Original Research Article

EFFECT OF OESTRUS SYNCHRONISATION ON REPRODUCTIVE TRAITS IN MERINO SHEEP USING COMBINATIONS OF INTRAVAGINAL SPONGES, ESTRUMATE AND PREGNANT MARE SERUM GONADOTROPIN FOR ARTIFICIAL INSEMINATION

ABSTRACT

The study carried out to evaluate the comparative effectiveness of four different synchronization protocols on reproductive performance of Merino ewes during breeding season. 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. Total of 204 ewes were randomly assigned to four treatments: TRT A (Control), TRT B (14 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 14 days, single injection of prostaglandin at 12 days, and intravaginal sponges together with a single injection of pregnant mare serum gonadotropin Hormone). The experimental treatments did not have significant effect ($p > 0.05$) effect on conception rate, returned rate, pregnancy rate, lambing rate, ewes aborted, sex of lambs and ewe lambing during breeding season. Merino sheep farmers are recommended to implement estrus synchronization and artificial insemination techniques in their flocks in order to improve reproductive performance of ewes during breeding season.

Keywords: Merino, oestrus, synchronization, Prostaglandin.

1. INTRODUCTION

The merino sheep are the most dominant breed kept by farmers throughout the country of Lesotho and its dominance is enabled by regular upgrading with high genetic rams that for a long time were mostly imported from South Africa (Phoofolo et al., 2023). Sheep production plays a critical part in the economy of the Lesotho as it improves the economic well-being of people in the rural areas (Moiloa et al., 2021). Reproduction in sheep is one critical factor for production and success operation at the sheep farm. Reproductive performance is evaluated on the basis of lamb crop for given lambing season (Ishfasq-Ahamad et al., 2022). Reproductive efficiency in livestock depends on multiple factors including insemination, conception, embryonic development and lambing as key determinants of the farm productivity and profitability (Hodge et al., 2023). According to Ataman and Akoz (2006), $PGF_2\alpha$ consists of 2 doses with a 9-11-day interval and conception rates and prolificacy after mating was comparable to unsynchronized ewes. Long interval prostaglandin protocols followed by cervical timed artificial insemination improved genetic merit in 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, and Olivera-Muzante et al. (2011) said decreased pregnancy rates could also be caused by low progesterone concentrations during the luteal phase of the previous estrus cycle. 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 are said to be more effective treatment than any other synchronization protocols that result in successful breeding, increased conception rates and in shortening the breeding period (Abdalla et al., 2014). Naderipour et al. (2011) observed that, the combination of PMSG, P_4 and $PGF_2\alpha$ reduces the intervals between the onset of estrus and ovulation. Pregnant mare serum gonadotropin is also said be effective in increasing the ratio of ovulation and twinning rates, and that the difference of 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 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 decreased fertility. Quereda et al. (2020) observation is supports the use of intravaginal sponges which may cause low fertility rates and prolificacy, because sponges secrete abnormal discharges which results in changes in the vaginal bacterial microbiota, thus leading to vaginitis with consequent reduction in fertility rates. According to Nebel and Roth (2011), low oestrus detection rate is strongly linked with poor fertility, long lambing interval and reduced genetic progress. 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 have 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 (control), 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. 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 were bred by AI. Most of the treated ewes showed estrus 36-96 h after the second ($PGF_2\alpha$) injection. 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 (PMSG).

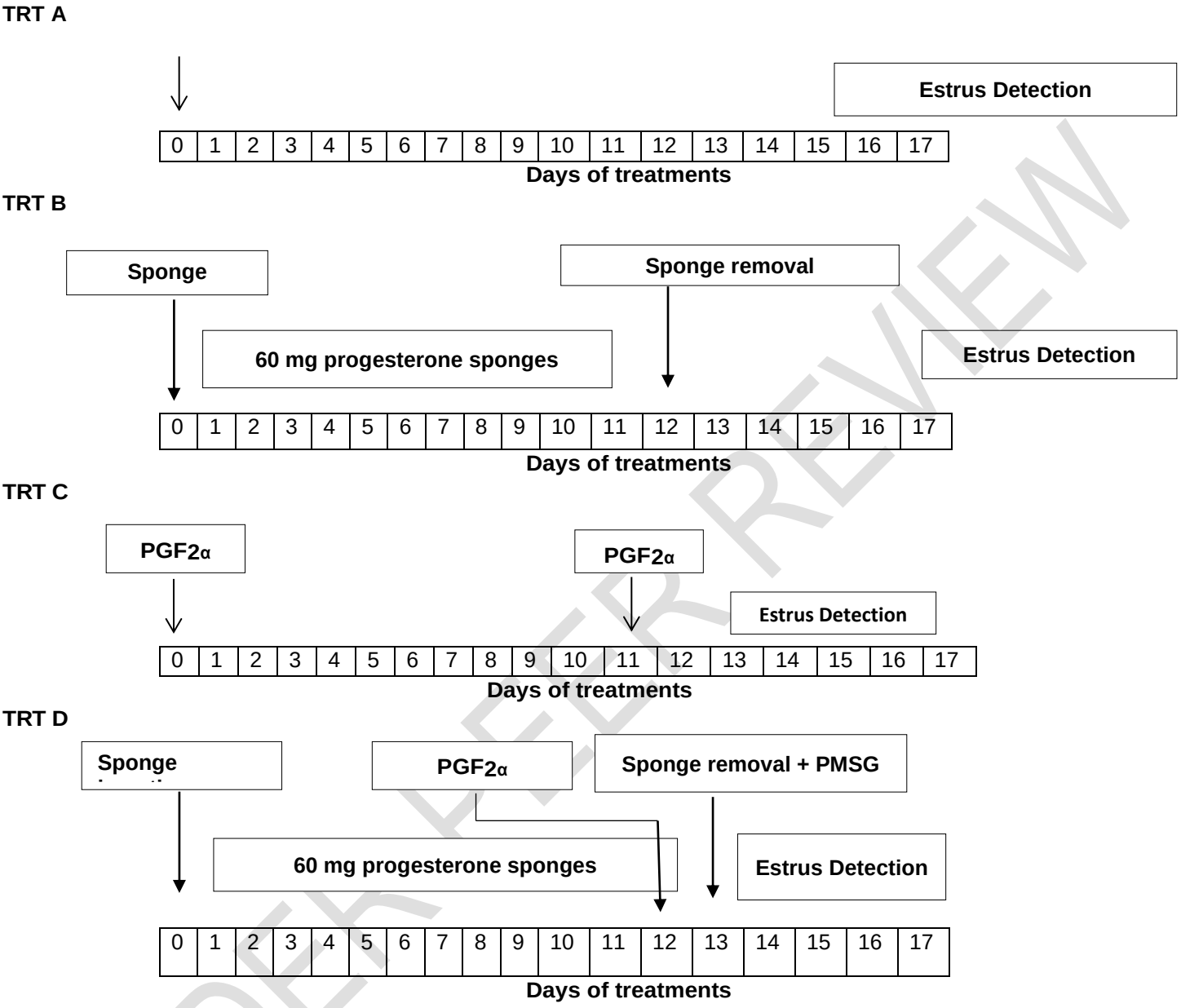

Figure. 1 Merino sheep estrus synchronization with or without hormones

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 $PGF_2\alpha$ injection and 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.

Conception rate = $\frac{Total\ number\ of\ ewes\ \ lambed}{Total\ number\ of\ ewes\ inseminated}$ x 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.

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

2.2.4 LAMBING RATE

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

Lambing rate = $\frac{Total\ Number\ of\ lambed\ ewes}{Total\ number\ of\ ewes\ inseminated}$ x 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 (GarcíaPérez and Núñez-Antón, 2003) and the p-values determined for each cell less than or equal to adjusted p-values were considered to be significant.

3. RESULTS AND DISCUSSION

The result presented in Table I gives the effect of different synchronisation protocols on reproductive parameters of Merino ewes during breeding season. The total number of ewes inseminated was 46 in TRT A (Control) and TRT C (double injection PGF2α) while, 48 and 50 were reported in TRT B (P_4) and TRT D (P_4 + PGF2α + PMSG), respectively. The synchronization treatments did not differ significantly ($p > 0.05$) based on conception rate, return rate, pregnancy rate, lambing rate and ewes aborted during the breeding season. The findings based on sex of lambs born and number of lambs born per ewe per lambing did not vary significantly ($P > 0.05$) with different synchronisation protocols during breeding season.

The insignificant ($P > 0.05$) result based on conception rate was higher compared to results obtained by Abecia et al. (2012) that ewes synchronized with a protocol involving progesterone had a conception rate of 65.00 %, while those synchronized using a combination of progesterone and eCG had a conception rate of 75.00 %. The finding in the current study does not agree with result obtained by Martemucci et al. (2017) that ewes synchronized with progesterone-impregnated sponges achieved a conception rate of 60.00 %, while those synchronized with combined protocol had higher conception rate of 70.00 %. Furthermore, Menchaca et al. (2015) indicated that, ewes synchronized with progesterone-impregnated sponges achieved a conception rate of 55.00 % while, those synchronized with prostaglandin F2α injections alone had a conception rate of 50.00 %, and ewes synchronized with the combination protocol had highest conception rate of 65.00 %. The variation could be as result of different breeds of sheep used in the studies. Smith et al. (2015) reported return rate of 20.00 % in ewes inseminated which were synchronized using the Ovsynch protocol, and ewes synchronized with CIDR had a return rate of 15.00 % (Jones and Brown, 2018) similar to the current result Gonzalez et al. (2017) reported return rate of 25.00 % in ewes treated with the double PGF2α protocol which is comparable to 26.09 % observed in the current study. Ewes synchronized using a progestogen-based protocol were found to have a return rate of 18.00 % in a study by Martinez et al. (2016) and was reported lower as compared to the return rate of ewes treated with intravaginal sponges in the study as reported 25.00 %. The variation could be as result of nutrition and health status of the ewes used in various studies.

The proportion of ewes pregnant was insignificantly different possibly as a result of poorly performed AI, lower FSH and LH, failed ovulation after synchronization by some ewes and presence of reproductive diseases. The current result is not in line with the report by Aboagla et al. (2017) which showed that, the pregnancy rate was 60.00 % for ewes synchronized with the progesterone-based protocol and 55.00 % for ewes synchronized with the prostaglandin-based protocol. Martemucci et al. (2015) investigated the pregnancy rates of ewes synchronized using controlled internal drug release (CIDR) devices compared to those synchronized using PGF2α and observed a pregnancy rate of 70.00 % for ewes synchronized with CIDR devices and 65.00 % for ewes synchronized with PGF2α. Menchaca et al. (2016) reported a pregnancy rate of 58.00 % for ewes synchronized with intravaginal sponges and 62.00 % for ewes synchronized with CIDR devices. The insignificant ($P > 0.05$) result on lambing rate could be as result of different genetic makeup of the ewes even though they are merinos but from different farmers. The percentage ewe's abortion was lower than result reported by Smith et al. (2018) that 15.00 % of ewes synchronized using protocol A aborted after being inseminated during the breeding season. However, 10.00 % of ewes synchronized using protocol B experienced abortion. This could be as result of unique physiological characteristics of merino ewes and good managemental practices associated with wool producing animals. The lambing rate in the current study is in line with the report by Aboagla et al. (2018) of a lambing rate of 70.00 % in ewes synchronized using the Ovsynch protocol. However, the current lambing rate was higher than 65.00 % by Abecia et al. (2016) in ewes synchronized using the Double PGF2α protocol. Bartlewski et al. (2017) observed a lambing rate of 68.00 % in ewes synchronized using the Short-Term Protocol which agrees with 69.56 % in this study.

The result of the current study in in line with 70.00 % reported by Sánchez-Dávila et al. (2015) in ewes synchronized using a progestogen-based protocol. The lambing rate was reported higher in TRT D and TRT C and agrees with the result by Menchaca et al. (2014) that ewes synchronized with a combination protocol exhibited a lambing rate of 75.00 %. The current result agrees with the results obtained by Viñoles et al. (2008) who investigated the use of progestagen and prostaglandin-based synchronization protocols in sheep and observed insignificant result based on sex ratio of lambs born. The percentage of ewes that lambed twins and triplets were found statistically insignificant ($P > 0.05$). In a study by Aboagye et al. (2016) ewes synchronized using a protocol involving progesterone and gonadotropin-releasing hormone (GnRH) had a higher percentage of male lambs born, 55.00 % compared to 45.00 % for those synchronized using PGF2α. Smith et al. (2018) compared the sex ratio of lambs born from ewes synchronized with two different protocols; progesterone and eCG (equine chorionic gonadotropin), progesterone and PMSG (pregnant mare serum gonadotropin) and reported 52.00 % male and 48.00 % female, respectively. Bartlewski et al. (2012) indicated that, the double prostaglandin protocol can result in a

higher percentage of twins and triplets' births. Abecia et al. (2013) found that the CIDR-based protocol resulted in a higher percentage of twin births compared to the FGA-based protocol in Rasa Aragonesa ewes while, El-Sherif et al. (2016) reported that, the percentage of single births from ewes synchronized with a progesterone-based protocol was 42.00 %, while the percentage of twin births was 49.00 % and the percentage of triplet births was 9.00 %. Martemucci et al. (2012), El-Sherif et al. (2018), Muñoz-Gutiérrez et al. (2017), Abecia et al. (2013), Menchaca et al. (2009) all studied the effects of different synchronization protocols on reproductive performance and all observed that, the percentage of twin births were higher than percentage of single births and percentage of triplet births was the lowest.

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 [a]	63.04 [a]	78.00 [a]
Lambing rate (%)	80.43 [a]	79.17 [a]	69.56 [a]	82.00 [a]
Ewes Aborted (%)	2.17 [a]	2.08 [a]	8.70 [a]	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 [a]	8.00 [a]
Triple lambing (%)	0.00 [a]	0.00 [a]	2.17 [a]	2.00 [a]

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.

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