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Estrous Synchronization Protocols in South African Merino Ewes

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Abstract: The South African Merino breed is the most important sheep breed in South Africa due to its superior wool quality. With an increasing wool demand from China and Europe, the need for wool production in South Africa has risen significantly. To meet this high demand, it is crucial to enhance the reproduction rate of the Merino sheep using assisted reproduction technologies during the spring breeding season when their reproductive performance is low. This study aimed to compare the effects of short- and long-term progesterone treatments with or without eCG on estrus response and hormonal profiles of South African Merino ewes during the spring breeding season. Seventy-six South African Merino ewes aged between 2 and 5 years were divided into two groups: Short (n = 36) and long (n = 40) term progesterone treatment. Controlled Internal Drug Release devices (CIDR) were inserted for 11 days in the short-term group and 14 days in the long-term group. At CIDR withdrawal, half of each group received intramuscular injections of eCG (300 IU) and the other half did not receive it. Blood samples were collected at CIDR insertion and withdrawal and 48 h following CIDR withdrawal to measure progesterone and estrogen levels. One-way Analysis Of Variance (ANOVA) of the SPSS® software was used to analyze the data. Ewes treated with both short and long-term progesterone either with or without eCG exhibited similar estrus response, onset, and duration ($p>0.05$). Progesterone levels remained consistent across all treatment groups at CIDR insertion, removal, and 48 h following removal ($p>0.05$). However, estrogen levels were significantly higher at CIDR removal and 48 h following removal ($p<0.05$) compared to insertion. In conclusion, estrus synchronization with the use of short- or long-term progesterone protocols with or without eCG is effective in synchronizing estrus in South African Merino ewes during the spring breeding seasons.

Keywords: Equine Chorionic Gonadotropin, Estrogen, Estrus, Long-Term Progesterone, Sheep, Short-Term Progesterone

Introduction

Sheep are important to South Africa's agribusiness economy serving as key contributors to meat and wool production (Salama *et al.*, 2024). Their significance extends beyond economic value such as supporting the livelihoods of people (Burutaran *et al.*, 2024). The preference for sheep among farmers is due to their manageable nature and suitability for small-scale farming (Santos-Jimenez *et al.*, 2022). The South African Merino breed is the most important sheep breed in South Africa (Nel *et al.*, 2024) due to its superior wool quality compared to other white wools. With increasing wool demand from China and Europe, the need for wool production in South Africa has risen significantly (Zenda *et al.*, 2024). To meet this high demand, it is crucial to enhance the reproduction rate of the Merino sheep. This could be accomplished by using assisted reproduction technologies to manipulate the reproductive cycle more especially during the spring breeding season when their reproductive performance is low (Takci and Dinc, 2023).

Sheep are seasonal breeders and are influenced by the photoperiod (Magawana *et al.*, 2021). The two main breeding seasons for sheep in South Africa are autumn

and spring (Wentzel, 2012; Magawana *et al*., 2021). The autumn breeding season is characterized by peak sexual activities in sheep due to the short-day photoperiod (Wentzel, 2012; El-Mokadem *et al*., 2019). On the other hand, the spring breeding season has average sexual activities compared to autumn due to the long-day photoperiod (Wentzel, 2012; Luridiana *et al*., 2015; Nakafeero *et al.*, 2020; Magawana *et al.*, 2021). Generally, when compared to the autumn breeding season, breeding sheep in spring leads to low estrus activities, ovulation, conception, and lambing rates due to the photoperiod effect (Luridiana *et al*., 2015; El-Mokadem *et al.*, 2019; Magawana *et al*., 2021). Moreover, in South Africa, most sheep farmers use the spring breeding season because there is enough grazing in autumn to feed the lambing ewes and their lambs (Magawana *et al.*, 2021). Assisted reproductive technologies such as estrus synchronization have emerged as valuable tools for enhancing reproductive performance, especially during the spring breeding season (Santos-Jimenez *et al.*, 2022).

Estrus synchronization involves the artificial regulation of estrus and ovulation using hormonal treatments (Martinez-Ros and Gonzalez-Bulnes, 2019). Common protocols include the use of intravaginal devices such as CIDR containing Progesterone (P4) which are often combined with equine Chorionic Gonadotropin (eCG) (Rosasco *et al.*, 2019; Santos-Jimenez *et al.*, 2022). This protocol mimics the luteal phase of the estrus cycle and has been effective in inducing estrus, particularly during the non-breeding season in South Africa (Takci and Dinc, 2023).

Different protocols have been used to synchronize estrus in small stocks and yielded different results. The administration of progesterone for 12-14 days using the CIDR device with or without the administration of eCG at device removal is the commonly used estrus synchronization protocol that mimics the luteal phase of the estrous cycle in small stock in general (Martinez-Ros and Gonzalez-Bulnes, 2019; El-Mokadem *et al*., 2019). Long-term progesterone treatment leads to low levels of progesterone towards the end of treatment. This is because of the absorption reduction of progesterone from the exogenous source such as CIDR (Takci and Dinc, 2023). There is a further report that when the corpus luteum degenerates, exogenous progesterone becomes inadequate to suppress the pulse frequency of Luteinizing Hormone (LH), and the follicle turnover is slowed (Martinez-Ros and Gonzalez-Bulnes, 2019). Therefore, this results in ovulation of follicles that are aged at device removal, which may pose a threat to fertility (López-García *et al.*, 2021). Contradictory, short-term progesterone (5-11 days) is favored in some studies due to better fertility because of sufficient suppression of pulse frequency of the LH and ovulation of the newly recruited follicles (Martinez-Ros and Gonzalez-Bulnes, 2019; Nakafeero *et al*., 2020). This study aimed to evaluate and compare the effect of different estrus synchronization protocols on South African Merino ewes during the spring breeding season to increase their population.

Materials and Methods

Ethical approval (UZREC 171110-030) for the study was obtained from the University of Zululand's animal ethics committee. The research was conducted during the spring breeding season (September and November 2019) at Cedara Research Station, approximately 16 km from Pietermaritzburg, KwaZulu-Natal, South Africa. A total of 76 Merino ewes aged between 2 and 5 years were selected for this study. The ewes were allowed to graze on natural pastures and had continuous access to water throughout the experiment.

The 76 Merino ewes were stratified into four groups based on their average age:

- Group 1: Short-term progesterone and eCG (CIDR + eCG), CIDRs were inserted for 11 days (n = 18)
- Group 2: Long-term progesterone and eCG (CIDR + eCG), CIDRs were inserted for 14 days (n = 20)
- Group 3: Short-term progesterone (CIDR only), CIDRs were inserted for 11 days (n = 18)
- Group 4: Long-term progesterone (CIDR only), CIDRs were inserted for 14 days (n = 20)

Each ewe was inserted with a CIDR device (Pfizer, New Zealand) containing 0.3 g of progesterone hormone. The duration of treatment was 11 days for the short-term group and 14 days for the long-term group. At the time of CIDR withdrawal, 18 ewes (short-term group) and 20 ewes (long-term group) received an intramuscular injection of eCG (300 IU) (Intervet Schering-Plough Animal Health Pty Ltd., SA) (Martinez-Ros and Gonzalez-Bulnes, 2019). Moreover, the remaining 18 ewes (short-term group) and 20 (long-term group) were not administered eCG upon CIDR removal.

Estrus detection was done with the aid of two teaser rams per group for 72 h post-CIDR removal at 12 h intervals. The primary estrus signs targeted were tail twitching and standing to be mounted (Ekiz and Ozcan, 2006; Gore, 2016; Burutaran *et al*., 2024). Parameters recorded included estrus response (expressed as the percentage of ewes exhibiting estrus among synchronized ewes), onset of estrus (measured as the time from CIDR removal to first acceptance of mounting and tail twitching) and duration of estrus (defined as the time from the first acceptance of mounting and tail twitching to the first refusal) (Martinez-Ros *et al*., 2018; Nakafeero *et al*., 2020).

Blood samples were collected from six ewes per group to determine serum progesterone and Estrogen (E2) hormone levels. The blood samples were collected at CIDR insertion, withdrawal, and 48 h following CIDR withdrawal. In total, 72 blood samples were collected. Blood samples were collected by puncturing the jugular vein into 4 mL sterile BD vacutainer tubes. Following blood collection, serum was harvested through centrifugation (3000× g for 20 min at 4°C) and stored at -20°C until analysis (Nogueira *et al.*, 2016; Santos-Jimenez *et al.*, 2022).

Serum progesterone and estrogen levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Progesterone ELISA Kit: Catalog Number KA2323, 96 assays, Version: 05 and estradiol ELISA Kit: Catalog Number KA2318, 96 assays, Version: 04). The assay procedure included adding 50 µL of sample to each well followed by the addition of 50 µL of Biotinylated Detection Ab and incubation for 45 min at 37°C. After aspirating and washing the samples three times, 100 µL of Horseradish Peroxidase (HRP) conjugated with avidin was added per well and incubated for 15 min at 37°C. Subsequently, 90 µL of the kit's substrate reagent was added followed by another 15 min incubation at 37°C. The addition of 50 µL of the kit's stop solution halted the reaction. Absorbance readings were taken at 450 nm using a Multiskan microplate reader immediately after plate preparation (Martinez-Ros and Gonzalez-Bulnes, 2019).

The estrus response, onset of estrus, duration of estrus, and hormonal profiles were analyzed using One-Way Analysis Of Variance (ANOVA) of the SPSS® software (IBM SPSS Statistics, Version 23.0, 2015, Armonk, NY., USA). Treatment means were separated using Tukey's post-hoc test and were considered significantly different at $p<0.05$.

Results

Table (1) shows the estrus response, onset, and duration of estrus under different estrus synchronization protocols. Irrespective of the hormonal (P4 & eCG) protocol used, there was no significant difference between short and long-term protocol on estrus response, onset, and duration of estrus in South African Merino ewes. There was no significant difference between South African Merino ewes that were synchronized with short- and long-term progesterone in conjunction with eCG on estrus response, onset, and duration of estrus. Moreover, there was no significant difference between South African Merino ewes that were synchronized with short-term progesterone used without eCG and in conjunction with eCG on estrus response, onset, and duration of estrus. There was no significant difference between long-term progesterone used without eCG and with eCG on estrus response, onset, and duration of estrus in South African Merino ewes. Additionally, irrespective of CIDR insertion duration, there was no significant difference between South African Merino ewes that were synchronized with progesterone used in conjunction with eCG and without eCG on estrus response, onset, and duration of estrus.

Table (2) shows the number of ewes showing estrus at different time intervals. Irrespective of the hormonal (P4 & eCG) protocol used, there was no significant difference between short- and long-term protocols on the number of South African Merino ewes showing estrus signs from 24-72 h. There was no significant difference between South African Merino ewes that were synchronized with short- and long-term progesterone used in conjunction with eCG on the number of ewes showing estrus signs from 24-72 h. Moreover, there was no significant difference between short- and long-term progesterone used without eCG. Additionally, irrespective of the CIDR insertion duration, there was no significant difference between South African Merino ewes that were synchronized with progesterone used in conjunction with eCG and progesterone without eCG from 24-72 h.

Table 1: The estrus response, onset, and duration of South African Merino ewes during the spring breeding season

Treatments	Estrus Response (%)	Onset of estrus (h) (mean ± SE)	Duration of estrus (h) (mean ± SE)
Short (11 d)	34/36 (94.4)	48.0±2.4	27.5±0.9
Long (14 d)	39/40 (97.5)	43.2±1.5	25.5±0.5
CIDR + eCG short (11 d)	18/18 (100)	47.3±3.3	26.3±1.1
CIDR + eCG long (14 d)	20/20 (100)	51.0±2.1	25.6±0.8
CIDR + eCG short (11 d)	18/18 (100)	47.3±3.3	26.3±1.1
CIDR short (11 d)	16/18 (88.9)	45.8±3.5	28.8±1.4
CIDR + eCG long (14 d)	20/20 (100)	51.0±2.1	25.6±0.8
CIDR long (14 d)	19/20 (95)	53.7±2.7	25.4±0.6
CIDR + eCG (11&14 d)	38/38 (100)	48.0±1.9	25.9±0.7
CIDR only (11&14 d)	35/38 (92.1)	48.0±2.1	27.1±0.8

[a,b] Values with different superscripts within the same cell on the same column differ significantly ($p<0.05$). Short-CIDRs were inserted for 11 days (irrespective of the hormones used), Long-CIDRs were inserted for 14 days (irrespective of the hormones used), CIDR + eCG short-CIDRs were inserted for 11 days and eCG was injected, CIDR + eCG long - CIDRs were inserted for 14 days and eCG was injected, CIDR short - CIDRs were inserted for 11 days and no eCG was injected, CIDR long - CIDRs were inserted for 14 days and no eCG was injected, CIDR + eCG - CIDRs were used with eCG (irrespective of CIDR insertion duration) and CIDR only - CIDRs were used without eCG (irrespective of CIDR insertion duration)

Table 2: Number of South African Merino ewes starting to show estrus signs at different time intervals during spring breeding season

Number of ewes starting to show estrus signs at different time intervals (%)					
Treatments	24 h	36 h	48 h	60 h	72 h
Short (11 d)	3 (8.8)	12 (35.3)	8 (23.5)	8 (23.5)	3 (8.8)
Long (14 d)	0	4 (10.3)	19 (23.1)	13 (33.3)	3 (7.7)
CIDR + eCG short (11 d)	2 (11.1)	5 (27.8)	4 (22.2)	6 (33.3)	1 (5.6)
CIDR + eCG long (14 d)	0	3 (15)	9 (45)	7 (35)	1 (5)
CIDR + eCG short (11 d)	2 (11.1)	5 (27.8)	4 (22.2)	6 (33.3)	1 (5.6)
CIDR short (11 d)	1(6.3)	7 (43.8)	4 (25)	2 (12.5)	2 (12.5)
CIDR + eCG long (14 d)	0	3 (15)	9 (45)	7 (35)	1 (5.6)
CIDR long (14 d)	0	1 (5.3)	10 (62.5)	6 (31.6)	2 (10.5)
CIDR + eCG (11&14 d)	2 (5.3)	8 (21.1)	13 (34.2)	13 (34.2)	2 (5.3)
CIDR only (11&14 d)	1 (2.9)	8 (22.9)	14 (40)	8 (22.9)	4 (11.4)

[a,b] Values with different superscripts within the same cell on the same column differ significantly ($p<0.05$). Short-CIDRs were inserted for 11 days (irrespective of the hormones used), Long-CIDRs were inserted for 14 days (irrespective of the hormones used), CIDR + eCG short-CIDRs were inserted for 11 days and eCG was injected, CIDR + eCG long - CIDRs were inserted for 14 days and eCG was injected, CIDR short-CIDRs were inserted for 11 days and no eCG was injected, CIDR long - CIDRs were inserted for 14 days and no eCG was injected, CIDR + eCG-CIDRs were used with eCG (irrespective of CIDR insertion duration) and CIDR only-CIDRs were used without eCG (irrespective of CIDR insertion duration

Table (3) shows the progesterone and estrogen levels for South African Merino ewes at different treatment periods. Irrespective of hormonal (P4 & eCG) protocol used when short and long-term protocols were used, there was no significant difference between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of progesterone. There was no significant difference in progesterone levels at CIDR insertion, removal, and 48 h post-CIDR removal whether South African Merino ewes were synchronized with short- or long-term protocol used in conjunction with or without eCG. There was no significant difference in progesterone levels on South African Merino ewes synchronized with CIDR + eCG short, CIDR + eCG long, CIDR short, CIDR long, CIDR + eCG and CIDR only at CIDR insertion, removal, and 48 h post-CIDR removal.

Irrespective of the hormonal (P4 & eCG) protocol used when the short-term protocol was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen. However, the short-term protocol resulted in the same level of estrogen at CIDR removal and 48 h post removal. Irrespective of the hormonal (P4 & eCG) protocol used when the long-term protocol was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal (206.5±18.8 ng/mL) on the levels of estrogen. However, long-term protocol resulted in the same level of estrogen at CIDR removal and 48 h post removal.

At CIDR insertion there was a significant difference ($p<0.05$) between short and long-term protocols in terms of estrogen levels. However, there was no significant difference between short- and long-term protocols in terms of estrogen levels at CIDR removal and 48 h post-removal. When short-term in conjunction with eCG was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen. However, short-term eCG protocol resulted in the same level of estrogen at CIDR removal and 48 h post removal. When long-term in conjunction with eCG was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen. However, long-term protocol with eCG resulted in the same level of estrogen at CIDR removal and 48 h post removal. There was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen when short-term without eCG protocol was used. However, short-term without eCG protocol resulted in the same level of estrogen at CIDR removal and 48 h post removal. When long-term without eCG protocol was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen. However, long-term without eCG protocol resulted in the same level of estrogen at CIDR removal and 48 h post removal.

At CIDR insertion there was a significant difference ($p<0.05$) between CIDR short + eCG, CIDR long + eCG**,** CIDR short, and CIDR long protocols in terms of estrogen levels in South African Merino ewes. However, there was no significant difference between CIDR short + eCG and CIDR long + eCG protocols in terms of estrogen levels at CIDR removal and 48 h post removal. Moreover, at CIDR removal there was a significant difference ($p<0.05$) between CIDR long + eCG and CIDR long protocols in terms of estrogen levels. There was no significant difference between CIDR short and long protocols in terms of estrogen levels at CIDR removal and at 48 h post removal. At CIDR removal, CIDR long + eCG was significantly different ($p<0.05$) from CIDR short and CIDR long in terms of estrogen levels.

Table 3: Effect of estrus synchronization protocols on the progesterone and estrogen level of SA Merino ewes during spring breeding season (Mean ± SE)

Hormones	Treatments	CIDR insertion	CIDR removal	48 h post-CIDR removal
Progesterone (ng/ml)	Short	9.5±0.2	10.0±0.08	9.5±0.3
	Long	9.0±0.4	11.0±0.02	9.5±0.4
	CIDR + eCG short (11 d)	9.0±0.2	11.0±0.06	10.0±0.2
	CIDR + eCG long (14)	10.0±0.3	11.0±0.02	10.0±0.1
	CIDR short (11 d)	10.0±0.1	11.0±0.09	9.0±0.3
	CIDR long (14 d)	8.0±0.4	11.0±0.01	9.0±0.6
	CIDR + eCG (11&14 d)	9.5±0.3	11.0±0.04	10.0±0.2
	CIDR only (11&14 d)	9.0±0.3	11.0±0.05	9.0±0.5
Oestrogen (pg/ml)				
	Short	32.2±14.3 ^{a,1}	182.5±41.7 ^{b}	185.2±61.2 ^{b}
	Long	73.0±45.1 ^{a,2}	190.2±49.8 ^{b}	206.5±18.8 ^{b}
	CIDR + eCG short (11 d)	30.0±16.9 ^{a,1}	188.7±49.1 ^{b,1,2}	202.5±16.9 ^{b,1,2}
	CIDR + eCG long (14)	81.8±42.8 ^{a,2}	210.1±57.6 ^{b,2}	234.5±17.7 ^{b,2,}
	CIDR short (11 d)	34.4±11.7 ^{a,1}	176.2±34.3 ^{b,1,2}	167.9±44.3 ^{b,1}
	CIDR long (14 d)	64.1±47.4 ^{a,2}	170.3±42.0 ^{b,1}	178.4±19.9 ^{b,1}
	CIDR + eCG (11&14 d)	55.9±29.9 ^{a}	199.4±53.4 ^{b}	281.5±17.3 ^{c,1}
	CIDR only (11&14 d)	49.3±29.6 ^{a}	173.3±38.2 ^{b}	173.2±31.9 ^{b,2}

^{a,b} Values with different superscripts within the same cell on the same row differ significantly ($p<0.05$). ^{1,2} Values with different superscripts within the same cell on the same column differ significantly ($p<0.05$). Short–CIDRs were inserted for 11 days (irrespective of the hormones used), Long-CIDRs were inserted for 14 days (irrespective of the hormones used), CIDR + eCG short-CIDRs were inserted for 11 days and eCG was injected, CIDR + eCG long-CIDRs were inserted for 14 days and eCG was injected, CIDR short-CIDRs were inserted for 11 days and no eCG was injected, CIDR long-CIDRs were inserted for 14 days and no eCG was injected, CIDR + eCG-CIDRs were used with eCG (irrespective of CIDR insertion duration) and CIDR only-CIDRs were used without eCG (irrespective of CIDR insertion duration)

Additionally, irrespective of CIDR insertion duration, when progesterone in conjunction with eCG protocol was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen. Irrespective of CIDR insertion duration when progesterone only without eCG protocol was used, there was a significant difference ($p<0.05$) between CIDR insertion, removal, and 48 h post-CIDR removal on the levels of estrogen. However, irrespective of CIDR insertion duration, progesterone only without eCG protocol resulted in the same level of estrogen at CIDR removal and 48 h post removal.

At CIDR insertion and removal there was no significant difference between progesterone in conjunction with eCG and progesterone without eCG protocols in terms of oestrogen levels irrespective of CIDR insertion duration. However, at 48 h post-removal, there was a significant difference ($p<0.05$) between progesterone in conjunction with eCG and progesterone without eCG protocols in terms of estrogen levels.

Discussion

The estrus response in the current study when South African Merino sheep were synchronized during the spring breeding season ranged from 88.9-100% in all treatment groups. These results are similar to the previous results reported by Swelum *et al.* (2018) (95-100%); Rosasco *et al.* (2019) (82-94%); Takci and Dinc (2023) (76-96%), when Awassi, Rambouillet, and Kangal breeds were used respectively, during the unfavorable breeding season.

Irrespective of the hormonal (P4 & eCG) treatment, both short- and long-term protocols had similar estrus response (94.4 and 97.5% respectively), onset of estrus (48.0 and 43.2 h, respectively) and duration of estrus (27.5 and 25.5 h, respectively) when South African Merino ewes were used during spring breeding season. The results of the current study are similar to the estrus response reported by Nakafeero *et al.* (2020) when both short (97.5%) and long (100%) term protocols were used in the South African Mutton Merino breed during the natural breeding season. However, the current study contradicts the estrus response of 90% (short-term protocol) which was higher compared to the estrus response of 60% (long-term protocol) when Ossimi ewes were used during the unfavorable breeding season (Salama *et al*., 2024). Breed differences between the current and previous studies might have contributed to these variations (Nakafeero *et al.*, 2020). According to Burutaran *et al.* (2024), different breeds have different reproductive performance. Moreover, nutritional

differences might have contributed to these variations, (Nakafeero *et al.*, 2020), as in the current study they grazed on natural pasture with unknown levels of nutrients, and in the previous study, they were fed a balanced diet. According to Joshi (2022), balanced dietary nutrients stimulate the programming and expression of metabolic pathways that allow animals to reach their genetic reproductive potential.

The onset of estrus in both short and long protocols of the current study started late at 48.0 and 43.2 h, respectively compared to the onset of estrus at 30.8 and 24.9 h when the same protocols were used in South African Mutton Merino during the breeding season (Nakafeero *et al.*, 2020). This might be because in the current study, ewes were synchronized during the unfavorable breeding season (spring) whereas in the previous study, they were synchronized during the favorable (autumn) breeding season. The unfavorable breeding seasons during spring and winter in South Africa have average to low sexual activities compared to the autumn breeding season due to the long-day photoperiod (Nakafeero *et al.*, 2020). On the other hand, the onset of estrus in the short protocol in the current study started earlier when compared to the onset of estrus (53.9 h) of the Ossimi breed during the same unfavorable breeding season (Salama *et al*., 2024). The differences might be attributed to different intravaginal devices that were used. In the current study, CIDRs were used whereas in the previous study, Flurogestone Acetate (FGA) devices were used. According to Ramukhithi *et al.* (2012), the use of CIDRs is advantageous as they do not absorb vaginal fluids that induce bacteria contamination as compared to other vaginal devices.

The duration of estrus in both short- and long-term protocols of the current study was shorter (27.5 and 25.5 h, respectively) compared to the duration of estrus (short = 35 and long = 43.4 h) reported by Martinez-Ros *et al.* (2018) during unfavorable breeding season when mixed sheep breeds were used. The differences might also be due to different intravaginal devices and breeds that were used. In the current study, CIDRs and South African Merinos were used whereas in the previous study, FGA and mixed sheep breeds were used.

Both South African Merino ewes that were synchronized using CIDR + eCG and CIDRonly protocols resulted in the same estrus response (100 and 92.1%, respectively). The estrus response of the current study is similar to the estrus response of 97.5 and 100% reported by Nakafeero *et al*. (2020) when same the CIDR + eCG and CIDR-only protocols were used, respectively during the breeding season. Moreover, the estrus response of South African Merino ewes (92.1%) when CIDR only protocol was used was also similar to the estrus response of 100% reported by Martinez-Ros *et al.* (2018) when the same protocol was used in mixed sheep breeds during the same spring breeding season. On the other hand, the estrus response of South African Merino ewes (100%) when CIDR + eCG protocol was used, was higher than the estrus response of 76% reported by Takci and Dinc (2023) during the same unfavorable breeding season when Kangal breed was used. The differences between the current and previous studies might be due to the type of intravaginal devices and the time they spent inside the ewes' vagina (Martinez-Ros and Gonzalez-Bulnes, 2019).

Both South African Merino ewes that were synchronized using CIDR + eCG and CIDR-only protocols resulted in the same onset of estrus (48.0 and 48.0 h, respectively). The onset of estrus for ewes synchronized with CIDR + eCG and CIDR-only protocols in the current study started late at 48.0 h when compared to the onset of estrus of 28.9 h and 26.8 h when the same protocols were used in South African Mutton Merino during the breeding season (Nakafeero *et al.*, 2020). The difference might be due to different breeds and feeds (Burutaran *et al.*, 2024) that were used. Moreover, this might be due to the exposure of the rams to those ewes that were synchronized with CIDR only. According to Nakafeero *et al*. (2020), the exposure of rams to ewes increases the secretion of LH and stimulates the LH surge in cyclic ewes and it also induces estrus in ewes that are anovulatory.

Both South African Merino ewes that were synchronized using CIDR + eCG and CIDR-only protocols resulted in the same duration of estrus (27.1 and 25.9 h, respectively). The duration of estrus when CIDR only protocol was used in the current study was longer (27.1 h) when compared with the duration of estrus of 19 and 18 h during the unfavorable and favorable breeding season, respectively when Kıvırcık breed was used (Ekiz and Ozcan, 2006). These differences might be attributed to that Ekiz and Ozcan (2006) sheep were supplemented with pellets which contain 13% crude protein whereas ewes of the current study were grazing on natural pasture. This agrees with Gore (2016) who indicated that adequate nutrition is necessary as it boosts the female reproductive activities of an animal. On the other hand, the duration of estrus when the CIDR + eCG protocol was used in the current study was shorter (25.9 h) when compared with the duration of estrus of 34.1 h reported by Martinez-Ros and Gonzalez-Bulnes (2019) when the same protocol was used in Segureña breed during the breeding season. This might be due to several factors such as season, breed, and nutrition (Rosasco *et al.*, 2019; El-Mokadem *et al.*, 2019; Magawana *et al.*, 2021).

Irrespective of hormonal (P4 & eCG) protocols, both short- and long-term protocols had similar levels of progesterone at CIDR insertion (9.5 and 9.0 ng/mL, respectively), withdrawal (10.0 and 11.0 ng/mL, respectively) and 48 h post-withdrawal (9.5 and 9.5 ng/mL,

respectively) when South African Merino ewes were used during spring breeding season. This result contradicts the literature, as it has been documented that the level of progesterone in the blood increases and decreases after CIDR insertion and withdrawal (Takci and Dinc, 2023). In the current study, the progesterone levels for short- and long-term protocols at CIDR insertion (9.5 and 9.0 ng/mL, respectively) were lower than the level of progesterone in Corriedale multiparous ewes when the same protocols were used (14.0 and 16.6 ng/mL, respectively) (Vilariño *et al*., 2013) during the breeding season. The difference between the current and previous study might be due to nutrition, ewes in the previous study were supplemented with concentrate and alfalfa hay (Vilariño *et al*., 2013) while in the current study, ewes were not supplemented. As already mentioned, adequate nutrition is necessary as it boosts female reproductive activities (Joshi, 2022).

However, the level of progesterone for short- and long-term protocols in the current study was higher at CIDR insertion (9.5 and 9.0 ng/mL, respectively) and withdrawal (10.0 and 11.0 ng/mL, respectively) compared to the level of progesterone in Farahani breed when the same protocols were used at CIDR insertion (0.1 and 0.1 ng/mL, respectively) and withdrawal (3.69 and 2.56) ng/mL, respectively) during unfavorable breeding seasons (Moghaddam *et al*., 2021). The differences might also be due to different CIDR insertion periods which affect hormone release and absorption (Graves *et al.*, 2004; Sotgiu *et al*., 2021). In the study conducted by Moghaddam *et al*. (2021), the CIDRs were inserted for 6 and 12 days (short and long, respectively) while in the current study, the CIDRs were inserted for 11 and 14 days.

In the current study, administration of progesterone did not increase the level of serum progesterone, at CIDR insertion (9.5 ng/mL short protocol and 9.0 ng/mL long protocol), withdrawal (10.0 ng/mL short protocol and 11.0 ng/mL long protocol) and 48 h post withdrawal (9.5 ng/mL short protocol and 9.5 ng/mL long protocol). Contradictory, according to Swelum *et al*. (2018); and Santos-Jimenez *et al*. (2020), CIDR insertion led to an effective increase in the serum progesterone levels of sheep for 3 or 4 days, followed by a decrease from 48 h (Rosasco *et al*., 2019; Takci and Dinc, 2023) to 6 days after treatment (Swelum *et al*., 2018; Santos-Jimenez *et al*., 2020). This turns to results in ovarian activity inhibition via increasing the negative feedback mechanism on the hypothalamus, which further inhibits pituitary LH secretion (Martinez-Ros and Gonzalez-Bulnes, 2019; Santos-Jimenez *et al*., 2022). The possible reason why the current study did not follow the same progesterone pattern as previous studies could be due to sample size, which could have affected the ability to detect significant changes and statistical power. Smaller sample sizes result in greater variability and less consistent results (Rusticus and Lovato, 2019).

Both South African Merino ewes that were synchronized using CIDR + eCG and CIDR-only protocols resulted in the same level of progesterone at CIDR insertion (9.5 and 9.0 ng/mL, respectively), withdrawal (11.0 and 11.0 ng/mL, respectively) and 48 h post-withdrawal (10.0 and 9.0 ng/mL, respectively) when South African Merino ewes were used during spring breeding season. The level of progesterone in the current study was higher than the progesterone level average reported by Swelum *et al*. (2018) at CIDR insertion (5. 25 ng/mL), withdrawal (5.75 ng/mL) and 48 h post-withdrawal (1.5 ng/mL) when Awassi breed was used during the breeding season. Moreover, the level of progesterone in the current study was higher than the progesterone level of 5.7 and 3.9 ng/mL of CIDR + eCG and CIDR-only protocols respectively, reported by Martinez-Ros and Gonzalez-Bulnes (2019) when Segureña breed was used during the breeding season. The differences might also be attributed to breed differences. Moreover, individual differences in the physiological response to hormone treatments among sheep could have led to variations in serum progesterone levels (La Salles *et al*., 2017; Sotgiu *et al*., 2021; Joshi, 2022).

Irrespective of hormonal (P4 & eCG) protocols, in the current study long-term protocol (73.0 pg/mL) had higher ($p<0.05$) serum estrogen level than the short-term protocol (32.2 pg/mL) at CIDR insertion when South African Merino ewes were used during spring breeding season. The reason for this difference might be due to CIDR insertion timing (López-García *et al*., 2023). CIDR insertion timing may have coincided with different stages of the estrous cycle or follicular development in the two protocols, influencing estrogen levels (Kasimanickam, 2021; López-García *et al*., 2023). However, the estrogen levels in short- and long-term protocols were similar at CIDR withdrawal (182.5 and 190.2 pg/mL, respectively) and 48 h post-withdrawal (185.2 and 206.5 pg/mL, respectively). The level of estrogen in the current study for the long-term protocol was higher than the estrogen level reported by Swelum *et al*. (2015) at CIDR withdrawal (142 pg/mL) and 48 h post-withdrawal (142 pg/mL) when the same protocol was used in Najdi breed during the breeding season. These differences might be due to environmental temperatures (Bhimte *et al*., 2018; La Salles *et al*., 2017). In the current study, the temperature ranged from 20-28°C (Thipe *et al*., 2020; Ncoyini-Manciya and Savage, 2022) whereas in the previous study, the temperature ranged from 14.4-21.1°C (Swelum *et al*., 2015). Low temperatures could have resulted in vasoconstriction, reducing blood flow to the ovaries and decreasing estrogen production (Usman *et al*., 2022). Moreover, low temperatures

could have also inhibited the release of Gonadotropin-Releasing Hormone (GnRH)), which controls estrogen production (Mohammed *et al.*, 2019).

In the current study, CIDR + eCG and CIDR-only protocols had the same estrogen levels at CIDR insertion (55.9 and 49.3 pg/mL, respectively) and withdrawal (199.4 and 173.3 pg/mL, respectively). The average level of estrogen reported in the current study was higher than the average estrogen level of 45.5 pg/mL reported by Martinez-Ros *et al*. (2018) when the Segureña meat breed was used during the breeding season. This might be because in Martinez-Ros *et al*. (2018) study, the progesterone treatment was used in conjunction with prostaglandin F2α while in the current study, progesterone was used in conjunction with eCG. However, the use of prostaglandin F2α causes the level of progesterone to decline, which leads to estrus (Vilariño *et al.*, 2013; Swelum *et al.*, 2015; 2018; Farahavar *et al.*, 2020).

However, the level of estrogen in the CIDR + eCG protocol (281.5 pg/mL) was higher ($p<0.05$) than the level of estrogen in CIDR only protocol (173.2 pg/mL) at 48 h post-CIDR removal when the South African Merino ewes were used during spring breeding season. The level of estrogen in the current study at 48 h post-withdrawal was higher than the estrogen levels of 110 pg/mL at 48 h post-withdrawal reported by Ekiz and Ozcan (2006) when Kıvırcık ewes were used during breeding and unfavorable breeding seasons. These differences might be due to the injection of eCG at CIDR withdrawal, which might have stimulated the increase of estrogen level at 48 h post-withdrawal while in the study by Ekiz and Ozcan (2006), eCG was not injected. The eCG injections are administered in addition to progesterone treatment, as a supplementary measure to improve ovulation rates during synchronization (Ramukhithi *et al.*, 2012; Santos-Jimenez *et al.*, 2022).

The levels of estrogen in the current study were significantly higher at the CIDR removal and 48 h post-withdrawal in all treatment groups compared to estrogen levels at CIDR insertion. This is due to the stimulation of ovarian activity by decreasing the negative feedback mechanism on pituitary LH secretion following the decrease in progesterone levels after the CIDR device's withdrawal (Alvarado-Espino *et al*., 2019; Sotgiu *et al*., 2021; Santos-Jimenez *et al.*, 2022; Joshi, 2022). These results are in agreement with those of Swelum *et al.* (2015; 2018), who observed that serum estrogen levels were higher at the CIDR removal and 48 h post-withdrawal, providing an excellent advantage for fixed-time artificial insemination. It is well known that estrogen is considered an excellent quality follicle marker during the estrus cycle. This is because the level of estrogen is directly proportional to the number of persistent or large follicles (Año-Perello *et al*., 2020).

Moreover, hormonal profiles should have revealed expected trends in progesterone and estrogen levels, with CIDR insertion increasing progesterone levels and subsequent withdrawal stimulating ovarian activity and estrogen production (Swelum *et al*., 2018; Santos-Jimenez *et al.*, 2020).

Conclusion

The use of short and long CIDR treatment can be used to synchronize estrus of South African Merino sheep during the spring breeding season. Moreover, the use of CIDR in conjunction with eCG may not be significant during the spring breeding season. This was shown by a high level of estrogen at 48 h post-CIDR removal. It is recommended that further studies should be conducted using a larger sample size, which would strengthen this study's conclusions. Furthermore, future studies should inject eCG at 48 h before CIDR removal.

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Author's Contributions

Nhlakanipho Sam Zulu: Conceptualisation of the study, data collection, analysis, and interpretation; and manuscript write-up.

Fhulufhelo Vincent Ramukhithi and Khoboso Christine Lehloenya: Conceptualisation and supervision of the study.

Ethics

The content of the article is unique and includes material that has not been previously published. The corresponding author has verified that all co-authors have reviewed and consented to the manuscript and there are no ethical concerns present.

Conflict of Interest Declaration

There is no conflict of interest to declare.

References

Alvarado-Espino, A. S., Menchaca, A., Meza-Herrera, C. A., Carrillo-Moreno, D. I., Zúñiga-García, S., Arellano-Rodríguez, F., Mellado, M., & Véliz, F. G. (2019). Ovarian response is not affected by the stage of seasonal anestrus or breed of goats when using a progesterone injection plus human chorionic gonadotropin-based protocol. *Animal Reproduction Science*, *204*, 60–65. https://doi.org/10.1016/j.anireprosci.2019.03.005

Año-Perello, A., Santos-Jimenez, Z., Encinas, T., Martinez-Ros, P., & Gonzalez-Bulnes, A. (2020). Use of GnRH for Synchronization of the Follicular Wave in Assisted Reproductive Technologies in Sheep: A Preliminary Study. *Animals*, *10*(7), 1208. https://doi.org/10.3390/ani10071208

Bhimte, A., Thakur, N., Lakhani, N., Yadav, V., Khare, A., & Lakhani, P. (2018). Endocrine changes in livestock during heat and cold stress. *Journal of Pharmacognosy and Phytochemistry*, *7*(4), 127–132.

Burutaran, M., Fierro, S., Negrín, F., Minteguiaga, M., Gil, J., & Olivera–Muzante, J. (2024). Estrous, ovulation, and reproductive responses of ewes synchronized with a long interval prostaglandin–based protocol for timed AI. *Theriogenology*, *214*, 187–191. https://doi.org/10.1016/j.theriogenology.2023.10.027

Ekiz, E. E., & Ozcan, M. (2006). Sexual behavior and hormone levels of Kıvırcık ewes after estrus synchronization during and out of the breeding season. *Archives Animal Breeding*, *49*(6), 583–592. https://doi.org/10.5194/aab-49-583-2006

EL-Mokadem, M. Y., Nour El-Din, A. N. M., Ramadan, T. A., Rashad, A. M. A., Taha, T. A., & Samak, M. A. (2019). Alleviation of reproductive seasonality in Barki ewes using CIDR-eCG with or without melatonin. *Small Ruminant Research*, *174*, 170–178. https://doi.org/10.1016/j.smallrumres.2019.03.019

Farahavar, A., Rostami, Z., Alipour, D., & Ahmadi, A. (2020). The effect of pre-breeding vitamin E and selenium injection on reproductive performance, antioxidant status, and progesterone concentration in estrus-synchronized Mehraban ewes. *Tropical Animal Health and Production*, *52*(4), 1779–1786. https://doi.org/10.1007/s11250-019-02183-8

Gore, D. L. M. (2016). *Effect of β Carotene Supplementation on Oestrous Synchronisation and Milk Production of Saanen Goats*.

Graves, W., McLean, A., Smith, R., & Rosenberg, J. (2004). Physiology and endocrinology: Reproductive technologies and management. *Poultry Science*, *83*, 366–373.

Joshi, P. (2022). Nutrition and reproduction in sheep. *Food & Agribusiness Management (FABM)*, *3*(2), 40–44. https://doi.org/10.26480/fabm.02.2022.40.44

Kasimanickam, R. (2021). Pharmacological intervention of estrous cycles. *Bovine Reproduction*, 458–470. https://doi.org/10.1002/9781119602484.ch37

La Salles, A. Y. F. de, Batista, L. F., de Souza, B. B., da Silva, A. F., & Correia, É. L. de B. (2017). Growth and Reproduction Hormones of Ruminants Subjected to Heat Stress. *Journal of Animal Behaviour and Biometeorology*, *5*(1), 7–12. https://doi.org/10.14269/2318-1265/jabb.v5n1p7-12

López-García, S., Sánchez-Torres Esqueda, M. T., Cordero-Mora, J. L., Figueroa-Velasco, J. L., Martínez-Aispuro, J. A., & Salinas Ríos, T. (2023). Sincronización de estros en ovejas mediante protocolo de 6 días con CIDR de primera, segunda y tercera reutilización. *Revista Mexicana de Ciencias Pecuarias*, *14*(3), 610–621. https://doi.org/10.22319/rmcp.v14i3.6309

López-García, S., Sánchez-Torres, M. T., Cordero-Mora, J. L., Figueroa-Velasco, J. L., Martínez-Aispuro, J. A., García-Cué, J. L., Martínez-Cruz, I., & Cárdenas-León, M. (2021). Estrous synchronization in sheep with reused progesterone devices and eCG. *Revista Brasileira de Zootecnia*, *50*. https://doi.org/10.37496/rbz5020200176

Luridiana, S., Mura, M. C., Daga, C., Farci, F., Di Stefano, M. V., Zidda, F., & Carcangiu, V. (2015). Melatonin treatment in spring and reproductive recovery in sheep with different body condition scores and age. *Animal Reproduction Science*, *160*, 68–73. https://doi.org/10.1016/j.anireprosci.2015.07.004

Magawana, M., Dugmore, T. J., de Villiers, J.F., & Gcumisa, S. T. (2021). Effect of lambing season, year, sex, and birth status on weaning and post-weaning growth performance of merino lambs. *Applied Animal Husbandry & Rural Development*, *14*, 62–68.

Martinez-Ros, P., & Gonzalez-Bulnes, A. (2019). Efficiency of CIDR-Based Protocols Including GnRH Instead of eCG for Estrus Synchronization in Sheep. *Animals*, *9*(4), 146. https://doi.org/10.3390/ani9040146

Martinez-Ros, P., Astiz, S., Garcia-Rosello, E., Rios-Abellan, A., & Gonzalez-Bulnes, A. (2018). Effects of short-term intravaginal progestagens on the onset and features of estrus, preovulatory LH surge, and ovulation in sheep. *Animal Reproduction Science*, *197*, 317–323. https://doi.org/10.1016/j.anireprosci.2018.08.046

Moghaddam, A., Kohram, H., Rahimi-Feyli, P., & Sohrabi, G. (2021). Effect of intravaginal device type and treatment length on estrus synchronization and reproductive performance of farahani ewes out of breeding season. *Songklanakarin Journal of Science & Technology*, *43*(1), 80–86.

Mohammed, T., Al-Ani, A., & Munther, A. (2019). Effect of arginine on physiological and reproductive performance of Awassi ewes. *Iraqi Journal of Veterinary Sciences*, *33*(2), 213–220. https://doi.org/10.33899/ijvs.2019.162878

Nakafeero, A., Hassen, A., & Lehloenya, K. C. (2020). Investigation of ram effect and eCG usage in progesterone based oestrous synchronization protocols on fertility of ewes following fixed time artificial insemination. *Small Ruminant Research*, *183*, 106034. https://doi.org/10.1016/j.smallrumres.2019.106034

Ncoyini-Manciya, Z., & Savage, M. J. (2022). The Assessment of Future Air Temperature and Rainfall Changes Based on the Statistical Downscaling Model (SDSM): The Case of the Wartburg Community in KZN Midlands, South Africa. *Sustainability*, *14*(17), 10682. https://doi.org/10.3390/su141710682

Nel, C., Gurman, P., Swan, A., van der Werf, J., Snyman, M., Dzama, K., Olivier, W., Scholtz, A., & Cloete, S. (2024). Including genomic information in the genetic evaluation of production and reproduction traits in South African Merino sheep. *Journal of Animal Breeding and Genetics*, *141*(1), 65–82. https://doi.org/10.1111/jbg.12826

Nogueira, D. M., Cavalieri, J., Fitzpatrick, L. A., Gummow, B., Blache, D., & Parker, A. J. (2016). Effect of hormonal synchronisation and/or short-term supplementation with maize on follicular dynamics and hormone profiles in goats during the non-breeding season. *Animal Reproduction Science*, *171*, 87–97. https://doi.org/10.1016/j.anireprosci.2016.06.003

Ramukhithi, F. V., Nedambale, T. L., Sutherland, B., Greyling, J. P. C., & Lehloenya, K. C. (2012). Oestrous synchronisation and pregnancy rate following artificial insemination (AI) in South African indigenous goats. *Journal of Applied Animal Research*, *40*(4), 292–296. https://doi.org/10.1080/09712119.2012.685280

Rosasco, S. L., Beard, J. K., Hallford, D. M., & Summers, A. F. (2019). Evaluation of estrous synchronization protocols on ewe reproductive efficiency and profitability. *Animal Reproduction Science*, *210*, 106191. https://doi.org/10.1016/j.anireprosci.2019.106191

Rusticus, S. A., & Lovato, C. Y. (2019). Impact of sample size and variability on the power and type i error rates of equivalence tests: A simulation study. *Practical Assessment, Research, and Evaluation*, *19*(1), 11.

Salama, M. S., Ashour, M. A., Taher, E. S., El-kon, I., Sayed, S., Alkeridis, L. A., Stefan, B., Ana-Maria, I., Al-Shuraym, L. A., & Shukry, M. (2024). Evaluating estrus synchronization and early pregnancy detection in Ossimi sheep: The influence of fluorogestone acetate treatment duration and dosage. *Veterinary and Animal Science*, *24*, 100351. https://doi.org/10.1016/j.vas.2024.100351

Santos-Jimenez, Z., Martinez-Herrero, C., Encinas, T., Martinez-Ros, P., & Gonzalez-Bulnes, A. (2020). Comparative efficiency of oestrus synchronization in sheep with progesterone/eCG and progesterone/GnRH during breeding and non-breeding season. *Reproduction in Domestic Animals*, *55*(7), 882–884. https://doi.org/10.1111/rda.13698

Santos-Jimenez, Z., Martínez-Ros, P., Encinas, T., Morales-Cruz, J. L., Guerrero-Gallegos, H. Z., Gonzalez-Avalos, R., Gonzalez-Bulnes, A., & Guillen-Muñoz, J. M. (2022). Ovarian Response and Fertility after Short-Term Progestagen/eCG Treatments Are Compromised in Nulliparous Sheep during Non-Breeding Season. *Veterinary Sciences*, *9*(12), 663. https://doi.org/10.3390/vetsci9120663

Sotgiu, F. D., Porcu, C., Pasciu, V., Dattena, M., Gallus, M., Argiolas, G., Berlinguer, F., & Molle, G. (2021). Towards a Sustainable Reproduction Management of Dairy Sheep: Glycerol-Based Formulations as Alternative to eCG in Milked Ewes Mated at the End of Anoestrus Period. *Animals*, *11*(4), 922. https://doi.org/10.3390/ani11040922

Swelum, A. A.-A., Alowaimer, A. N., & Abouheif, M. A. (2015). Use of fluorogestone acetate sponges or controlled internal drug release for estrus synchronization in ewes: Effects of hormonal profiles and reproductive performance. *Theriogenology*, *84*(4), 498–503. https://doi.org/10.1016/j.theriogenology.2015.03.018

Swelum, A. A.-A., Saadeldin, I. M., Moumen, A. F., Ali, M. A., & Alowaimer, A. N. (2018). Efficacy of controlled internal drug release (CIDR) treatment durations on the reproductive performance, hormone profiles, and economic profit of Awassi ewes. *Small Ruminant Research*, *166*, 47–52. https://doi.org/10.1016/j.smallrumres.2018.07.018

Takci, A., & Dinc, D. A. (2023). Stimulation of Estrus and Ovulation by Resynchronization in Kangal Sheep during Early Anestrus. *Veterinary Sciences*, *10*(8), 499. https://doi.org/10.3390/vetsci10080499

Thipe, E. L., Workneh, T. S., Odindo, A. O., & Laing, M. D. (2020). Effects of winter greenhouse microclimate on butterhead lettuce (*Lactuca sativa* var. *capitata*). *Acta Horticulturae*, *1271*, 17–24. https://doi.org/10.17660/actahortic.2020.1271.3

Usman, M., Sarwar, A. S., Shahid, R. U., Rehman, Sajjad Ur, & Khamas, W. A. (2022). Histological and ultrastructural studies of female reproductive vasculature of one humped camel in relation to possible thermoregulation and ovarian hormones. *Veterinary Research Forum*, *13*(2), 177–186. https://doi.org/10.30466/vrf.2021.129861.2991

Vilariño, M., Rubianes, E., & Menchaca, A. (2013). Ovarian responses and pregnancy rate with previously used intravaginal progesterone releasing devices for fixed-time artificial insemination in sheep. *Theriogenology*, *79*(1), 206–210. https://doi.org/10.1016/j.theriogenology.2012.10.007

Wentzel, D. (2012). Managerial aspects affect reproduction in woolled sheep. *Merino Breeders Journal*, *40*(3).

Zenda, M., Malan, P. J., & Geyer, A. C. (2024). An analysis of the wool characteristics that determine wool price for Merino wool in South Africa. *Scientific African*, *23*, e02005. https://doi.org/10.1016/j.sciaf.2023.e02005