Investigation of ram effect and eCG usage in progesterone based oestrous synchronization protocols on fertility of ewes following fixed time artificial insemination

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Highlights

- Short term progesterone treatment in oestrous synchronization protocols improves conception rates.
- Inclusion of male effect or eCG in a progesterone based oestrous synchronization protocol leads to similar response.
- Male effect can replace eCG in a progesterone based synchronization protocols.

Abstract

Improving oestrus synchronization protocols is important, particularly to ensure that protocols which guarantee reduced hormonal use and environmental safety also result in acceptable reproductive efficiency. The aim of this study was to compare 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. Seventy-eight South African Mutton Merino ewes 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 intramuscular 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. Ultrasound was 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. 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 synchronized ewes, with no significant difference among treatment groups. Overall, conception rate (CR) and the proportion of ewes lambing to synchronized oestrus were (74.4% and 52.6%, respectively). There was no significant difference among treatment groups in oestrous response, onset of oestrus, number of follicles, diameter of the largest follicle, CR and AI to lambing interval ($P > 0.05$). When data were pooled, CIDR-14 d protocols showed a significantly shorter interval to onset of oestrus (24.9 ± 1.6 vs 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% vs 63.2%, P < 0.05) than CIDR-14 d protocols when data were pooled, but CR between eCG and Ram protocols was not statistically different (P > 0.05). Mean AI to lambing interval was 158.2 ± 1.2 d, ranging from 147 to 154 d and 166 to 186 d post AI. Ewes lambing to synchronized oestrus per treatment group were 52.6% (eCGshort), 42.9% (Ramshort), 63.2% (eCGlong) and 52.6% (Ramlong), respectively (P > 0.05). In conclusion, the 4 protocols investigated were effective in synchronizing oestrus with similar response to synchronization of oestrus and fertility among treatment groups. Of the 4 protocols, the Ramlong protocol offers the benefit of being less costly because of reduced hormonal use in addition to the adequate fertility obtained.

Keywords: Long-term progesterone protocol; Short-term progesterone protocol; Ram effect; eCG; Sheep; South African mutton merino

1. Introduction

Management of reproduction using oestrus synchronization in small ruminants involves the use of natural and/or pharmacological methods to manipulate/modify the oestrous cycle (Martin et al., 2004; Abecia et al., 2012). Oestrus synchronization is usually combined with artificial insemination to increase the efficiency of breeding (Donovan et al., 2004). Methods commonly used include; use of intravaginal sponges impregnated with progestagens (Abecia et al., 2012) or CIDR (Wheaton et al., 1993). The use of prostaglandin ($PGF_{2\alpha}$) or its analogues is also popular during the breeding season (Abecia et al., 2012) and is sometimes combined with P4 to achieve greater reproductive efficiency (Ali, 2007; Martemucci and D'Alessandro, 2011).

The most commonly used progestagens/progesterone (P4) protocols for synchronization of oestrus involve a 12–14 d treatment period (Olivera-Muzante et al., 2011; Swelum et al., 2015). 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 P4 from the exogenous source is insufficient to suppress LH pulse frequency, the turnover of follicles is slowed (Driancourt, 2001; Viñoles et al., 2001). Therefore, this leads to ovulation of aged follicles at device withdrawal, which may affect fertility (Johnson et al., 1996; Viñoles et al., 2001). On the other hand, short-term treatment periods (Ali, 2007; Fleisch et al., 2012) result in better fertility due to adequate suppression of the LH pulse frequency and ovulation of young (newly recruited) follicles (Viñoles et al., 2001).

Progesterone/progestagens are often combined with equine chorionic gonadotropin (eCG) at device withdrawal in a protocol (Donovan et al., 2004; Zonturlu et al., 2018; Martinez-Ros et al., 2019). Moreover, reports both in small ruminants (Bodin et al., 1997; Drion et al., 2002; Anel et al., 2005) and in cattle (Drion et al., 2001) indicate that when eCG is used, fertility is lower with each breeding, due to accumulation of residual antibodies from previous treatments (Bodin et al., 1997). Therefore, use of other possible alternatives to eCG such as the ram effect and have been evaluated (Hawken et al., 2007; Meilán and Ungerfeld, 2014).

The ram effect increases LH secretion and advances the LH surge in randomly cyclic ewes (Hawken et al., 2007). It also induces oestrus in anovulatory ewes (Hawken et al., 2008). When used in combination with oestrus synchronization treatments, the ram effect advances oestrus (Contreras-Solís et al., 2009; Ungerfeld, 2011) and also results in higher fertility compared to unexposed ewes (Contreras-Solís et al., 2009). Since the ram effect is a cheap non-pharmacological method for induction and synchronization of oestrus and ovulation, its use is particularly important.

Therefore, due to the rising concern from the consumer with regards to the use of high quantities of P4 in food producing animals (Martin et al., 2004), and the concern of depositing unsafe products into the environment, there is need to improve P4 based protocols. The efficiency of short-term P4 treatment periods (Fleisch et al., 2012) and re-use of the intravaginal device (Vilarino et al., 2013), as well as the use of the ram effect in combination with P4 (Romano et al., 2000) have been studied. 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. This study therefore had two objectives; to compare oestrous response and fertility of ewes treated with CIDR for 9 d with those treated for 14 d, and also to determine oestrous response and fertility rates of ewes treated with eCG and those exposed to the ram effect following treatment with CIDR.

2. Materials and methods

2.1. Study area and general management of animals

The procedures used for this study was approved by the University of Pretoria ethics committee (Reference no: EC108-14). Data was collected during 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). Data were collected from South African Mutton Merino (SAMM) ewes 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). Chocolate maize (125−250 g per sheep per day) was also provided based on body condition of the ewes.

2.2. Synchronization of oestrus and treatment groups

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 balanced by body weight and parity and randomly assigned to 4 treatment groups in a 2 × 2 factorial design. The ewes had not been synchronized for oestrus before and had been isolated from rams (distance of 5 km) for a period of one month before the beginning of the experiment to ensure effectiveness of the ram effect stimulus.

Oestrus synchronization was accomplished by administration of progesterone using a controlled internal drug release (CIDR) vaginal device (0.3 g progesterone; EAZI-BREED™ CIDR-G®), Zoetis (Ltd), New Zealand) primed for 9 d (short) or 14 d (long). Controlled internal drug release was inserted with an applicator, which was dipped in a disinfectant (LUTALYSE® sterile solution, Ramsem, South Africa) 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.

2.3. Teasing and oestrus detection

Oestrous behaviour was monitored daily from 12−84 h post CIDR withdrawal, at intervals of 12 h (7.00 a.m. 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.

2.4. Semen collection and semen 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) and pooled. 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, cleaned with distilled water and dried with paper towel. 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. 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. Only ejaculates of volume over 0.5 ml and mass motility over 3 [determined under a light microscope (Olympus C × 21, on a pre-warmed stage (37 °C] were used. Examination was performed by placing a 10 µl drop of semen on a 76 × 26 × 1 mm glass slide (LASEC Pty Ltd, South Africa) and evaluated at 10x magnification.

2.5. 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 fresh undiluted semen with sperm concentration of approximately 400×10^6 (a minimum dose for artificial insemination based on minimum sperm concentration of 2000×10^6/ml) 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.

2.6. Ultrasonographic evaluation of follicular response and pregnancy diagnosis

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. Ultrasonographic evaluation for pregnancy diagnosis (PD) was also performed on all ewes by transrectal ultrasonography at 35 d post AI using the same procedure and scanner as with follicular response.

2.7. Data collection at lambing

Data on lambing were obtained from daily observation and recording of lambing events Ewes lambed from 147 to 185 d post AI. There were no ewes lambing between 154 and 165 d post AI.

2.8. Reproductive parameters

Reproductive parameters were calculated as; Oestrous response- proportion of ewes observed in oestrus at any one time from 12 to 84 h post CIDR withdrawal, onset of oestrus-interval from CIDR withdrawal to first signs of observed oestrus, 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 synchronized as determined by ultrasound at 48 h post CIDR withdrawal. It was calculated by number of ewes confirmed pregnant/number of ewes synchronized *100, ewes lambing- proportion of ewes lambing of all ewes synchronised. It was calculated by number of ewes lambing/number of ewes synchronized *100, AI to lambing interval - number of days between AI and lambing and lambing to the first service period - number of ewes lambing within 147–154 d post AI.

2.9. Statistical analysis

Variables analysed were; oestrous response, onset of oestrus, number of follicles, diameter of the largest follicles, conception rate and AI to lambing interval. Continuous data were analysed with analysis of variance (ANOVA; One-Way ANOVA; SAS and Version, 2003), depending on the values of the classification variable. 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. synchronized 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 and conception 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. Results were either expressed as percentages or mean ± SEM. Statistical significance was accepted from $P < 0.05$.

3. Results

3.1. Effect of synchronization treatment on oestrus response and interval to onset 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 synchronization of oestrus except one, hence it was omitted from the analysis. The overall oestrous response obtained was 98.7%. A greater proportion of ewes showed oestrous signs at 36 and 48 h post CIDR withdrawal (Fig. 1). There was no significant difference ($P > 0.05$) in oestrous response between treatment groups (Table 1). Overall, mean interval to onset of oestrus in this study was 27.9 ± 1.4 (Table 1). Most ewes began exhibiting oestrus at 24 and 36 h post CIDR withdrawal (Fig. 2) There was no significant difference ($P = 0.16$) in the mean interval to onset of oestrus between treatment groups. When data for all ewes treated with CIDR for 9 d or 14 d were pooled, CIDR-14 d ewes were seen to exhibit oestrus earlier ($P = 0.03$) than CIDR-9 d ewes (Fig. 3, Table 1). 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.

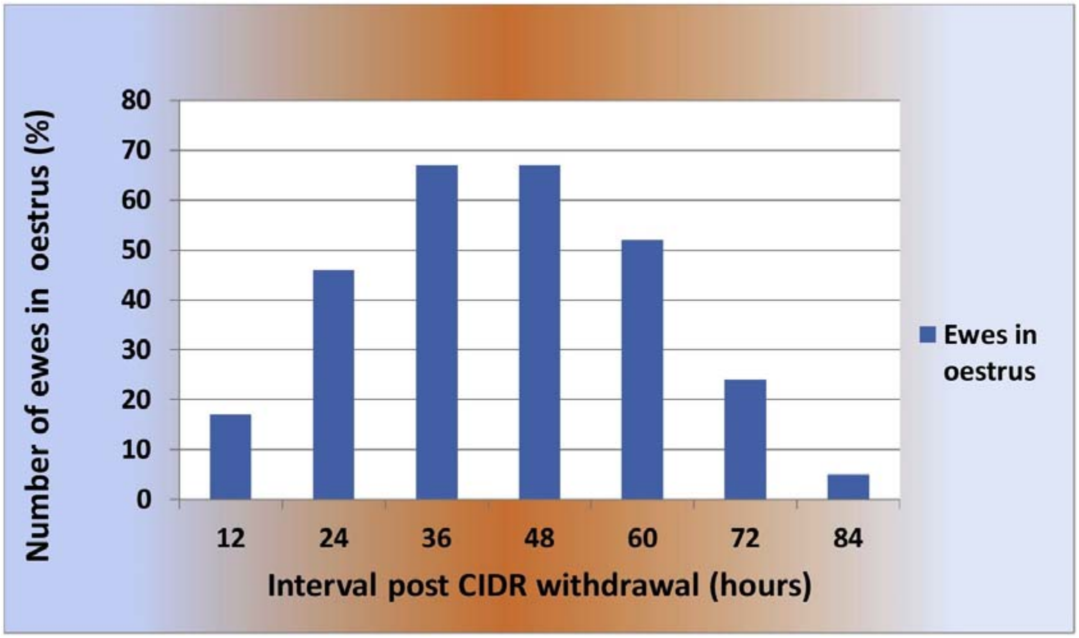

Fig. 1. Number of ewes in oestrus at different intervals following 9 or 14 d CIDR treatment and eCG or ram exposure at CIDR withdrawal.

Table 1. Oestrous response, onset of oestrus, number of follicles and diameter of the largest follicle (mean ± SEM) following different oestrus synchronization protocols and artificial insemination in South African Mutton Merino ewes during the breeding season.

Treatments	Oestrous Response (%)	Onset of Oestrus (h)	Number of follicles 48 h post CIDR withdrawal (n = 21)	Diameter of the largest follicle 48 h post CIDR withdrawal (n = 21)
eCGshort	19/19 (100)[a]	30.3 ± 2.8 [a]	2.5 ± 0.6 [a]	7.2 ± 0.7 [a]
Ramshort	20/21 (95.2)[a]	31.2 ± 2.1 [a]	1.8 ± 0.6 [a]	5.9 ± 0.5 [a]
eCGlong	19/19 (100) [a]	23.4 ± 2.1 [a]	2.5 ± 0.3 [a]	6.5 ± 1.0 [a]
Ramlong	19/19 (100) [a]	26.5 ± 2.4 [a]	1.8 ± 0.5 [a]	6.4 ± 0.9 [a]
eCG	38/38 (100) [a]	26.8 ± 1.8 [a]	2.5 ± 0.4 [a]	6.9 ± 0.5 [a]
Ram effect	39/40 (97.5) [a]	28.9 ± 2.0 [a]	1.8 ± 0.4 [a]	6.2 ± 0.5 [a]
CIDR-9d	39/40 (97.5) [a]	30.8 ± 2.1 [a]	2.2 ± 0.4 [a]	6.6 ± 0.4 [a]
CIDR-14d	38/3/ (100) [a]	24.9 ± 1.6 [b]	2.1 ± 0.3 [a]	6.5 ± 0.6 [a]
Overall	77/78 (98.7)	27.9 ± 1.4	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, Ramshort; 9 d CIDR treatment & ram effect, eCGlong; 14 d CIDR treatment & eCG, Ramlong; 14 d CIDR treatment & ram effect.

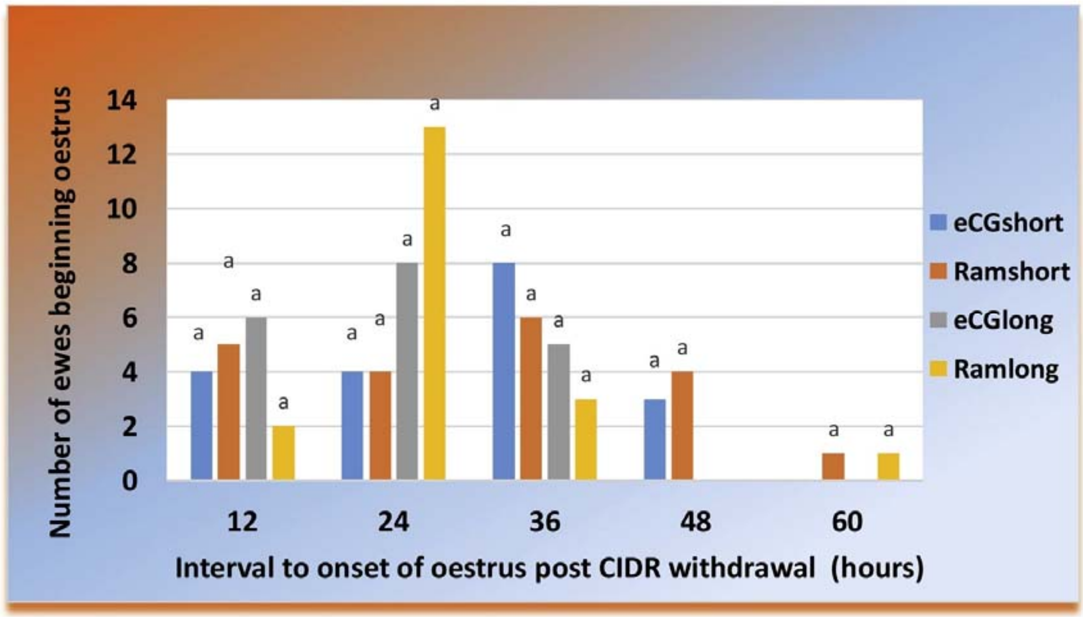

Fig. 2. Number of ewes per treatment group beginning oestrus at different intervals following 9 or 14 d CIDR treatment and eCG or ram exposure. eCGshort (n = 19, Ramshort (n = 21), eCGlong (n=19), Ramlong (n = 19, P > 0.05). Only ewes which responded to oestrus synchronization (n = 77) are represented in the figure. Different superscripts on clustered treatments are significantly different (a, b, P < 0.05).

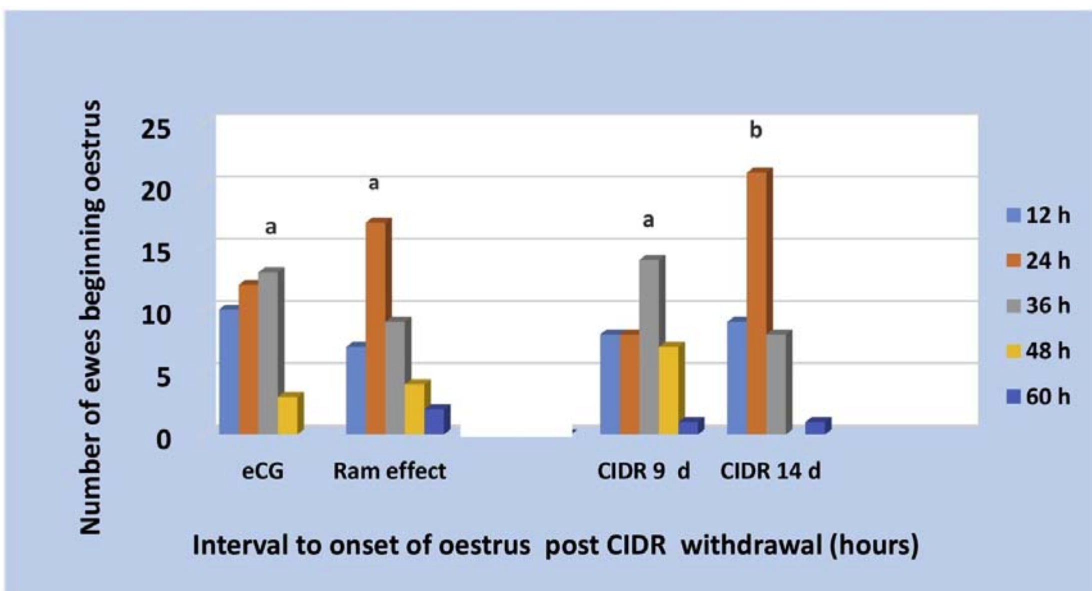

Fig. 3. Number of ewes beginning oestrus at different intervals following 9 (n = 39) or 14 d (n = 38) CIDR treatment (pooled data). Different superscripts on clustered treatments are significantly different (a, b, P < 0.05). Only ewes which responded to oestrus synchronization (n = 77) are represented in the figure.

3.2. Effect of synchronization 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 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. Overall, mean diameter of the largest follicle was 6.5 ± 0.4 mm (Table 1). 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.

3.3. Effect of synchronization treatment on conception rate and interval from artificial insemination to lambing

The overall conception rate (CR) was 74.4% (n = 58/78), Table 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. When data 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 ($P > 0.05$). The mean interval from artificial insemination (AI) to lambing in this study was 158.6 ± 1.2 d, $P > 0.05$, Table 2 with a 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. 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 to the first service period was 150.2 ± 0.3 d while that of ewes lambing to other services was 170.1 ± 0.9 d.

Table 2. Conception rate and corresponding lambing performance of South African Mutton Merino ewes following different oestrus synchronization protocols and artificial insemination during the breeding season.

Treatments	Conception rate (%)	Ewes which lambed to the first service period of all ewes synchronized (%)	AI to lambing
eCGshort	17/19 (89.5)^{a}	10/19 (52.6)^{a}	157.8 ± 2.7^{a}
Ramshort	17/21 (81.0)^{a}	9/21 (42.9)^{a}	161.4 ± 2.9^{a}
eCGlong	12/19 (63.2)^{a}	10/19 (52.6)^{a}	157.4 ± 2.3^{a}
Ramlong	12/19 (63.2)^{a}	12/19 (63.2)^{a}	158.1 ± 2.2^{a}
eCG	29/38 (76.3)^{a}	22/38 (57.9)^{a}	157.6 ± 1.7^{a}
Ram effect	29/40 (72.5)^{a}	19/40 (47.5)^{a}	159.7 ± 1.8^{a}
CIDR-9d	34/40 (85.0)^{a}	19/40 (47.5)^{a}	159.6 ± 2.0^{a}
CIDR-14d	24/38 (63.2)^{b}	22/38 (57.9)^{a}	157.8 ± 1.6^{a}
Overall	58/78 (74.4)	41/78 (52.6)	158.6 ± 1.2

Different superscripts in the same column of clustered treatments are significantly different (a, b, $P < 0.05$). eCGshort; 9 d CIDR treatment & eCG, Ramshort; 9 d CIDR treatment & ram effect, eCGlong; 14 d CIDR treatment & eCG, Ramlong; 14 d CIDR treatment & ram effect. Controlled internal drug release (CIDR), equine chorionic gonadotropin (eCG).

4. Discussion

A high oestrous response was obtained with all protocols used in this study as shown by the proportion of ewes responding to oestrus synchronization. A similar response has been observed in studies conducted during the breeding season with progestagens/progesterone (P4; Fleisch et al., 2012; Fierro et al., 2016; Zonturlu et al., 2018), which was possibly due to the efficient delivery of P4 by the controlled internal drug release (CIDR) device during the treatment period (Abecia et al., 2012). The tight synchrony of oestrus observed at 36 and 48 h post CIDR withdrawal in the present study was also reported by other researchers (Vilarino et al., 2013; Swelum et al., 2015). A tight synchrony of oestrus indicates that the ovulatory follicles originated from a single wave (Barrett et al., 2004), which is of particular importance in fixed time insemination (FTAI).

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 (Evans et al., 2004; Vilarino 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. Harl (2014) also observed that long-term protocols result in a shorter interval to oestrus compared to short-term protocols.

The similar interval to onset of oestrus between eCG and ram exposed ewes when data were pooled implies that equine chorionic gonadotropin (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. The similar response to oestrus synchronization as observed by the oestrous response and onset 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.

A low dosage of eCG was used in this study which may perhaps explain the similarity in the mean number of follicles between groups treated with eCG and the ram effect. 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), which is most often related to the number of follicles (Scaramuzzi et al., 1993).

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., 2005; Letelier et al., 2011) and with studies where prostaglandin ($PGF_{2\alpha}$) was used (Gonzalez-Bulnes et al., 2005; 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.

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; Vilarino et al., 2013; Swelum 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).

Although use of short-term P4 treatments would reduce quantities of hormone used, offer the advantage for re-use of the intravaginal device (Vilarino et al., 2013) and also result in ovulation of high quality follicles (Vinoles et al., 1999; Flynn et al., 2000; Viñoles et al., 2001), recent studies still employ long-term P4 treatments and have obtained acceptable CR (Olivera-Muzante et al., 2011; Swelum et al., 2015). Addition of eCG to a protocol, which is known to increase the recruitment of small (new) follicles could perhaps offset the negative effect of long-term P4 treatment on fertility (Bister et al., 1999). 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 synchronization protocols without affecting CR.

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, 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 season (Boscos et al., 2002; Swelum et al., 2015), perhaps due to the different breeding methods used.

It is unclear why the CIDR-9 d group which was diagnosed with high CR resulted in a low proportion of ewes lambing from 147 to 154 d post AI (period 1). It is however 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 used $PGF_{2\alpha}$ at the end of the treatment period (Martemucci and D'Alessandro, 2011; Fleisch et al., 2012) and 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; Ali et al., 2009), suffice to say that benefits of oestrus synchronization using various protocols can only be fully realised when ewes which conceive carry their foetuses to term (Mukasa-Mugerwa et al., 1994).

In conclusion, this study has shown that eCGshort, eCGlong, Ramshort and Ramlong oestrus synchronization protocols had similar effectiveness on oestrus synchronization and fertility, although the proportion of ewes lambing to the first service period in the Ramshort group was lower. Given the above results, the ram effect can replace eCG in a P4 based oestrus synchronization protocol to avoid 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 lower proportion of ewes lambing to the first service period.

Declaration of Competing Interest

None.

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