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Response to different oestrus synchronisation protocols and fertility of ewes following artificial insemination

by

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Declaration

I Angella Nakafeero, declare that the dissertation which I hereby submit for the degree of MSc (Agric) Animal Science: Production Physiology at the University of Pretoria is my own work and has not been previously submitted at this or any other tertiary institution

Signature……

Date…….

Dedication

To the Blessed Virgin Mary mother of God and my mother.

To the memory of my late grandparents Peter and Anastasia Kaggwa.

To my mother Bernadine Kayongo for the great gift of education which she has worked hard for and nurtured.

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Abstract

The response of South African Mutton Merino (SAMM) ewes to synchronisation of oestrus and fertility following different protocols and artificial insemination (AI) were studied using data collected during the autumn breeding season. The study was aimed at comparing the effect of long and short-term progesterone (P4) treatment and their combination with either equine chorionic gonadotropin (eCG) or the ram effect (Ram) on oestrous response and fertility of ewes. Ewes (n = 78) were randomly allocated to four treatment groups in a 2×2 factorial design and primed with controlled internal drug release (CIDR) for a 9 (short) or 14 d (long) period. At CIDR withdrawal, ewes in each group received either a single intramascular injection of equine chorionic gonadotrophin (eCG; 300 IU) or exposure to the ram effect; eCGshort (n=19), Ramshort (n=21), eCGlong (n=19) and Ramlong (n=19). Oestrous behaviour was monitored from 12-84 h post CIDR withdrawal and ultrasound performed at 48 h post CIDR withdrawal to examine number and diameter of follicles. Artificial insemination (AI) was performed twice at 48 and 60 h post CIDR withdrawal with fresh undiluted semen using the cervical method. Non-return rate (NRR) was monitored 15-21 d post AI while pregnancy diagnosis was performed by transrectal ultrasound at 35 d post AI and confirmed by lambing data. Oestrous behaviour was observed in 98.7% of all synchronised ewes, with no significant difference between treatment groups. Overall, CR and the proportion of ewes lambing to synchronised oestrus were (74.4% and 52.6%, respectively). There was no significant difference between treatment groups in oestrus response, onset of oestrus, duration of oestrus, number of follicles, diameter of the largest follicle, NRR, conception rate (CR), AI to lambing interval, twinning rate and number of lambs born. When data were pooled, CIDR-14 d protocols showed a significantly shorter interval to onset of oestrus (24.9 ± 1.6 versus 30.8 ± 2.1, $P < 0.05$) than CIDR-9 d protocols but there was no difference ($P > 0.05$) between eCG and Ram protocols when data were pooled. CIDR-9 d protocols resulted in a significantly higher CR (85.0% versus 63.2%, $P < 0.05$) than CIDR-14 d protocols when data were pooled but there was no difference ($P > 0.05$) in CR between eCG and Ram

protocols. Mean AI to lambing interval was 158.2 ± 1.2 d, ranging from 147-154 d and 166-186 d post AI. The proportion of ewes lambing to synchronised oestrus per treatment group were (eCGshort (52.6%), Ramshort (42.9%), eCGlong (63.2%), Ramlong (52.6%), $P > 0.05$). Therefore, it can be concluded that the 4 protocols investigated were effective in synchronising oestrus with similar response to synchronisation of oestrus and fertility between treatment groups. Of the 4 protocols, the Ramlong protocol offers the benefit of reduced cost, reduced hormonal use and adequate fertility compared to other protocols. In addition, the cost of labour involved is foregone and a safer product is disposed of to the environment compared to use of the eCGlong and Ramshort or eCGshort protocols respectively.

Key words: CIDR, eCG, Ram effect, South African Mutton Merino, short-term progesterone treatment, long-term progesterone treatment.

List of abbreviations

AI: Artificial insemination:

ANOVA: Analysis of variance

ARTs: Assisted reproductive techniques

CIDR: Controlled internal drug release

CIDR-9 d: 9 d CIDR treatment

CIDR-14 d: 14 d CIDR treatment

CL: Corpus luteum

CR: Conception rate

eCG: Equine chorionic gonadotropin

eCGlong: 14 d CIDR treatment + eCG

eCGshort: 9 d CIDR treatment + eCG

FGA: Flurogestone acetate

FSH: Follicle stimulating hormone

FTAI: Fixed time artificial insemination

GLM: Generalized linear model

GnRH: Gonadotropin releasing hormone

LH: Luteinising hormone

MAP: Medroxyprogesterone acetate

NRR: Non-return rate

oEGP: oestrus-associated glycoprotein

P4: Progesterone/progestagens

$PGF_{2\alpha}$: Prostaglandin

Ram: Ram effect

Ramlong: 14 d CIDR treatment + ram effect

Ramshort: 9 d CIDR treatment + ram effect

SAMM: South African Mutton Merino

SEM: Standard error mean

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CHAPTER ONE

1. INTRODUCTION

1.1 Overview

Sheep production is an important economic activity in South Africa as it is largely supported by the existence of the vast and semi-arid areas of the Eastern Cape, Northern Cape, Free State and the Western Cape, where sheep thrive, given their low production requirements (Cloete and Olivier, 2010). Although modest in terms of their total contribution to gross domestic product (Agriculture, 2013), small stock especially sheep are of strategic importance in the rural parts of South Africa, where they contribute to food security and are also an alternative source of income in crop-pasture regions (Cloete and Olivier, 2010). The South African Mutton Merino (SAMM) is one of the important dual-purpose sheep breeds in South Africa, grown for wool and meat (Schoeman et al., 2010). The breed earns more than 200 million dollars in foreign exchange annually, and also clothes, feeds, and provides employment opportunities to many (Erasmus, 2001). Returns from sheep production largely depend upon the efficiency of reproduction as it affects the number of ewes which lamb per year, as well as the number of lambs born (Wheaton et al., 1992).

Oestrus synchronisation is one of the technologies in sheep used to increase reproductive efficiency in different parts of the world (Greyling et al., 1997; Fukui et al., 1999; Vilariño et al., 2017). Management of reproduction using oestrus synchronisation in small ruminants both during the breeding and non-breeding season (Greyling et al., 1997) involves the use of natural and/or pharmacological methods to manipulate/modify the oestrous cycle (Martin et al., 2004; Abecia et al., 2012). Methods commonly used for oestrus synchronisation include; use of intravaginal sponges impregnated with progestagens; (Flurogestone acetate (FGA) or Methyl acetoxyprogesterone (MAP), which are analogues of progesterone

(Abecia et al., 2012), and CIDR, an intravaginal device which contains 0.3 g of progesterone (Wheaton et al., 1993). The most popular prostaglandin ($PGF_{2\alpha}$) products are Lutalyse and the $PGF_{2\alpha}$ analogue cloprostenol (Abecia et al., 2012). To achieve greater efficiency, progesterone is sometimes combined with $PGF_{2\alpha}$ (Ali, 2007; Martemucci and D'Alessandro, 2011), whereas melatonin, a hormone used during the non-breeding season is also commonly combined in a protocol with progesterone/progestagens (P4) and/or prostaglandin (Uslu et al., 2012).

Prostaglandin acts by causing regression of the corpus luteum (CL), an ovarian structure which produces progesterone (Boscos et al., 2002; Martemucci and D'Alessandro, 2011). Hence its use is only limited to the breeding season when the CL is active (Abecia et al., 2012). However, when P4 is used in oestrus synchronisation protocols, the luteinising hormone (LH) pulse frequency is suppressed (Driancourt, 2001), which prevents the occurrence of oestrus, the LH surge and ovulation, until the device is withdrawn (Rubianes et al., 1996; Evans, 2003; Gonzalez-Bulnes et al., 2005b). Furthermore, gonadotropin releasing hormone (GnRH) and gonadotropins such as follicle stimulating hormone (FSH) and equine chorionic gonadotropin (Abecia et al., 2012), as well as the ram effect (Delgadillo et al., 2009) are also used in combination with either P4 or $PGF_{2\alpha}$ to stimulate follicular development and synchronise oestrus and ovulation (Abecia et al., 2012). A combination of these products in a protocol is usually based on the desired effects, season or availability.

Oestrus synchronisation facilitates the use of AI which has the potential to impact on genetic improvement and thus increase production (Faigl et al., 2012). Furthermore, the technology has proved to be particularly useful during the non-breeding season because it offers the advantage of out of season breeding (Barrett et al., 2004; Ungerfeld et al., 2005; Martinez et al., 2015), and hence lambs can be produced twice a year. On the other hand, when used during the breeding season, synchronisation of oestrus reduces the breeding time (Ali, 2007), which shortens the

lambing period and ensures that lambs of uniform weight are born. A reduction in the breeding and lambing period would further permit efficient use and concentration of labour and resources especially during lambing in order to reduce lamb mortalities (Abecia et al., 2011). In addition, some protocols increase ovulation rate which is the basis for improved conception rate, lambing rate and litter size (Mutiga and Mukasa-Mugerwa, 1992). Moreover, induction of oestrus in peri-pubertal ewe lambs, which would bring their first lambing forward offers the benefit of increased on-farm profitability (Abecia et al., 2011).

It is reported that use of P4 with fixed time artificial insemination (FTAI) results in lower fertility compared to natural oestrus (Greyling et al., 1997). However, inspite of this, P4 is an essential tool and is also a more practical method for oestrus synchronisation both during the non-breeding and breeding season compared to other methods (Abecia et al., 2012). For this therefore, application of P4 has further been extended to other assisted reproductive techniques (ARTs) such as superovulation and embryo transfer (Menchaca et al., 2009; Lehloenya and Greyling, 2010). Commonly used P4 protocols for synchronisation of oestrus involve a 12-14 d treatment period combined with equine chorionic gonadotropin (eCG) at device withdrawal (Donovan et al., 2004; Zeleke et al.,2005; Moakhar et al., 2012).

The effectiveness of 12-14 d (long-term) has been proven, thus when used, a high percentage of ewes respond with oestrus (over 90%) and with high pregnancy rates of over 70% (Greyling et al., 1997; Zeleke et al., 2005; Moakhar et al., 2012). However, it is reported that long-term P4 treatment periods result in low P4 concentration towards the end of the treatment, due to a reduction in the absorption of P4 from the exogenous source (Greyling et al., 1994). It is also reported that when the CL regresses and the exogenous source of P4 is insufficient to suppress LH pulse frequency, the turnover of follicles is slowed (Driancourt, 2001; Viñoles et al., 2001), therefore when the device is withdrawn, ewes will ovulate an aged oocyte which may affect fertility or the ability of the fertilised ova to grow into a viable

embryo capable of developing into a pregnancy (Johnson et al., 1996; Viñoles et al., 2001). Conversely, Evans et al. (2001) found that although follicles of up to 14 d were older, they had similar fertility to young follicles.

On the other hand, short-term treatment periods (Ali, 2007; Fleisch et al., 2012; Jackson et al., 2014) expose follicles to high P4 concentration which leads to adequate suppression of the LH pulse frequency and hence result in better fertility due to ovulation of young (newly recruited) follicles (Viñoles et al., 2001). Therefore, , short-term treatments have an advantage over long-term treatments in the control of follicular dynamics and in improving fertility. It has also been reported that when P4 is combined with eCG, fertility can be improved (Leyva et al., 1998; Driancourt, 2001; Vilariño et al., 2010). Equine chorionic gonadotropin synchronises follicular wave development (Ali, 2007) thus inducing a tighter synchrony of oestrus and ovulation in cyclic ewes (Driancourt, 2001), which is necessary for FTAI (Greyling et al., 1997; Letelier et al., 2011). Equine chorionic gonadotropin is also superior in increasing ovulation rates, (Letelier et al., 2011), which is the basis for multiple births and higher lambing rates (Mutiga and Mukasa-Mugerwa, 1992).

Moreover, with the above benefits to the use of eCG, reports both in small ruminants (Bodin et al., 1997; Drion et al., 2001b; Anel et al., 2005) and in cattle (Drion et al., 2001a) indicate that fertility is lower with each breeding. This is attributed to the accumulation of residual antibodies from previous treatments (Bodin et al., 1997). Hence to achieve tighter synchrony of oestrus, and also induce and synchronise ovulation, researchers have evaluated the use of GnRH (Titi et al., 2010; Hashem et al., 2015), FSH (Knights et al., 2001; Boscos et al., 2002) and the ram effect (Hawken et al., 2007; Meilán and Ungerfeld, 2014; Jackson et al., 2014) as possible alternatives to the use of eCG.

The ram effect is a stimulus which has been observed to increase LH secretion and advance the LH surge in randomly cyclic ewes (Hawken et al., 2007). It has also been observed to induce oestrus in anovular ewes (Ungerfeld et al., 2004; Hawken et al., 2008). When used in combination with oestrus synchronisation treatments, the ram effect advances oestrus (Ungerfeld and Rubianes, 1999b; Contreras-Solis et al., 2009; Ungerfeld, 2011) and also results in higher fertility compared to unexposed ewes (Contreras-Solis et al., 2009). There is evidence that pheromones produced by the ram, as well as other non-olfactory cues are responsible for stimulation of the LH pulse frequency (Delgadillo et al., 2009). Increase in the LH pulse frequency results in rapid growth of preovulatory follicles, increased secretion of oestradiol, behavioural oestrus, the LH surge and ovulation (Delgadillo et al., 2009).

The ram effect is a cheap, non-pharmacological method for induction and synchronisation of oestrus and ovulation, whose use is particularly important at the present time when the use of high quantities of P4 in food producing animals is a health concern to the consumer (Martin et al., 2004). Considering the risk of residues, countries such as the US, have enacted and enforced regulations for maximum limits of chemical residues in food, and as such, the use of high quantities of progestagens has been abolished (Macrì et al., 2006). Similarly, use of progestagens is minimised in the EU, whereas funding the research and development of the most efficient and clean products for use in farm animals is into force. This drive would ensure safety of animals, humans and the environment (Macrì et al., 2006; Martin and Kadokawa, 2006). Hence this creates the need to develop alternative protocols which would reduce the use of hormones. In this regard, commercial intravaginal devices containing lower doses of progestagens (20-30 mg) have been researched (Greyling et al., 1994; Letelier et al., 2009), and studies on short-term treatments and re-use of P4 intravaginal devices (Fleisch et al., 2012; Vilariño et al., 2013; dos Santos-Neto et al., 2015) have been conducted. For the above reasons therefore, the present study investigated whether use of a

protocol involving the ram effect for synchronisation of oestrus and ovulation following a 9 or 14 d P4 treatment period would result in better or similar response to synchronisation of oestrus and fertility to a protocol where eCG is used following the same P4 treatment durations.

1.2 Problem statement

Previous studies in South Africa (Greyling et al., 1997; Zeleke et al., 2005) and in other parts of the world (Barrett et al., 2004; Viñoles et al., 2011; Moakhar et al., 2012) have used long-term P4 in combination with eCG. However, reduction in the quality of ovulated follicles following long-term P4 treatment (Johnson et al., 1996; Viñoles et al., 2001; Menchaca and Rubianes, 2004) and the repeated use of eCG in sheep (Bodin et al., 1997; Drion et al., 2001b; Anel et al., 2005) are reported to be detrimental to fertility (Johnson et al., 1996; Viñoles et al., 2001). Moreover, excessive use of hormones in food producing animals is both a consumer and an environmental concern (Macrì et al., 2006; Martin and Kadokawa, 2006). Short-term P4 treatments would therefore offer the advantage of re-use of the intravaginal device and can still result in high CR (over 70%) at third use (Vilariño et al., 2013). This also provides that the device which has been used thrice would be safer when disposed of to the environment. Hence a short-term P4 protocol in combination with the ram effect would offer a cheaper protocol with reduced hormonal use. For the above reasons therefore, studies have focused on short-term P4 treatment periods (Fleisch et al., 2012; Vilariño et al., 2013; dos Santos-Neto et al., 2015) and use of the ram effect both in combination with P4 (Ungerfeld and Rubianes, 1999b; Romano et al., 2000), as well as $PGF_{2\alpha}$ (Ungerfeld, 2011; Meilán and Ungerfeld, 2014). However, no studies have yet compared short-term and long-term P4 protocols in combination with eCG or the ram effect on the same flock/under the same conditions. In light of the aforementioned problems associated with the traditional long-term P4-eCG protocol, this study therefore compared response to

oestrus synchronisation and fertility following short-term and long-term P4 protocols in combination with eCG or the ram effect.

1.3 Overall aim

The overall aim of the present study was to improve response to synchronisation of oestrus and fertility of ewes following artificial insemination.

1.4 Specific objectives

a) To compare response to oestrus synchronisation (oestrous response, interval to onset of oestrus, duration of oestrus, number of follicles and diameter of the largest follicle (at 48 h post CIDR withdrawal) and fertility (conception rate, AI to lambing interval, number of lambs born and twinning rate), of ewes treated with protocols of CIDR for 9 d with those treated for 14 d.

b) To compare response to oestrus synchronisation (oestrous response, interval to onset of oestrus, duration of oestrus, number of follicles and diameter of the largest follicle (at 48 h post CIDR withdrawal) and fertility (conception rate, AI to lambing interval, number of lambs born and twinning rate) of ewes treated with protocols of CIDR combined with eCG with protocols combined with the ram effect.

1.5 Hypotheses

This study hypothesises that the use of a short-term P4 protocol for oestrus synchronisation with the ram effect can be a possible alternative to the conventional long-term P4 treatment combined with eCG.

H1: Protocols for oestrus synchronisation with 9 d progesterone treatment will result in better response to synchronisation of oestrus and fertility than the protocols with 14 d treatment period.

H2: Progesterone based protocols for oestrus synchronisation combined with the ram effect will result in similar response to synchronisation of oestrus and fertility as the protocols combined with eCG.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction of literature

Profitability in sheep production largely depends upon the efficiency of reproduction (Martin et al., 2004). Unlike tropical sheep breeds which reproduce all year round, breeds which originate from high latitudes or temperate regions are seasonal breeders (Rosa and Bryant, 2003). This implies that ovarian activity in these breeds is controlled by photoperiod (Malpaux and Karsch, 1990; Rosa and Bryant, 2003; Zarazaga et al., 2003), while in tropical breeds, the pattern of rainfall in relation to food availability is the main determinant of reproduction (Mukasa-Mugerwa and Lahlou-Kassi, 1995). Given that these factors reduce the reproductive window, reproduction should be managed to make the most of the period during which ewes are most likely to reproduce. Management of reproduction involves use of hormonal and non-hormonal methods to control ovarian activity, of which the method of oestrus synchronisation employed may depend on the season of breeding and/or the desired effects (Abecia et al., 2012). Knowledge of the female oestrous cycle and hormonal control therefore is important, given that it is the basis for oestrus synchronisation.

The most common methods of oestrus synchronisation include; use of prostaglandin ($PGF_{2\alpha}$) or its analogues, progesterone (P4) or its analogues, P4 in combination with equine chorionic gonadotropin (eCG) and use of the ram effect (Wildeus, 2000; Abecia et al., 2012). It is important to note that oestrus synchronisation is commonly applied alongside fixed time artificial insemination (FTAI) which is an important tool for faster genetic progress with the potential to impact positively on production (Faigl et al., 2012). However, it has been reported that fertility in sheep following oestrus synchronisation and artificial insemination (AI) is lower compared to breeding at

natural oestrus and at natural service (Boscos et al., 2002; Donovan et al., 2004). The main factors responsible for the lower fertility include; inadequate timing of AI in relation to ovulation (Driancourt, 2001) and the method of AI used (Anel et al., 2005; Fair et al., 2005).

One of the ways by which fertility could be improved following oestrus synchronisation and AI is to improve the protocols used. Most recent research on P4 based protocols is focused on developing short-term protocols (Vilariño et al., 2013), whereas other protocols with reduced use of hormones (Ungerfeld, 2011, Meilán and Ungerfeld, 2014) or hormone free methods for synchronisation of oestrous (Martin et al., 2004; Scaramuzzi et al., 2014) have also been researched. Reasons for the above trend is that excessive use of hormones in food producing animals is both a consumer and environmental safety concern (Macrì et al., 2006; Martin and Kadokawa, 2006). In addition, short-term P4 treatment has been shown to result in better fertility compared to long-term P4 treatment (Johnson et al., 1996; Viñoles et al., 2001), whereas repeated use of eCG is associated with reduced fertility with subsequent breeding (Bodin et al., 1997; Drion et al.,2001b; Anel et al., 2005). Hence the short-term-P4 protocol combined with the ram effect could be a possible alternative to the conventional 12-14 d P4 treatment combined with eCG.

2.2 Seasonality and breeding in sheep

Most sheep breeds exhibit a seasonal pattern of breeding and are thus known as short day breeders because they breed in late summer-late winter when the period of day light shortens (Malpaux and Karsch, 1990; Rosa and Bryant, 2003; Cole and Cupps, 2013). During this period, ewes will display a pattern of subsequent oestrous cycles (polyoestrous), if they have attained puberty, are not pregnant or under lactational anoestrous (Rosa and Bryant, 2003). This period is known as the ovulatory or breeding period and is preceded by a period of arrest of ovulatory activity which is known as seasonal anoestrous (Rosa and Bryant, 2003). This

pattern of seasonal breeding ensures that lambs are born in spring and hence can survive better as they would be born at a time when the temperature is optimal and pasture resources are in abundance (Malpaux and Karsch, 1990). Changes in photoperiod therefore act as signals for animals to predict the availability of food at the time of parturition (Zarazaga et al., 2003; Malpaux, 2006).

Although photoperiod is the main signal controlling the onset of breeding, other signals for breeding may be nutritional, social/pheromonal or a combination (Rosa and Bryant, 2003). The effect of photoperiod is more pronounced in sheep breeds which originate from high latitudes or temperate regions (Rosa and Bryant, 2003) such as the Suffolk, Finnish Landrace and the Ile de France breeds. These breeds are highly seasonal, and so the timing of their annual reproductive cycle depends on changes in day length (Rosa and Bryant, 2003). On the other hand, breeds from low to intermediate latitudes such as the Australian merinos, SAMM and Menz breeds are either aseasonal or they exhibit a short anoestrous period (Mukasa-Mugerwa and Lahlou-Kassi, 1995; Rosa and Bryant, 2003). Hence the key determinant for breeding in these breeds is the pattern of rainfall in relation to food availability (Malpaux, 2006). These breeding patterns therefore call for a need to manipulate the oestrous cycle with hormonal, nutritional or pheromonal (ram effect) means. This would ensure better management of reproduction during the breeding season, and also stimulate ewes into breeding during the anoestrous period so that more lambs are produced per year.

2.3 The female oestrous cycle and hormonal control

The female oestrous cycle refers to the number of days between two consecutive periods of oestrus. It is characterised by a series of well coordinated events between the hypothalamus, anterior pituitary and the ovaries, which are controlled by hormonal and neural mechanisms (Bartlewski et al., 1999a; Smith and Jennes, 2001; Barrett et al., 2004). Briefly, the oestrous cycle is controlled by gonadotropin

releasing hormone (GnRH) produced by the hypothalamus, which stimulates the pituitary gland of the anterior pituitary to secrete hormones follicle stimulating hormone (FSH) and luteinising hormone (Rawlings and Cook, 1993). Large follicles secreting high levels of oestrogen (Rawlings and Cook, 1993; Bartlewski et al., 1999a) cause increased release of luteinising hormone (LH) and an LH surge (Leyva et al., 1998; Caraty and Skinner, 1999). The LH surge promotes final development and rapture of the follicle, which results in release of the oocyte. (Van Cleeff et al., 1998; Frandson et al., 2009). Whereas both FSH and LH are important for the recruitment and growth of follicles (McNeilly et al., 1990; Adams, 1999), FSH is specifically important at the early stage of the cycle for the recruitment of follicles, while LH is critical for the final growth of the dominant follicle and ovulation, (Bartlewski et al., 2011; Fatet et al., 2011).

In sheep, the oestrous cycle varies between 13 -19 d (Leyva et al., 1998; Rosa and Bryant, 2003). Researchers who studied several oestrous cycles in sheep found that a higher percentage of ewes had a cycle of about 17 d (Mukasa-Mugerwa and Lahlou-Kassi, 1995; Lopez-Sebastian et al.,1997; Ravindra and Rawlings, 1997), with a few ewes having short (13-15 d) and others longer (more than 17d) cycles (Lopez-Sebastian et al., 1997). The oestrous cycle is divided into 2 phases, the follicular and the luteal phase (Souza et al., 1997; Fatet et al., 2011), with the follicular phase ranging from day 14-1, (day 0 = oestrus), while the luteal phase extends from day 2-13 (Bearden and Fuquay, 1992). The follicular phase commences with proestrus, a stage where growth of the preovulatory follicle (s) occurs (Karaca et al., 2008; Fatet et al., 2011). It is followed by oestrus, a stage of sexual receptivity which involves final growth and maturation of the preovulatory follicle (s), as well as ovulation (Fatet et al., 2011). The luteal phase on the other hand commences with metoestrus, a stage where development of the corpus luteum (CL) occurs (Leyva et al., 1998; Niswender et al., 2000; Fatet et al., 2011). Dioestrus follows metoestrus and it is during this stage that the CL produces high levels of

progesterone, and will regress towards the end if a pregnancy is not established (Cole and Cupps, 2013).

2.3.1 Follicular phase

The follicular phase begins around the time of luteolysis, when progesterone concentration decreases (Gonzalez-Bulnes et al., 2005a). As the phase advances, follicles ≥ 2mm in diameter are recruited (Rubianes and Menchaca, 2003; Cueto et al., 2006). Follicles develop early at the foetal stage and at birth, thousands of primary follicles await recruitment to continue their development and to be selected further during a specific oestrous cycle (Driancourt et al., 1990; Rawlings et al., 2003). In sheep, follicles of various sizes can be found on the surface of the ovary at any stage of the oestrous cycle (Souza et al., 1997; Bister et al., 1999).

2.3.1.1 Patterns of follicle growth

When studied through ultrasonography, ovarian follicles are seen to follow a wavelike pattern of growth (Ginther et al., 1995; Lopez-Sebastian et al., 1997; Evans et al., 2000). A wave refers to the emergence and synchronous growth of a group of antral follicles (Adams, 1999; Vilariño et al., 2013), where one or two follicles continue to grow to a diameter of 3 mm or more (Viñoles and (Rubianes, 1998; Menchaca et al., 2004; Vilariño et al., 2013). The selected follicle (s) will either become atretic or proceed to ovulation (Leyva et al., 1998). The number of waves is variable, with three to four waves commonly occurring during the oestrous cycle (Ginther et al., 1995; Leyva et al., 1998; Viñoles, 2000) marked by peaks of oestradiol (Wathes and Hamon, 1993). Wave 1 emerges around the day of ovulation (Leyva et al., 1998), with other waves emerging every 4-7 d depending on the number of waves (Leyva et al., 1998; Bister et al., 1999; Seekallu et al., 2010). In their study of follicular dynamics following a 12 d treatment with medroxyprogesterone acetate (MAP) intravaginal sponges, Leyva et al. (1998)

observed that most ewes had 3 waves, with a new wave emerging every 5-5.5 d. Emergence of follicles occurs following an increase in secretion of FSH (Ginther et al., 1995; Bister et al., 1999; Evans et al., 2002), and is followed by selection of the ovulatory follicle, based on size, ability to suppress other follicles as well as the ability to secrete high amounts of oestradiol (Johnson et al., 1996). Hence during follicular growth, follicles either undergo atresia or proceed to ovulation (Leyva et al., 1998).

2.3.1.2 Follicular growth and dominance

After selection, ovulatory follicle (s) will continue growing and become dominant (Bister et al., 1999; Gonzalez-Bulnes et al., 2005b). In a study by Gonzalez-Bulnes et al. (2005b), the dominance effect was expressed during the follicular phase of ewes treated with progestagen, where an increase in diameter of the largest follicle occurred as number of smaller follicles decreased following withdrawal of the insert. This dominance effect is of importance based on the study by Gonzalez-Bulnes et al. (2005b), who observed that ovulation failed to occur in ewes where dominance effect was not expressed. To maintain dominance, the ovulatory follicle will secrete inhibin and thus suppress the growth of smaller follicles which will become subordinate and undergo regression/atresia (Evans et al., 2000; Gonzalez-Bulnes et al., 2005b; Cueto et al., 2006). However, some studies in sheep (Driancourt, 1994; Letelier et al., 2009) and in goats (Gonzalez-Bulnes et al., 2005a) have demonstrated a lack of inhibitory effect of the dominant follicle on the growth of the smaller follicles. New follicles have been observed to emerge, increase in size, and finally ovulate in presence of a large follicle (Johnson et al., 1996; Evans et al., 2001), which aspect does not occur in cattle (BK et al., 1995).

2.3.1.3 Number and diameter of preovulatory follicles

Number of follicles selected is determined by the ovulation rate and this varies based on breed and/or individual animal (Scaramuzzi et al., 1993; Driancourt, 2001). A high rate of atresia in nonprolific sheep breeds such as the Merinos limits ovulation rate (Scaramuzzi et al., 1993; Driancourt, 2001). A rapid turnover of the largest follicle was observed in the Merino del pais breed during the oestrous cycles studied (Lopez-Sebastian et al., 1997). However, in prolific breeds such as the Finnish Landrace and Romanov breeds (BK et al., 1995), it is common to have more than 3 different follicles growing to the size of the largest follicle during the oestrous cycle (Driancourt, 2001).

Size of the dominant follicle (s) does not change much before oestrus but increases significantly on the day of oestrus (Lopez-Sebastian et al., 1997). In their study, Lopez-Sebastian et al. (1997) observed that size of the dominant follicle did not change much during the last 7 d before oestrus, but significantly increased on the day of oestrus. In addition, as the follicular phase advanced, the number of large follicles increased whereas a steady decline in number of small and medium follicles was observed as oestrus approached, which observation was also evident in the study of Gonzalez-Bulnes et al. (2005b). Both number and size of the largest follicle influence fertility (Gonzalez-Bulnes et al., 2005b; Reyna et al., 2007). In the study by Gonzalez-Bulnes et al. (2005b), the number of medium follicles decreased with an increase in number of large follicles from 5.2 ± 0.2 to 6.6 ± 0.2 in ewes which ovulated. Additionally, the number of fertilised ova tended to be higher in ewes which had a high number and size of the largest follicle.

Size of the follicle is related to the amount of oestradiol produced (Gonzalez-Bulnes et al., 2005b; Letelier et al., 2009), although it is also reported that sheep breeds with high ovulation rates maintain small sizes of the preovulatory follicles with altered functionality (Driancourt et al., 1991; Driancourt et al., 1996). Large follicles

generally produce large amounts of oestradiol, and also result in production of high amount of progesterone by the CL formed after ovulation (Gonzalez-Bulnes et al., 2005b). In their study, Gonzalez-Bulnes et al. (2005b) observed that ewes which had a lower concentration of progesterone after ovulation registered more degenerated embryos. Similarly, deficiency in the secretion of oestradiol during the preovulatory phase affects the ability of the ovulated oocyte to be fertilised and to develop into a viable embryo (Gonzalez-Bulnes et al., 2005b). Oestradiol has a role in inducing secretory cells of the oviduct which in response secrete an estrus associated glycoprotein (oEGP) around the time of estrus (Murray, 1992; Nancarrow and Hill, 1994). This glycoprotein is crucial for fertilisation and embryo development (Nancarrow and Hill, 1994).

2.3.1.4 Oestrus, LH surge and ovulation

As follicles grow, they begin to secrete estrogens, which act on them to increase FSH and LH receptors (McNeilly et al., 1990). FSH and LH together further promote follicular growth and secretory capacity, hence forming a local positive effect (Bearden and Fuquay, 1992; Frandson et al., 2009). Oestrogens also enter the systemic circulation and affect other sites in the body, which leads to behavioural oestrus (Frandson et al., 2009). They stimulate increase in vascularity of the vulva which results in swelling and reddening, followed by discharge of mucus and thickening of the vaginal wall in preparation for copulation (Bearden and Fuquay, 1992; Hafez and Hafez, 2000). On the other hand, oestrogens circulating in the blood have a negative feedback on FSH secretion from the anterior pituitary but promote an increase in LH secretion (Menegatos et al., 2003; Letelier et al., 2011). Presence of large follicles producing high levels of oestrogen increases the LH pulse frequency and leads to an LH surge (Hafez and Hafez, 2000; Frandson et al., 2009). The LH surge will promote final development and rapture of follicle, which results in ovulation (Frandson et al., 2009; Letelier et al., 2011).

2.3.2 Luteal phase

The luteal phase begins after ovulation, by the action of LH on granulosa cells of the raptured follicle (s), which transform from oestrogen producing cells to progesterone producing cells called luteal cells (Niswender et al., 2000). This phenomenon is known as luteinisation, and it results in formation of the CL (Niswender et al., 2000; Frandson et al., 2009), an ovarian structure which produces progesterone (Boscos et al., 2002). In their study, Johnson et al. (1996) observed that the CL appeared 7 d after oestrus, whereas Bartlewski et al. (1999b) detected the CL by day 5 after ovulation and reported it to have a lifespan of 11.6 ± 1.1 d.

The concentration of progesterone increases as the CL increases in size during the early to the mid luteal phase (Leyva et al., 1998; Vilariño et al., 2013). High levels of progesterone produced by the CL inhibit further secretion of LH from the anterior pituitary through negative feedback to the hypothalamus (Souza et al., 1997). If conception has occurred, maternal recognition of pregnancy takes place, which prevents regression of the CL so that it continues producing progesterone (Frandson et al., 2009). The mechanism involves release of embryonic secretory products by the embryo, hence inhibiting uterine secretion of $PGF_{2\alpha}$, which would otherwise cause regression of the CL (Hafez and Hafez, 2000; Frandson et al., 2009).

When a pregnancy is established, progesterone will inhibit further oestrous cycles, increase uterine gland secretion and inhibit uterine motility in order that implantation occurs, and pregnancy is maintained (Frandson et al., 2009). However, if conception does not occur, the CL will regress, followed by a reduction in the concentration of progesterone (Bearden and Fuquay, 1992; Frandson et al., 2009), which stimulates follicular growth. Gradual reduction in secretion of progesterone is said to occur at 11-13 d of oestrus (Leyva et al., 1998; Niswender et al., 2000), while structural luteolysis in the study by Leyva et al. (1998) occurred at 14 d of the oestrous cycle.

The different stages of the oestrous cycle and hormonal control are illustrated in Fig.2.1.

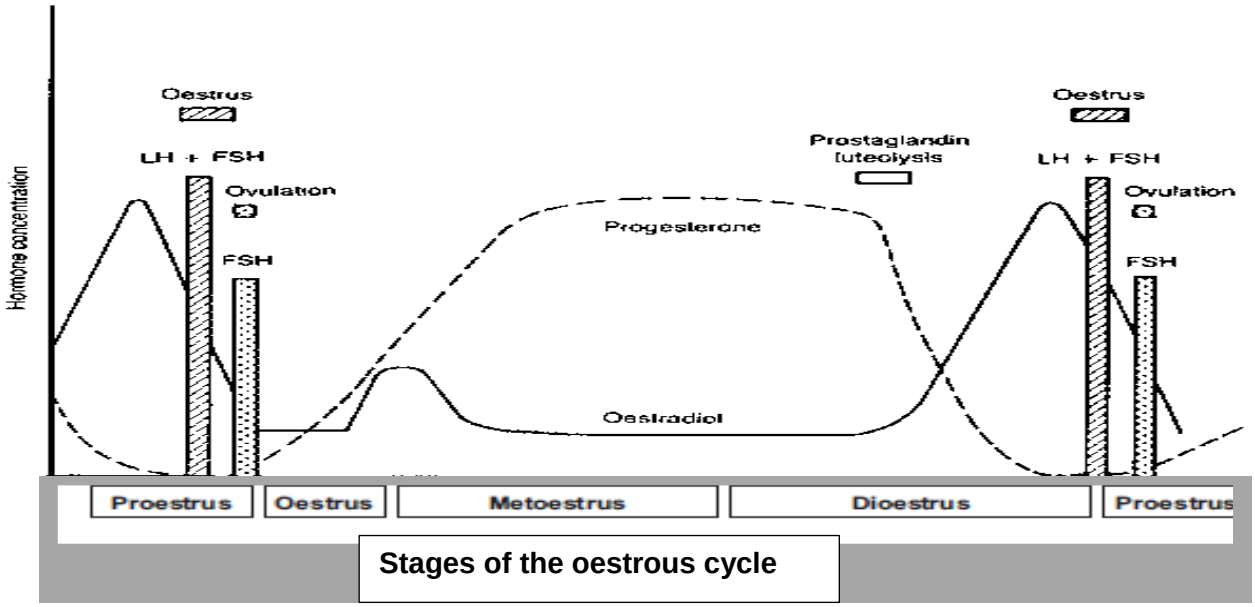

Fig. 2.1. Illustration of different stages of the oestrous cycle and hormonal control (Peters and Lamming 1983; Fatet et al., 2011). Luteinising hormone (LH), folllicle stimulating hormone (FSH).

2.4 Oestrus synchronisation

Oestrus synchronisation is a tool for management of reproduction (Haresign, 1992; Wildeus, 2000). It involves treatment of animals with exogenous hormones (Abecia et al., 2012) or use of hormone free treatments (Martin et al., 2004, Scaramuzzi et al., 2014). Thus, the luteal phase of the oestrous cycle can be manipulated either by prolonging it with P4 or by shortening it with $PGF_{2\alpha}$ (Wildeus, 2000; Abecia et al., 2012). The oestrous cycle can also be manipulated by light control (Pellicer-Rubio et al., 2008) or treatment with melatonin during the anovulatory season (Nowers et al., 1994; Uslu et al., 2012). Other common applications for oestrus synchronisation involve the use of gonadotropins and/or the ram effect in combination with P4 or $PGF_{2\alpha}$ based protocols (Evans et al., 2004; Hawken et al., 2005; Ungerfeld, 2011).

The ram effect can also be applied without prior hormonal treatment (Hawken et al., 2005; Scaramuzzi et al., 2014).

2.4.1 Methods of oestrus synchronisation

The most common methods of oestrus synchronisation involve use of P4 and/or $PGF_{2\alpha}$ used combination with or without eCG or the ram effect (Wildeus, 2000; Abecia et al., 2012).

2.4.1.1 Prostaglandin or its analogues

The effectiveness of $PGF_{2\alpha}$ depends on presence of the CL or refractoriness of the existing CL (Leyva et al., 1998; Abecia et al., 2012). Hence use of prostaglandin is only recommended during the ovulatory season (Gonzalez-Bulnes et al., 2005b) or during early to mid-luteal phase when the CL is considered to be less refractory and hence responsive to the luteolytic effect of $PGF_{2\alpha}$ (Rubianes et al., 2003; Contreras-Solis et al., 2009). However, in tropical sheep breeds with all year round breeding, $PGF_{2\alpha}$ can be administered at any time of the year, with 2 injections given 7-14 d apart (Gonzalez-Bulnes et al., 2005b; Abecia et al., 2012; Fierro et al., 2016). At the second injection, most ewes have a responsive CL and therefore regression will be induced and consequently rapid follicular growth, oestrous and ovulation will follow (Godfrey et al., 1997; Gonzalez-Bulnes et al., 2005b; Contreras-Solis et al., 2009).

Although some studies have found fertility following treatment with $PGF_{2\alpha}$ to be lower than treatment with P4 (Barrett et al., 2002; Olivera-Muzante et al., 2011), $PGF_{2\alpha}$ has the advantage of improving welfare of the animals as it does not cause discomfort as would be the case with intravaginal devices i.e. handling during intravaginal insertion, frequent monitoring for insert retention, handling at insert withdrawal and possible vaginitis or related complications (Fierro et al., 2013). In addition, unlike P4 which is associated with deposits of residues in food products (Wheaton et al., 1993; Rowe et al., 2010; Reyes et al., 2012), $PGF_{2\alpha}$ is metabolised

at a fast rate and completely (Light et al., 1994). Furthermore, there is a risk of liberation of sex steroids to the environment when P4 inserts are disposed of after use (Martin and Kadokawa, 2006).

2.4.1.2 Progesterone or its analogues

Progesterone/progestagens are the most commonly used treatments in oestrus synchronisation programmes because they can be used both in the breeding as well as the non-breeding season, and they are also readily available on the market (Wildeus, 2000; Abecia et al., 2012). Exogenous P4 mimics the action of natural P4 produced by the CL after ovulation, by increasing plasma P4 concentration during treatment (Rubianes et al., 1996; Husein and Kridli, 2002). High levels of P4 suppress the LH pulse frequency and follicular growth (Rubianes et al., 1996; Flynn et al., 2000) through negative feedback to the hypothalamus (Scaramuzzi et al., 1993). Once the device is withdrawn, increase in FSH and LH secretion induces recruitment of a new wave of follicular growth (Campbell et al., 1999; Evans, 2003; Driancourt, 2001). Consequently, the development of a new dominant follicle will be synchronised in all females, which will ovulate at a predetermined time depending on the protocol used (Driancourt, 2001; Vilariño et al., 2013).

Presence of a viable dominant follicle at the time of device withdrawal will lead to it being selected to become the preovulatory follicle, hence it will continue growing to its maximum size and later ovulate (Flynn et al., 2000). Hence the variability in the interval between device withdrawal and oestrus arises from the difference in the wave from which the preovulatory follicle has originated (Viñoles and Rubianes, 1998). Synchronising follicular wave development is therefore necessary to reduce variability in time to oestrus which is often accomplished by addition of gonadotropins and/or the ram effect to a protocol (Rajamahendran and Raniowski, 1993; Barrett et al., 2004; Evans et al., 2004). Hence the variability in the interval between device withdrawal and oestrus arises from the difference in the wave from which the preovulatory follicle has originated (Viñoles and Rubianes, 1998).

Synchronising follicular wave development is therefore necessary to reduce variability in time to oestrus which is often accomplished by addition of gonadotropins and/or the ram effect to a protocol (Rajamahendran and Raniowski, 1993; Barrett et al., 2004; Evans et al., 2004).

2.4.1.3 Different sources of progesterone or progestagens and combinations with prostaglandin

Sponges i.e. MAP and flurogestone acetate (FGA) or controlled internal drug release (CIDR) are the most popular treatments for oestrus synchronisation in sheep (Greyling et al., 1997; Martemucci and D'Alessandro, 2011; Swelum et al., 2015). Various studies have reported similar fertility following use of CIDR, FGA or MAP in cyclic ewes (Fukui et al., 1993; Luther et al., 2007), and similar oestrous response (Ungerfeld and Rubianes, 2002; Hashemi et al., 2006), as well as fertility (Ungerfeld and Rubianes, 2002) in anoestrous ewes. It is believed that the rate at which P4 is released from the different sources during treatment does not differ (Luther et al., 2007). However, earlier onset of oestrus in ewes following treatment with CIDR (Fukui et al., 1993) as well as higher oestradiol concentration, conception rate (CR) and lambing rates than sponges have been reported (Swelum et al., 2015).

Moreover, it has been reported that intravaginal sponges alter the characteristics of cervical mucus, which affects the viability of ram spermatozoa (Manes et al., 2016), and which may be responsible for the lower CR which has been reported in sponges compared to CIDR (Swelum et al., 2015). Treatment with sponges has also been shown to affect sperm transport and survival, due to residues of progestagens left on the vaginal wall following withdrawal of the sponge (Greyling et al., 1997). In addition, sponges are associated with high bacterial load which causes vaginitis, thus affecting fertility (Manes et al., 2016).

Some P4 based protocols have $PGF_{2\alpha}$ administered at the beginning of device insertion (Martemucci and D'Alessandro, 2011) to guarantee that ewes ovulate follicles of the same age, when new follicles are recruited in a synchronous manner following luteolysis (Evans et al., 2001). On the other hand, when $PGF_{2\alpha}$ is administered at the end of a P4 based protocol, (Dixon et al., 2006; Ali, 2007; Vilariño et al., 2013), it eliminates the CL, thus the period over which the animal is exposed to progesterone is reduced, which would otherwise interfere with LH release and prolong the onset of events of the follicular phase (Husein and Kridli, 2002).

2.4.1.4 Duration of treatment with progesterone or progestagens

Long-term P4 treatments of 12-14 d are reported to have detrimental effects on follicular dynamics, especially towards the end of the treatment period when the concentration of P4 acquired from the intravaginal device is low (Greyling et al., 1994; Ungerfeld and Rubianes, 1999a). A low concentration of P4 during treatment leads to an increase in the LH pulse frequency, although these subluteal P4 levels do not result in an LH surge (Flynn et al., 2000; Menchaca and Rubianes, 2004). Consequently, the turnover of follicles will be affected, with the largest follicle (s) remaining dominant for a longer period and hence will be older when ovulation occurs (Johnson et al., 1996; Flynn et al., 2000; Viñoles et al., 2001). Therefore, presence of large follicles on the day of insert withdrawal is an indication of follicles having grown faster under low P4 concentration and are thus considered to be older (Johnson et al., 1996; Flynn et al., 2000).

Age of the follicle in respect to ovulation is the number of days from emergence of the follicle to ovulation (Johnson et al., 1996; Vilariño et al., 2013). In their experiment, Johnson et al. (1996) observed that ewes with low concentration of P4 (< 1 ng/ml) had large ovulatory follicles which remained dominant for more days (Johnson et al., 1996). In addition, peripheral concentration of oestradiol in these ewes was higher and first service CR was lower than in ewes whose P4

concentration was high during the treatment period (Johnson et al., 1996). A high concentration of P4 during treatment suppresses LH secretion and pulse frequency (Driancourt, 2001; Viñoles et al., 2001), hence inducing a faster follicle turnover (Driancourt, 2001). In this case, regression of the largest follicle is induced early and emergence of the next follicular wave is accelerated, which results in the ovulation of a young and high quality follicle (Rubianes et al., 1996). Therefore, this indicates that short-term P4 treatments which ensure high P4 concentration throughout the treatment period result in better control of follicular dynamics than long-term treatments, hence resulting in better CR (Menchaca and Rubianes, 2004).

2.4.1.5 Progesterone in combination with eCG

Progesterone/progestagens are more effective when combined with gonadotropins as they provide a more precise synchrony of oestrus and consequently ovulation in the flock (Driancourt, 2001; Vilariño et al., 2010). Equine chorionic gonadotropin is the most widely used gonadotropin for improvement of fertility in sheep (Abecia et al., 2012), given that use of other gonadotropins such as FSH have limitations related to the process of preparation and the cost (Boscos et al., 2002). Equine chorionic gonadotropin is a glycoprotein hormone secreted by the trophoblastic cells of a pregnant mare and circulates in the blood around 40 –120 d of pregnancy (Noronha and Antczak, 2010). It is said to increase ovulation rate by reducing the rate of atresia of preovulatory follicles (Bister et al., 1999), which results in more lambs born when high doses of eCG (450-600 IU) are used (Zaiem et al., 1996; Husein and Kridli, 2002). It has an outstanding capacity to function as FSH and LH even in other species (Murphy and Martinuk, 1991; Stewart and Allen, 1995). For this reason, it has been used in oestrus synchronisation programmes to improve reproductive performance.

Ewes treated with eCG at device withdrawal demonstrate a shorter interval to oestrus and ovulation (Barrett et al., 2004; Ali, 2007), and also have higher ovulation rates than untreated ewes (Johnson et al., 1996; Boscos et al., 2002; Luther et al.,

2007). Assessment of increased ovulation rate following eCG treatment as determined by the number of embryos 25 d post mating and the number of lambs born was higher in eCG treated ewes compared to ewes which were not treated (Johnson et al., 1996). In addition, the study of Boscos et al. (2002) which compared eCG with FSH treatment (10 IU), found that ewes treated with eCG had the highest mean number of CL per ewe ovulating (2.8 ± 0.2 versus 2.1 ± 0.3). Equine chorionic gonadotropin is often administered at withdrawal of P4 insert, with the dose varying from 250-500 IU, depending on age (250-300 IU for ewe lambs, 350-500 IU for adult ewes), season (400-500 IU during the anoestrous season, 300-350 IU during the breeding season and breed (lower dose for prolific breeds; Husein and Kridli, 2002). The combination of P4 with eCG has also achieved high pregnancy rates in cattle (Macmillan and Peterson, 1993).

2.4.1.6 Use of the ram effect

The ram effect is a sexual stimulus released by a sexually active ram, which stimulates reproductive activity in ewes (Iglesias et al., 1991). The ram effect involves modulation of the gonadotropin-pituitary axis by pheromones and /or socio-sexual stimuli which interact to induce reproductive activity in ewes via neural pathways (Delgadillo et al., 2009). Pheromones are chemical substances released by animals which cause specific reactions when received by another animal of the same species (Cohen-Tannoudji et al., 1994). Pheromonal stimulus reaches the hypothalamus through the nasal epithelium along the main olfactory bulb or the vomeronasal organ and accessory olfactory bulb (Delgadillo et al., 2009). This was confirmed by the fact that exposure of ewes to the ram effect induced activation of neurones in both the main and accessory olfactory bulbs (Gelez and Fabre-Nys, 2006).

Mediation of the ewe's reproductive axis by the ram via pheromones is an established phenomenon and has been demonstrated in studies where wool or wool extracts applied onto the nose of anoestrous ewes increased LH and FSH secretion

as well as number and size of follicles (Signoret, 1991; Cohen-Tannoudji et al., 1994). In these studies, the LH surge and ovulation also occurred in some ewes. Nevertheless, research over the years has established that the male effect is not purely controlled by pheromones (Vielma et al., 2005; Rivas-Muñoz et al., 2007). Stimuli involved in the male effect are: olfactory, auditory, visual and tactile (Delgadillo et al., 2009). Physical presence of a sexually active male among females produces a greater response since it combines both the stimulus from pheromones and sexual behaviour (Iglesias et al., 1991). Hence the complete socio-sexual stimulus which includes visual-audio and tactile signals such as mounting, kicking and leaning over is important for inducing a greater response in ewes (Iglesias et al., 1991; Delgadillo et al., 2009; Hawken et al., 2009).

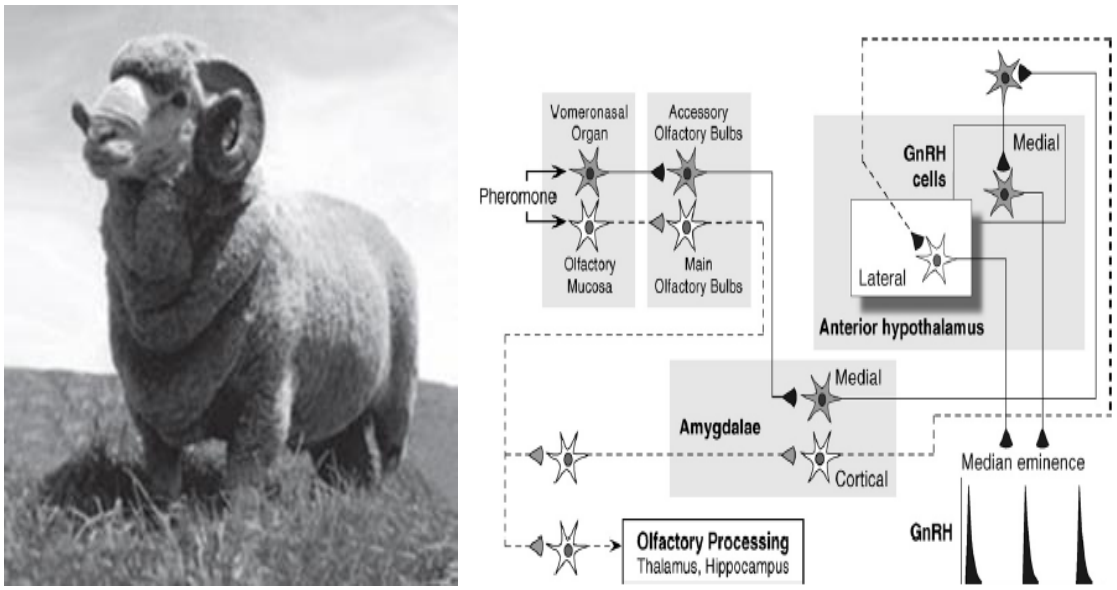

Fig.2.2. Illustration of the pathway of pheromones to the hypothalamus (Delgadillo et al., 2009). Gonadotropin releasing hormone (GnRH).

This was demonstrated in an experiment where both awake and sedated males caused an initial increase in LH secretion which only lasted up to 24 h with sedated males (Vielma et al., 2005). Therefore, the ability of a male to induce a greater

response in ewes depends on the intensity of sexual behaviour exhibited, male libido as well as pheromone production, which aspects depend on the concentration of circulating androgens (Perkins and Fitzgerald, 1994; Delgadillo et al., 2009). Ewes have also been exposed to rams with differing levels of libido, where by high libido rams induced ovulation in 95% of ewes, whereas low libido rams only induced 78% of ewes (Perkins and Fitzgerald, 1994). A high incidence of ovulation has also been reported in anovular Corriedale ewes in physical contact with rams exhibiting high intensity of sexual behaviour (Knights et al., 2001). Hence it is important to consider levels of sexual activity of rams when selecting candidates for vasectomy to be used in the stimulation of reproductive activity in females. This is particularly crucial for AI programmes since rams which exhibit high sexual activity induce a greater reproductive response in ewes.

Auditory stimuli such as vocalisations which sexually active rams emit during courtship also contribute to the male effect (Rivas-Muñoz et al., 2007). This is supported by the observation that “real time” vocalisations from sexually active males transmitted through a loud speaker stimulated behavioural oestrus in anoestrous ewes (Vielma et al., 2005). Although 5 out of 6 anoestrous females exhibited behavioural oestrus, only 2/5 females ovulated, which indicates that vocalisations from sexually active males alone are not sufficient to induce a full reproductive response in ewes and therefore cannot substitute the complete socio-sexual stimuli required to stimulate maximum reproductive response (Vielma et al., 2005).

It is presumed that the ram effect to acts by overcoming the negative feedback exerted by oestradiol (Tilbrook et al., 1991). When the ram effect is applied to anovular ewes with no prior treatment for synchronisation of oestrus, the immediate response to ram introduction is an increase in LH secretion, which may culminate in an LH surge and ovulation (Ungerfeld et al., 2004). The first ovulation is often silent and is not preceded by oestrus hence it is not fertile (Ungerfeld and Rubianes,

2002). This is followed by a short luteal phase (4-5 d) caused by early regression of the CL (Chemineau et al., 1993; Lassoued et al., 1997), which occurs as a result of reduced lifespan of the ram-induced CL caused by premature induction of $PGF_{2\alpha}$ in response to low progesterone concentration (Lassoued et al., 1997). A second ovulation without oestrus occurs, followed by a luteal phase of normal length, oestrus and ovulation (Cushwa et al., 1992; Ungerfeld et al., 2004).

On the other hand, when applied to randomly cycling ewes, the ram effect induces an increase in LH secretion (Hawken et al., 2007), which may culminate in oestrus, an LH surge and ovulation (Martin et al., 2004). In addition, the ram effect has been observed to induce oestrus (Contreras-Solis et al., 2009; Ungerfeld and Rubianes, 1999b; Ungerfeld, 2011) increase the LH pulse frequency (Hawken et al., 2007) and advance ovulation (Contreras-Solis et al., 2009) in ewes synchronised with P4 or $PGF_{2\alpha}$ during the breeding season. Various studies have demonstrated that incorporating the ram effect into protocols for oestrus synchronisation results in high reproductive response (Evans et al., 2004; Contreras-Solis et al., 2009; Ungerfeld, 2011), therefore the ram effect has been introduced at different times during oestrous synchronisation (Romano et al., 2000; Evans et al., 2004), and also used in combination with gonadotropins (Evans et al., 2004) to achieve a high response in ewes. A reduced interval to onset of oestrus, LH surge and ovulation (26.4 ± 0.9 vs 28.8 ± 0.8; 30.2 ± 1.4 vs 36.6 ± 2.5; 56.2 ± 0.7 vs 60.4 ± 0.4) respectively have been observed when the ram effect was added to a protocol with progestagen treatment and eCG (Evans et al., 2004). Ungerfeld et al. (2005) also obtained a higher CR (87.5% vs 76.5%), among ewes exposed to the ram effect compared to unexposed ewes bred naturally following a 12 d priming with MAP and immediate ram exposure at sponge withdrawal during the mid-breeding season.

Immediate exposure of ewes to the ram effect following device withdrawal induces a greater reproductive response in ewes when the ram is left continuously with the ewes (Ungerfeld and Rubianes, 1999b; Romano et al., 2000). Ewes treated with

MAP impregnated sponges and exposed to the ram continuously from the time of sponge withdrawal had a mean oestrus onset of 32.9 h compared to 53.2 h for ewes exposed to the ram 48 h after sponge withdrawal (Romano et al., 2000). In their experiment (Romano et al., 2000) observed that 95.6% of the ewes exposed to the ram continuously from the time of sponge withdrawal were in oestrus at 48 h compared to 63.4% of ewes exposed 48 h after sponge withdrawal. This suggests that if breeding at FTAI had been performed, a greater proportion of ewes exposed continuously to the ram effect at sponge withdrawal would have conceived compared to ewes exposed at 48 h after sponge withdrawal. In studies where the ram effect has been used in a protocol with $PGF_{2\alpha}$, 2 injections were administered 10-13 d apart, and the ram effect applied coincidentally with the second $PGF_{2\alpha}$ injection (Contreras-Solis et al., 2009; Ungerfeld, 2011).

The ram effect has also been observed to induce oestrus in peripubertal ewe lambs (Donovan et al., 1991). It is recommended that ewes are isolated for a period of at least one month (out of sight, smell and sound; minimum 1km) prior to ram introduction as it results in a greater response when the ram is introduced (Ungerfeld and Rubianes, 1999b) However, if the rams are novel, isolation may not be necessary, as the stimulus is only rendered ineffective when rams used for teasing have been in close contact with the ewes and are therefore familiar (de St Jorre et al., 2012).

2.5 Artificial insemination in sheep

Artificial insemination (AI) is an important tool for faster genetic progress in animals, which was developed over 50 years ago in the Soviet Union and is now being applied in the breeding of sheep in other of the world (Donovan et al., 2004; Luther et al., 2007; Viñoles et al., 2011). It is often applied in combination with oestrus synchronisation (Donovan et al., 2004; Viñoles et al., 2011; Vilariño et al., 2017), especially with protocols where FTAI is used (Godfrey et al., 1999; Luther et al.,

2007; Vilariño et al., 2013). Hence it eliminates the need to observe for oestrus which creates more efficiency in breeding.

Artificial insemination makes it possible to use superior sires for genetic improvement and also to reduce the cost of purchasing and maintaining breeding rams. Furthermore, the use of superior sires under AI programmes ensures that they produce many offsprings and hence spread good genes across a wider range. The use of AI also ensures that lambing is batched, which reduces incidences of high lamb mortality associated with handling many lambs at a time. With the use of AI, accidents which result from ram fights and low CR due to poor fertility of rams can be avoided. In addition, the behaviour of preference of rams and ewes to specific breeding mates which would reduce CR is also done away with. Moreover, ewes which are small or incapacitated, as well as maiden lambs can be bred with much ease, as well as avoid inbreeding (Faigl et al., 2012).

2.5.1 Fertility in sheep following oestrus synchronisation and artificial insemination

It has been observed that fertility is lowered when breeding is done at induced oestrus compared to breeding at natural oestrus and at AI compared to natural service (Boscos et al., 2002). Factors which affect fertility following synchronisation of oestrus and AI include; reproductive failure due to improper timing of AI in relation to ovulation (Driancourt, 2001). Poor synchronisation of ovulation results from failure to achieve synchrony of follicular wave development, which means that dominant follicles will be at different stages of development at insert withdrawal (Driancourt, 2001). Therefore, P4 treatments have been used in combination with $PGF_{2\alpha}$, eCG and the ram effect to improve synchrony of oestrus and ovulation (Evans et al., 2004; Ali, 2007; Vilariño et al., 2010).

In addition, treatments with P4 are known to alter hormonal profiles (Gonzalez-Bulnes et al., 2005b) and the properties of cervical mucus (Manes et al., 2016), all

which have a bearing on fertility. Ram factors such as the fertility of breeding rams have also been shown to influence the fertility of females following oestrus synchronisation and AI (Gil et al., 2003; Donovan et al., 2004). Certain rams perform better than others when used in oestrus synchronisation programmes and AI, therefore it is important that rams used for AI following oestrus synchronisation are carefully selected (Donovan et al., 2004). If the quality of semen collected is insufficient, this may result in fewer females being served in order to obtain high conception rates (Paulenz et al., 2004). On the other hand, volume of semen collected per breeding ram as well as quality could be affected when the electro ejaculation method is used for semen collection as compared to the artificial vagina method (Marco-Jiménez et al., 2005; Marco-Jiménez et al., 2008). Hence to maintain the best samples for AI, semen is usually pooled (Paulenz et al., 2002) if it does not affect the breeding programme. It is also important to note that use of sexed semen in oestrus synchronisation programmes is not a common practice, given that egg quality and hence fertility in females is reduced following synchronised than with spontaneous oestrus (Donovan et al., 2004; Bodmer et al., 2005).

Other factors such as poor semen handling/insemination technique (Faigl et al., 2012), nutritional status of the ewe at breeding (Scaramuzzi et al., 2014) and exposure of females to stress conditions such as high temperatures (Hansen, 2009) have also been reported to affect fertility. Furthermore, efficiency of AI following oestrus synchronisation is affected by the method of AI (Anel et al., 2005; Fair et al., 2005) which most often is dictated by the type of semen used i.e fresh undiluted, fresh diluted, chilled or cryopreserved/frozen-thawed (Donovan et al., 2004). It has also been demonstrated in the study by Fair et al. (2005) that type of breed can affect fertility by its influence on the transport of spermatozoa across the reproductive tract.

2.5.2 Methods of artificial insemination

Methods of AI are defined by the site of deposition of spermatozoa, and/or gadgets used in the process. The methods are; vaginal, cervical, transcervical intrauterine and laparoscopic intrauterine (Faigl et al., 2012).

2.5.2.1 Vaginal

Vaginal insemination involves depositing fresh semen deep into the vagina of the ewe without attempting to locate the cervix (Faigl et al., 2012). Conception rates are often lower with this method compared to cervical AI (Faigl et al., 2012). The volume of semen and number of progressively motile spermatozoa recommended for use under this method are 0.2 ml and 400 × 10^6 (minimum), respectively (Faigl et al., 2012).

2.5.2.2 Cervical

Artificial insemination via the cervix with fresh semen is the most practical method of AI, given the ease of application, coupled with the relatively good fertility results obtained when employed (Greyling et al., 1997; Zeleke et al., 2005; Contreras-Solis et al., 2009). Cervical AI is performed by inserting a speculum fitted with a light source into the cervix to a depth of 5-12 mm (Faigl et al., 2012). After locating the mouth of the cervix and positioning the catheter through the speculum, semen is deposited through (Faigl et al., 2012). Alternatively, specialised equipment has been developed for small ruminants to enable depositing of semen deep into the cervix (Intracervical; Donovan et al., 2004; Houdeau et al., 2008; Fierro et al., 2017). This method has been used to improve CR following AI with frozen-thawed semen (Donovan et al., 2004), since fresh/chilled semen has a short shelf life. Fresh undiluted fresh diluted (Donovan et al., 2004; Zeleke et al., 2005) and chilled (Greyling et al., 1997) semen have all been used under the cervical method, and have produced adequate CR. The volume of fresh semen and number of motile

spermatozoa recommended for insemination are 0.2 ml and 200 × 10^6 (minimum) respectively (Faigl et al., 2012).

Use of frozen-thawed semen under the cervical AI method is associated with lower fertility (Donovan et al., 2004; Fair et al., 2005). This is due to the fact that a limited number of viable spermatozoa reach the uterine body, since most of them are damaged during cryopreservation and thawing (Salamon and Maxwell, 1995). However, addition of seminal plasma to frozen-thawed spermatozoa can overcome effects of impaired function caused by cryopreservation, which would improve fertility after cervical AI (Maxwell et al., 1999). In their study, (Donovan et al., 2004) obtained low conception rates of 52% and 37% using frozen-thawed semen collected and frozen in Ireland and Norway respectively at synchronised AI (Donovan et al., 2004), although a better CR of 58% had been earlier obtained in Norway on a bigger flock using cervical AI with frozen-thawed semen (Olesen, 1993). Development of cryopreservation, thawing and non-surgical AI techniques to further improve fertility with the use of frozen-thawed semen is therefore necessary since the quantity of semen which can be obtained from a ram at a given time is limited, (Medeiros et al., 2002).

2.5.2.3 Laparoscopic- intrauterine

With laparoscopic-intrauterine AI, semen is deposited into the uterus and is performed with the aid of a laparoscope (Gourley and Riese, 1990). Unlike other animal species, ewes have a long cervical canal of approximately 6–7 cm (Faigl et al., 2012), with a closed cervix which limits penetration with a catheter or insemination pipette via the cervix into the uterus during AI (Halbert et al., 1990; Faigl et al., 2012). This particularly limits insemination with frozen-thawed semen through the cervix given the difficulty in the transport of spermatozoa across the cervix to the uterine body (Fair et al., 2005), which is thought to be breed specific (Fair et al., 2005).

The laparoscope has therefore made the use of frozen-thawed semen possible, effective and economical, since high fertility rates can be achieved with relatively low doses of semen if insemination is performed at a precise time in relation to ovulation (Luther et al., 2007; Vilariño et al., 2013; dos Santos-Neto et al., 2015). However, although insemination with frozen-thawed semen using laparoscopic-Intrauterine insemination has proved to be technically feasible, its use is limited by the fact that the method is not practical for use at farm level (Godfrey et al., 1999). In addition, laparoscopic AI is forbidden in some countries such as Norway and Sweden for reasons relating to animal welfare (Gordon, 2004). Furthermore, the necessary equipment are costly and the method also requires well qualified technicians (Vilariño et al., 2013). Therefore, a transcervical-intrauterine AI procedure has been developed for use with frozen-thawed semen (Windsor et al., 1994; Wulster-Radcliffe et al., 2004; Faigl et al., 2012), as an alternative method.
The recommended timing of AI with laparoscopic insemination is 48-65 h following device withdrawal (Faigl et al., 2012), whereas the volume of inseminate and the number of motile spermatozoa required are 0.05 ml and 20–40 × 10^6 (minimum), respectively (Faigl et al., 2012).

2.5.2.4 Transcervical –intrauterine

Transcervical-intrauterine AI involves depositing of semen into the uterus via the cervix using specialised instruments (Windsor et al., 1994; Faigl et al., 2012). One such example is the Guelph system of transcervical AI (Wulster-Radcliffe et al., 2004). Although it is reported that experienced inseminators can penetrate the cervix and deposit semen into the uterus in a high percentage of ewes without causing injury, the method has been reported to cause trauma /injury to the vaginal/ cervical wall which in turn affects fertility (Faigl et al., 2012).

Conception rates of 70-89% have been reported under the transcervical-intrauterine AI method with frozen-thawed semen (Husein et al., 1998; Godfrey et al., 1999). If penetration using the transcervical-intrauterine method are improved,

it is a feasible method which can be employed with frozen-thawed semen, especially in the case where the laparoscopic-intrauterine method is not applicable (Windsor et al., 1994). In the study by Husein et al. (1998), cervical penetration was achieved under 7 min in all ewes inseminated. The major limitation to the transcervical-intrauterine AI method is variation in the anatomy of the cervix between ewes (Husein et al., 1998). The recommended minimum volume and concentration of spermatozoa for use under this method is 0.2-0.5 ml and 100-200 $\times 10^6$ mm, respectively (Faigl et al., 2012).

2.5.3 Ways to improve fertility following oestrus synchronisation and artificial insemination

2.5.3.1 Provision of shade

Provision of shade or confining of animals during the treatment period reduces the effect of heat stress and improves fertility (Jordan, 2003). Heat stress is known to affect follicular development and intensity of oestrus and is a common cause of reproductive failure in stock (Jordan, 2003).

2.5.3.2 Use of quality semen

Quality of semen used for AI is an important determinant of fertility. Improvement of cryopreservation and thawing procedures aimed at reducing damage to spermatozoa is one of the ways aimed at improving fertility following insemination with frozen-thawed semen (Salamon and Maxwell, 1995). Nutritional supplementation and treatment of rams with melatonin especially during the non-breeding season are important for the improvement of semen quality of rams. In addition, studies in small ruminants recommend supplementation of AI sires with vitamin E or selenium to reduce oxidative stress and hence improve quality of spermatozoa, especially when the electro ejaculator method is used for semen collection (Bucak et al., 2008).

2.5.3.3 Double insemination

Performing double insemination although costly in terms of the need for more doses of semen, it extends the period of availability of viable spermatozoa when performed, which caters for ewes ovulating late, since duration over which spermatozoa are viable and are capable of fertilising ova is limited (Menchaca and Rubianes, 2004). Hence double insemination can improve fertility and reduce the percentage of ewes returning to oestrus.

2.5.3.4 Appropriate insemination technique

Matching the method of insemination with the appropriate semen type ensures high CR. Cervical AI is the most commonly used method of insemination in small ruminants for fresh or chilled semen (Faigl et al., 2012), of which better CR has obtained when semen is deposited deep into the cervix (intracervical) compared to the vaginal method (Faigl et al., 2012) or the cervical method where semen is deposited at the cervical mouth or as deep into the cervix as the speculum/catheter can get (Houdeau et al., 2008). Since excessive dilation of the vagina with the speculum in an attempt to locate the cervix and penetrate the cervical canal has been reported to cause intensive uterine motility especially in maiden ewes, rapid depositing of semen is recommended in order to obtain high fertility (Houdeau et al., 2008).

On the other hand, use of frozen-thawed semen with cervical AI has resulted in low fertility, whereas high fertility is achieved when frozen-thawed semen is used with the laparoscopic-intrauterine method (Godfrey et al., 1999). Although improvement of fertility following use of frozen-thawed semen is important, the laparoscopic AI method which results in high fertility is not only non-practical under farm conditions but also an animal welfare concern (Faigl et al., 2012). Hence studies into developing suitable non-surgical methods to deposit spermatozoa into the uterine

body via the cervix as replacement for the laparoscopic intrauterine AI method are being undertaken (Wulster-Radcliffe et al., 2004).

2.5.3.5 Improving protocols

Use of short-term treatment protocols in oestrus synchronisation programmes which result in ovulation of young follicles containing good quality ova has been suggested for improvement of fertility (Rubianes et al., 1996; Viñoles et al., 2001; Martemucci and D'Alessandro, 2011). In addition, recent research is focused on developing protocols with reduced hormonal use (Ungerfeld and Rubianes, 2002; Vilariño et al., 2010) or development of hormone free protocols which would result in acceptable fertility (Martin et al., 2004). These recent developments are both consumer driven, and a concern for environmental safety (Scaramuzzi et al., 2014).

2.6 Summary

It is evident from the review of literature that there is a shift from the traditional 12-14 d P4-eCG protocol which is sometimes combined with $PGF_{2\alpha}$ for efficiency of oestrus synchronisation. Most current research is geared towards development of protocols with reduced quantities of hormones or protocols which involve hormone-free methods (nutritional and ram effect) for management of reproduction. Halving or reducing the quantities of progestagens in sponges, re-use of intravaginal P4 devices and studies involving protocols with shorter treatment periods are the focus of most recent research. It is worth noting that research into improvement of $PGF_{2\alpha}$ based protocols and their use in combination with the ram effect has also been conducted for breeding during the ovulatory season, possibly since use of P4 is associated with residues, animal welfare and environmental safety concerns. Protocols which are both efficient and which offer the opportunity for reduced cost and reduced hormonal use are important both for South Africa and other African countries, where oestrus synchronisation particularly involving use of the male effect in both sheep and goats has not been fully exploited. In addition, use of the ram

effect presents as a more sustainable practice for most sheep production systems in Africa since the availability of hormones, cold chain or expertise, all which are crucial for the use of hormonal methods to synchronise oestrus may be limiting. More efficient and sustainable protocols for oestrus synchronisation would contribute to increased use of AI and genetic improvement in small ruminants, which would improve production. The present study therefore compared response to oestrus synchronisation and fertility following short-term and long-term P4 protocols.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study area and general management animals

The study used a data set collected during the 2016 March-May (autumn) breeding season at the small stock unit of the University of Pretoria experimental farm. The farm is located in Gauteng, South Africa (latitude: 25°44'30''S, longitude 28°15'30''E) with an average annual rainfall of 650 mm and dry autumn and winter seasons (Van Niekerk and Hassen, 2009). The sheep used for data collection were South African Mutton Merinos (SAMM). The SAMM is not a highly seasonal breed (Botha, 1980; Robinson et al., 1985), and therefore it has two breeding seasons; autumn (March-May) and Spring (September-November). The sheep were maintained under the paddock system, and grazed on native and grown pastures during the day for 8 h. During the night, the sheep were housed in pens, where phosphate mineral lick and water were provided *ad libitum.* The predominant native pasture species at the farm were: *Panicum maximum, Anthephora pubescens, Eragrostis spp, Digitaria eriantha, Chloris gayana* (Van Niekerk and Hassen, 2009). Grown pasture grasses for summer included; Kikuyu grass and smuts-finger grass whereas rye grass is grown for winter (Swanepoel, C. 2016, personal communication, 25 July). Chocolate maize (125-250 g per sheep per day) was also provided based on body condition of the ewes. All breeding ewes (maiden and mature) grazed and also camped together during the night (Swanepoel, C. 2016, personal communication, 25 July).

Seventy-eight (78) non-lactating maiden [parity = 0, weight = 47.5 ± 0.9 kg (n = 24] and mature [primiparous and multiparous; parity = 1- 6, weight = 63.1 ± 1.2 kg (n = 54] ewes were randomly assigned to 4 treatment groups in a 2 × 2 factorial design (Table 3.1). Weight and parity were balanced across the groups i.e. eCGshort (parity

= 2.0 ± 0.5 units, weight = 57.4 ± 2.4 kg; Ramshort (parity = 2.0 ± 0.4 units, weight = 58.6 ± 2.1 kg); eCGlong (parity = 2.5 ± 0.5 units, weight = 59.0 ± 2.9 kg); Ramlong (parity = 2.0 ± 0.4 units, weight = 58.0 ± 2.4 kg). The ewes had not been synchronised for oestrus before and had been isolated from rams (distance of 1 km) for a period of one month before the beginning of the experiment.

Oestrus synchronisation was accomplished by administration of progesterone using a controlled internal drug release (CIDR) vaginal device (0.3 g progesterone; EAZI-BREED™ CIDR-G®), Zoetis, New Zealand Ltd) primed for 9 d or 14 d; Table 3.1. Controlled internal drug release was inserted with an applicator, which was dipped in a disinfectant (LUTALYSE® sterile solution) before insertion into the vagina. Ewes and rams were kept separate as they grazed during the day and in the night pens. Ewes were also monitored every second day for any dropping of the CIDR insert. At CIDR withdrawal, ewes were separated, with some of the ewes (n= 38) receiving an intramuscular injection of eCG (300 IU; Chronogest, Intervet Pty (Ltd), South Africa), while others (n = 40) were continuously exposed to a vasectomised ram (ratio, 1: 26 ewes maximum) up to 84 h post CIDR withdrawal.

Table 3.1. Number of ewes per treatment group following treatment of South African Mutton Merinos with different oestrus synchronisation protocols during the breeding season.

Method for stimulation of ovulation	**Period of CIDR treatment**		
	9 d	**14 d**	**Total number of ewes**
eCG	eCGshort (19)	eCGlong (19)	38
Ram effect	Ramshort (21)	Ramlong (19)	40
Number of ewes	40	38	**78**

eCGshort; 9 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramshort; 9 d CIDR treatment & ram effect at CIDR withdrawal, eCGlong; 14 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramlong; 14 d CIDR treatment & ram effect at CIDR withdrawal. Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG).

3.2 Teasing and oestrous detection

Oestrous behaviour was monitored daily from 12-84 h post CIDR withdrawal, at intervals of 12 h (7.00 am and 7.00 pm). Four (4) SAMM entire rams fitted with aprons were enclosed in iron netted pens while ewes were allowed to freely access the pens. Ewes were confirmed to be in oestrus when they showed sexual receptivity by moving to the individual ram pens and touching the ram with their face. Other signs such as tail wagging or displaying an immobilisation reflex while accepting a mount from other ewes were also observed and regarded as confirmation signs for oestrus. Ewes confirmed in oestrus were immediately recorded and removed to clearly observe for more ewes which exhibited oestrous behaviour.

3.3 Semen collection and artificial insemination procedures

3.3.1 Semen collection and analysis

Semen used for artificial insemination (AI) was collected from 4 SAMM breeding rams at the time of AI (48 h and 60 h post CIDR withdrawal). Collection of semen was by electro ejaculator (Ramsem, South Africa). During semen collection, rams were restrained in a lateral recumbence position to perform electrical stimulation via the rectum similar to the procedure used by (Lukusa and Lehloenya, 2017). Briefly, the penile opening was shaved and cleaned with distilled water. The penis was then guided from the sheath and firmly held in position. A 15 ml graduated tube (Minitube®, South Africa) was suspended close to the penile tip for collection of the ejaculate. The rectum was cleared of faeces and the rectal probe was lubricated with K-Y jelly (Johnson & Johnson Pty Ltd, South Africa) and inserted into the rectum. A power output of 3-5 volts was generated using the manual control knob of the electro ejaculator. Manual electric stimulation was held for 4-5 sec and then brought to 0. The procedure was repeated after a period of rest equivalent to the duration of electrical stimulation. Ejaculates of volume not less than 2 ml and mass

motility over 80% [determined under a light microscope (Olympus C×21, on a pre-warmed stage (37°C] were passed. Mass motility was determined by examining a 10 µl drop of semen on a 76×26×1 mm glass slide, covered with a cover slip 18×18 mm (LASEC Pty Ltd, South Africa). Semen collection was performed in an enclosed environment and the collection tube was held tightly in the palm to protect the ejaculate from temperature shock and exposure to direct sunlight.

3.3.2 Artificial insemination

All treated ewes were artificially inseminated twice at fixed times of 48 and 60 h post CIDR withdrawal. Ewes were restrained by placing the hind quarters over a rail, supported by an assistant, with the head facing downwards and fore limbs standing on the floor, similar to the procedure used by Karagiannidis et al. (2001). Prior to insemination, semen was maintained at 35-37°C. The vulva of the ewe was wiped with a paper towel to prevent contamination of the semen and the reproductive tract. Cervical AI was then performed using a speculum with a light source which was lubricated with K-Y jelly (Johnson & Johnson (Pty) Ltd, South Africa) and carefully inserted into the vagina to locate the cervix (Fair et al., 2005). A volume of 0.2 ml of fresh undiluted semen with sperm concentration of approximately 200 × 10^6 was drawn into the catheter (I.M.V, France) using a syringe, and deposited into the opening of the cervix. Ejaculates which had passed the quality test were pooled during AI, based on the planned rams for use on specific ewes. Both semen collection and AI procedures were performed by experienced personnel.

3.4 Ultrasonographic evaluation of follicular response

Transrectal ultrasonography was performed on 21 ewes with at least 4 ewes selected at random from each of the treatment groups. The procedure was conducted at 48 h post CIDR withdrawal (time of first AI) to examine the number and size of preovulatory follicles. This was done by an experienced operator using a real-time B-mode ultrasound scanner (Aloka SSD 500® – Aloka CO, Tokyo, Japan)

equipped with a transrectal 7.5-MHz linear array probe (UST-660-7.5 model). Ewes were restrained in a standing position to perform the ultrasound procedure. The rectal probe lubricated with K-Y jelly was inserted into the rectum and sketches of ovaries were viewed on the monitor. Measurements of diameter and number of follicles on the ovaries were recorded. The design of the experiment is illustrated in Fig.3.1.

3.5 Non-return rate and ultrasonographic evaluation for pregnancy diagnosis

Non-return to oestrus was monitored from 15-21 d post AI by observing for ewes which exhibited oestrous behaviour in the morning (7.00 am) and evening (7.00 pm) for 30 min. Since ewes were joined with rams after the experimental period, ewes which exhibited oestrus and were served naturally were recorded as having returned to oestrus. In addition, ultrasonographic evaluation for pregnancy diagnosis (PD) was performed by transrectal ultrasonography at 35 d post AI using the same procedure and scanner as with follicular response.

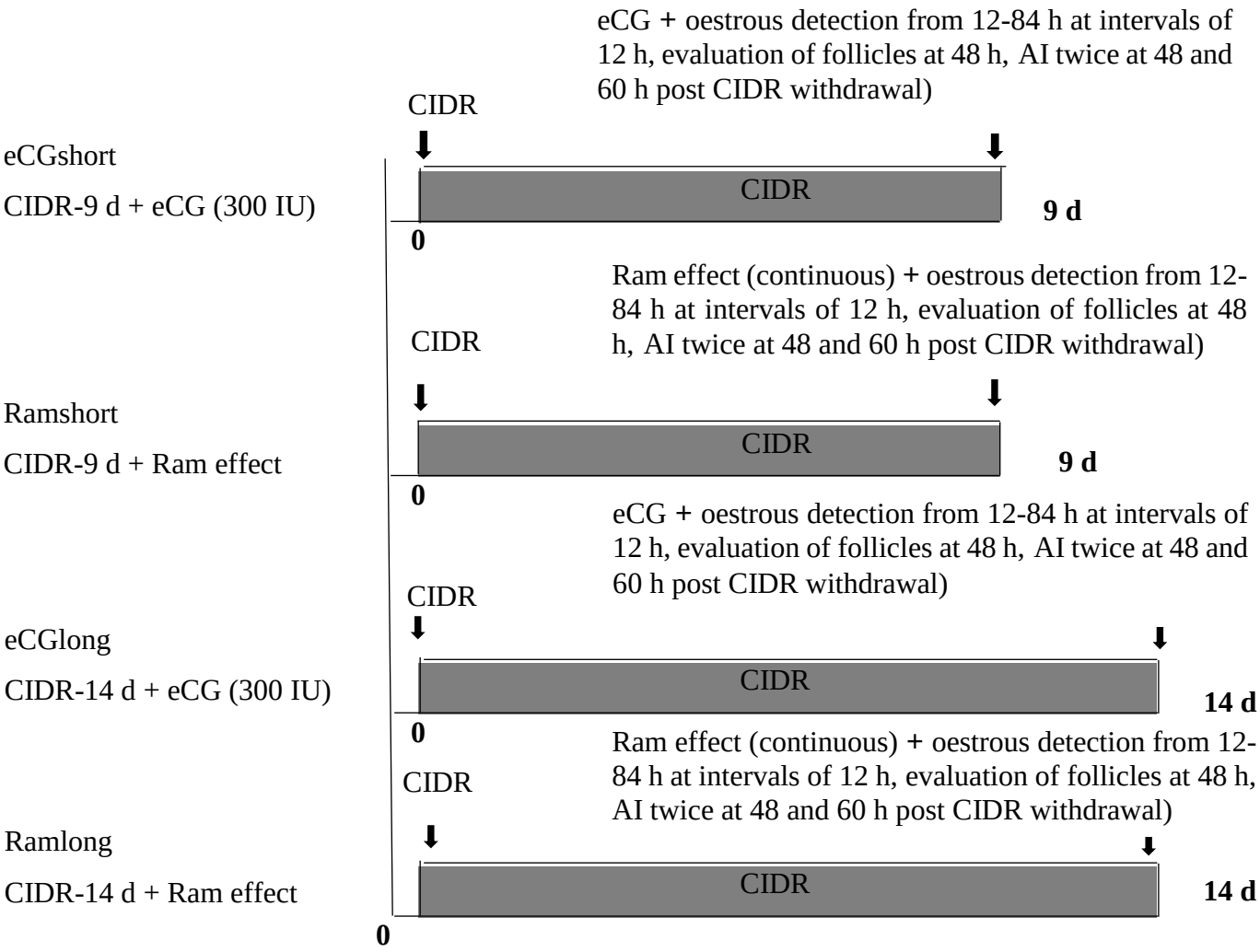

Fig 3.1. Illustration of the experimental design of the present study. Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG), artificial insemination (AI).

3.6 Data collection at lambing

Data on lambing were obtained from daily observation and recording of lambing events i.e. identity of ewes lambing, dates of lambing, number of lambs born per ewe, sex and weight of lambs, lambs born alive and lambs born dead were recorded. Ewes lambed from 147 to 185 d post AI. There were no ewes lambing between 154 and 165 d post AI.

Statistical analysis

Variables for response to oestrus synchronisation and fertility evaluated are presented in Table 3.2, with treatment group (eCGshort, Ramshort, eCGlong and Ramlong) as the main explanatory variable. Data were also pooled to analyse the effect between treatments i.e. eCG and ram effect, 9 and 14 d P4 treatment. Continuous data (onset of oestrus, duration of oestrus, number of follicles, diameter of the largest follicle, AI to lambing interval and number of lambs born) were analysed with analysis of variance (ANOVA; One-Way ANOVA or t-Test; SAS and Version, 2003), depending on the values of the classification variable. Other confounding factors such as weight and parity were classified as weight group (1 = 35-50 kg, 2 = 51-60 kg, 3 = 61-70 kg, 4 = 71-80 kg) and parity group (0 = parity 0, 1 = parities 1-3 and 2 = parities 4-1) and used in modelling with generalized linear model (GLM) procedures; SAS and Version, 2003). Data on follicles were grouped as small (2.0-3.9 mm), medium (4.0-5.9 mm) and large (≥ 6.0 mm), based on the ranking of the diameter of follicles by (Noel et al., 1993), to run the necessary analyses. Data for AI to lambing interval were clustered into 2 periods of lambing; 147-154 d post AI (period 1) and 165-185 d post AI (period 2), which were considered to correspond with lambing to first service i.e synchronised oestrus and AI (Kridli and Al-Khetib, 2006; Evans et al., 2001) and lambing to spontaneous oestrus and natural service, respectively. Differences between means were compared using the Tukey test. Analyses for categorical variables (oestrous response, conception rate, non-return rate and twinning rate) were performed with Chi-Square (SAS and Version, 2003). If the analysis indicated that Chi-Square was not a valid test, Fisher's Exact test was used. Data were grouped as:12 and 24 h (early); 36 and 48 h (late) to analyse the effect of early or late onset of oestrus on conception rate. Correlation between number of follicles and onset of oestrus, number of follicles and number of small, medium and large follicles, number of follicles and average diameter of follicles per ewe were analysed using Pearson correlation analysis (SAS and Version, 2003). Results were either expressed as percentages or mean ± SEM. Statistical significance was accepted from $P < 0.05$.

Table 3.2. A description of variables in the analysis of reproductive performance of South African Mutton Merino ewes following different oestrous synchronisation protocols and artificial insemination.

Variable	Description
Oestrous response	Proportion of ewes observed in oestrus at any one time from 12–84 h post CIDR withdrawal.
Onset of oestrus	Interval from CIDR withdrawal to first signs of observed oestrus.
Duration of oestrus	Interval from onset of oestrus to cessation of oestrus behaviour.
Number of follicles	Number of preovulatory follicles observed by ultrasound at 48 h post CIDR withdrawal.
Diameter of the largest follicle	Diameter of the largest preovulatory follicle (mm) observed by ultrasound at 48 h post CIDR withdrawal.
Conception rate	Proportion of ewes confirmed pregnant of all ewes synchronised as determined by ultrasound at 48 h post CIDR withdrawal. It was calculated by number of ewes confirmed pregnant/number of ewes synchronised *100.
Non-return rate	Proportion of ewes not returning to oestrus (as observed from 15 – 21 d post AI) of all ewes synchronised. It was calculated by number of ewes which did not return to oestrus/ number of ewes synchronised * 100.

Ewes lambing	Proportion of ewes lambing of all ewes synchronised. It was calculated by number of ewes lambing/number of ewes synchronised *100.
AI to lambing interval	Number of days between AI and lambing.
Lambing to the first service period	Number of ewes lambing within 147-154 d post AI.
Lambs born	Number of lambs per ewe lambing. It was calculated by number of lambs born/number of ewes
Twinning	Proportion of ewes lambing with twins. It was calculated by number of ewes lambing with twins/number of ewes lambing *100.

CHAPTER FOUR

4. RESULTS

4.1 Effect of synchronisation treatment on oestrous response, interval to onset of oestrus and duration of oestrus

Three (3) ewes dropped the controlled internal drug release (CIDR) device during the experimental period but they were replaced. These ewes responded to synchronisation of oestrus except one, hence it was omitted from the analysis. The overall oestrous response obtained was 98.7%. The proportion of ewes which responded with oestrous signs per treatment group were [100% (eCGshort), 95.2% (Ramshort), 100% (eCGlong) and 100% (Ramlong]. There was no significant difference ($P > 0.05$) in oestrous response between treatment groups (Table 4.1, Fig 4.2). A greater proportion of ewes [87.0%, (n = 67/77), Fig.4.1] showed oestrous signs at 36 and 48 h post CIDR withdrawal.

Overall, mean interval to onset of oestrus in this study was 27.9 ± 1.4 (Table 4.1). Most ewes began exhibiting oestrus at 24 and 36 h post CIDR withdrawal, with a greater proportion of ewes (above 65%) beginning oestrus during this period (Fig 4.3). There was no significant difference ($P = 0.16$) in the mean interval to onset of oestrus between treatment groups. A large proportion of ewes (96.2%, n = 75/78) had begun oestrus by 48 h following CIDR withdrawal, and by 60 h, all ewes had attained oestrus except one which did not respond to synchronisation during the experiment. When data for all ewes treated with CIDR for 9 d or 14 d (CIDR-9 d and CIDR-14 d, respectively) were pooled, CIDR-14 d ewes were seen to exhibit oestrus earlier ($P = 0.03$) than CIDR-9 d ewes (Table 4.1; Fig. 4.4). However, equine chorionic gonadotropin (eCG) and ram exposure had no effect ($P > 0.05$) on the mean interval to onset of oestrus when data were pooled. The overall mean duration of oestrus in this study was 31.8 ± 1.6 (Table 4.1), with most ewes (57.1%) in oestrus

for 24-36 h. There was no difference in duration of oestrus (P > 0.05) between treatment groups.

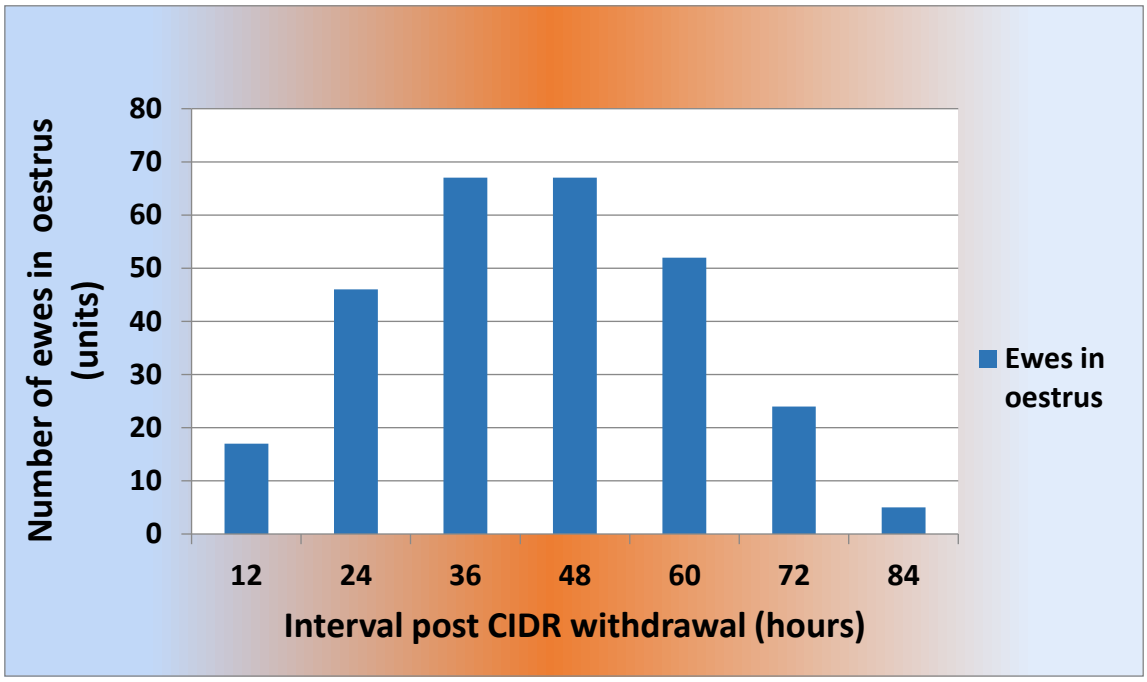

Fig. 4.1. Number of ewes in oestrus at different intervals following 9 or 14 d CIDR treatment and eCG (300 IU) or ram exposure at CIDR withdrawal (P > 0.05). Only ewes which responded to oestrus synchronisation (n = 77) are represented in the figure. Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG).

4.2 Effect of synchronisation treatment on follicular response at 48 h post CIDR withdrawal

Overall, mean number of follicles at 48 h post CIDR withdrawal was 2.1 ± 0.3 (Table 4.1). The maximum number of follicles observed was 5 and the minimum was 1. There was no significant difference (P > 0.05) in the mean number of follicles between treatment groups. Similarly, there was no significant difference (P > 0.05) between treatments in the number follicles when data were pooled, although eCG treated ewes registered a numerically higher number of follicles than ewes exposed to the ram effect (2.5 ± 0.4 versus 1.8 ± 0.4, P = 0.19). Parity and weight also had

no effect on number of follicles. A moderate positive correlation was observed between mean number of follicles and interval to onset of oestrus, which tended towards significance (r= 0.45; *P* = 0.05). Number of follicles tended to increase with the increase in interval to onset of oestrus from 24-48 h.

Overall, mean diameter of the largest follicle was 6.5 ± 0.4 mm (Table 4.1). Diameter of the largest and smallest follicles observed were 9.3 and 2.0 mm, respectively. Mean diameter of the largest follicle did not differ between treatment groups (*P* > 0.05). Similarly, there was no significant difference (*P* > 0.05) between treatments in the mean diameter of the largest follicle when data were pooled. The average diameter of all follicles per ewe was (5.6 ± 0.4 mm, *P* > 0.05). It was observed that there was a strong negative correlation between number of follicles and average diameter of follicles per ewe (r = -0.59, *P* = 0.005). Number of follicles reduced with increase in average diameter of follicles. Furthermore, most ewes [57.1% (n = 12/21) versus 42.8% (n = 9/21), *P* > 0.05] were observed with a single large follicle or with divergence in diameter i.e. a difference of ≥ 2.5 mm between the dominant (large) follicle and the largest subordinate follicle.

In addition, when follicles were classified by diameter as small (2.0-3.9 mm), medium (4.0-5.9 mm) and large (≥ 6.0 mm), based on the ranking of the diameter of follicles by (Noel et al., 1993), it was observed that mean number of small, medium and large follicles were; 0.7± 0.2, 0.6 ± 0.2, 0.8 ± 0.1, *P* > 0.05, respectively. In addition, analysis showed that mean number of follicles had a strong positive correlation with number of medium follicles (r = 0.7, *P* = 0.0001, respectively). Number of follicles increased with an increase in the number of medium follicles.

4.3 Effect of synchronisation treatment on non-return rate and conception rate

Non-return rate (NRR) in this study was 66.7%. There was no significant difference in NRR between treatment groups (*P* > 0.05, Table 4.2). Parity group, weight group,

number of follicles and diameter of the largest follicle also had no effect on NRR (P > 0.05). The overall conception rate (CR) was 74.4% (n = 58/78), Table 4.2. Although a higher CR was observed in eCGshort and Ramshort compared to eCGlong and Ramlong treatment groups, (84.2% and 81.0% versus 57.9% and 68.4%, respectively), there was no significant difference (P = 0.15) in CR between the treatment groups.

However, when data for period of CIDR treatment were pooled, CIDR-9 d ewes had a significantly higher CR (P = 0.03) than CIDR-14 d ewes [85% (n = 34/40) versus 63.2% (n = 24/38, respectively] whereas there was no difference in CR between eCG treated ewes and ram exposed ewes (P > 0.05). There was a strong relationship between NRR and CR (P < 0.001). A proportion of [77.6 %, (n = 45/58] ewes which did not return to oestrus was confirmed pregnant by ultrasound. Parity group, weight group, number of follicles and diameter of the largest follicle also had no effect on CR. Similarly, there was no significant difference in CR between maiden and older ewes [79.2% (n = 19/24) versus 72.2% (n = 39/54], respectively. However, there was a tendency for ewes confirmed pregnant to begin oestrus later (29.4 ± 1.6 versus 23.4 ± 2.3; P = 0.06) than ewes which did not conceive. When data for interval to onset of oestrus were pooled and grouped as: 12 and 24 h (early); 36 and 48 h (late), it was observed that there was a tendency for a higher proportion of ewes which began oestrus late to conceive than ewes which began oestrus early [86.2% (n = 25/29) versus 67.4% (n = 31/46), P = 0.07, Fig. 4.5]. The proportion of CIDR-9 d and CIDR-14 d ewes beginning oestrus early and late which conceived were: [CIDR-9 d, 76.5% (n = 13/17) versus CIDR-14 d, 62.1% (n = 18/29), P = 0.31] and [CIDR-9 d, 95.2% (n = 20/21) versus CIDR-14 d, 62.5% (n = 5/8), P = 0.05, respectively]. The results of NRR and CR were later confirmed with lambing data.

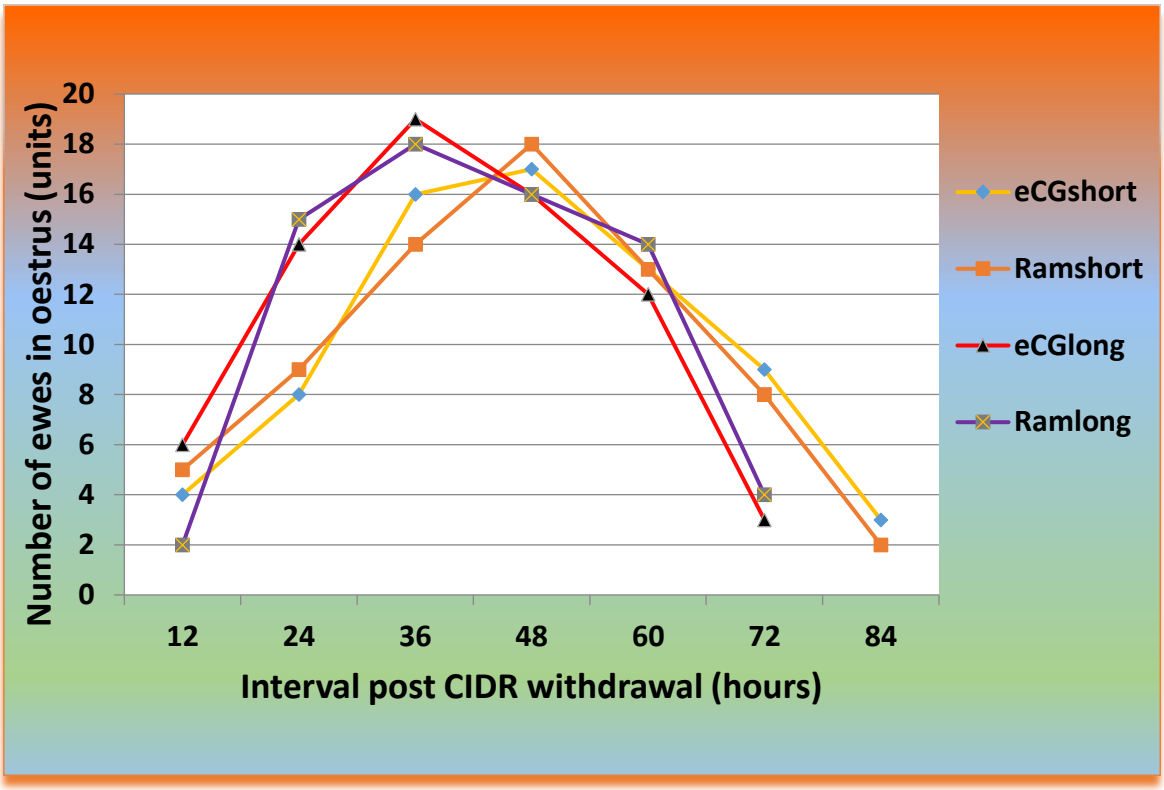

Fig. 4.2. Number of ewes in oestrus per treatment group at different intervals following 9 or 14 d CIDR treatment and eCG (300 IU) or ram exposure at CIDR withdrawal. eCGshort (n = 19); 9 d CIDR treatment & eCG, Ramshort (n = 21); 9 d CIDR treatment & ram exposure, eCGlong (n =19); 14 d CIDR treatment & eCG (300 IU), Ramlong (n = 19); 14 d CIDR treatment & ram exposure ($P > 0.05$).

4.4 Effect of synchronisation treatment on interval from artificial insemination to lambing, twinning rate and number of lambs born

Overall, 91.0% (n = 71/78) of all ewes in this study lambed. Seven ewes did not lamb, of which 85.7% (n = 6/7) were either maiden ewes or ewes of parity 1. The older ewe (parity 3) which did not lamb was limping at the time of breeding.

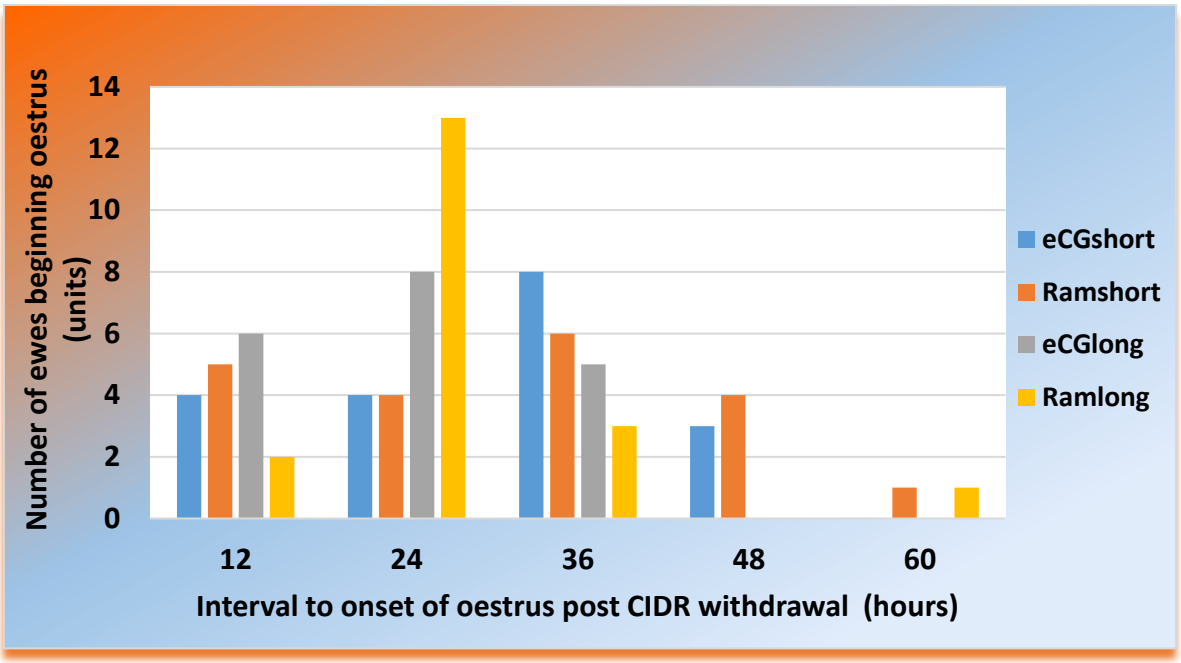

Fig. 4.3. Number of ewes per treatment group beginning oestrus at different intervals following 9 or 14 d CIDR treatment and eCG or ram exposure at CIDR withdrawal. eCGshort (n = 19); 9 d CIDR treatment & eCG (300 IU), Ramshort (n= 21); 9 d CIDR treatment & ram effect, eCGlong (n =19); 14 d CIDR treatment & eCG (300 IU), Ramlong (n = 19); 14 d CIDR treatment & ram effect ($P > 0.05$). Only ewes which responded to oestrous synchronisation (n = 77) are represented in the figure. Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG).

The mean interval from artificial insemination (AI) to lambing in this study was 158.6 ± 1.2 d, $P > 0.05$, Table 4.2, with range of 147-186 d post AI. There was no significant difference ($P > 0.05$) between treatment groups in the interval from AI to lambing. Similarly, parity and weight had no effect ($P > 0.05$) on the interval from AI to lambing. The proportion of ewes confirmed pregnant which lambed was 93.1% (n = 54/58). Based on the clustering of data into period 1 (147-154 d post AI) and period 2 (166 -186 d post AI), the mean AI to lambing interval for ewes lambing in period 1 was 150.2 ± 0.3 d while that of ewes lambing in period 2 was 170.1 ± 0.9 d. The proportion of ewes confirmed pregnant which lambed in period 1 and period 2 were; 75.9% (n = 41/54) and 24.1% (n = 13/54), Table 4.3, Fig. 4.6, respectively. Four (4) ewes diagnosed as pregnant did not lamb.

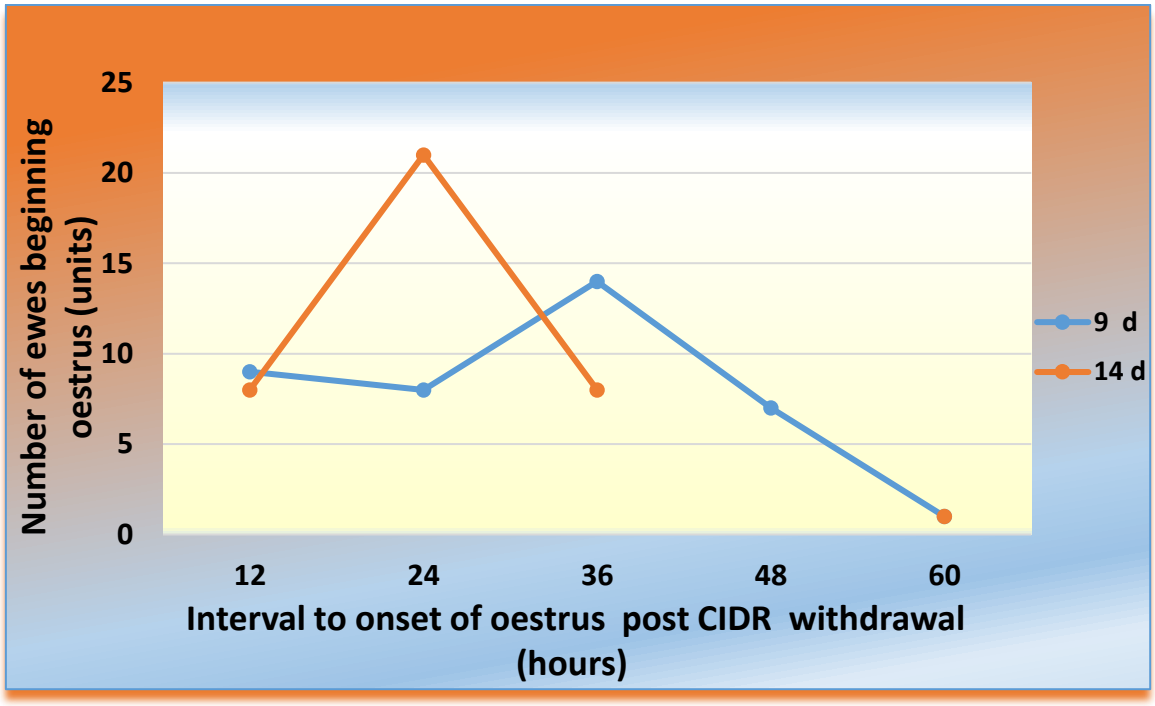

Fig. 4.4. Number of ewes beginning oestrus at different intervals following 9 (n = 40) or 14 d (n = 38) CIDR treatment (pooled data; P < 0.05). Oestrous behaviour was monitored using apron fitted rams in pens from 12 to 84 h post CIDR withdrawal at an interval of 12 h for 30 min. Only ewes which responded to oestrus synchronisation (n = 77) are represented in the figure. Controlled internal drug release (CIDR).

There was a significant difference (P = 0.03) between treatment groups in the proportion of ewes confirmed pregnant which lambed in period 1 and period 2 (Table 4.3). A significantly higher (P < 0.05) proportion of ewes confirmed pregnant in the eCGlong and Ramlong treatment groups lambed in period 1 than in period 2 whereas in the eCGshort and Ramshort treatment groups, the proportion of ewes confirmed pregnant which lambed in period 2 was significantly higher (P < 0.05) than in period 1. Similarly, when data for period of CIDR treatment were pooled, it was observed that the proportion of ewes confirmed pregnant in the CIDR-14 d group which lambed in period 1 was significantly higher (P = 0.004) than in period 2, whereas in the CIDR-9 d group, the proportion of ewes confirmed pregnant which lambed in period 1 was significantly lower than in period 2 (P < 0.05). However, there was no significant difference (P > 0.05) between eCG treated ewes and ram

exposed ewes in the proportion of ewes confirmed pregnant which lambed in period 1 and 2 when data were pooled (Table, 4.3). The overall proportion of ewes which lambed in period 1 of all ewes synchronised was 52.6% (n=41/78*), P* > 0.05. The proportion of ewes per treatment group which lambed in period 1 of all ewes synchronised are represented in Table 4.3.

The overall twinning rate in this study was 42.3% (n = 30/71), Table 4.2. There was no significant difference (*P* > 0.05) in twinning rate between treatment groups. There were more twin births in lambing period 1 than in period 2 [63.3% (n = 19/30) versus 36.7% (n = 11/ 30), respectively] although the difference was not significant *(P* > 0.05). When twinning rate between eCG treated ewes and ram exposed ewes which lambed in period 1 and period 2 were compared, it was observed that twinning rate was not affected by any of the above variables in the 2 lambing periods. Analysis also showed that twin births among eCG treated ewes were higher in ewes which lambed in period 1 than in period 2 (11/17 (64.7%) versus 6/17 (35.3%; *P* > 0.05, respectively) and among eCG treated ewes than in ram exposed ewes lambing in period 1 (11/19 (57.9% versus 8/19 (42.1%; *P* > 0.05, respectively), although the difference was not significant.

Table 4.1. Oestrous and follicular response (mean ± SEM) following different oestrus synchronisation protocols and artificial insemination in South African Mutton Merino ewes during the breeding season.

Treatments	Oestrous response (%)	Onset of oestrus (h)	Duration of oestrus (h)	Number of follicles at 48 h post CIDR withdrawal (n = 21)	Diameter of the largest follicle (mm) at 48 h post CIDR withdrawal (n = 21)
eCGshort	19/19 (100) [a]	30.3 ± 2.8 [a]	32.8 ± 3.3 [a]	2.5 ± 0.6 [a]	7.2 ± 0.7[a]
Ramshort	20/21 (95.2) [a]	31.2 ± 3.3 [a]	30.0 ± 3.6 [a]	1.8 ± 0.6 [a]	5.9 ± 0.5[a]
eCGlong	19/19 (100) [a]	23.4 ± 2.1 [a]	32.8 ± 3.3 [a]	2.5 ± 0.3 [a]	6.5 ± 1.0[a]
Ramlong	19/19 (100) [a]	26.5 ± 2.4 [a]	31.6 ± 3.4 [a]	1.8 ± 0.5 [a]	6.4 ± 0.9[a]
eCG	38/38 (100) [a]	26.8 ± 1.8[a]	32.8 ± 2.3 [a]	2.5 ± 0.4 [a]	6.9 ± 0.5 [a]
Ram effect	39/40 (97.5) [a]	28.9 ± 2.0 [a]	30.8 ± 2.3 [a]	1.8 ± 0.4 [a]	6.2 ± 0.5 [a]
CIDR-9 d	39/40 (97.5) [a]	30.8 ± 2.1 [a]	31.4 ± 2.5 [a]	2.2 ± 0.4 [a]	6.6 ± 0.4[a]
CIDR-14 d	38/38 (100) [a]	24.9 ± 1.6[b]	32.2 ± 2.1[a]	2.1 ± 0.3 [a]	6.5 ± 0.6[a]
Overall	77/78 (98.7)	27. 9 ± 1.4	31.8 ± 1.6	2.1 ± 0.3	6.5 ± 0.4

Different superscripts in the same column of clustered treatments are significantly different (a,b, $P < 0.05$). eCGshort; 9 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramshort; 9 d CIDR treatment & ram effect at CIDR withdrawal, eCGlong; 14 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramlong; 14 d CIDR treatment & ram effect at CIDR withdrawal. eCG, data for all eCG treated ewes pooled; Ram effect, data for all ewes exposed to the ram effect pooled; CIDR-9 d, data for all ewes treated with CIDR for 9 d pooled; CIDR-14, data for all ewes treated with CIDR for 14 d pooled.

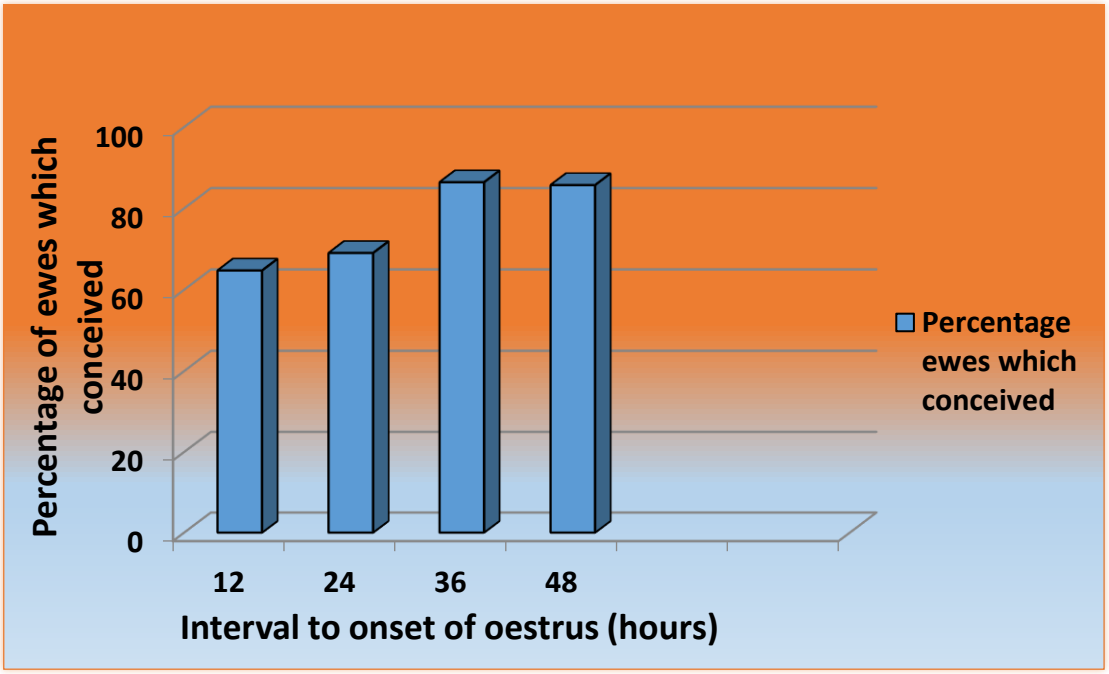

Fig. 4.5. Proportion of ewes which conceived from different intervals of onset of oestrus following 9 or 14 d treatment with CIDR and eCG (300 IU) or ram exposure at CIDR withdrawal. AI was performed twice at 48 and 60 h post CIDR withdrawal (P > 0.05). Pregnancy diagnosis was performed by ultrasound (35 d post AI). Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG), artificial insemination (AI).

However, the proportion of twin births among ram exposed ewes lambing in period 1 and period 2 and among eCG and ram exposed ewes lambing in period 2 were very close. Parity, weight and number of follicles also had no effect on twinning rate. In addition, when data for older ewes which had twin births in period 1 were pooled and compared with maiden ewes which had twins in the same period, the twinning rate in older ewes tended to be higher [84% (n = 16/19) versus 16% (n = 3/19), $P < 0.05$] than in maiden ewes.

The mean number of lambs born per ewe lambing in this study was 1.5 ± 0.1 (Table 4.2). There was no significant difference ($P > 0.05$) in number of lambs born between treatment groups. In addition, the mean number of lambs born per ewe lambing in period 1 and period 2 did not differ (1.5 ± 0.1 versus 1.4 ± 0.1, $P > 0.05$, respectively).

Table 4.2. Least square means (± SEM) and percentages of fertility parameters following different oestrus synchronisation protocols and artificial insemination in South African Mutton Merino ewes during the breeding season.

Treatments	Non-return rate (%)	Conception rate (%)	AI to lambing interval (d)	Twinning rate per ewe lambing (%)	Lambs born per ewe lambing (units)
eCGshort	12/19 (63.3) [a]	17/19 (89.5) [a]	157.8 ± 2.7[a]	8/17 (47.1) [a]	1.5 ± 0.1 [a]
Ramshort	16/21 (76.2) [a]	17/21 (81.0) [a]	161.4 ± 2.9[a]	6/17 (35.3) [a]	1.4 ± 0.1 [a]
eCGlong	13/19 (68.4) [a]	12/19 (63.2) [a]	157.4 ± 2.3 [a]	9/19 (47.4) [a]	1.5 ± 0.1 [a]
Ramlong	11/19 (57.9) [a]	12/19 (63.2) [a]	158.1 ± 2.2 [a]	7/18 (38.9) [a]	1.5 ± 0.1 [a]
eCG	25/38 (65.8) [a]	29/38 (76.3) [a]	157.6 ± 1.7[a]	17/36 (47.2) [a]	1.5 ± 0.1 [a]
Ram effect	27/40 (67.5) [a]	29/40 (72.5) [a]	159.7 ± 1.8 [a]	13/35 (37.1) [a]	1.4 ± 0.1 [a]
CIDR-9 d	28/40 (70.0) [a]	34/40 (85.0) [a]	159.6 ± 2.0 [a]	14/34 (41.2) [a]	1.4 ± 0.1 [a]
CIDR-14 d	24/38 (63.2) [a]	24/38 (63.2) [b]	157.8 ± 1.6 [a]	16/37 (43.2) [a]	1.5 ± 0.1 [a]
Overall	52/78 (66.7)	58/78 (74.4)	158.6 ± 1.2	30/71 (42.3)	1.5 ± 0.1

Different superscripts in the same column of clustered treatments are significantly different (a,b, $P < 0.05$). eCGshort; 9 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramshort; 9 d CIDR treatment & ram effect at CIDR withdrawal, eCGlong; 14 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramlong; 14 d CIDR treatment & ram effect at CIDR withdrawal. eCG, data for all eCG treated ewes pooled; Ram effect, data for all ewes exposed to the ram effect pooled; CIDR-9 d, data for all ewes treated with CIDR for 9 d pooled; CIDR-14, data for all ewes treated with CIDR for 14 d pooled. AI was performed twice at 48 and 60 h post CIDR withdrawal. Monitoring for non-return was performed 21 d post AI. Non-return rate (number of ewes which did not return to oestrus/ number of ewes synchronised * 100. Pregnancy diagnosis was by ultrasound (35 d post AI). Conception rate (Number of ewes confirmed pregnant/number of ewes synchronised *100). Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG), artificial insemination (AI).

Similarly, the number of lambs born by eCG treated ewes and ram exposed ewes lambing in period 1 (1.5 ± 0.1 versus 1.4 ± 0.1, $P > 0.05$, respectively) was not significantly different. Parity group, weight group and number of follicles also had no effect on number of lambs born. However, when data on number of lambs born by older ewes which lambed in period 1 were pooled and compared to the number of lambs born by maiden ewes which lambed in the same period, it was observed that older ewes had significantly more number of lambs born ($P < 0.05$) compared to maiden ewes (1.6 ± 0.1 versus 1.2 ± 0.1, $P = 0.04$, respectively). A total of 103 lambs were born in this study, of which the proportion of single, twin and triplet births was 56.3% (n = 40/71), 42.3% (n = 30/71) and 1.4% (n = 1/71), respectively.

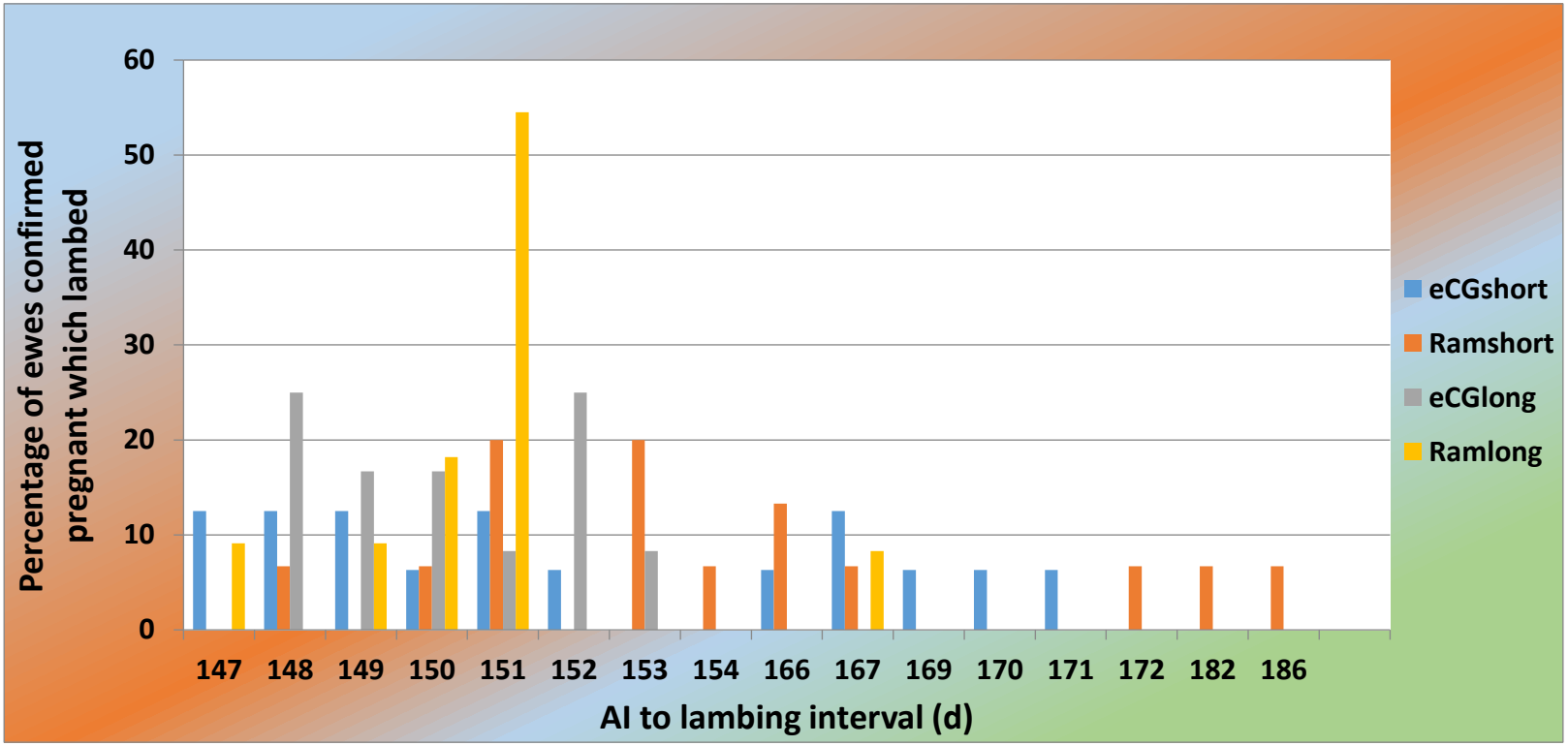

Fig.4.6. Percentage of ewes per treatment group confirmed pregnant by ultrasound 35 d post AI which lambed at different intervals following oestrus synchronisation and AI. eCGshort (n = 16); 9 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramshort (n= 15); 9 d CIDR treatment & ram effect at CIDR withdrawal, eCGlong (n =12); 14 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramlong (n = 11); 14 d CIDR treatment & ram effect at CIDR withdrawal ($P < 0.05$). AI was performed twice at 48 and 60 h post CIDR withdrawal. Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG), artificial insemination (AI).

Table 4.3. Conception rate and corresponding lambing performance of South African Mutton Merino ewes following different oestrus synchronisation protocols and artificial insemination during the breeding season.

Treatments	Conception rate (%)	Ewes confirmed pregnant which lambed to the first service period (%)	Ewes confirmed pregnant which lambed to spontaneous oestrous and natural service (%)	Ewes confirmed pregnant which did not lamb (%)	Ewes which lambed to first the service period of all ewes synchronised (%)
eCGshort	17/19 (89.5) [a]	10/16 (62.5) [a]	6/16 (37.5) [a]	1/1 (100) [a]	10/19 (52.6) [a]
Ramshort	17/21 (81.0) [a]	9/15 (60.0) [a]	6/15 (40.0) [a]	2/2 (100) [a]	9/21 (42.9) [a]
Ramlong	12/19 (63.2) [a]	10/11 (90.9) [b]	1/11 (9.1) [b]	1/1 (100) [a]	10/19 (52.6) [a]
eCGlong	12/19 (63.2) [a]	12/12 (100) [b]	0/12 (0.0) [ab]	-	12/19 (63.2) [a]
eCG	29/38 (76.3) [a]	22/28 (78.6) [a]	6/28 (21.4) [a]	1/1 (100) [a]	22/38 (57.9) [a]
Ram effect	29/40 (72.5) [a]	19/26 (73.1) [a]	7/26 (26.9) [a]	3/3 (100) [a]	19/40 (47.5) [a]
CIDR- 9 d	34/40 (85.0) [a]	19/31 (61.3) [a]	12/31 (38.7) [a]	3/3 (100) [a]	19/40 (47.5) [a]
CIDR- 14 d	24/38 (63.2) [b]	22/23 (95.7) [b]	1/23 (4.3) [b]	1/1 (100) [a]	22/38 (57.9) [a]
Overall	58/78 (74.4)	41/54 (75.9)	13/54 (24.1)	4/4 (100)	41/78 (52.6)

Different superscripts in the same column of clustered treatments are significantly different (a,b, $P < 0.05$). eCGshort; 9 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramshort; 9 d CIDR treatment & ram effect at CIDR withdrawal, eCGlong; 14 d CIDR treatment & eCG (300 IU) at CIDR withdrawal, Ramlong; 14 d CIDR treatment & ram effect at CIDR withdrawal. eCG, data for all eCG treated ewes pooled; Ram effect, data for all ewes exposed to the ram effect pooled; CIDR-9 d, data for all ewes treated with CIDR for 9 d pooled; CIDR-14, data for all ewes treated with CIDR for 14 d pooled.

CHAPTER FIVE

5. DISCUSSION

5.1 Effect of synchronisation treatment on oestrous response, interval to onset of oestrus and duration of oestrus

A high oestrous response was obtained with all protocols used in this study as shown by the proportion of ewes responding to oestrus synchronisation. A similar response has been observed in studies conducted during the breeding season with progestagens/progesterone (Greyling et al.,1997; Fleisch et al., 2012; Fierro et al., 2016) and prostaglandin (Godfrey et al.,1999; Fierro, 2016), as well as during the non-breeding season (Greyling et al., 1994; Ungerfeld and Rubianes, 2002). The high oestrous response obtained in the present study was possibly because the controlled internal drug release (CIDR) device was efficient in delivering progesterone during the treatment period (Abecia et al., 2012). Progesterone levels during treatment could have adequately suppressed luteinising hormone (LH) secretion and led to synchrony of follicular wave development (Driancourt, 2001; Evans, 2003) and subsequent synchrony of oestrus when equine chorionic gonadotropin (eCG) or the ram effect were applied at CIDR withdrawal.

The tight synchrony of oestrus observed at 36 and 48 h post CIDR withdrawal in the present study was also reported by other researchers (Vilariño et al., 2013; Swelum et al., 2015). A tight synchrony of oestrus indicates that the ovulatory follicles originated from a single wave (Viñoles and Rubianes, 1998; Barrett et al., 2004), which is of particular importance in fixed time insemination (FTAI), given that a greater variability in response to oestrus synchronisation results in reduced fertility (Fukui et al., 1993).

Treatment group in the present study had no effect on the mean interval to onset of oestrus, which is known to determine the timing of the LH surge (Fabre-Nys and

Martin, 1991). The mean interval to onset to oestrus obtained in the present study is in the range obtained by other researchers who studied short-term (Fukui et al., 1993; Martemucci and D'Alessandro, 2011) and long-term treatment of P4 (Greyling et al., 1997; Evans et al., 2004; Vilariño et al., 2010), during the breeding season. The lack of significance between treatment groups was possibly due to the small number of ewes. However, the earlier onset of oestrus in ewes treated with CIDR for 14 d (CIDR-14 d) compared to those treated with CIDR for 9 d (CIDR-9 d) when data were pooled is perhaps an indicator of faster follicular growth (Evans et al., 2004) in the former than in the latter, whereas a longer interval to onset of oestrus may indicate slower follicular growth following CIDR withdrawal. Harl, (2014) also observed that long-term protocols result in a shorter interval to oestrus compared to short-term protocols.

It is reported that the rate of progestagen absorption from medroxyprogesterone acetate (MAP) sponges towards the end of a 14 d treatment is low (Greyling et al., 1997). The decrease in endogenous progesterone secretion, coupled with reduced P4 supply from the exogenous source towards the end of the treatment period in long-term P4 treatments (Johnson et al., 1996) induces an increase in the LH pulse frequency (Flynn et al., 2000; Evans et al., 2001). Therefore, follicles which grow under low P4 concentration remain dominant for a longer period than follicles which grow under high P4 concentration (Leyva et al., 1998; Viñoles et al., 2001). For the above reason, it be speculated that follicles in the CIDR-14 d were larger than in the CIDR-9 d group at the time of device withdrawal, and that the follicles grew even faster following treatment with eCG or ram exposure, which could have led to an earlier peak of oestradiol and onset of oestrus (Flynn et al., 2000).

Researchers who compared long-term and short-term P4 treatments also reported an earlier onset of oestrus in long-term compared to short-term P4 treatments (Viñoles et al., 2001; Ustuner et al., 2007). The mean duration of oestrus obtained in the present study was longer than what has been reported by previous studies

(Greyling et al., 1997; Zeleke et al., 2005), but shorter than what Evans et al. (2004) obtained. The contrast could possibly be due to the fact that the sheep breeds or the protocols (Evans et al., 2004) used in the above studies were different from the present study. When the interval to onset of oestrus and duration of oestrus are shortened, the timing of the LH surge and ovulation also occur earlier (Evans et al., 2004), which increases the efficiency of FTAI programmes.

The similar interval to onset of oestrus between eCG and ram exposed ewes when data were pooled implies that eCG and the ram effect induced similar patterns of follicular growth in ewes pre-treated with CIDR. No studies have compared eCG and the ram effect on P4 treated ewes under the same conditions/on the same flock. However, in the study by Evans et al. (2004), the ram effect was applied in a protocol with progestagens 3 d before sponge withdrawal while eCG was administered at sponge withdrawal. Their results showed a significant increase in LH secretion, an earlier onset of oestrus, LH surge and ovulation, and a reduced duration of oestrus in ram exposed compared to unexposed ewes. Similarly, other studies have reported a high synchrony of oestrus and an earlier onset of oestrus in P4 pre-treated ewes exposed to the ram effect following device withdrawal (Ungerfeld and Rubianes, 1999b; Romano et al., 2000). A recent study in Boer goats has also reported a similar observation (Romano et al., 2016). However, exposure of ewes to the ram effect during anoestrous without prior P4 treatment resulted in lower oestrous response (Knights et al., 2001). The similar response to oestrus synchronisation as observed by the oestrous response, onset of oestrus and duration of oestrus between treatment groups observed in the present study and especially with regards to eCG treatment and ram exposure at the end of P4 treatment suggests that eCG can be replaced with the ram effect in a P4 based protocol.

5.2 Effect of synchronisation treatment on follicular response at 48 h post CIDR withdrawal

The mean number of follicles at 48 h post CIDR withdrawal in the present study was slightly higher than the mean number of ovulated follicles (about 1.5 units) reported in synchronised (Takada et al., 2012) and unsynchronised ewes (Ginther et al., 1995). It can be considered that the number of follicles present at 48 h post P4 withdrawal (preovulatory stage) is a reflection of whether a single follicle or multiple follicles would be ovulated. The number of follicles at the preovulatory stage aligns with the ovulation rate which is breed specific if superovulation is not induced (Bartlewski et al., 1999a). A low dosage of eCG was used in this study which may explain the similarity in the mean number of follicles between groups treated with eCG and the ram effect. Although not significant, a numerically higher number of follicles observed in eCG treated ewes than ram exposed ewes when data were pooled suggests that eCG treatment played a role in influencing the number of follicles, even though the dosage was low. In studies where a dosage higher than 400 IU has been used, increased ovulation rate was observed (Husein et al., 1998; Zeleke et al., 2005). The observation in the present study that number of follicles tended to increase with increase in interval to onset of oestrus from 24 to 48 h implies that a greater proportion of ewes which began oestrus at or close to 48 h post CIDR withdrawal had more follicles than ewes which began oestrus much earlier. Gonzalez-Bulnes et al. (2005b) observed a total of 10.3 ± 0.3 follicles 24 h before onset of oestrus which number is known to decrease as the follicular phase advances close to the time of ovulation due to the dominance effect exerted by larger follicles (Ginther et al., 1995; Gonzalez-Bulnes et al., 2005b).

Mean diameter of the largest follicle obtained in this study agrees with findings of Ali (2007), who used a higher dosage of eCG (500 IU) than what was used in the present study. However, Barrett et al. (2004) who also used a high dosage of eCG (500 IU) obtained a larger diameter of the ovulatory follicle in Western White-Faced ewes treated with MAP for 12 d. In general, diameter of the largest follicle obtained

in the present study does not differ with results of other researchers who used P4 combined with eCG (Gonzalez-Bulnes et al., 2005b; Letelier et al., 2011) and with studies where prostaglandin ($PGF_{2\alpha}$) was used (Gonzalez-Bulnes et al., 2005b; Letelier et al., 2011; Fierro et al., 2016). The implication from the above observation is that different treatments (treatment type, length of P4 treatment and treatment of eCG or no treatment) may result in similar diameter of the ovulatory follicle which could possibly explain why diameter of the largest follicle in the present study was similar between treatment groups.

It is reported in cattle that a larger follicle diameter indicates follicle maturity (Vasconcelos et al., 2001). Moreover, size of the ovulatory follicle is known to influence ovulation rate, volume of the CL and progesterone secretion (Perry et al., 2007). On the other hand, a study by Viñoles et al. (2001) in sheep has shown that follicles with prolonged dominance have larger preovulatory diameters and in another study that not all follicles which grow under subluteal P4 levels persist (Viñoles et al., 1999). Bartlewski et al. (1999) also report that the preovulatory follicle diameter of non-prolific breeds is larger compared to that of prolific breeds. in the present study. This could possibly explain the manifestation of oestrous behaviour in a large proportion of ewes in the present study, which is induced by high oestradiol concentration produced by mature follicles (Gonzalez-Bulnes et al., 2005b). Besides the observation of large follicles at 48 h post CIDR, follicles with a diameter less than 4 mm were also observed, which diameter is considered not to be of a preovulatory stage (Ginther, 2000). In addition, the observation that ewes which had small follicles also had a high number of follicles could imply that these ewes possibly had ovulated earlier or that they would ovulate multiple follicles. According to Bartlewski et al. (1999b), ewes with multiple ovulations have a mechanism of ovulating mature follicles at different sizes. This was confirmed by the lambing data in the present study which showed that despite not having large follicle diameters, these ewes lambed to the first service period with twins. However, since mean diameter of the largest follicle at 48 h post CIDR withdrawal in the present study

compares with diameter of the ovulatory follicle in a number of studies with different treatments, used on different sheep breeds (Gonzalez-Bulnes et al., 2005b; Letelier et al., 2011; Fierro et al., 2016), suffice to say that most follicles were mature at 48 h post CIDR withdrawal, following treatment with protocols used.

A strong positive correlation between number of follicles and medium size follicles which was observed in the present study shows that ewes which had a high number of follicles mostly had medium size follicles. A further observation that number of follicles reduced with increase in average diameter of follicles per ewe implies that most ewes which had a large average diameter of follicles had fewer follicles than ewes which had a small average diameter. This observation is an indicator of dominance known to be associated with non-prolific breeds (Castonguay et al., 1990). In addition, the observation that some ewes had a single large follicle or that there was divergence in diameter between the dominant (large) follicle and the largest subordinate follicle in most ewes also suggests an expression of dominance (Ginther et al., 1995), which means that a high ovulation rate would not be expected of this flock.

5.3 Effect of synchronisation treatment on non-return rate and conception rate

The overall non-return rate (NRR) obtained in the present study was higher than what is reported by Olivera-Muzante et al. (2011) and Fierro et al. (2017), who used 2 injections of $PGF_{2\alpha}$ treatments for oestrus synchronisation. However, the high NRR of 75% following a 13 d treatment with MAP and 300 IU of eCG at sponge withdrawal which was reported by Olivera-Muzante et al. (2011) in the same study where $PGF_{2\alpha}$ was used is similar to the NRR obtained in CIDR-9 d ewes of the present study when data were pooled. It is thought that the CIDR-9 d treatment resulted in ovulation of younger and hence better quality follicles (Viñoles et al., 1999; Flynn et al., 2000; Viñoles et al., 2001), which perhaps explains why there was a higher NRR than in the CIDR-9 d than CIDR-14 d ewes, although the

difference was not significant. The reason for the lower NRR obtained in the present study compared to what was reported by Olivera-Muzante et al. (2011) could be attributed to use of the cervical AI method which results in lower fertility compared to intrauterine artificial insemination (AI), which method is known to improve conception (Fair et al., 2005).

A conception rate (CR) above 70% observed in the present study concurs with most previous studies conducted during the breeding season, where P4 was used in combination with eCG and FTAI (Olivera-Muzante et al., 2011; Vilariño et al., 2013; Swelum et al., 2015). A high CR obtained in the present study could be due to the fact that AI was performed twice at 48 and at 60 h post CIDR withdrawal, which ensured that spermatozoa were present in the uterine body close to the time of ovulation, for ewes ovulating early as well as those which would ovulate late, as in the studies where intrauterine AI was used (Fair et al., 2005; dos Santos-Neto et al., 2015). There was no significant difference between treatment groups in CR perhaps due to the limited number of animals used in the present study. However, the significantly high CR in the short-term P4 treated ewes than in ewes treated with long-term P4 when data were pooled could be attributed to ovulation of young follicles of high quality (Viñoles et al., 2001).On the other hand, long-term P4 treatment may negatively affect CR due to the effect of prolonged dominance of the ovulatory follicle on oocyte and embryo quality (Viñoles et al., 2001), which reason possibly explains the higher CR in CIDR-9 d compared to CIDR-14 d. Similar findings have been obtained by other researchers in sheep (Viñoles et al., 2001; Karaca et al., 2009), goats (Vilariño et al., 2011) and in cattle (Mihm et al., 1994).

Although use of short-term P4 treatments would reduce quantities of hormone used, offer the advantage for re-use of the intravaginal device (Vilariño et al., 2013; dos Santos-Neto et al., 2015) and also result in ovulation of high quality follicles (Viñoles et al., 1999; Flynn et al., 2000; Viñoles et al., 2001), recent studies still employ long-term P4 treatments and have obtained acceptable CR (Viñoles et al., 2011; Olivera-

Muzante et al., 2011; Swelum et al., 2015). The effect of long-term P4 treatment on fertility could be offset by addition of eCG, which increases the recruitment of small (new) follicles (Bister et al., 1999). In addition, the method of AI used could also influence CR following long-term P4 treatment, since it has been shown that not all follicles growing under long-term P4 treatment persist (Viñoles et al., 1999), which may affect the quality of follicles ovulated (Viñoles et al., 2001). Therefore, given that some researchers differ in their views on the effect of duration of P4 treatment on fertility (Evans et al., 2001; Viñoles et al., 2001), fertility following long-term P4 treatment appears to still be a contentious matter.

A lower CR observed in the present study in ewes which began oestrus early (12 and 24 h) compared to those which began oestrus late (36 and 48 h) may have been due to inadequate timing of AI in relation to the time of ovulation in the former. It seems that ewes which began oestrus early could have been served long after occurrence of the LH surge which may explain the difference in CR observed between the 2 groups. Timing of ovarian and endocrine events following oestrus synchronisation is variable and is affected by many factors, one of them being method of oestrus synchronisation (Evans et al., 2004; Ali et al., 2009). A further observation that there was a tendency for ewes confirmed pregnant to begin oestrus later could suggest that adequate timing of AI may have favoured the CIDR-9 d group (onset of oestrus in this group occurred later) and probably that is why the group had a significantly higher CR than the CIDR-14 d group. On the other hand, although the difference was not significant, it was observed that for the ewes which began oestrus early, CR was higher in the CIDR-9 d than the CIDR-14 d group, which implies that possibly the quality of oocyte was an important factor in the determination of CR between CIDR-9 d and CIDR-14 d ewes besides adequate timing of AI.

The present study observed no significant difference in the effect of eCG treatment and ram exposure on CR of P4 pre-treated ewes when data were pooled. There is

no published work which has evaluated CR and compared the effect of eCG treatment and ram exposure on P4 pre-treated ewes using the same flock/under the same conditions. However, reports from studies which either used the ram effect or eCG in P4 based oestrus synchronisation protocols show that fertility is improved when P4 or $PGF_{2\alpha}$ are combined with eCG (Luther et al., 2007) or the ram effect (Ungerfeld et al., 2005; Contreras-Solis et al., 2009) respectively, compared to protocols where P4 (Luther et al., 2007) or $PGF_{2\alpha}$ (Godfrey et al., 1999; Viñoles et al., 2011; Fierro et al., 2016) alone are used. Acceptable levels of CR have also been obtained during the anoestrous season where P4 has been combined with eCG (Ungerfeld and Rubianes, 2002) or the ram effect (Knights et al., 2001). Therefore, given the implication from the present study that the use of eCG or the ram effect in combination with P4 has similar effect on CR, it can be concluded that eCG could be replaced by the ram effect in P4 based oestrus synchronisation protocols without affecting CR. This could be done in light of the fact that repeated use of eCG has been reported in previous studies to result in reduced fertility with each breeding (Anel et al., 2005), due to accumulation of residual antibodies from previous treatments (Bodin et al., 1997; Drion et al., 2001b).

It is important to note that in the study by Fierro et al. (2017), NRR was very close to pregnancy rate (59.7% and 56.9%, respectively), whereas in the present study, the difference between NRR and CR was not very close, although analysis showed that the 2 variables were strongly related. A higher value of CR than NRR could indicate that some ewes which returned to oestrus were pregnant. The study of Morris et al. (2008), shows that not all ewes which exhibit oestrus could be repeats. Therefore, while NRR as one of the methods used to assess conception in small ruminants (Olivera-Muzante et al., 2011), may be reliable, hormonal (Godfrey et al., 1999) and ultrasound methods (Viñoles et al., 2011; Swelum et al., 2015) seem to be superior over NRR in the assessment of conception.

5.4 Effect of synchronisation treatment on interval from artificial insemination to lambing, twinning rate and number of lambs born

The mean AI to lambing interval obtained in the present study indicates that not all ewes lambed to the first service period. The range of the AI to lambing interval for ewes which lambed to the first service period is in agreement with the report of Evans et al. (2001) of 142-152 d post breeding and with the mean AI to lambing interval reported by Fleisch et al. (2012) of 149.8 ± 1.4 d (mean ±SD). Results of the present study show that a greater proportion of ewes lambed to the first service period. The proportion of ewes lambing to the first service period in the CIDR-9 d group was lower than the CIDR-14 d when data were pooled. A similar proportion to what was observed in the CIDR-9 d group was reported by Knights et al. (2001) in anoestrous ewes treated with CIDR for 5 d and exposed to the ram effect prior to natural mating.

However, other studies where short-term P4 treatments were used reported a higher proportion of ewes lambing to the first service period (above 50%) both in the breeding (Martemucci and D'Alessandro, 2011; Fleisch et al., 2012) and non-breeding season (Fukui et al., 1993; Fukui et al., 1994), than what was obtained in the CIDR- 9 d group of the present study. The proportion of ewes lambing to the first service period obtained in the CIDR-14 d group is in agreement with the report of Greyling et al. (1997), who used a similar protocol (long-term) as in the present study on Merino ewes. However, other researchers obtained a higher proportion (above 65%) of ewes lambing to the first service period with long-term P4 treatment during the breeding (O'Doherty and Crosby, 1990; Boscos et al., 2002; Swelum et al., 2015), and in the non-breeding season (Greyling et al., 1994).

It is unclear why the CIDR-9 d group which was diagnosed with high NRR and CR resulted in a low proportion of ewes lambing from 147-154 d post AI (period 1). Reasons for failure of ewes to lamb to first service include; fertilisation failure at first

service and embryonic or foetal losses during gestation (Olivera-Muzante et al., 2011). Embryonic losses are known to occur mainly due to disruption of progesterone levels following ovulation, which hormone is important in the maintenance of pregnancy (Kleeman et al., 1994). In the present study, overall gestational losses [CR - proportion of ewes lambing to the first service period/CR; Olivera-Muzante et al., 2011] were estimated at 29.3%, whereas when data were pooled, gestational losses in the CIDR-9 d and the CIDR-14 d groups were 44.1% and 6.2% respectively.

Gestational losses similar to what was obtained in the present study (29.3%) were reported by Mukasa-Mugerwa et al. (1994) and Knights et al. (2001). However, the percentage gestational loss estimated in the CIDR- 9 d group was very high. It is expected that gestational losses mostly occur at the embryonic stage rather than at a later stage, given the weak functionality of the placenta at the early stages of pregnancy (Chavatte-Pamer et al., 2012). Late gestational losses are attributed to a reduced supply of nutrients to the placenta and high temperature during pregnancy (Vatnick et al., 1991). However, it is unlikely that any of the above factors were responsible for the gestational losses after 35 d given that all animals in the present study were managed under the same conditions after the treatment period. Moreover, losses were mostly observed in the CIDR-9 d group, which points to the fact that the losses had to do with the treatment rather than management factors.

In their study, Olivera-Muzante et al. (2011) obtained significantly higher gestational losses following use of 2 injections of $PGF_{2\alpha}$, 7 d apart than in 13 d treatment of ewes with MAP combined with eCG (300 IU). Hence it is logical to imagine that gestational losses may be influenced by the type of protocol used. Short-term treatment in combination with eCG or the ram effect in this study could have been detrimental to fertility as shown in the study by Viñoles et al. (2001). The difference in the percentage gestational losses estimated beyond 35 d between the CIDR-9 d and CIDR-14 d treatment groups in the present study may be due to factors related

to the different patterns of follicular development induced by the 2 treatments. This could have had an effect on oestradiol production during the follicular phase, thus affecting the uterine environment (Murray, 1992; Nancarrow and Hill, 1994).

In addition, it was observed that gestational losses within the CIDR-9 d group were more pronounced in the ram exposed than eCG treated ewes, perhaps due to the fact that use of eCG is known to result in multiple ovulations (Ali, 2007). Hence in ewes with multiple embryos or foetuses, one embryo or foetus may be lost other than a total loss of the pregnancy (Schrick and Inskeep, 1993). It is worth noting that studies which used short-term P4 and reported a higher proportion of ewes lambing to the first service period than what was obtained in the present study also used $PGF_{2\alpha}$ at the end of the treatment period (Martemucci and D'Alessandro, 2011; Fleisch et al., 2012). In addition, these studies used intrauterine or natural methods of breeding (Fukui et al., 1994; Martemucci and D'Alessandro, 2011; Fleisch et al., 2012), which was possibly the reason for better lambing performance observed in these studies. Whereas other researchers who studied fertility following short-term P4 treatment did not report on the proportion of ewes which lambed to the first service period (Viñoles et al., 2001; Karaca et al., 2009; Ali et al., 2009), suffice to say that benefits of oestrus synchronisation using various protocols can only be fully realised when ewes which conceive carry their foetuses to term (Mukasa-Mugerwa et al., 1994).

A similar twinning rate as what was obtained in the present study has been reported in tropical Menze ewes (Mukasa-Mugerwa et al., 1994 and Merinos (Viñoles et al., 2011). The proportion of singletons compared to twins which was reported in Merino ewes by Viñoles et al. (2011) also concurs with the results of the present study. This implies that Merinos are generally non-prolific as has been reported in an earlier study on a larger Merino flock (Kilgour, 1992). Results of the twinning rate obtained in period 1 compared to ewes which lambed from 166-186 d post AI (period 2) and the twinning rate in ram exposed ewes which lambed in period 1 compared to period

2 when data were pooled indicate that twinning and consequently number of lambs born was to some degree influenced by eCG treatment in ewes which lambed in period 1 rather than the ram effect. Although a low dosage of eCG was used in the present study, it is widely known that eCG increases ovulation rate in small ruminants (Mutiga and Mukasa-Mugerwa, 1992; Letelier et al., 2011) and in cattle (Sa Filho et al., 2010), which may affect twinning and number of lambs born, especially when a high dosage is used (Ali, 2007). On the other hand, it is reported in sheep Mukasa-Mugerwa et al. (1994) and in goats Romano et al. (2016) that the male effect does not affect ovulation rate. However, it has been shown that nutritional methods which ensure that ewes have a high body condition score could improve ovulation rate and number of lambs born in response to the ram effect (Scaramuzzi et al., 2014).

The mean number of lambs born in this study was similar between treatment groups and was also similar when data were pooled, which implies that length of P4 treatment, administration of eCG and ram exposure did not influence the number of lambs born. However, Zeleke et al. (2005) who used the same dosage of eCG as in the present study on anoestrus Dorper ewes and Ali (2007) who used a high dosage of eCG (500 IU) on cyclic Ossimi ewes obtained a significant difference between eCG treated and untreated ewes. Mean number of lambs born in the present study is similar to what was reported in studies with long-term (Greyling et al., 1997; Boscos et al., 2002; Zeleke et al., 2005) and short term (Martemucci and D'Alessandro, 2011) P4 treatments combined with eCG or short-term P4 treatments combined with the ram effect (Knights et al., 2001). Although a higher mean number of lambs born than in the present study has been reported where a high dosage of eCG was used (Ali, 2007), it is important to note that treatment with a high dosage of eCG may not influence lambs born as shown in study of Swelum et al. (2015). A higher ovulation rate observed in anoestrous Finn ewes (Husein et al., 1998) than in cyclic Suffolk ewes (Evans et al., 2001), where the same dosage of eCG was

used further suggests that there could be breed differences in the response to stimulation of ovulation rate by eCG.

A lower mean number of lambs born (less than 1.3) than what was obtained in the present study has been reported in trials where $PGF_{2\alpha}$ alone has been used (Mutiga and Mukasa-Mugerwa, 1992; Olivera-Muzante et al., 2011) and in P4 protocols where eCG has not been used (O'Doherty and Crosby, 1990; Greyling et al., 1994; Zeleke et al., 2005). This could possibly be due to variability in the timing of ovarian and endocrine events leading to oestrus and ovulation, which affects CR (Wildeus, 2000) and thus reduce the chances of having more lambs born. In other studies, a higher number of lambs born has been observed where natural mating was used (Knights et al., 2001; Ali, 2007; Fleisch et al., 2012), compared to studies which used cervical AI (Greyling et al., 1997; Zeleke et al., 2005; Swelum et al., 2015). It has also been observed in a recent study that ovulation rate can be increased by oestrous synchronisation (Letelier et al., 2011), due to lower expression of follicle dominance in treated than untreated ewes, which is known to favour ovulation rate (Mandiki et al., 2000).

Therefore, oestrus synchronisation protocols and breeding methods which improve conception should also influence number of lambs born. The significantly higher mean number of lambs born observed in older ewes compared to maiden ewes is in agreement with the findings of O'Doherty and Crosby (1990), who observed that eCG did not increase number of lambs born in maiden ewes. Ovulation rate is generally known to increase with age, reaching a maximum between 3-6 yrs and then decline (Hafez, 1993). It is also important to note that increased ovulation rate in maiden ewes following eCG treatment may not be realised by the increase in number of lambs born, given the limitation of their uterine capacity and competition between embryos or foetuses for access to the endometrium (Dixon et al., 2007).

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, all protocols investigated in the present study were effective in synchronising oestrus, with tight synchrony observed between 36 and 48 h post controlled internal drug release (CIDR) withdrawal. Oestrous response, onset of oestrus, duration of oestrus, number of follicles, diameter of the largest follicle, non-return rate, conception rate (CR), artificial insemination (AI) to lambing interval, number of lambs born, and twinning rate were similar between treatment groups. A significantly longer interval to onset of oestrus and higher CR were observed in ewes primed with CIDR for 9 d (CIDR-9 d) than in ewes primed with CIDR for 14 d (CIDR-14 d) when data were pooled, but were similar in eCG and ram exposed ewes. Short-term P4 protocols led to higher gestational losses 35 d post AI than long-term P4 protocols, given that a lower proportion of the CIDR-9 d ewes lambed to the first service compared to the CIDR-14 d ewes although the former had higher CR. Hence, short-term P4 treatment combined with the ram effect might not be efficient, as the proportion of ewes which lambed to the first service was low compared to short-term P4 treatment combined with eCG and long-term P4 treatment combined with eCG or the ram effect. Therefore, it can be concluded that the 4 oestrus synchronisation protocols investigated in this study were effective in synchronising oestrus with similar response to synchronisation of oestrus and fertility between treatment groups, although the proportion of ewes lambing to the first service period in the Ramshort group was low. Given the above results, the ram effect can replace eCG in a progesterone based oestrus synchronisation protocol due to the negative effect of repeated use of eCG on fertility. However, short-term P4 treatment

combined with the ram effect may be used with caution given the observation in the present study that it resulted in a small proportion of ewes lambing to the first service period.

6.2 Recommendations

- Based on the high response to oestrus synchronisation and adequate fertility obtained with eCGshort, Ramlong and eCGlong protocols, they can be recommended for use in fixed time artificial insemination to improve synchronisation of oestrus and ovulation during the breeding season.

- Of the 4 protocols, the Ramlong protocol offers the benefit of reduced cost, reduced hormonal use and adequate fertility compared to other protocols.

- Further investigation involving endocrine data to improve the protocols.

- Further investigation to establish possible causes of high reproductive wastage after 35 d post AI associated with CIDR-9 d protocols.

- Further research into development of short-term progesterone treatment (perhaps shorter than 9 d) in combination with prostaglandin and the ram effect. This would ensure reduction in the quantity of hormones used for treatment and also offer the benefit of re-use of the intravaginal device.

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