA}	45.0^{aA}	

Within a column means followed by different small letter superscript significantly differ ($p < 0.05$) whereas within a row means followed by different capital letter superscript significantly differ ($p < 0.05$); P4: Progesterone, eCG: equine chronic gonadotrophin

Chapter 5
Discussion

Due to limited literature on the effect of Se supplementation and oestrous synchronisation on response to oestrus, onset and duration of oestrus, number of follicles, size of the largest follicle, pregnancy rate, gestation length, kid birth weight and litter size in goats. Findings from other related goat breeds were used to compare and contrast the results of the present study. Furthermore, analysis of reproductive hormones was not part of the present study. Reports from previous studies on reproductive hormones were used to explain and link findings of the present study with regard to follicular response and oestrous parameters.

5.1 The effect of selenium supplementation and oestrous synchronisation protocols on the number of follicles and size of the largest follicle of South African indigenous goats

Previous studies (Sa`-Filho *et al*., 2010; Monteiro *et al*., 2016; Mpebe *et al*., 2017) reported that the size of the largest follicle does not only influence ovulation rate but also indicate the competency of the oocyte, embryo development and high pregnancy rate. Compared to other studies (Gore, 2016), the number of follicles and size of the largest follicle following oestrous synchronisation obtained in the present study was low. A possible explanation for the poor follicular activity could be attributed to the body condition score (BCS), body weight, breed and poor nutrition.

Although numerically no significant difference was recorded the diameter of the largest follicle in the P4 + male effect group was larger compared to P4 + eCG and male effect. The difference between the present study and other studies in terms of size of the largest follicle may be attributed to the addition of the hormone $PGF2_{\alpha}$ at CIDR withdrawal during the breeding season. Prostaglandin is a luteolytic hormone that was not used in the present study during oestrous synchronisation. Previous studies Lucy *et al*. (1992) have reported that LH pulses and follicles grew larger when $PGF2_{\alpha}$ was administered along with P4 in cattle.

In addition, González-Bulnes *et al*. (2005) reported that synchronisation of oestrous and ovulation during the breeding season without $PGF2_{\alpha}$ induce lower quality pre-ovulatory follicles. Thereby, resulting in poor luteal function and viability of early embryo which might have been a cause of the poor pregnancy rate in the present study. The beneficial effect of the largest follicle is often associated with greater display of oestrus (Sa`-Filho *et al*., 2010).

Larger follicles secrete an increased concentration of the hormone oestradiol-17β which is associated with increased display of oestrous behaviour. Although numerically no significance difference was recorded, results of the present study revealed that the P4 + male effect group also had the highest oestrus response.

The present study did not evaluate oestradiol-17β concentration it can be assumed that the concentration of oestriadiol-17β may have been greater in the P4 + male effect group hence larger diameter of the largest follicle and higher oestrus response. In accordance with previous studies the present study revealed that diameter of the largest follicle was larger in P4 treated groups compared to the male effect group (Gonzalez-Bulnes *et al*., 2005; Fernandez-Moro *et al.,* 2008). The size of the largest follicle following P4 + male effect in this study agrees with the findings by Adib *et al*. (2014), the authors reported the positive effect of P4 priming on the growth and maturation of pre-ovulatory follicles induced by the male effect.

The lack of difference amongst the oestrous synchronisation protocol on the number of follicles in the present study may be attributed to the similar effect of both eCG and male effect to induce LH surge (Gore, 2016). Previous studies reported that oestrous synchronisation using the male effect only had no effect on follicle development (Peciller-Rubio *et al*., 2016) which is in line with results of the present study. A possible reason for the poor response with regard to ovulatory response following male induced oestrous in the present study might be caused by poor quality of the first ovulations, short cycles with low and unstable luteal activity (Gonzalez-Bulnes *et al*., 2006; Nogueira *et al*., 2015) when oestrus is synchronised without prior administration of P4.

Results of the present study with regard to the effect of eCG on the number of follicles and diameter of largest follicle are not in line with results from previous studies (Gore, 2016) in other goat breeds using similar oestrous synchronisation protocols. However, results of the present were in accordance with the findings reported by Barret *et al.* (2004) following 12 days P4 treatment and 500 IU of eCG. In line with the findings of the present study, the authors reported that treatment with eCG had limited effect on the dynamics of ovarian activity.

The present findings that Se supplementation had no effect on the number of follicles and size of the largest follicle agrees with results reported by Cerny *et al*. (2016). However, results of the present study contradicts previous findings by, the authors reported that Se supplementation increased the number of follicles (Ratto *et al*., 2008; Vazquez-Hernandez *et al.,* 2017). In agreement with previous findings by (Cerri *et al*., 2009), results of the present finding also shows that there was no interaction between Se supplementation and oestrous synchronisation protocols on the number of follicle and size of the largest follicle.

5.2 The effect of selenium supplementation and oestrous synchronisation protocols on the onset of oestrous, duration of oestrous and oestrous response of South African indigenous goats

In agreement with the present finding previous studies, Kuru *et al*. (2017) reported that Se supplementation had no effect on oestrous parameters (oestrus response, oestrus onset and duration). A possible reason for the lack of beneficial effect of Se on oestrous parameters in the present study may

be due to the failure of Se to exert impact on hormones regulating the oestrus cycle such as oestradiol-17β and LH (Ganie *et al*., 2015; Cerny *et al*., 2016).

Although there was no significant difference, the oestrous duration in the present study following P4 + eCG treatment was longer compared to oestrous duration recorded by Lehloenya *et al*. (2005) following the use of P4 analogue and 300 IU eCG treatment in Boer goats. The difference in results between the present study and previous studies in terms of oestrous duration may be attributed to form of P4 treatment used (CIDR vs progestagen sponges). Previous studies have reported a difference in retention rate and metabolization in P4 devices mainly CIDR and progestagen sponges (MAP/ FGA) (Romano, 1996). The oestrous duration in the present study following P4 + male effect was longer than oestrus duration reported by Chemineau (1985). The difference in results may be attributed to breed as some studies emphasized breed has a significant effect on oestrous response following oestrous synchronisation (Ngere & Dzamuka, 1975; Fabre-Nys *et al*., 2015). In support with observations from previous studies does that were not treated with P4 in the male effect group displayed short oestrous cycles (Chemineau, 1985; Restall, 1988;). The oestrous duration from day seven in the male effect group was shorter. The shorter oestrous duration may be as a result of silent heat or inconsistency of short cycles due to the exclusion of P4 treatment in the male effect group.

In agreement with previous studies results of the present study revealed that male introduction shortened the interval to onset of oestrous during the breeding season (Ungerfeld & Rubianes, 1999; Moeni *et al*., 2015). In this study, the onset of oestrus for the P4 + male effect was earlier compared to the P4 + eCG and male effect. A possible explanation for the male introduction to reduce the time interval to onset of oestrus might be attributed by the fact that the male effect provokes an increase in tonic secretion of LH which stimulates follicular growth and oestradiol-17β. Thereby, causing a more rapid onset of oestrus by rapidly increasing LH pulse frequency (Chemineau, 1985; Evans *et al*., 2004). Previous studies on the male effect have reported that buck introduction following P4 treatment hasten the onset of oestrus activity (Rekwot *et al*., 2001).

Eight does did not display any signs of oestrous behaviour following oestrous synchronisation. However, three of those does were able to conceive, it can be assumed that these does experienced silent heat (oestrus without visible signs). A similar observation was also reported by Ramukhiti *et al.* (2012) in South African indigenous goats.

Compared to other studies (Lehloenya *et al.*, 2005) the oestrous response following oestrous synchronisation using P4 treatment in the present study was low. Previous studies reported oestrous response of higher than 90 % on average in goats treated with P4 + eCG (Amarantidis *et al*., 2004; Lehloenya *et al*., 2005; Dogan *et al*., 2005). The oestrous response following oestrous synchronisation using the male effect only in the present study was also low (Fitz-Rodriguez *et al*., 2009). The reason

for the low oestrous response maybe due to the high P4 concentration during the natural breeding season is usually associated with suppression of pulsatile LH secretion and follicular growth and therefore, leading to poor oestrous response (Hawkens *et al*., 2007; Saganuma *et al.,* 2017).

The pregnancy rate in the present study is comparable with the findings reported by Lehloenya *et al*. (2005) in South African indigenous goats following treatment with P4 and eCG treatment. The authors reported conception rate of 52 % and 53% in Boer and Nguni goats respectively. According to Chao (2008), low concentration of P4 following 12 days' treatment increase size of the largest follicle and thus concentration of oestradiol-17β consequently, resulting in low pregnancy rate. Therefore, it can be assumed that long P4 treatment of 11 days or more may have negative effect on the fertility. In agreement with previous studies (Araya *et al*., 2017), the pregnancy rate following male effect oestrous synchronisation was low. A possible explanation for the low pregnancy rate following the 13 days' male effect protocol in the present study may, be due to possible early embryonic mortality. Therefore, maintaining the does with the bucks for a period longer than 13 days might provide an opportunity for the does that had not been fertilized to be mated again (Araya *et al*., 2017).

The findings of the present study with regard to Se supplementation has failed to confirm the hypothesis that Se can have an effect on number of follicles and diameter of the largest follicle, pregnancy rate and oestrous parameters. However, the diameter of the largest follicle and onset of oestrus were positively affected by the addition of male effect following P4 treatment. The positive influence of the male effect on the size of the largest follicle and oestrus onset may possibly encourage the use of the male effect when synchronizing oestrous in goats treated with P4. Thereby limiting the wide application of eCG which has been implicated to hinder fertility in goats when administered repeatedly. Studies regarding the interaction of Se supplementation and oestrous synchronisation protocols on the number of follicles, diameter of the largest follicle, oestrous onset and duration as well as oestrous response of indigenous goats we could not be found to compare and contrast with the present findings

5.3 The effect of selenium supplementation and oestrous synchronisation on the gestation length, litter size and kid birth weight of South African indigenous goats

In line with findings of previous studies, results of the present study revealed that the gestation length, litter size and kidding weight were not affected by Se supplementation (Hammer *et al*., 2011; Yavuzer & Bengisu, 2014). However, the results of the present study contradict results observed by Farrag *et al.* (2017), the authors reported that Se supplementation and oestrous synchronisation increased lamb birth weight, average daily gain (ADG) and weaning weight. Oestrous synchronisation protocols did not have any positive effect on the litter size and kidding weight. These present findings concur with the reports from previous studies in which oestrous synchronisation protocols had no effect on the litter size and kid birth weight (Gore, 2016). However, it was noticed that oestrous synchronisation protocol had a significant effect on the mean gestation length. The male effect had a longer gestation length. Therefore,

it can be assumed that oestrous synchronisation by hormonal treatment may have an effect of the gestation length.

The overall, gestation length in the present study was not in line with the average gestation length of 145 - 153 days in goats reported by Rekik *et al.* (2012) and the mean natural gestation length of 148 days reported by other studies (Lehloenya *et al.*, 2005). The present study recorded an extended gestation length of 155 days in the P4 treated groups which is slightly longer than the findings reported by Lehloenya *et al.* (2005) in South African indigenous goats. It has been reported that breed may have influence on gestation length (Khanum *et al*., 2007). Other studies have recorded shorter gestation length of 144 -146 days (Khanum *et al*., 2007; Chowdhury *et al*., 2002) in small size breeds such as Pakistan Dwarf goat, Black Bengal goat and South African dwarf goat compared to large breeds such as Saanen, Toggenburg and Alpine.

Mellado *et al.* (2000) suggested that prolonged gestation in goats may be advantageous because it does not only results in heavier kids but also decreases the likelihoods of stillbirths and neonatal survival of kids. The authors also suggested that gestations approaching 160 days should be considered normal in goats. The reason being that the incomplete maturation of some foetal organs in those kids carried less than 148 days may be the main reason for perinatal mortality.

Optimum litter size in sheep and goats is the primary reproductive concern (Fatet *et al*., 2011). The average litter size of unimproved indigenous goats of 1.7 kids per doe reported by Webb and Mamabolo (2004) following a survey is not comparable with the findings of the present study. The litter size in the present study following oestrous synchronisation using P4 was comparable with the mean litter size obtained by kor *et al*. (2011) in Raieni goats.

Following P4 + eCG oestrous synchronisation protocols in goats, previous studies (Lehloenya *et al*., 2005) reported higher litter size in goats. The litter size in the present study following male effect and P4 + male effect was less than litter size reported by Chemineau (1985) following the male effect and P4 + male effect. The variation in the observation between the present and previous studies in terms of litter size may be attributed to breed.

Kid birth weight is an important reflection of potential growth. Low birth weight than optimum is the primary factor determining pre-weaning losses (Assan, 2013). Following oestrous synchronisation protocols, previous studies (Lehloenya *et al*., 2005) reported that the mean kidding weight in South African indigenous goats was 2.7 kg which is higher than findings in the present study. Other studies Chowdhury *et al*. (2002) observed birth weight as low as 1.24 kg and 1.19 kg in males and females, respectively in Black Bengal kids.

There was no literature that could explain the interaction between Se and oestrous synchronisation on the kidding weight observed in the present study. The difference in litter varies among breed (Epstein & Herz, 1964; Amoah *et al*., 1996), nutritional and environmental factors. In agreement with findings of the present study.

Chapter 6
Conclusions and recommendations

6.1 Conclusions

Neither dietary Se supplementation nor the interaction effect between Se and oestrous synchronisation had a significant effect on the number of follicles, diameter of the largest follicle, oestrous onset and duration, pregnancy rate, gestation length and litter size. However, there was a significant interaction effect between Se and oestrous synchronisation on the kidding weight and oestrus response. The addition of the male effect following P4 treatment improved the diameter of the largest follicle and also resulted in the early onset of oestrous. It can be concluded that the addition of the male effect in P4 synchronised goats could improve reproduction performance. The male effect can therefore be used as a co-treatment to replace the use of eCG in order to limit the use of hormones in synchronising oestrous in South African indigenous goats.

6.2 Recommendations

Based on the present study, the following are recommended:

- More studies should be conducted with a focus on evaluating the effect of different levels and sources of Se on the reproductive performance of South African indigenous goats.
- More studies should be conducted on improving the simplicity of the male effect protocol as an independent oestrous synchronisation strategy. The male effect can be an efficient reproductive technique that can be used for the induction and synchronisation of oestrus for farmers with limited financial inputs.
- A further detailed understanding of the role of oxidative stress in female reproduction which will allow the development of specific anti-oxidant intervention strategies that might improve reproductive performance in livestock.

6.3. Critical Evaluation

Below are the aspects of the research that could ascertain the highest degree of confidence and accuracy but were not addressed or negatively influenced results of the present study.

- The exclusion of reproductive hormone analysis (oestradiol-17β and progesterone), GSH-Px or Se analysis. Analysis of these hormones could have added more value to the results and overall conclusion of the study regarding Se supplementation
- The sample size was not sufficient to obtain substantial conclusions and obtain accurate results, the exclusion of several animals due to poor health severely affected the sample size and results obtained.

- The duration of Se supplementation and interval between supplementation might have contributed to the lack of significance of Se on reproductive efficiency.
- Observing for oestrus response and signs of oestrus is a subjective task, to allow accurate oestrus response results, the task could have been carried out by one individual or by recording from CCTV cameras mounted in strategic places.
- The addition of the hormone $PGF_{2\alpha}$ as part of the synchronisation protocols could have made a difference in terms of follicular number and size as well as oestrous parameters. Based on existing literature the addition of a luteolytic hormone such as $PGF_{2\alpha}$ when synchronising oestrus in the natural breeding season when goats are actively cycling is essential due to the presence of the active CL.
- Blood samples could have been collected from the experimental does before the start of the trial to analyse the concentration of Se prior any supplementation to adjust the dose of Se as sodium selenite given if needs be.

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