Original Research Article

EFFECT OF USING DIFFERENT ESTRUS SYNCHRONIZATION PROTOCOLS ON REPRODUCTIVE PERFORMANCE IN MERINO EWES

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ABSTRACT

Sheep production system in Lesotho is based on natural mating hence reproduction performance of these sheep falls below the expected lamb crop per year. The study was carried out to evaluate the comparative effectiveness of four different synchronization protocols on the reproductive performance of Merino ewes during the autumn breeding season. The ewes were inseminated immediately after withdrawal of the treatments as they come on estrus. The indices determined after artificial insemination with semen collected from ram were (a) conception rate and return rate of ewes in different synchronization protocols (b) pregnancy rate and lambing rate of ewes in different synchronization protocols and, (c) sex of lambs and number of lambs born per ewe in different synchronization protocols. A total of 204 ewes were randomly assigned to four treatments: TRT A (Control), TRT B (12 days' intravaginal sponges [0.3 g P4] insert), TRT C (Double injection of prostaglandin at day 0 and 11), and TRT D (intravaginal sponges for 13 days, single injection of prostaglandin at 12 days together with single injected of pregnant mare serum gonadotropin at day 13. The experimental treatments did not have a significant effect ($p \geq 0.05$) on conception rate, returned rate and sex of lambs while, pregnancy rate, lambing rate, ewes aborted and number of lambs born per ewe differed significantly ($p \leq 0.05$) between treatments. Merino sheep farmers are recommended to implement estrus synchronization and artificial insemination techniques in their flocks in order to improve the reproductive performance of ewes during the autumn breeding season.

Keywords: Merino, oestrus, synchronization, Prostaglandin.

1. INTRODUCTION

The dominance of Merino sheep as the primary breed among farmers in Lesotho presents several challenges. The natural reproductive cycle of these sheep is closely linked to environmental cues, particularly the length of daylight. However, the lack of scientific synchronization among ewes leads to logistical and managemental difficulties for farmers. This situation necessitates meticulous planning and resource allocation to effectively manage breeding and ensure optimal productivity. Abecia et al. (2012) indicates that, reproduction of small ruminants can be controlled by several methods developed in recent decades and some of these involve administration of hormones that modify the physiological chain of events involved in the estrus cycle. Reproduction in sheep is one critical factor for production and successful operation at the sheep farm. Artificial insemination technique is an assisted reproduction technology and consists in the application of a dose of semen into the female reproductive tract by means of instruments as reported by Gibbons et al. (2019). According to Ataman and Akoz (2006), $PGF_2\alpha$ consists of 2 doses with a 9-11-day interval conception rates and prolificacy after mating were comparable to unsynchronized ewes. Long interval prostaglandin protocols followed by cervical timed artificial insemination improved genetic merit in the sheep industry with pregnancy rates ranging between 60-70 % (Gibbons et al., 2019). Fierro et al. (2017) reported that, pregnancy rates may be decreased due to alterations in the contractions of the myometrium, sperm transport and variability in the timing of onset estrus and ovulation. In addition, Dursun (2019) indicated that protocols

based on $PGF_{2}\alpha$ resulted in high estrus and ovulation but the fertility of ewes became poor. Use of PMSG in collaboration with $PGF_{2}\alpha$ and progesterone is said to be a more effective treatment than any other synchronization protocol that result in successful breeding, increased conception rates and in shortening the breeding period (Abdalla et al., 2014). Pregnant mare serum gonadotropin is also be effective in increasing the ratio of ovulation and twinning rates, and the difference in estrus interval after the removal of the sponges can be reduced by PMSG to achieve the consistency of estrus time of ewes (Yu et al., 2022). Estrus synchronization by progestogen is reported by Martines-Ros et al. (2018) to be low as compared to natural estrus and this is due to prolonged treatment durations. Manes et al. (2014) reported that the use of intravaginal sponges is a predisposing factor for vaginal contamination, and in prolonged treatment duration, sponges tend to cause inflammation thus reducing conception rates and decreasing fertility. This study focused on evaluating the reproductive performance of Merino ewes under different estrus synchronization protocols during the breeding season.

2. MATERIAL AND METHODS

2.1 STUDY AREA

The study was done in Lesotho a country located in southern Africa. It covers about 30, 355 km^2, with an elevation of between 1, 388 m and 3, 482 m above sea levels and lies between latitudes 28° and 31° S and longitudes 27° and 30° E (Ministry of Energy, Meteorology and Water Affairs, 2013). Data was collected during breeding season from November 2022 to October 2023 at National University of Lesotho (NUL) Animal Farm. The NUL Farm is situated at Roma, which is about 35 kilometers southeast of Maseru. The climate of Roma alternates between the hot and cold months. The winter being the coldest season is from May to August during which temperatures may drop as low as -1 ˚C and summer the hottest season from September to April during which the temperature can be as high as 28 ˚C (Ministry of Energy, Meteorology and Water Affairs, 2013). Roma is in the Lowlands agroecological zone with an altitude ranging from 1500 – 1800 m above sea level and the annual mean rainfall ranging from 600 – 900 mm (Ministry of Energy, Meteorology and Water Affairs, 2013).

2.2 EXPERIMENTAL DESIGN AND SAMPLE SIZE DETERMINATION

A longitudinal study design was conducted for a period of 1 year (November 2022 to October 2023). A simple random sampling technique was used to select the ewes, with respect to sheep age. Sheep age was supplied by the Farmers and confirmed by dentition and records. The experimental animals were divided in to four treatments (TRT), namely TRT A (CONTROL), TRT B, TRT C and TRT D. A total of 204 ewes were used as experimental units composed of ewes that were lambed at least once. Each treatment was composed of 51 ewes replicated three times and each has 17 ewes. The experimental animals were marked with ear-tags for identification.

2.3 SYNCHRONIZATION PROTOCOLS

The synchronization methods schedule for the ewes is presented in Fig. 1. For TRT A was regarded as control (untreated ewes), oestrus cycles were synchronized using three different protocols. In TRT B, progesterone sponges containing 60 mg of medroxyprogesterone were inserted intra-virginally for a period of 12 days, and after sponge removal, the ewes came on heat between 36-72 hours later and were bred by AI immediately after showing estrus sings. In TRT C, ewes received two injections of prostaglandin (estrumate); the first given on day 0 and the second on day 11 and treated ewes came on heat after either the first or second injection and all the ewes were bred by AI after the second injection following estrus signs. Most of the treated ewes showed estrus 36-96 h after the second ($PGF_{2}\alpha$) injection (1ml per ewe). In TRT D**,** progesterone sponges containing 60 mg of medroxyprogesterone were inserted intra-virginally for a period of 13 days, and ewes received single injection of $PGF_{2\alpha}$ intramuscularly on Day 12. The intra-virginally sponge removal was done on day 13 and on the same day ewes received single injection of pregnant mare serum gonadotropin Hormone (2ml per ewe).

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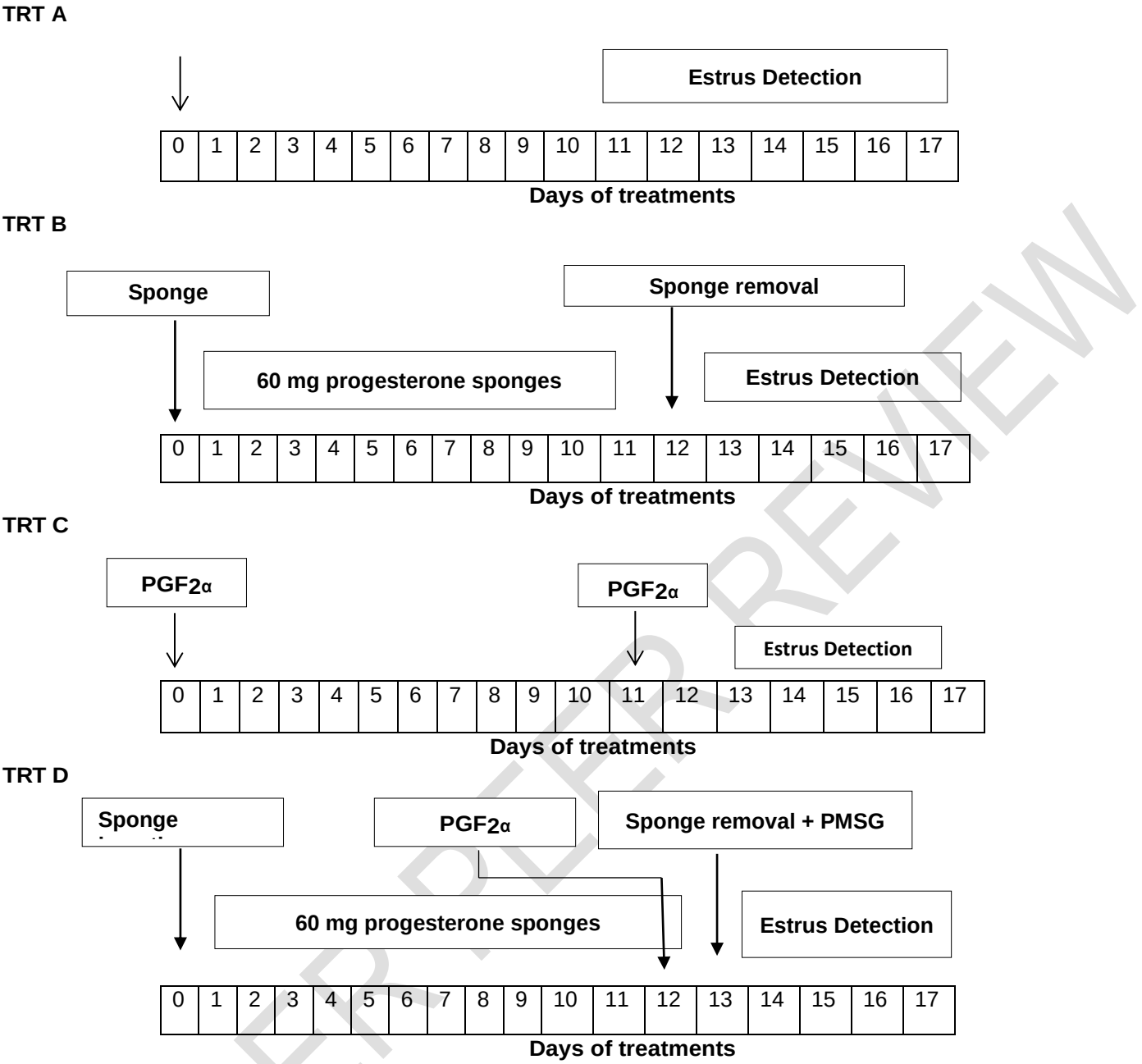

Figure. 1 Merino sheep estrus synchronization with or without hormones

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2.4 MANAGEMENT AND FEEDING OF EXPECTED ANIMALS

Merino rams between 3-7 years of age with BCS of 3.0 and above were used in this experiment. Rams selected for semen collection were housed night and day for about two weeks before semen collection started. Donor rams (n= 2) used were healthy, sexually matured have good body configuration, well developed testes, good vigor, good libido and high adaptability to artificial vagina semen collection technique. To achieve maximal fertility, rams were physically examined (Breeding Soundness Evaluation) for reproductive fitness before breeding to detect any abnormalities that may affect reproductive ability. The experimental males were trained to mount on dummies (oestrus/anestrous ewes) for collection of semen by artificial vagina (AV) in the early morning at 6.30 to 7.00 am once per day for two weeks. The rams were fed on quality hay. The rams accessed water ad libitum and were supplemented with 50- 300 grams per day of pellets or cereal grains. Non-pregnant ewes were used in the experiment with BCS of 2.5 and above. All experimental ewes were housed in the night and allowed to graze during the day on confined natural pasture for 6-9 hours daily. The ewes had access to water at any

time and they were supplemented with cereal grains in the afternoon. As a routine flock health management practice at NUL Farm, the experimental animals were dosed against internal parasites (Prodose Orange at 2 ml/10 kg body weight, Virbic RSA (Pty) Ltd). The ewes were injected with Solution ® 3.5% L. A at 1 ml/10 kg body weight, Intervet South Africa (Pty) Ltd and vaccinated against blackleg and anthrax with Blanthrax at 2 ml/sheep, Intervet South Africa (Pty) Ltd., in the different seasons of the year before the beginning of the experiment. The experimental animals were also dipped against ectoparasites using Cooperzon® 30 at 1 L/1000 L of clean water, Cooper Veterinary Products (Pty) Ltd.

2.5 ESTRUS DETECTION

Oestrus detection was one of the key mechanisms in fertility management programmes in the sheep industry. Oestrus detection using teaser rams equipped with crayons or marking harness was done twice daily morning and evening, for two hours at each time. This was performed six hours after withdrawal of the treatments lasted for 96 hours thereafter according to Ferdowsi et al. (2018).

2.2 DATA COLLECTION

The following reproductive parameters were collected:

2.2.1 CONCEPTION RATE

Conception rate was calculated as percentage of the number of ewes that lambed compared to the number of ewes exposed to the ram.

$$\text{Conception rate} = \frac{Total\ number\ of\ ewes\ \ lambed}{Total\ number\ of\ ewes\ inseminated} \times 100$$

2.2.3 PREGNANCY RATE

Pregnancy rate was calculated as percentage of the number of pregnancies (including live births, still births and abortions) to total number of ewes exposed to the ram in a single breeding season.

$$\text{Pregnancy rate} = \frac{Total\ Number\ of\ pregnant\ ewes}{Total\ number\ of\ ewes\ inseminated} \times 100$$

2.2.4 LAMBING RATE

Lambing rate can be calculated as number of ewes lambed per total number of ewes inseminated.

$$\text{Lambing rate} = \frac{Total\ Number\ of\ lambed\ ewes}{Total\ number\ of\ ewes\ inseminated} \times 100$$

2.3 STATISTICAL ANALYSIS

SPSS (2020) Version 20.00 was used to analyze the data. The data were analyzed by descriptive statistics within the crosstabs to tabulate the percentages. A chi-square test was employed to show the significant association between different treatments based on the following reproductive parameters (i) Conception rate, (ii) Pregnancy rate, (iii) Abortions, (iv) lambing rate, (v) number of lambs born: singles or twins, and (vi) sex of the lambs. Probability values less than or equal to ($P \leq 0.05$) were considered to be significant. The different synchronization treatments and reproductive parameters was tested using the Bonferroni post- hock cell-wise adjusted standardized residuals analysis and the p-values determined for each cell less than or equal to adjusted p-values were considered to be significant.

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3. RESULTS AND DISCUSSION

The results in Table I illustrate the impact of various synchronization protocols on the reproductive parameters of Merino ewes during the autumn breeding season. The synchronization treatments showed no significant differences in conception rates, return rates, or the sex of lambs born. However, notable differences were observed in pregnancy rates, abortion rates, lambing rates, and the number of lambs born per ewe during this season. The insignificant result (P≤0.05) based on conception rate was higher than reported by Manes et al. (2014), who found that progesterone (P4) protocols were associated with vaginal contamination and inflammation, leading to reduced conception rates and fertility in ewes. In contrast, the current study showed higher conception rates and the results aligning with those of Abdalla et al. (2014), which indicated 100% conception rates in ewes treated with a combination of (P4 + PGF2α + PMSG). This variation may be due to the different sheep breeds used in the studies. The current study's findings are consistent with those of Yu et al. (2022) and Ataman et al. (2006), who reported conception rates of 75% and 86.6%, respectively. This discrepancy may be attributed to differences in the nutrition and health status of the ewes across various studies.

The proportion of pregnant ewes showed significant difference, likely due to lower levels of FSH and LH, failed ovulation post-synchronization in some ewes, and the presence of reproductive diseases. The lambing rate was reported insignificance and may stem from the varying genetic backgrounds of the ewes, despite all being Merinos from different farmers. In this study, pregnancy rates were comparable to those reported by Amer and Hazzaa (2009), who found a 66% rate in ewes treated with a 12-day progesterone protocol. These results are consistent with Moghaddam (2012), who reported a 77.5% pregnancy rate in ewes treated with CIDR and PMSG. Additionally, Dursun (2019) and Ataman et al. (2006) reported pregnancy rates of 82.5% and 86.7%, respectively, in ewes treated with a combination of progesterone, prostaglandin, and PMSG.

In contrast to the current study's findings, Fierro et al. (2017) reported pregnancy rates of 28.8%, 30.3%, 46%, 56.9%, and 56% from ewes treated with double injections of prostaglandin at intervals of 7, 10, 12, 14, and 16 days. These differences may stem from the unique physiological traits of Merino ewes and effective management practices associated with wool-producing animals. The current study's findings on abortion rates align with Dursun (2019), which reported rates of 13.6% and 15% in ewes treated with progesterone combined with prostaglandins, and those treated with a combination P4 + PGF2α + PMSG. However, the current study contradicts Dursun (2019) results, which indicated higher abortion rates of 44% in ewes treated with progesterone alone and 35.4% in ewes receiving a double injection of prostaglandin. The current study results on lambing rate differed significantly ($p \leq 0.05$) align with findings from Ataman et al. (2006), which reported rates of 64.6% and 80% in ewes given double injections of prostaglandin. Additionally, the results are consistent with Ataman and Akoz (2006), who found an 81.8% lambing rate in ewes treated with two PGF2α injections, and Kuru et al. (2020), who reported a 100% lambing rate in ewes treated with progestogen and eCG for 10 to 14 days. In contrast, Amer and Hazzaa (2009) reported lower lambing rates of 33.3%, 58.3%, and 45.7% for various treatments involving intravaginal sponges with progesterone and PGF2α injections.

The percentage of ewes that lambed twins and triplets was statistically significant ($P \leq 0.05$). The current study's findings on twinning rates align with those reported by Sherry et al. (2012), which indicated higher percentages of single births (50%), followed by 42.9% twin births and 7.1% triplet births in ewes treated with progesterone alone. Additionally, the results are consistent with Sherry et al. (2012), who noted high single birth rates of 92.9%, with 0% twins and 7.1% triplets in ewes treated with a double injection of prostaglandins. The current study's findings regarding the sex ratio of lambs align with those of Hajibemani et al. (2023), who reported a female-to-male ratio of 57.1% to 42.9% in nulliparous Ghezel ewes treated with double injections of prostaglandins. In contrast, Ahilmaidi et al. (2022) found a higher number of males than females in ewes treated with CIDR + PMSG (62.85% to 37.15%) and P4 + GnRH (55% to 45%).

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Table 1. Effect of different synchronisation protocols on reproductive parameters during breeding season.

Indices	Different synchronization protocols			
	TRT A	TRT B	TRT C	TRT D
No. Ewes inseminated	46	48	46	50
Conception rate (%)	84.78 [a]	75.00 [a]	73.91 [a]	80.00 [a]
Return rate (%)	15.22 [a]	25.00 [a]	26.09 [a]	20.00 [a]
Pregnancy rate (%)	78.26 [a]	70.83 [b]	63.04 [c]	78.00 [a]
Lambing rate (%)	80.43 [a]	79.17 [a]	69.56 [b]	82.00 [a]
Ewes Aborted (%)	2.17 [a]	2.08 [a]	8.70 [b]	2.00 [a]
Males lambs (%)	36.73 [a]	38.00 [a]	31.25 [a]	39.29 [a]
Females lambs (%)	46.94 [a]	44.00 [a]	47.92 [a]	46.43 [a]
Single lambing (%)	76.09 [a]	77.08 [a]	76.09 [a]	74.00 [a]
Twinning lambing (%)	6.52 [a]	4.17 [a]	0.00 [b]	8.00 [c]
Triple lambing (%)	0.00 [a]	0.00 [a]	2.17 [b]	2.00 [b]

Means within a row with different superscripts are significantly different (P< 0.05). Means within a row with same superscripts do not differ (P > 0.05) significantly. Control (TRT A) - Untreated ewes. TRT B- ewes treated with intra-vaginal sponges alone on day = 0 to day = 12.TRT C- ewes received double injection of prostaglandin on day = 0 to day =11. TRT D- ewes treated with intra-vaginal sponges on day = 0 to day = 13, injected with prostaglandin on day = 12 and sponge removed at day =13 plus PMSG injection on same day.

4. CONCLUSION

From the study, it was concluded that estrus synchronization with combination treatment D resulted in comparatively higher lambing rate of 82.00 % than 80.43 %, 79.17 % and 69.56 % from treatment A, treatment B and treatment C respectively. The majority of lambs born were females in all treatments 46.94 % (TRT A), 44.00 % (TRT B), 47.92 % (TRT C) and 46.43 % (TRT D). The proportion of lambs born as singles was highest in treatment B followed by 76.09 % in both treatments A and C and 74.00 % in treatment D.

5. Disclaimer (Artificial intelligence)

Authors hereby declare that this manuscript has been prepared entirely without the assistance of artificial intelligence tools or technologies. All content, including text and data analysis, has been produced solely by the authors. Specifically, no generative AI models such as OpenAI's ChatGTP, Google's Bard, or any other AI-based writing or content generation tools have been utilized in the preparation of this manuscript. The authors affirm that all research and writing processes were conducted manually to ensure authenticity and originality.

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