



# **Effect of the Duration of Progesterone Treatment on Synchronising Oestrus Outside the Natural Breeding Season in Merino Ewes**

By

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## Declaration

I, Nhlakanipho Sam Zulu, declare that this dissertation, which I hereby submit for the degree MSc (Agriculture) (Animal Science) at the University of Zululand, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:



Date: 03/02/2022

## **Dedication**

This work is dedicated to my mother, Ntombenhle Zulu, and my sisters and brothers.

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## Abstract

This study aimed to evaluate the effect of the duration of progesterone treatment on the reproductive performance of South Africa Merino ewes during the spring breeding season. The study compared the effect of long and short-term progesterone treatment and its combination with or without equine chorionic gonadotropin on oestrous response and hormonal profiles. Seventy-six (76) Merino ewes aged between 2–5 years were allocated into two groups, 40 for long-term and 36 for short-term progesterone treatments. For the long and short-term progesterone treatment groups, controlled internal drug release devices were inserted for 14 and 11 days, respectively. At the withdrawal of the controlled internal drug release device, half of each group were injected intramuscularly with 300 IU of equine chorionic gonadotropin, resulting in four treatment groups. The oestrous response was monitored using teaser rams. Blood samples were collected to measure the serum progesterone and oestradiol concentrations. One-way ANOVA of the SPSS® software (IBM SPSS Statistics, Version 23.0, 2015, Armonk, NY., USA) was used for statistical analysis. The means were considered significant at  $P < 0.05$ . The overall oestrous response obtained was 96%. A greater proportion of the ewes showed oestrous signs at 48 and 60 hours post controlled internal drug release withdrawal. There was no significant difference ( $P > 0.05$ ) in oestrous response and duration between the treatment groups. Overall, the mean interval to the onset of oestrus in this study was  $46.8 \pm 1.4$  hours. Most ewes began exhibiting oestrus at 24 and 36 h post controlled internal drug release withdrawal. There was no significant difference ( $P = 0.07$ ) in the mean interval to the onset of oestrus between the treatment groups. Progesterone hormone did not differ significantly ( $P > 0.05$ ) between the treatment groups at controlled internal drug release insertion and removal and 48 hours post removal. However, oestradiol was

significantly higher at controlled internal drug release removal and 48 hours post removal ( $P < 0.05$ ) than at insertion in all treated groups. Therefore, it was concluded that using the progesterone synchronisation protocol, regardless of the duration or conjunction with eCG treatment, can effectively synchronise oestrus during the spring breeding season in South Africa Merino ewes. For seasonal breeders who use South Africa's spring breeding season, the progesterone oestrous synchronisation protocol is recommended to improve the reproduction activity in Merino ewes.

**Keywords:** oestrous synchronisation, long-term progesterone, short-term progesterone, controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG), sheep, oestradiol, reproductive hormone

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## **List of Abbreviations**

CIDR: Controlled internal drug release

eCG: Equine chorionic gonadotropin

FGA: Fluorogestone acetate

LH: Luteinising hormone

MAP: Medroxyprogesterone

SEM: Standard error mean

SA: South Africa

SAn: South African

# 1 CHAPTER ONE: INTRODUCTION

## 1.1 Overview

One of the expanding segments of the agricultural economy is the sheep livestock sector, particularly in developing countries (Food and Agriculture Organization, 2009). Interest in meat and dairy products keep expanding due to the consistently growing population, and questions emerge concerning how this demand will be met and by who (United Nations, 2013). Sheep are used for meat and wool production (Haenlein, 2004; Hasani *et al.*, 2018). Therefore, sheep play a vital role in supporting the livelihood of the human population as a whole (Hasani *et al.*, 2018). Farmers in many countries favour keeping sheep over other livestock mainly because sheep are easy to manage and dwell in a small area (Gore, 2016). Furthermore, sheep are a significant part of the agribusiness economy of South Africa (SA) (Hasani *et al.*, 2018).

Sheep are seasonal breeders and influenced by the photoperiod. The two main mating seasons for sheep in SA are autumn and spring (Wentzel, 2012). The autumn breeding season is characterised by peak sexual activities in sheep due to the short-day photoperiod (Wentzel, 2012). On the other hand, the spring mating season has average sexual activities due to the long-day photoperiod (Wentzel, 2012). Therefore, the natural breeding season for sheep is autumn, and their reproductive performance is higher in autumn than in spring.

Advancements, such as oestrous synchronisation (Piotroski *et al.*, 2013; López-García *et al.*, 2021), can be used for the reproductive management of sheep to improve reproduction performance in the non-breeding season (Santos-Jimenez *et al.*, 2020). Using reproductive technology, sheep can reproduce during breeding and non-breeding seasons (Dogan *et al.*, 2005; Santos-Jimenez *et al.*, 2020). Oestrous

synchronisation is a powerful management tool that has been effectively utilised for artificial insemination and natural mating, particularly in ruminants (Oliveira *et al.*, 2016). This technique applies hormones to stimulate animals to express oestrus simultaneously. Oestrous synchronisation is practical and successful when used in sheep to stimulate ovulation in the non-breeding season (Martinez *et al.*, 2015). The administration of progesterone for between 12–14 days through controlled internal drug release (CIDR) devices, with or without the administration of equine chorionic gonadotropin (eCG) at device removal, is the commonly used oestrous synchronisation protocol that mimics the luteal phase of the oestrous cycle (Kusina *et al.*, 2000; Martinez *et al.*, 2015; Santos-Jimenez *et al.*, 2020). Generally, a single 300 IU dose of eCG is injected intramuscularly at progesterone removal (Martinez-Ros & Gonzalez-Bulnes, 2019). This oestrous synchronisation protocol is also practised in SA. However, most South African (SA) studies examine long-term progesterone treatment (12–14 days) (Gore, 2016).

Currently, short-term progesterone (5–11 days) is favoured due to better fertility, resulting from the sufficient suppression of luteinising hormone (LH) pulse frequency and ovulation of the newly recruited follicles (Martinez-Ros & Gonzalez-Bulnes, 2019; Nakafeeroa *et al.*, 2020). Therefore, this study evaluated the effect of the duration of progesterone treatment on the reproductive activity of SA Merino ewes during the spring breeding season.

## **1.2 Problem statement**

Breeding season is the most important factor affecting reproduction in sheep. Generally, when compared to autumn breeding, breeding sheep in spring leads to low oestrous exhibition, ovulation, conception and lambing rates due to the photoperiod effect, and causes complications for spring season natural and artificial

insemination breeding. Furthermore, using the spring breeding season leads to reduced reproduction performance, reducing the lambing percentage and agribusiness economy compared to the autumn breeding season. Unfortunately, in SA, most sheep farmers prefer using the spring breeding season because there is enough grazing in autumn to feed the lambing ewes and their lambs. Oestrous synchronisation improves reproductive performance and induces oestrus in the spring breeding season by mimicking physiological processes that occur during the natural autumn breeding season. Synchronisation of oestrus and induction of ovulation is mainly based on the progesterone protocol, which can be either long-term (12–14 days) or short-term (5–11 days), followed by the intramuscular injection of eCG at CIDR withdrawal (López-García *et al.*, 2021).

There are different results reported for the effectiveness of progesterone duration. Some studies favour short-term progesterone treatment and suggest it is more effective at inducing oestrus outside the natural breeding season. Other studies find that short and long-term progesterone treatments are similarly effective at stimulating oestrus outside the natural breeding season. Therefore, this study evaluated the effect of the duration of progesterone treatment on Merino ewes' reproductive activity during the spring breeding season.

### **1.3 Aim of the study**

This study aimed to evaluate the effect of the duration of progesterone treatment on reproductive activity during the spring breeding season in SA Merino ewes.

### **1.4 Objective of the study**

The overarching objective of this study was to compare the effect of oestrous synchronisation on oestrous response and hormonal profiles of SA Merino ewes

treated with progesterone for 11 days with those treated for 14 days.

### **1.5 Hypotheses of the study**

H1: Merino ewes treated with progesterone for 14 days will have improved oestrous response and hormonal profiles than those treated for 11 days.

## **2 CHAPTER TWO: LITERATURE REVIEW**

### **2.1 Introduction**

Reproductive technologies, such as oestrous synchronisation in farm animals, have allowed farmers to advance the management efficiency and biology of their animals (Martemucci & D'Alessandro, 2011). Oestrous synchronisation is a management strategy used to give tightly synchronised oestrus and satisfactory fertility in the wake of mating outside or amid the breeding season (Martinez-Ros *et al.*, 2018b; Nogueira *et al.*, 2016). Oestrous synchronisation assists seasonal breeders with managing reproduction and has enabled the scientific study of the reproductive endocrine processes (Vasantha, 2016). In addition, utilising oestrous synchronisation allows the possibility to time the lambing and breeding seasons to take benefit of seasonal variations in market demand, labour resources and forage availability (Swelum *et al.*, 2018). This strategy is also useful in preparing for fixed-time artificial insemination (Godfrey *et al.*, 1997; Swelum *et al.*, 2018). Sheep oestrous synchronisation has been accomplished using several methods with varying degrees of success. A common protocol is progesterone treatment for 12–14 days via CIDR with or without the injection of eCG at the device's removal (Martinez *et al.*, 2015). During sheep's breeding and non-breeding seasons, this protocol or method is used frequently to synchronise oestrous (Swelum *et al.*, 2015). However, there is a need to address the impediments and improve the effectiveness of existing protocols and methods (Martemucci & D'Alessandro, 2011).

### **2.2 Oestrous synchronisation methods in sheep**

Proficient oestrous synchronisation in sheep ordinarily includes control of the oestrous cycle's luteal phase. Luteal phase control relies on either expanding the life

expectancy of the corpora lutea (Smith *et al.*, 2010) or regressing or relapsing it rashly. Generally, to broaden the luteal stage progesterone is used, while prostaglandin is applied to shorten it (Cox *et al.*, 2012). The two hormones stimulate natural control amid the oestrous cycle. Progesterone is produced by the corpus luteum amid the luteal phase while the uterus produces prostaglandin towards the end of the luteal stage to relapse the corpus luteum. Progesterone inhibits the arrival of LH, which is responsible for ovulation by sending a negative feedback mechanism to the hypothalamus (Piotroski *et al.*, 2013).

Although the technique of oestrous synchronisation has been a valuable and successful management tool that has been employed to improve reproductive efficiency (Swelum *et al.*, 2015), there are some reported disadvantages. It is understood that long-term progesterone treatment duration leads to low concentrations of progesterone towards the treatment end, resulting from the reduction in progesterone absorption from the exogenous source (Martinez-Ros *et al.*, 2018a). There is further evidence that when the corpus luteum relapses and the exogenous source progesterone is inadequate to suppress pulse frequency of the LH, the follicles turnover is slowed (Martinez-Ros *et al.*, 2018a; Martinez-Ros & Gonzalez-Bulnes, 2019; Piotroski *et al.*, 2013). Therefore, this results in ovulation of follicles that are aged at device removal, which may pose a threat to fertility (Oliveira *et al.*, 2016). Alternatively, short-term progesterone treatment durations (Martinez-Ros *et al.*, 2018a) leads to improved fertility because of sufficient suppression of the pulse frequency of the LH and ovulation of newly recruited follicles (López-García *et al.*, 2021; Martinez-Ros *et al.*, 2018a; Nakafeero *et al.*, 2020). Furthermore, short-term treatment durations are recognised as effectively similar to long-term durations for inducing oestrus, ovulation and a corpora lutea that is completely functional

(Martinez-Ros *et al.*, 2019).

Oestrous synchronisation protocols utilise progesterone, such as fluorogestone acetate (FGA), norgestomet inserts, CIDR, melengestrol acetate and medroxyprogesterone (MAP), incorporate intravaginal device. CIDR is considered one of the most efficient methods to synchronise oestrus and improve the reproductive performance of sheep (Swelum *et al.*, 2015; Swelum *et al.*, 2018). This progesterone protocol can be applied in conjunction with co-treatments, such as human chorionic gonadotropins and eCG for oestrous synchronisation to induce oestrus onset and improve the rate of ovulation. The protocol using progesterone and its analogues are broadly used for oestrous synchronisation in sheep. It has been demonstrated that proficient oestrous synchronisation is regularly accomplished when progesterone, or its analogues, is utilised (Biehl *et al.*, 2019; Romano, 2004) in small ruminants outside the breeding season (Dogan *et al.*, 2005; Santos-Jimenez *et al.*, 2020). Therefore, in this study, only the progesterone protocol was used, with and without eCG treatment, but with two different treatment lengths, namely 14 days for long-term and 11 days for short-term treatment.

### **2.2.1 Type of progesterone pessary in conjunction with eCG**

A intravaginal device impregnated with progesterone in combination with eCG treatment is generally utilised for sheep oestrous synchronisation (López-García *et al.*, 2021). The progesterone intravaginal devices usually used incorporate FGA, MAP, and CIDR (Kusina *et al.*, 2000; Swelum *et al.*, 2015); all these devices are similarly powerful (Kusina *et al.*, 2000; Romano, 2004; Romano *et al.*, 2000; Swelum *et al.*, 2015). Some investigations of oestrous synchronisation in sheep have tried the adequacy of intravaginal devices without co-treatments. Intravaginal devices impregnated with progesterone embedded in sheep for 14 days amid the breeding

season demonstrated no difference in the length of oestrus, reaction to oestrus and the lambing rate between CIDR, FGA and MAP (Swelum *et al.*, 2015). Additionally, Kusina *et al.* (2000) reported the earlier beginning of oestrus in the CIDR treatment group than in the FGA and MAP groups in sheep.

Furthermore, in synchronising oestrus in non-lactating does and ewes amid the regular breeding season, FGA and MAP intravaginal progestagen sponges containing progesterone treatments or in combination with eCG were found to be similarly proficient (Gore, 2016; Nogueira *et al.*, 2016; Oliveira *et al.*, 2016). A progesterone-based protocol in conjunction with eCG treatment may improve follicular advancement, trigger ovulation relating to hormonal profiles, and increase oestrous response (Abecia *et al.*, 2012; El-Mokadem *et al.*, 2019; Motlomelo *et al.*, 2002). For the most part, the prescribed dosages are from 200 to 600 IU of eCG. The dosage may differ per breeding season and the breed, age and weight of every animal. In previous studies in sheep, the intravaginal impregnated devices (FGA, CIDR and MAP) were embedded for 12–14 days, and at the pessaries' withdrawal, 300–500 IU of eCG was infused intramuscularly (Gore, 2016; Martemucci & D'Alessandro, 2011). These authors found that the reaction to oestrus was comparable for FGA, CIDR and MAP. The oestrus reaction in ewes between the MAP and CIDR treatments impregnated with progestin was comparable when pessaries were embedded for 14 days and infused with 500 IU of eCG amid the non-breeding season (Kusina *et al.*, 2000; Gore, 2016). Moreover, when intravaginal pessaries were embedded for 14 days, and 350 IU of eCG administered intramuscularly at removal, comparable reactions to oestrus for FGA and CIDR were observed (Gore, 2016).

### **2.2.2 Duration of progesterone treatment in conjunction with eCG**

Oestrus and ovulation are effectively stimulated by eCG (Martemucci & D'Alessandro, 2011; Romano, 2004). Recently, the practicality of different short-term (5–11 days) protocols using CIDR devices, with or without eCG injection at CIDR removal, has been evaluated (Martinez-Ros *et al.*, 2018a; Martinez-Ros & Gonzalez-Bulnes, 2019; Nakafeero *et al.*, 2020). Martinez-Ros and Gonzalez-Bulnes (2019) found that after 5 days without eCG treatment, the fertility and pre-ovulatory events seemed improved over CIDR device's insertion for 6 or 7 days. In addition, these authors reported that the results for 5 days of treatment were similar to those when the CIDR device was inserted for 14 days and eCG was injected at removal. These results may indicate that the follicle that is dominantly stimulated by the CIDR insertion reaches its growing phase height 5 days later, and is capable of ovulating without eCG induction when the CIDR is withdrawn (Martinez-Ros *et al.*, 2018a). However, after 1 or 2 days the follicle would be in the regressing or static phase and, therefore, its capability to ovulate without eCG stimulation would be compromised (Martinez-Ros *et al.*, 2018a; Martinez-Ros & Gonzalez-Bulnes, 2019). The results of studies by Martinez-Ros *et al.* (2018a, 2019) showed that when eCG was used, more than 80% of the ewes exhibited oestrus, and the earlier and more synchronised onset of oestrus. Among the eCG treatment groups, the sheep treated for 14 days showed earlier oestrus onset than those in the short-term treatment groups (Martinez-Ros & Gonzalez-Bulnes, 2019).

In addition, a previous study found that there was similar fertility under field circumstances between treatments of 14 days of CIDR in conjunction with eCG and 5 days of CIDR treatment without eCG. However, when eCG was used in conjunction with the 5 days CIDR treatment, fertility increased by approximately

20% (Martinez-Ros *et al.*, 2018a). These results highlight the benefits of using eCG regardless of the duration of CIDR insertion.

Furthermore, Martinez-Ros *et al.* (2018a) indicated that the treatment based on CIDR- protocols, independent of the duration of the treatment and the eCG addition, stimulate the oestrous response after CIDR removal in most of the treated sheep. Both the treatment length and the injection of eCG have important effects on the grouping and timing of oestrus and successive ovulation ( Martinez-Ros *et al.*, 2018a; Nakafeero *et al.*, 2020; Piotroski *et al.*, 2013). Briefly, eCG injection grouped and advanced the oestrous response and stimulated ovulation entirely in ewes that displayed oestrus (López-García *et al.*, 2021; Martinez-Ros & Gonzalez-Bulnes, 2019; Nakafeero *et al.*, 2020; ). In the eCG treated sheep, the onset of oestrus appeared later in the short-term than in the long-term treatment groups; such a dissimilarity was not observed in sheep without eCG injection (Martinez-Ros *et al.*, 2018a; Martinez-Ros & Gonzalez-Bulnes, 2019).

Synchronisation of oestrus with progesterone in combination with eCG improves the hormonal profiles in sheep. This improvement in hormonal profiles has been attributed to the way that eCG induces the beginning of oestrus, advances the follicular improvement and triggers ovulation (Nogueira *et al.*, 2016), and consequently may lead to the higher odds of pregnancy following artificial insemination or natural breeding (Martinez *et al.*, 2015; Romano, 2004; Swelum *et al.*, 2015). The serum progesterone concentration followed a similar pattern in both long and short-term progestin treatments from the insertion of CIDR until removal; it was reduced by 71–75% within 6–12 hours following removal in the study conducted by Martinez-Ros *et al.* (2018a). There was a linear and marked increase in oestradiol concentration in the short-term progesterone treatment from progesterone

withdrawal to 36 hours later but a lesser increase at 24 hours following removal (Martinez-Ros *et al.*, 2018a). In the study conducted by Ekiz *et al.* (2006), oestradiol concentration rose until 38 hours following progesterone withdrawal and then displayed a wavelike pattern; it began to rise one day before the onset of behavioural oestrus, and it appeared high throughout oestrus.

### **2.3 Advantages of using CIDR**

CIDR is the most used device to synchronise oestrus, mainly because resynchronisation is possible after breeding, and it is simple to use (Van Cleeff *et al.*, 1998). CIDR devices can effectively synchronise oestrus, enhancing a short and predictable period of bringing groups of ewes into oestrus ( Swelum *et al.*, 2018; Van Cleeff *et al.*, 1998; ). The response to oestrus was higher for CIDR when combined with eCG treatments and lower when CIDR was used without eCG, which allowed fixed-time artificial insemination to be successful with higher pregnancy rates (Awel *et al.*, 2009; López-García *et al.*, 2021).

### **2.4 Summary**

From the literature reviewed, it is evident that the effect of oestrous synchronisation on oestrus response and the hormonal profiles in sheep has been studied in breeding and non-breeding seasons. Oestrous synchronisation applying progesterone in conjunction with a co-treatment, such as eCG, is proficient to improve sheep's oestrous response and hormonal profiles. The literature reviewed highlighted that progesterone utilisation in combination with eCG has been the most common practice and a successful protocol in sheep. Oestrous synchronisation and ovulation for breeding in sheep is based chiefly on the insertion of intravaginal devices containing either progesterone CIDR inserts or progestagens (sponges impregnated with FGA

or MAP).

The literature examined identified CIDR as the most common device used for progesterone treatment to synchronise oestrus. Long-term progesterone treatment is the most used synchronisation protocol, but recently short-term progesterone has been reported as a valuable approach. Moreover, the use of progesterone improves the hormonal profiles of ewes. Furthermore, the CIDR-based progesterone protocols used in sheep stimulate the appearance of oestrus after CIDR withdrawal, regardless of eCG addition and the duration of the treatment. This study evaluated the effect of progesterone treatment's duration on oestrus response and hormonal profiles in SA Merino ewes during SA's spring breeding season.

## **3 CHAPTER THREE: MATERIALS AND METHODS**

### **3.1 Ethical approval**

The methodology and materials of this study were approved by the University of Zululand's animal ethics committee (Project no. UZREC 171110-030).

### **3.2 Experimental site and duration**

This experiment was conducted during the SAn spring breeding season. The experiment was conducted at Cedara Research Station in the uMgungundlovu District Municipality, approximately 16 km from Pietermaritzburg and 12 km from Howick., KwaZulu-Natal, SA. The site is located at latitude 29° 32' 52.12" S, longitude 30° 15' 58.19"E, at an elevation of 1037 m to 1448 mm above sea level. Cedara has a temperate climate; the winters are cold, with frost and the summers are warm to hot. The average rainfall in summer is approximately 900 mm/annum (Ndlovu *et al.*, 2021).

### **3.3 Experimental animals, feeding and management system**

A total of 76 Merino ewes aged 2–5 years were used in this study. All experimental animals followed the regular feeding program implemented at the research station by the farm manager, such as allowing them to graze in the station's different camps. In addition, the animals had free access to water at all times. Animals were ensured to be healthy, free of any disease and clinically normal during the experiment.

### 3.4 Experimental design

The experiment used a 2 by 2 factorial design, as shown in Table 3.1. Seventy six (76) Merino ewes were allocated into four groups based on oestrous synchronisation protocols. Animals were grouped based on age and then randomly allocated into treatment groups balancing the ages so that all the groups had a similar average age. Four groups were formed: 1. CIDR + eCG; long-term progesterone and eCG treated ewes (14 days, n = 20); 2. CIDR + eCG; short-term progesterone and eCG treated ewes (11 days, n = 18); 3. CIDR; long-term progesterone treated (14 days, n = 20); and 4. CIDR; short-term progesterone treated (11 days, n=18).

**Table 3.1** Experimental animals and the number of animals per group

| <b>Progesterone duration</b> | <b>Groups</b>       | <b>Groups</b> |
|------------------------------|---------------------|---------------|
| 14 days (n = 40)             | CIDR + eCG (n = 20) | CIDR (n = 20) |
| 11 days (n = 36)             | CIDR + eCG (n = 18) | CIDR (n = 18) |

### 3.5 Treatment

Each ewe received a CIDR device (Pfizer, New Zealand) containing 0.3 g progesterone. The duration of the treatment was 14 days for the long-term and 11 days for the short-term groups. At CIDR withdrawal, 20 of the long-term and 18 of the short-term treated ewes were injected intramuscularly with 300 IU of eCG (Intervet Schering-Plough Animal Health (Pty.) Ltd., SA). The other 20 of the long-term and 18 of the short-term treated ewes were only inserted with a CIDR device and were not injected with 300 IU of eCG at CIDR device removal. After that all ewes were subjected to oestrus detection.

## **3.6 Data collection and analysis**

### **3.6.1 Oestrus detection**

Oestrus was detected by introducing two teaser rams to each group for 72 hours immediately after CIDR removal. Thereafter, the ewes were visually monitored at 12 hour intervals following CIDR withdrawal until 72 hours (12, 24, 36, 48, 60 and 72 hours). Among the various signs of oestrus, tail twitching and standing to be mounted were the main signs targeted for (Ekiz et al., 2006). The parameters recorded were oestrus response (as the number of ewes showing oestrus/number of synchronised ewes × 100), onset of oestrus (as the time from CIDR removal to first acceptance of mounting), and also the duration of oestrus (as the time from the first acceptance of mounting to the first refusal).

### **3.6.2 Blood sampling**

Four experimental groups' blood samples were collected from six ewes per group. The blood samples were collected by puncturing the jugular vein using 4 ml sterile BD vacutainer tubes and the amount of blood collected was half or below half the tubes so that it provides enough serum for analysis. Blood samples were drawn at CIDR device insertion and withdrawal and at 12-hour intervals after CIDR withdrawal for 72 hours. After blood collection, the serum was harvested employing centrifugation (3000 × g for 20 min at 4°C) and stored at -20°C until analyses (Nogueira *et al.*, 2016).

### **3.6.3 Analysis of hormones**

The serum progesterone and oestradiol-17 $\beta$  concentrations were analysed using enzyme-linked immunosorbent assay (ELISA) kits, namely the Progesterone (Sheep) ELISA Kit (Abnova Corp., Taiwan, catalogue number KA2323, 96 assays, version: 05) and Estradiol (Sheep) ELISA Kit (Abnova Corp., Taiwan, catalogue number KA2318, 96 assays, version: 04).

The reproductive hormones were analysed using a Multiskan microplate reader. The manufacturer's instructions and procedures for analysis were followed for sample preparation (see the Elabscience® user manual (2018) for the Progesterone and estradiol ELISA kits, catalogue number E-EL-0154, 96T). First, 50 µL of the sample was added to each well and immediately 50 µL of Biotinylated Detection Ab was added, followed by incubation for 45 minutes at 37°C. The samples were then aspirated and washed three times, and 100 µL of horseradish peroxidase (HRP) conjugated with avidin was added to each well and incubated for a further 15 minutes at 37°C. The samples were aspirated again and washed five times, after which 90 µL of the kit's substrate reagent was added, followed by incubation for 15 minutes at 37°C. After incubation, 50 µL of the kit's stop solution was added. The well plate was inserted into the Multiskan microplate reader and read immediately at 450 nm.

### **3.7 Statistical analysis**

One-way analysis of variance (ANOVA) of the SPSS® software (IBM SPSS Statistics, Version 23.0, 2015, Armonk, NY., USA) was used to analyse the oestrous response, onset of oestrus, duration of oestrus and hormonal profiles between and within the experimental groups. After finding differences through one-way ANOVA, Tukey's post-hoc test was used to find which groups were significantly different from each another. The means were considered significant at  $P < 0.05$ .

## 4 CHAPTER FOUR: RESULTS

This chapter reports the study's results, including the effect of progesterone duration on oestrous response, the interval to the onset of oestrus after progesterone treatment, the typical duration of oestrus and the ewes' hormonal profiles before, during and post the treatment.

### 4.1 Effect of progesterone duration period on oestrous response

Four (4) ewes dropped the CIDR device during the experimental period and were excluded from the experiment. The SA Merino ewes' oestrous response from all treatment groups in the SAn spring breeding season was 96% (Table 4.1). There was no significant difference ( $P > 0.05$ ) in the oestrous response between the treatment groups following 11 or 14 days of CIDR treatment, with or without eCG.

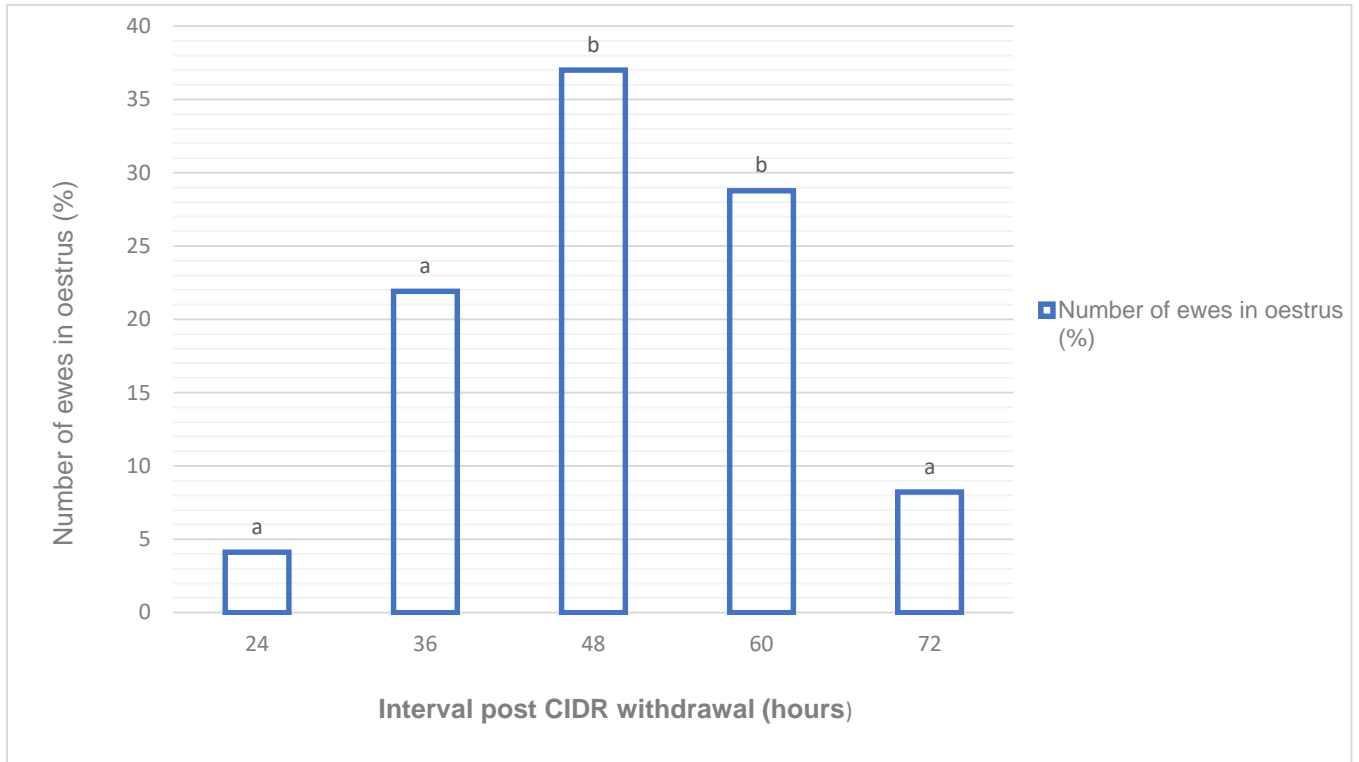
**Table 4.1** Oestrous response and the onset and duration of oestrus

| Treatments       | Oestrous Response (%)     | Onset of Oestrus (h) (mean $\pm$ SEM) | Duration of Oestrus (h) (mean $\pm$ SEM) |
|------------------|---------------------------|---------------------------------------|--|
| CIDR + eCG long  | 20/20 (100) <sup>a</sup>  | 51.0 $\pm$ 2.1 <sup>a</sup>           | 25.6 $\pm$ 0.8 <sup>a</sup>              |
| CIDR + eCG short | 18/18 (100) <sup>a</sup>  | 47.3 $\pm$ 3.3 <sup>a</sup>           | 26.3 $\pm$ 1.1 <sup>a</sup>              |
| CIDR + eCG long  | 20/20 (100) <sup>a</sup>  | 51.0 $\pm$ 2.1 <sup>a</sup>           | 25.6 $\pm$ 0.8 <sup>a</sup>              |
| CIDR long        | 19/20 (95) <sup>a</sup>   | 53.7 $\pm$ 2.7 <sup>a</sup>           | 25.4 $\pm$ 0.6 <sup>a</sup>              |
| CIDR + eCG short | 18/18 (100) <sup>a</sup>  | 47.3 $\pm$ 3.3 <sup>a</sup>           | 26.3 $\pm$ 1.1 <sup>a</sup>              |
| CIDR short       | 16/18 (88.9) <sup>a</sup> | 45.8 $\pm$ 3.5 <sup>a</sup>           | 28.8 $\pm$ 1.4 <sup>a</sup>              |
| Long             | 39/40 (97.5) <sup>a</sup> | 43.2 $\pm$ 1.5 <sup>a</sup>           | 25.5 $\pm$ 0.5 <sup>a</sup>              |
| Short            | 34/36 (94.4) <sup>a</sup> | 48.0 $\pm$ 2.4 <sup>a</sup>           | 27.5 $\pm$ 0.9 <sup>a</sup>              |
| CIDR + eCG       | 38/38 (100) <sup>a</sup>  | 48.0 $\pm$ 1.9 <sup>a</sup>           | 25.9 $\pm$ 0.7 <sup>a</sup>              |
| CIDR only        | 35/38 (92.1) <sup>a</sup> | 48.0 $\pm$ 2.1 <sup>a</sup>           | 27.1 $\pm$ 0.8 <sup>a</sup>              |
| Overall          | 73/76 (96)                | 46.8 $\pm$ 1.4                        | 26.5 $\pm$ 0.5                           |

The means with the same superscripts indicates no significant differences between treatments ( $P > 0.05$ ). Long-term progesterone treatment = 14 days; short-term progesterone treatment = 11 days

## 4.2 Interval to the onset of oestrus

Most ewes showed oestrus signs between 48 and 60 hours post the CIDR device's withdrawal (Figure 4.1; Table 4.1 & 4.2).



The bars with the different superscripts indicates significant differences between treatments ( $P < 0.05$ ).

**Figure 4.1** Oestrous distribution of ewes at different intervals (hours) following CIDR withdrawal.

**Table 4.2** Number of ewes per treatment group beginning oestrus at different intervals

| Number of ewes beginning oestrus per hour interval |                |                 |                 |                 |                |  |
|--|----------------|-----------------|-----------------|-----------------|----------------|--|
| Treatments   | 24h            | 36h             | 48h             | 60h             | 72h            |  |
| CIDR + eCG long                                    | 0 <sup>a</sup> | 3 <sup>a</sup>  | 9 <sup>a</sup>  | 7 <sup>a</sup>  | 1 <sup>a</sup> |  |
| CIDR + eCG short                                   | 2 <sup>a</sup> | 5 <sup>a</sup>  | 4 <sup>a</sup>  | 6 <sup>a</sup>  | 1 <sup>a</sup> |  |
| CIDR long  | 0 <sup>a</sup> | 1 <sup>a</sup>  | 10 <sup>a</sup> | 6 <sup>a</sup>  | 2 <sup>a</sup> |  |
| CIDR short   | 1 <sup>a</sup> | 7 <sup>a</sup>  | 4 <sup>a</sup>  | 2 <sup>a</sup>  | 2 <sup>a</sup> |  |
| Long   | 0 <sup>a</sup> | 4 <sup>a</sup>  | 19 <sup>a</sup> | 13 <sup>a</sup> | 3 <sup>a</sup> |  |
| Short  | 3 <sup>a</sup> | 12 <sup>a</sup> | 8 <sup>a</sup>  | 8 <sup>a</sup>  | 3 <sup>a</sup> |  |
| CIDR + eCG   | 2 <sup>a</sup> | 8 <sup>a</sup>  | 13 <sup>a</sup> | 13 <sup>a</sup> | 2 <sup>a</sup> |  |
| CIDR only  | 1 <sup>a</sup> | 8 <sup>a</sup>  | 14 <sup>a</sup> | 8 <sup>a</sup>  | 4 <sup>a</sup> |  |
| Overall  | 3              | 16              | 27              | 21              | 6              |  |

The means with the same superscripts indicates no significant differences between treatments ( $P > 0.05$ ). Long-term progesterone treatment = 14 days; short-term progesterone treatment = 11 days

Although the highest number of ewes began oestrus at 48 hours, the values with the same superscripts (a) on the same row indicate that the treatments groups did not differ significantly ( $P > 0.05$ ).

#### 4.3 Duration of oestrus

There was no significant difference ( $P > 0.05$ ) in the duration of oestrus between the treatment groups following 11 or 14 days of CIDR treatment, with or without eCG (Table 4.1).

#### 4.4 The ewe's hormonal profiles

The experimental Merino ewes' reproductive hormones concentrations were measured following different synchronisation protocols in SA's spring breeding season. Table 4.3 shows that there was no significant difference ( $P > 0.05$ ) between

the ewes' progesterone hormone levels (ng/ml) of the treatment groups at the CIDR device's insertion, removal and 48 hours post removal.

**Table 4.3** Mean  $\pm$  SEM progesterone concentrations (ng/ml)

| <b>Treatments</b> | <b>CIDR Insertion</b>       | <b>CIDR Removal</b>          | <b>48 hours post CIDR removal</b> |
|-------------------|-----------------------------|------------------------------|-----------------------------------|
| CIDR + eCG long   | 10.0 $\pm$ 0.3 <sup>a</sup> | 11.0 $\pm$ 0,02 <sup>a</sup> | 10.0 $\pm$ 0,1 <sup>a</sup>       |
| CIDR long         | 8.0 $\pm$ 0.4 <sup>a</sup>  | 11.0 $\pm$ 0,01 <sup>a</sup> | 9.0 $\pm$ 0.6 <sup>a</sup>        |
| CIDR + eCG short  | 9.0 $\pm$ 0.2 <sup>a</sup>  | 11.0 $\pm$ 0.06 <sup>a</sup> | 10.0 $\pm$ 0.2 <sup>a</sup>       |
| CIDR short        | 10.0 $\pm$ 0.1 <sup>a</sup> | 11.0 $\pm$ 0,09 <sup>a</sup> | 9.0 $\pm$ 0.3 <sup>a</sup>        |

The means with the same superscripts indicates no significant differences between treatments ( $P > 0.05$ )

However, the oestradiol concentrations (pg/ml) were higher in all treatment groups following different oestrous synchronisation protocols at CIDR device removal and 48 hours post removal ( $P < 0.05$ ) (Table 4.4). Table 4.4 also shows that there was no significant difference ( $P > 0.05$ ) between the groups at CIDR device insertion. Mean  $\pm$  SEM values with different superscripts (a or b) on the same row indicate a significant difference ( $P < 0.05$ ).

**Table 4.4** Mean  $\pm$  SEM oestradiol-17 $\beta$  concentrations (pg/ml)

| <b>Treatments</b> | <b>CIDR Insertion</b>        | <b>CIDR Removal</b>           | <b>48 hours post CIDR removal</b> |
|-------------------|------------------------------|-------------------------------|-----------------------------------|
| CIDR + eCG long   | 81.8 $\pm$ 42.8 <sup>a</sup> | 210.1 $\pm$ 57.6 <sup>b</sup> | 234.5 $\pm$ 17.7 <sup>b</sup>     |
| CIDR long         | 64.1 $\pm$ 47,4 <sup>a</sup> | 170.3 $\pm$ 42.0 <sup>b</sup> | 178.4 $\pm$ 19.9 <sup>b</sup>     |
| CIDR + eCG short  | 30.0 $\pm$ 16.9 <sup>a</sup> | 188.7 $\pm$ 49.1 <sup>b</sup> | 202.5 $\pm$ 16.9 <sup>b</sup>     |
| CIDR short        | 34.4 $\pm$ 11.7 <sup>a</sup> | 176.2 $\pm$ 34.3 <sup>b</sup> | 167.9 $\pm$ 44.3 <sup>b</sup>     |

The means with different superscripts indicates significant differences between treatments ( $P < 0.05$ ).

## 5 CHAPTER FIVE: DISCUSSION

The oestrous response obtained in this study was 96%, as revealed by the number of ewes responding to oestrous synchronisation. A comparable oestrous response of 90% and upwards has been reported in studies during breeding (Martemucci & D'Alessandro, 2011; Oliveira *et al.*, 2016; Swelum *et al.*, 2018; Vilariño *et al.*, 2013; ) and non-breeding seasons (Cox *et al.*, 2012; Martinez *et al.*, 2015; Santos-Jimenez *et al.*, 2020). The positive oestrous response was due to the progesterone protocol and effective release of progesterone by the inserted CIDR device during the treatment period (Nakafeero *et al.*, 2020). Additionally, this high oestrous response may be credited to the CIDR device's nature; it is non-absorbent silicone that does not absorb vaginal secretions and holds properly without tightly adhering to the vaginal mucosa (Swelum *et al.*, 2018). The efficient synchrony of oestrus observed at 48 and 60 hours post CIDR removal in this study is supported by other researchers' results (Swelum *et al.*, 2015; Vilariño *et al.*, 2013). The CIDR device's effect on oestrous distribution can be related to the development stage of the follicle when the CIDR device is withdrawn. The efficient synchrony of oestrus shows that the ovulatory follicles are stimulated from a single wave (López-García *et al.*, 2021), which is vital for fixed-time insemination.

In this study, the mean interval observed to the onset of oestrus differs from previous studies on the use of short-term (López-García *et al.*, 2021; Martemucci & D'Alessandro, 2011; Martinez-Ros *et al.*, 2018; Vilariño *et al.*, 2013 ) and long-term progesterone treatments (Kaya *et al.*, 2013; Oliveira *et al.*, 2016; Santos-Jimenez *et al.*, 2020; Swelum *et al.*, 2015), during the breeding and non-breeding seasons. A possible reason for this might be because this study was conducted during the SAn spring breeding season, and a delayed interval to the onset to oestrus is expected.

Additionally, variations may be explained by differences in breed, nutrition and season. However, the lack of significance between the treatment groups was possibly due to the small number of ewes per treatment group. However, numerically there was a tendency of earlier onset of oestrus in ewes inserted with the CIDR device for 11 days compared to those treated with CIDR for 14 days; this perhaps shows a faster follicular growth (Hasani *et al.*, 2018) in the former than in the latter treatment.

This study's results support those of Harl (2014), who observed that short-term protocols resulted in a longer interval to oestrus compared to long-term protocols. Harl (2014) observed that ewes receiving a CIDR treatment for 14 days showed a shorter interval to oestrus. The shorter interval to oestrus observed in this study for all treatment groups could be because the more prolonged CIDR treatment period increased the possibility that the ewe's existing corpus luteum regressed during treatment. However, short-term progesterone treatments decrease hormone usage quantities, offer the benefit of the CIDR device's re-use (Vilariño *et al.*, 2013) and also lead to the ovulation of high-quality follicles ( Farahavar *et al.*, 2020; Gore, 2016; Martinez-Ros *et al.*, 2018a ).

Recent studies still employ long-term progesterone treatments and have obtained an acceptable oestrous response and onset to oestrus (Oliveira *et al.*, 2016; Swelum *et al.*, 2015). Nakafeero *et al.* (2020) reported 100% and 97.5% oestrous response in Merino ewes following long and short-term CIDR treatments, respectively. Furthermore, Nakafeero *et al.* (2020) reported that both long and short-term progesterone treatments effectively synchronise oestrus in sheep. The similar oestrus response to the synchronisation and onset of oestrus between this study's treatment groups, particularly regarding progesterone duration, suggests that long and short-term periods can effectively synchronise oestrus in a progesterone-based protocol

during the SAn spring breeding season.

The similar interval to the onset of oestrus between the treatments with progesterone CIDR devices, with and without eCG, implies that both treatments stimulated a similar follicular growth pattern in ewes. No studies have yet compared CIDR devices with eCG and without eCG at the end of treatment on progesterone treated ewes on the same flock or under the same conditions. A low dosage of eCG was used in this study, which may explain the similarity in the interval to onset of oestrus between the groups treated with CIDR plus and without eCG. In studies where a dosage higher than 300 IU has been used, an increment in ovulation rate was observed (Cox *et al.*, 2012; El-Mokadem *et al.*, 2019; López-García *et al.*, 2021), which resulted in an increase in the oestrous response and onset to oestrus (Swelum *et al.*, 2018). The addition of eCG to a protocol, which is known to increase the recruitment of new follicles, could perhaps stimulate an earlier onset to oestrus in both long and short-term progesterone treatments (Biehl *et al.*, 2019; Martinez-Ros *et al.*, 2018a; Oliveira *et al.*, 2016). This study's results imply that using CIDR with or without eCG at the end of the progesterone treatment has similar effects on oestrous response and the onset of oestrus between treatment groups. Therefore, the results suggest that using CIDR alone at the end of progesterone treatment can be effective in progesterone-based oestrous synchronisation protocols.

The progesterone treatments' duration had no significant effect on the duration of oestrus between the treated groups in this study. The lack of a significant effect between the oestrous synchronisation protocols on the duration of oestrus may be due to similar effects of CIDR with and without eCG to induce LH surge. The mean duration of oestrus ( $26.5 \pm 0.5$  hours) found in this study is slightly comparable to that ( $30.07 \pm 1.00$  hours) obtained by Gore (2016). However, when comparing this study's results

with other studies, the duration of oestrus was shorter (Oliveira *et al.*, 2016).

The duration of progesterone or CIDR treatment did not significantly alter the serum progesterone concentration of the ewes at CIDR withdrawal and 48 hours post withdrawal. The progesterone level was similar in all the treatment groups at CIDR device insertion. Similar results have been observed in a study conducted by Vilariño *et al.* (2013), who found that the progesterone levels were similar in all the treated groups at the CIDR device's insertion. Furthermore, the progesterone level within a group was not different at the CIDR device's removal in all groups. The absence of a significant difference between treatment groups was perhaps because of the small number of ewes used per group. In addition, it is typical that the level of progesterone in the blood increases rapidly after insertion of the CIDR device; it also decreases rapidly after the CIDR device's withdrawal with no accumulative effect.

Swelum *et al.* (2018) and Santos-Jimenez *et al.* (2020) observed that the CIDR insertion led to an effective increase in the serum progesterone concentration of sheep for 3 or 4 days, followed by a decrease 6 days after treatment. Furthermore, the increase in progesterone level due to insertion of the CIDR device resulted in ovarian activity inhibition via increasing the negative feedback mechanism on the hypothalamus, which further inhibits pituitary LH secretion (El-Mokadem *et al.*, 2019; Smith *et al.*, 2010). Hasani *et al.* (2018) had similar results and observed that continual exogenous progesterone administration adequately regressed the dominant follicles and suppressed the release of gonadotropin from the anterior pituitary until the time of progesterone withdrawal. Following the removal, the decline in progesterone concentration stimulated the initiation of ovarian activity through declining the negative feedback on LH secretion in the pituitary gland (Manes *et al.*, 2018; Martinez-Ros *et al.*, 2018b; Swelum *et al.*, 2015). However, this study's results disagree with those of

Biehl *et al.* (2019), who observed that the level of progesterone at the insertion and withdrawal time was significantly higher in long than in short-term CIDR treatments.

The level of oestradiol concentration was significantly higher at the CIDR device's removal and 48 hours post withdrawal; this is due to the stimulation of ovarian activity through decreasing the negative feedback mechanism on pituitary LH secretion following the decrease in progesterone concentration after the CIDR device's withdrawal (Swelum *et al.*, 2018). These results agree with those of Swelum *et al.* (2015; 2018), who observed that serum oestradiol levels were higher at the CIDR device's removal and 48 hours post withdrawal ( $P > 0.05$ ), providing an excellent advantage for fixed-time artificial insemination. It is well known that oestradiol is considered an excellent quality follicle marker during the oestrous cycle. This is because the level of oestradiol is directly proportional to the number of persistent or large follicles (Martinez-Ros & Gonzalez-Bulnes, 2019). The duration of the CIDR treatment did not significantly change the serum oestradiol concentration between the treatment groups at all stages of treatment. However, this may be due to the small number of ewes used for this study's hormonal profiles. Similar results have been observed in other studies during both the breeding and non-breeding seasons (Farahavar *et al.*, 2020; Hasani *et al.*, 2018; Olivera-Muzante *et al.*, 2013; Swelum *et al.*, 2015; Swelum *et al.*, 2018; Vilariño *et al.*, 2013).

## **6 CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS**

### **6.1 Conclusion**

This study evaluated different oestrous synchronisation protocols and found that long (14 days) and short-term (11 days) CIDR treatments, followed with or without eCG (CIDR + eCG long, CIDR + eCG short, CIDR long and CIDR short), showed similar effectiveness on the oestrous response, onset of oestrus, duration of oestrus and hormonal profiles in SA Merino ewes during the SAn spring breeding season. Given this study's results, both long and short-term progesterone durations are equally efficiently in synchronising oestrus in a progesterone-based protocol. Furthermore, this study's results imply that using a CIDR device alone without intramuscular injection of eCG at the device's withdrawal can synchronise oestrus during SA's spring breeding season. This protocol will minimise the cost of using eCG and reduce multiple hormonal use for oestrous synchronisation in SA Merino ewes. A high Estradiol concentration at 48 hours post CIDR removal, indicates the effectiveness of both progesterone durations with or without eCG in stimulating follicular growth during spring breeding season. Moreover, the CIDR treatment for hormonal analysis should be practised with caution given this study's observation of similar effects on the level of progesterone at the CIDR device's insertion, withdrawal and 48 hours post withdrawal. Therefore, it can be concluded that progesterone oestrous synchronisation protocols during the SAn spring breeding season may play an important role in stimulating oestrous response and hormonal profiles in SA Merino ewes for better reproduction performance.

### **6.2 Recommendations**

Based on the findings of this study, the following recommendations are made:

- Further studies should be conducted using a larger number of ewes, which would strengthen this study's conclusions.
- Future studies should focus on using different CIDR durations in the spring breeding season to relate them with the reproductive performance in ewes.
- A low dosage of eCG can result in similar oestrous parameters. Therefore, future studies should be conducted using higher dosages of eCG. Furthermore, future studies should inject eCG at 48 hours before CIDR removal for reliable results.
- Moreover, CIDR treatment on its own may reduce the cost and hence it should be used alone rather than in combination with eCG.

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