Research article

DOI: 10.48165/ijar.2024.45.02.10

The Indian Journal of Animal Reproduction

The official journal of the Indian Society
for Study of Animal Reproduction

Year 2024, Volume-45, Issue-2 (December)

ISSN 0970-2997 (Print)

ISSN 2583-7583 (Online)

Oestrus Synchronization in Merino Sheep Using Intravaginal Sponges, Estrumate PMSG on Oestrus Activity

Posane Slyvester Lebata*, Philip Makama Dawuda and Setsumi Molapo

Department of Animal Science, Faculty of Agriculture
National University of Lesotho, Roma 180, Lesotho

ABSTRACT

A study was conducted with 204 Merino ewes as experimental units to assess the effectiveness of four estrus synchronization protocols. The ewes were divided into four groups: TRT A (Control), TRT B (Intravaginal sponges for 14 days), TRT C (Double prostaglandin injections on days 0 and 11) and TRT D (Intravaginal sponges for 14 days, a single prostaglandin injection on day 12, plus PMSG). Six rams were used to detect heat, with standing to be mounted as the primary indicator of estrus. There were no significant differences ($p>0.05$) in estrus response rates among the groups: 94.10% for TRT B, 96.20% for TRT D, and 90.20% for both TRT A and TRT C. Additionally, the time from treatment withdrawal to the onset and end of estrus showed no significant variation ($p>0.05$). The duration of estrus differed significantly among the treatments (TRT D, TRT A, and TRT C) with notable differences at specific time intervals: 48h and 36h for TRT D, and 24h for TRT C ($p<0.05$). Significant differences were also found between TRT B and TRT D at 48h and 60h post-treatment withdrawal. Additionally, TRT D showed high significance at 24h and 36h intervals. At 72 hours, both TRT C and TRT D were significant, with TRT D remaining significant at 84h ($p<0.05$). Overall, combination treatment (TRT D) was more effective in synchronizing estrus in Merino ewes, resulting in a higher response rate and shorter interval from treatment withdrawal to estrus onset.

***Keywords*:** Estrus, Progesterone, Prostaglandin, PMSG, Synchronization.

How to cite: Lebata, P. S., Dawuda, P. M., & Molapo, S. (2024). Oestrus synchronization in Merino sheep using intravaginal sponges, Estrumate PMSG on oestrus activity. The Indian Journal of Animal Reproduction, 45(2), 59-66,10.48165/ijar.2024.45.02.10

INTRODUCTION

Domestic sheep, primarily used for meat, milk, and wool production, greatly contribute to economic and cultural aspects of farming. Estrus synchronization is an important and reliable method in managing ewe reproduction by manipulating their estrus cycles to induce standing heat and this practice helps farmers align breeding and lambing

*Corresponding author.

E-mail address: posanelebata@gmail.com (Posane Sylvester Lebata)

Received 17-08-2024; Accepted 26-11-2024

times within their flocks for optimal results (Gebreselassie et al., 2019). Breeders can use this technique to induce ewes to ovulate outside of the normal breeding season; however, implementing hormonal treatments is crucial for successful breeding and increasing the number of pregnant females (Habeeb et al., 2021). Exogenous hormones play a significant role in synchronization protocols (Hassanein et al., 2024). The success of synchronization programs relies on several factors beyond the use of exogenous hormones. Chaudhari et al. (2018) emphasized the importance of maintaining proper levels of nutrition, body condition, and health for the success of these programs. Therefore, this study was undertaken with the objective of evaluating the effectiveness of different synchronization protocols on estrus activity in Merino ewes during the breeding season. This study aimed to assess the effectiveness of various synchronization protocols on estrus activity in Merino ewes during the autumn breeding season. However, conducting the research in Lesotho adds a unique geographical aspect based on environmental conditions, altitude, and local farming practices can significantly affect reproductive outcomes.

MATERIALS AND METHODS

Study area: The study was conducted at the NUL laboratory of the National University of Lesotho, situated in Lesotho, a country covering approximately 30,355 km^2. Lesotho's elevation ranges from 1,388 m to 3,482 m and is positioned between latitudes 28° and 31° S and longitudes 27° and 30° E. The NUL experimental farm is located about 35 km southeast of Maseru. This area experiences winter temperatures as low as -1 °C and summer highs reaching up to 28 °C with an altitude of 1500-1800 meters above sea level with an annual rainfall ranging from 600-900 mm. The study was approved by the National University of Lesotho (NUL) Ethics Committee.

Selection of experimental animals, sample collection and synchronization protocols during autumn season: Ewes (= 204: Age = mature) were selected randomly based on their age as determined by the farm manager, which was then confirmed through dentition and records. The ewes were categorized into four treatment groups: TRT A (CONTROL), TRT B, TRT C, and TRT D. Each treatment group consisted of 17 ewes, replicated three times, resulting in a total of 51 ewes per treatment. In total, 204 ewes were utilized as experimental units. In Figure 1, TRT A was considered the control group. TRT B involved the use of progesterone sponges with medroxyprogesterone for 12 days, leading to the manifestation of heat signs after their removal. TRT C included double prostaglandin injections on Day 0 and Day 11. TRT D utilized progesterone sponges for 13 days, followed by a prostaglandin injection on Day 12 and a PMSG injection on Day 13.

Management and feeding of animals: The study used non-pregnant ewes with a body condition score (BCS) of 2.5 or higher. These ewes were provided continuous access to clean drinking water and received cereal grains as supplements in the afternoon. As part of the standard health care protocol at NUL Farm, the ewes were treatment for internal parasites using Prodose Orange and were vaccinated against Blackleg and Anthrax with Blanthrax before the commencement of the experiment. Additionally, they were administered Solution® 3.5 % L.A injections and treated for ectoparasites by dipping them in Cooperzon ® 30.

Estrus Detection: Apronized rams and visual observation were utilized for estrus detection.Sexually matured Apronized rams were introduced twice daily, in the morning and evening, for two hours each time from the beginning of the experiment and maintained throughout.

Statistical Analysis

Data were collected based on estrus response (%), onset of estrus (h), duration of estrus (h), interval from withdrawal of treatment to onset and end of estrus (h). The collected data were analyzed using the Statistical Package for Social Sciences (SPSS) Version 20.00. The mean time intervals were assessed through ANOVA in SPSS, with multiple comparisons carried out using Fisher's least significant difference (LSD) method and significance levels were set at ≤ 0.05. Descriptive statistics were utilized for data analysis within crosstabs to present percentages. A chi-square test was employed to show the significant relationships among different treatments concerning estrous response, duration of estrus, time interval from treatment to onset of estrus, and time interval from treatment to end of estrus. Adjusted standardized residuals were transformed in SPSS to derive chi-square values for further examination. The chi-square values were then transformed into p-values for each cell by dividing the standard p-value (0.05) by the number of observed analyses.

RESULTS AND DISCUSSION

The results represented in Table. 1 shows the lowest estrus response recorded in TRT A (Control) and TRT C (double injection PGF2α) at 90.20%, while the highest response was observed in TRT D (P_4 + PGF2α + PMSG) with 96.20%. TRT B (P4) had an estrus response of 94.10%. The differences in estrus response between treatments were not significant (p>0.05). The statistical analysis did not show

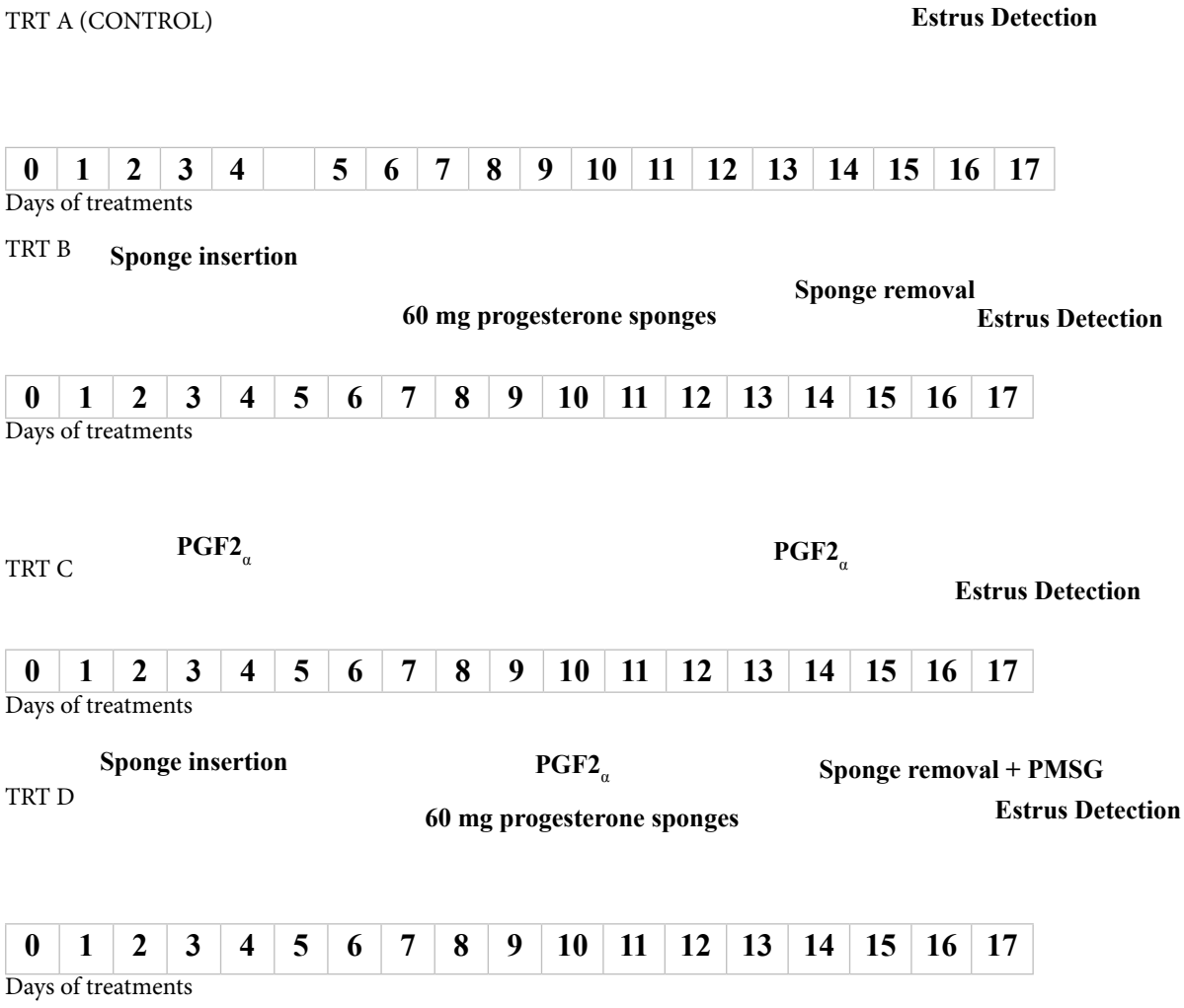

Fig. 1: Estrus synchronization with or without hormones

significant differences (p>0.05) in the intervals from treatment withdrawal to the onset and end of estrus during the breeding season. Despite TRT C having the longest insignificant interval to estrus and to the end of estrus. TRT D had the shortest intervals, with 33: 52h ± 1: 11 to onset of estrus and 79: 27 ± 1:41 to end of estrus. TRT B showed intervals of 42: 46h ± 2: 24 to onset of estrus and 85: 9h ± 2: 43 to end of estrus. The shortest estrus duration was observed in TRT A (Control), while the longest duration occurred in TRT D. The estrus duration in TRT C was 40: 43h ± 1: 31. Despite variations in estrus durations across treatments, TRT B showed a duration of 42: 46 ± 1: 27, which was not statistically significant (p>0.05) compared to other treatments. There were no statistically significant differences (p>0.05) between TRT B and TRT D or among TRT A, TRT B, and TRT D. However, statistical significance (p<0.05) was found when comparing TRT D to TRT C and TRT A based on estrus response. The overall estrus response rate above 90% across all treatments, with no statistically significant differences (p>0.05) during the breeding season in sheep (Table. 1) and this could be the surge in reproductive hormones during this season, triggered by changes in day length, leads to a higher proportion of ewes responding to synchronization. Bello *et al.* (2019) reported estrus response rates of 88.9%, 33.3%, and 37.5% for PGF2α, P_4 pessaries, and Control groups in ewes. The study aligns with previous research by Abdalla *et al.* (2014) showing lower estrus response in Karakul ewes compared to Barki ewes treated with $PGF_{2\alpha}$ and FGA impregnated sponges + PMSG (G2). The current study showed a 90.20% estrus response in ewes treated with double $PGF_{2\alpha}$ injection, contrasting with Rekik *et al.* (2016) who reported 93.3% and 79.3% estrus response, respectively. These findings align with Solomon *et al.* (2016b)

but differ from Shanbel (2019) regarding estrous response in Ethiopian sheep. Variations in estrous responses across studies may stem from breed, season, and overall animal management. Current findings contradict Martinez *et al.* (2015) on estrus rates in ewes treated with 400 IU of eCG, aligning with Waheeb *et al.* (2017) showing higher estrus induction using PGF2α in Barki ewes compared to controls. Bello *et al.* (2019) showed higher efficacy of PGF2α double injection over P4P for oestrus synchronization. The study showed longer estrus onset intervals (47:41h ± 2:05) in TRT C and shorter intervals in TRT D (33:52h ± 1.11), possibly due to animal age affecting estrus onset post-treatment withdrawal. Variations in reproductive traits and hormonal profiles among breeds were noted, aligning with Biehl *et al.* (2019) findings of ewes exhibiting estrous behavior within 48 hours post-progestogen sponge removal. The study's results contradict previous findings on estrus duration in Egyptian ewe lambs and Naeimi ewes, suggesting a potential link between elevated estrogen levels and prolonged estrus, impacting ovulation rates. The study contradicts the findings of Daghash *et al.* (2017) on estrus duration in ewes who reported the average duration of estrus in ewes ranging from 24 to 36 hours. Tharwat *et al.* (2020) found estrus lasted 41.60 ± 2.88 h with progesterone sponges, similar to double prostaglandin injection at 44.00 ± 2.63 h. Tharwat *et al.* (2020) show consistent estrus durations with different treatments.

Means within a row with different superscripts are significantly different ($p<0.05$). Means within a row with the same superscripts don't differ ($p>0.05$) significantly. Control (**A)** - Untreated ewes. **B**- ewes treated with intra-vaginal sponges alone on day = 0 to day = 12. **C**- ewes received double injection of prostaglandin on day = 0 to day =11. **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 the same day.

The study results in Fig. 1 analyzed estrus duration intervals of ewes under various synchronization treatments during the breeding season. The percentage of ewes in different synchronization treatments showed a significant association with estrus duration intervals (X^2 = 44.107, *df* =15, *n* = 190, *p* = 0.000). Standardized adjusted residuals analysis revealed that there was a higher-than-expected proportion of ewes in the 48h estrus duration interval within TRT D (*Z-score* = 4.216, *p* = 0.00002), which was statistically significant. Additionally, a higher-than-expected proportion of ewes in the 36h estrus duration interval within TRT D (*Z-score* = -3.520, *p* = 0.00043) was also found to be statistically significant. Conversely, a lower-than-expected proportion of ewes in the 24h estrus duration interval within TRT C (*Z-score* = 2.584, *p* = 0.00977) showed significant variation. In Fig. 1, statistically significant results ($p < 0.05$) were observed for different estrus durations of 48 hours (TRT D), 36 hours (TRT D), and 24 hours (TRT C). These significant findings may be attributed to adequate nutrition, which is crucial for maintaining normal reproductive functions in mammals. The ewes in TRT D experienced higher durations of estrus due to the combined effects of sustained progesterone release, timely prostaglandin administration leading to effective luteolysis and enhanced ovarian stimulation through inclusion of PMSG. Brown (2015) noted variability in estrus duration among synchronized ewes. Johnson and Williams (2017) investigated different synchronization protocols' effects on the percentage of ewes showing prolonged estrus. They found significant variations in long estrus durations across treatment groups.

The study Fig. 2 found a statistically significant association between the percentage of ewes in different synchronization treatments and the interval from treatment withdrawal to onset of estrus during the breeding season (X^2 = 54.726, *df* = 12, *n* = 144, *p* = 0.000). The significant results were likely due to: (i) a higher-than-expected pro-

Table 1: Effects of different synchronisation protocols on estrus activity.

Items	Different synchronisation protocols			
	A	B	C	D
Number of ewes	51	51	51	51
Estrus response number (%)	90.20 [a]	94.10 [a]	90.20 [a]	96.20 [a]
Interval from withdrawal of treatments to onset of estrus (h)	-	42:46 [a] ± 2:24	47:41 [a] ± 2:05	33:52 [a] ± 1.11
Interval from withdrawal of treatments to end of estrus (h)	-	85:09 [a] ± 2.43	88:37 [a] ± 1.41	79:27 [a] ± 1:41
Duration of estrus (h)	39:54 [a] ± 1:40	42:46 [ab] ±1:27	40:43 [a] ±1:31	45:57 [b] ± 1:13

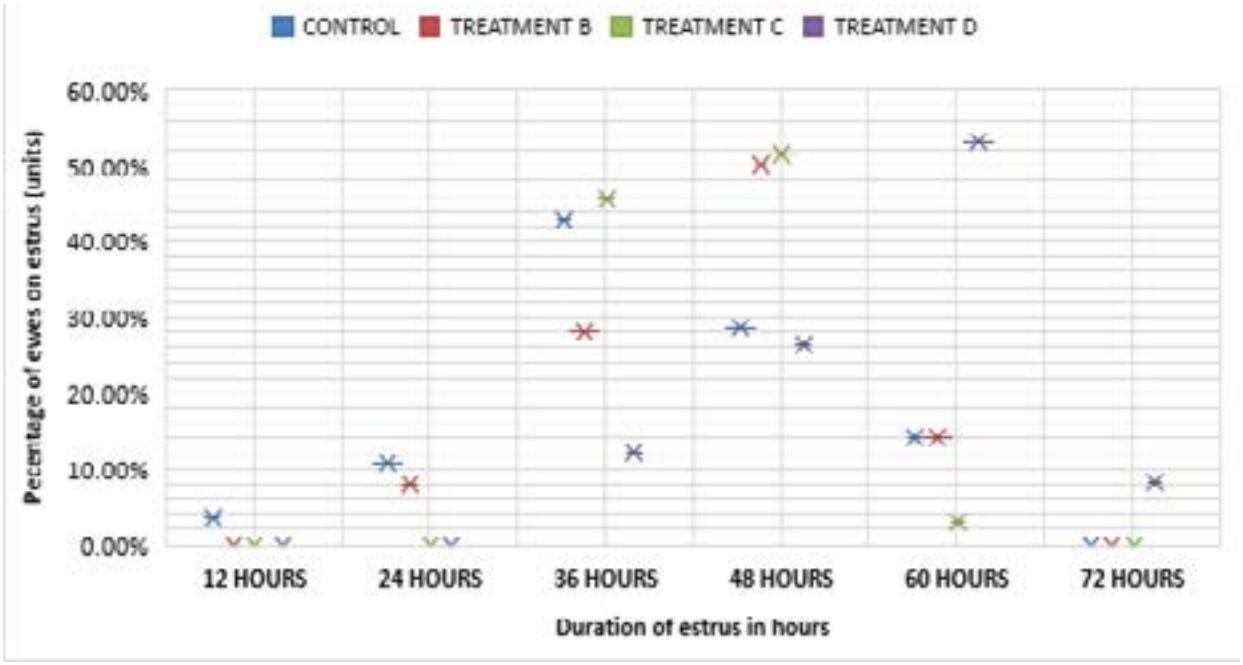

Fig. 2: Estrus duration under different synchronization protocols

portion of ewes showing estrus within 48h after treatment withdrawal in TRT B (*Z-score* = 3.744, p = 0.00018), (ii) a lower-than-expected percentage of ewes exhibiting estrus within 60 hours after treatment withdrawal in TRT B (*Z-score* = 3.744, p = 0.00018), (iii) a higher-than-expected percentage of ewes displaying estrus within 24h after treatment withdrawal in TRT D (*Z-score* = 3.118, p = 0.00182), and (iv) a lower-than-expected proportion of ewes showing estrus within 36h after treatment withdrawal in TRT D (*Z-score* = 4.080, p = 0.00005), (v) A higher-than-expected percentage of ewes showed estrus within 48h after treatment withdrawal in TRT D (*Z-score* = -4.049, p = 0.00005), and (vi) a lower-than-expected percentage of ewes exhibited estrus within 60 hours after treatment withdrawal in TRT D (*Z-score* = -4.049, p = 0.00005). The synergistic effect of removing the intra-vaginal sponge (which decreases progesterone), administering prostaglandin (which promotes luteolysis), and injecting PMSG (which stimulates follicular growth) hence this results in a more rapid transition into estrus compared to other treatments, this comprehensive approach maximized hormonal need for synchronization and stimulated ovarian activity in TRT D. The study results in Fig. 2 contradict Martinez-Ros *et al.* (2017), who found that 80 % of ewes exhibited estrus within 48 hours after progestagen-impregnated intravaginal sponges were removed. In contrast, Abecia *et al.* (2014) evaluated various synchronization protocols, including progestagen-impregnated sponges and eCG, and reported that 70 % of ewes showed estrus within 36 hours post-sponge removal. These findings align with the current study, showing the highest proportion of ewes in estrus in TRT D within 36 hours at 73.00 %. They found that 75 % of the ewes exhibited estrus within 72 hours after treatment withdrawal, which contradicts the current study's results showing the lowest percentage of ewes in estrus at 72 hours across various synchronization protocols. Palacín *et al.* (2019) investigated controlled internal drug release devices for estrus synchronization in ewes and reported that 90 % of ewes exhibited estrus within 72 hours after device removal.

The results in Fig. 3 show the time interval from treatment withdrawal to the end of estrus in ewes during the breeding season. This study found a significant relationship between different synchronization treatments and the duration from treatment withdrawal to the end of estrus in ewes (X^2 = 54.726, *df* = 12, n = 144, p = 0.000) during the breeding season. The significant findings may be attributed to a lower-than-expected proportion of ewes reaching the end of estrus within 72h after treatment withdrawal in TRT C (*Z-score* = -3.999, p = 0.00006), and a higher-than-expected percentage in TRT D (*Z-score* = 5.726, p = 0.0000). Additionally, there was a higher-than-expected percentage of ewes reaching the end of estrus within an 84-hour interval in TRT D (*Z-score* = -3.187, p = 0.00144). In Fig. 3, due to limited existing literature, comparing these findings with other studies on the impact of synchronization protocols on the interval from treatment withdrawal to the end of estrus in ewes during the breeding season was not feasible. The longer interval from onset of estrus to end of estrus observed in treated ewes can be attributed primarily by sustained influence of exogenous hormones introduced through various treatments however, these hormones alter

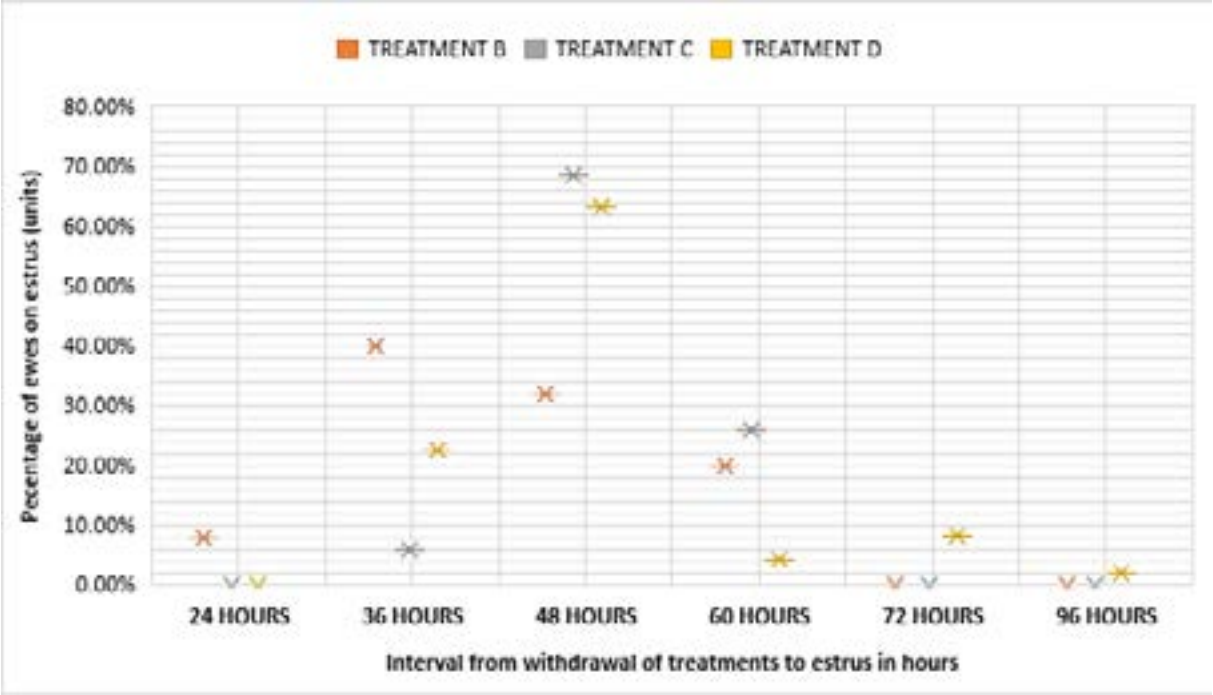

Fig. 3. Interval from withdrawal of treatments to onset of estrus in different synchronisation protocols.

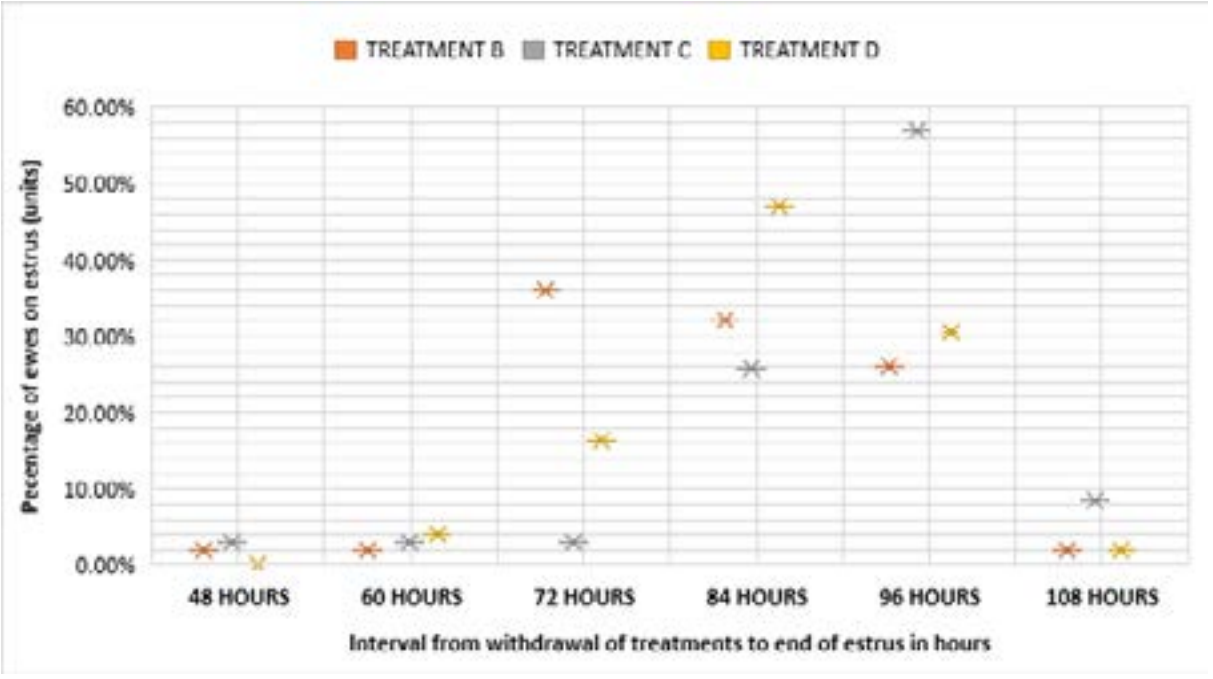

Fig. 4: Interval from withdrawal of treatments to end of estrus in different synchronisation protocols

normal endocrine feedback loops and prolong both behavioral signs and physiological readiness for mating. This study's results will serve as a foundational reference point for future research endeavors.

CONCLUSIONS

The study can be concluded that during the breeding season, TRT D was found to be the most effective estrus synchronization protocol, as evidenced by a higher estrus

response of 96.20 %. In comparison, TRT A, TRT B, and TRT C yielded estrus responses of 90.20 %, 90.20 %, and 94.10 % in their respective treated ewes. Moreover, ewes in TRT D exhibited a shorter time interval from the withdrawal of treatments to the onset of estrus (33:52 ± 1.11h), compared to those in TRT B (42:46 ± 2:24h) and TRT C (47:41 ± 2:05h). The duration of estrus for all treatment groups ranged from 36 to 48h, suggesting a prompt and synchronized response to the synchronization protocols.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to National University of Lesotho for funding this research.

CONFLICT OF INTEREST

The authors declare that they have no competing conflict of interest.

REFERENCES

Abdalla, E. B., Farrag, B., Hashem, A. L. S., Khalil, F. A. and Abdel-Fattah. (2014). Effect of progesterone, PGF2α, PMSG and PMSG on estrus synchronization and some reproductive traits in Barki ewes. *J. Agroaliment. Processes Technol.*, **20** (1): 93- 101.

Abecia, J. A., Forcada, F. and Gonzalez-Bulnes, A. (2014). Hormonal control of reproduction in small ruminants. *Anim. Reprod. Sci.*, **130**:173-179.

Bello, A., Abdullah, D., Salisi, M. S. and Jesse, F. F. A. (2019). Oestrus response rate in ewes treated with PGF2α and P4 pessaries during the non-breeding season. *J. Anim. Sci.*, **45**(3), 210-225.

Biehl, M. V., Ferraz Junior, M. V. C., Barroso, J. P. R., Susin, I., Ferreira, E. M., Polizel, D. M. and Pires, A. V. (2019). The reused progesterone device has the same effect on short or long estrus synchronization protocols in tropical sheep. *Trop. Anim. Health Prod.*, **51**: 1545-1549.

Brown, M. (2015). Principles of Sheep Reproduction: Synchronization Protocols and Estrus Duration Intervals. London: Springer.

Chaudhari, A., Haque, N., Jamnesha, N., Bhalakiya, N., Patel, G., Madhavatar, M., Patel, D. and P. Patel. (2018). Synchronization of Estrus. *Int. J. Curr. Microbiol. Appl. Sci.,* **7**: 1511-1519.

Daghash H., Fahmy S., Hassan, T. and Ali, M. (2017). Impact of PMSG, PMSG and hCG treatments on follicular diameter, conception and lambing rates of Egyptian ewe lambs using intravaginal sponges. *Egypt. J. Sheep Goats Sci*., **12** (1): 1-8.

Gebreselassie, G., Berihulay, H., Jiang, L. and Y. Ma. (2019). Review on Genomic Regions and Candidate Genes Associated with Economically important Production and Reproduction Traits in sheep (*Ovis aries*). *Animals.,* **10**(1).

Habeeb, H.M., Mohammed, M.H. and M.A. Kutzler. (2021). Estrus synchronization in the sheep and goat. Veterinary Clinics: *J. Small. Anim. Pract*., **37**(1): 125-137.

Hassanein, E. M., Szelényi, Z. and Szenci, O. (2024). Gonadotropin-Releasing Hormone (GnRH) and Its Agonists in Bovine Reproduction II: Diverse Applications during Insemination, Post-Insemination, Pregnancy, and Postpartum Periods. *Animals.*, **14** (11), 1575.

Johnson, D. and Williams, E. (2017). Impact of Synchronization Protocols on Estrus Duration Intervals in Sheep. *Reproduction Res.*, **18** (2): 123-135.

Martinez, M. F., McLeod, B., Tattersfield, G., Smaill, B., Quirke, L. D. and Juengel, J. L. (2015). Successful induction of estrus, ovulation and pregnancy in adult ewes and ewe lambs out of the breeding season using a PMSG + progesterone oestrus synchronisation protocol. *Anim. Reprod. Sci.*, **155**:28–35.

Martinez-Ros, P., Gonzalez-Bulnes, A., Palacios, C. and Menchaca, A. (2017). Estrous response and fertility in sheep treated with progestogen-impregnated intravaginal sponges during seasonal anestrus. *Anim. Reprod. Sci.*, **185**: 1-6.

Palacín, I., Vicente-Fiel, S., Palomares-Ruiz, A. and Martínez-Ros, P. (2019). Controlled internal drug release devices for oestrous synchronisation in sheep: a review on current knowledge and prospects for future research. *Anim. Reprod. Sci.*, **207**: 106-199.

Rekik, M., Aynalem, H., Ayele, A., Dagne, M., Shenkute, G., Salem, I. B., Hilali, M. E. D., Lassoued, N., Yaynshet, C. and Rischkowsky, B. (2016). PMSG and Prostaglandin- based synchronization protocols as alternatives to progestogen- based treatments in sheep. *Reprod. Domest. Anim.*, **51** (6): 924-929.

Shanbel, B. (2019). Evaluation of Artificial Insemination in Menz Sheep Following Estrus Synchronization with Progestogen and Prostaglandin-based Synchronization Protocols. MSc Thesis. *Hawassa University College of Agriculture Hawassa, Ethiopia.*

Solomon, G., Yayneshet, T., Yoseph, M., Zeleke, M., Tadesse, G., Ebro, A., Berhanu, G., Hoekstra, D. and Azage, T. (2016b).

Hormonal oestrus synchronization in four sheep breeds in Ethiopia: impacts on genetic improvement and flock productivity. LIVES Working Paper 25. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Tharwat, M., El-Bahr, S. and Al-Sobayil, F. (2020). Estrus synchronization and fertility response to different hormonal treatments in sheep. *Small Rumin. Res.*, **183**: 106021.

Waheeb, R. S., El-amrawi, G. A., Metwelly, K. K. and El-sabbagh, A. M. (2017). Synchronization of estrus in field conditions using progesterone sponge, PMSG, and $PGF_2\alpha$ in Barki ewes during breeding season. *Alex. j. vet. sci.*, **54** (2): 1-4.