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The Effect of β-Carotene + Vitamin E Injection on Fertility in Central Anatolian Merino Ewes Synchronized in the Breeding Season

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ABSTRACT: This study was conducted to investigate the effects of β-carotene + Vitamin E (β-Car+VitE) injection, which have anti-oxidant properties, on fertility in Central Anatolian Merino ewes. The ewes were synchronized with single dose of Prostaglandin F2α (PGF2α) during the breeding season. A total of 118 ewes were used in the study and randomly divided into three groups. Group 1 (n=46) ; PGF2α (1 mL, 263 µg cloprostenol sodium PGS, Alke, Turkey) + β-Car+VitE (0.5 mL/10 Kg, Ovostim, Provet, Turkey) were administered intramuscularly (IM) concurrently. Group 2 (n=39); ewes were administered only PGF2α. Group 3 (n=33) injected β-Car+VitE one week before PGF2α. Ewes which monitored estrus were mated with rams with known fertility by hand mating method. In the presented study, group 1, group 2 and group 3 had the onset to estrus and estrus rates 34.0±2.47h, 45.9±3.67h, 41.7±3.60h; 52.2%, 59.0%, 57.6% respectively. It was determined that the onset of estrus was shorter in group 1 than in group 2 and group 3 ($p<0.05$). Conception rate, twinning rate and litter size in group 1, group 2 and group 3 were 91.7%, 36.4%, 1.36%; 87.0%, 30.0%, 1.30 and 84.2%, 25.0%, 1.25 respectively ($p>0.05$). As a result, it was determined that β-Car+VitE injection reduced the onset to estrus but had no effect on the other reproductive parameters in ewes synchronized with single dose of PGF2α.

Keyword: Ewes; PGF2α; β-Carotene; Vitamin E; Reproductive performance

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INTRODUCTİON

In sheep farming systems, estrus synchronization is utilized to shorten the breeding season, lambing within a defined period, produce uniform offspring, reduce labor demands and induce estrous cycles in anestrous ewes (Fierro et al., 2013; Holm et al., 2008). Intravaginal progesterone-releasing devices, such as CIDRs or sponges, are commonly used methods for estrus synchronization (Wildeus, 2000). Additionally, within the breeding season, prostaglandins (PG) and their analogs represent as an alternative strategy to control reproduction in ewes by inducing luteolysis, eliminating the corpus luteum (CL) and initiating the subsequent follicular phase. Prostaglandin F2α is the primary luteolytic agent in ruminants and effective from the third day of the estrous cycle (Rubianes et al., 2003). The efficacy of prostaglandins depends on the presence of a functional corpus luteum (CL) on the ovary. Consequently, their application in sheep production systems is confined to the breeding season and commonly implemented through a single administration or two injections at an interval of 9 to 11 days (Fierro et al., 2013). Compared to alternative synchronization protocols, this method presents several advantages such as cost-effectiveness, improved animal welfare, rapid metabolic clearance and practicality (Abecia et al., 2012).

The presence of various vitamins and minerals is essential for maintaining reproductive health in animals (Zonturlu et al., 2017). In particular, β-carotene, which is a precursor of vitamin A and vitamin E are two important ones. β-carotene, which exists as a provitamin in green plants, is converted into vitamin A (retinol) within the intestinal mucosa, liver, and various body tissues of animals (Borel et al., 2005). It has been reported that there are positive correlations between the level of β-carotene in plasma and the amount of follicle fluid, luteal tissue and corpus luteum weight (Ayasan and Karakozak, 2010). β-carotene offers as the sole source of vitamin A in the ovaries, influences the rupture of the follicular wall during ovulation and contributes to the characteristic bright yellow pigmentation of the corpus luteum (Haliloglu et al., 2002). In addition, β-carotene has antioxidant properties and prevents the oxidation of unsaturated lipids, thereby inhibiting the formation of free radicals (Paiva and Russell, 1999). β-carotene deficiency has been reported to cause subestrus, delayed ovulation, impaired formation and reduction in size of the CL, decreased progesterone synthesis, luteal cyst formation and embryonic loss in early pregnancy (Çelik et al., 2009; Meza-Herrera et al., 2013). Vitamin E is one of the important vitamins that has different functions in ruminants. Vitamin E is naturally found in plants and the most important active tissue form is “Alpha-Tocopherol” (Bramley et al., 2000). Vitamin E is absorbed lymphatically, transported to the liver in the form of lipoprotein particles and stored there (Bjorneboe et al., 1990). Its main function is to protect the cell membrane by preventing the oxidation of free radicals formed as a result of lipid peroxidase reaction (Traber and Atkinson, 2007). It also boosts the immune system, increasing the body’s resistance to disease (Yang et al., 2002; Yazlik et al., 2020). Vitamin E, vitamin A, and their derivatives retinol and retinoids have been reported to enhance reproductive performance by exhibiting synergistic effects on the initiation of ovulation, steroidogenesis, and the regulation of pulsatile LH release (Meza-Herrera et al., 2013).

In conventional sheep farming, pasture-based feeding management is generally practiced. Nevertheless, due to the low yield and quality of pastures, ewes are unable to get sufficent nutrients (Abdelrahman and Al-Karablieh, 2002). It has been noted that there is a greater need for certain vitamins with antioxidant properties, especially during the transition season when dried grasses are prevalent. (Afshari et al., 2008; Liu et al., 2014). Therefore, it is important to obtain sufficient amounts of antioxidant vitamins during this period. Recent studies have reported that the administration of antioxidant vitamins in combination with synchronization protocols during the breeding season has a positive effect on fertility. (Ataman et al., 2023; Semra et al., 2023). Due to their antioxidant properties, β-carotene and vitamin E may enhance reproductive performance by reducing oxidative stress, regulating pulsatile LH release, optimizing the follicular and uterine environment when administered prior to PGF2α synchronization. The present study was conducted to investigate the effects of a single dose of PGF2α injection combined with β-Car+VitE on reproductive parameters in ewes during the breeding season.

MATERİALS AND METHODS

The study was carried out in the “T.C Ministry of Agriculture and Forestry Bahri Dağdaş International Agricultural Research Institute Directorate” located at an altitude of 1007 m in Konya province “37.868267 latitude and 32.553558 longitude”, during the breeding season (August-September), with the approval of the “Animal Experiments Lo-

cal Ethics Committee" within the institute dated 29.02.2024 and numbered 171.

Animals and management

A total of 118 multiparous Central Anatolian Merino ewes which were housed in a semi-open barn, clinically healthy, average age between 2-5 years and 55–60 kg live weight, were used. The ewes were fed mixture of barley (45%), corn (35%) and sunflower meal (20%) at 300g/ewe/day in addition to grazing prior to onset of breeding season. In order to stimulate to "Ram effect", teaser rams participated in flock for 10 days 45 minutes per day in begining of breeding season. Water was offered as *ad libitum*.

Treatment groups

The study was conducted on 118 ewes, which were randomly divided into 3 groups. Group 1 (n=46) ; PGF2α (1 mL, 263 µg cloprostenol sodium PGS, Alke, Turkey) + β-Car+VitE (0.5 mL/10 Kg, Ovostim, Provet, Turkey) were injected IM simultaneously. Group 2 (n=39); ewes were administered only PGF2α. Group 3 (n=33) were given single dose β-Car+VitE a week before PGF2α. All ewes were monitored for estrus with teaser rams for 5 days following PGF2α injections and hand-mated with fertile rams. In this method, each ewe was individually introduced to a fertile ram in a separate pen under supervision to ensure a single and controlled mating event (ewes: ram ratio of 5:1). After mating, the ewes were separated from the rams (Figure 1.).

Pregnancy diagnosis

Pregnancies were detected by transrectal real-time B Mod ultrasonography (Mindray DP-50) rang of 5–8 MHz frequency with a linear rectal probe on day 26 post-mating. Pregnancies were diagnosed by visualising the embryo in the vesicle and detecting embryonic heartbeat.

Reproductive parameters

In this study, reproductive parameters were calculated as follows.

- Estrus rate: Number of ewes showing estrus / Number of ewes in the group x 100
- The onset of estrus (hours) : Time of estrus onset - PGF2α injection
- Conception rate: Number of pregnant ewes/ Number of mated ewes in the group x 100
- Twinning rate: Number of twin lambing ewes/ Number of lambing ewes in the group ×100
- Litter size: Number of total lambs/ Number of lambing ewes in the group × 100

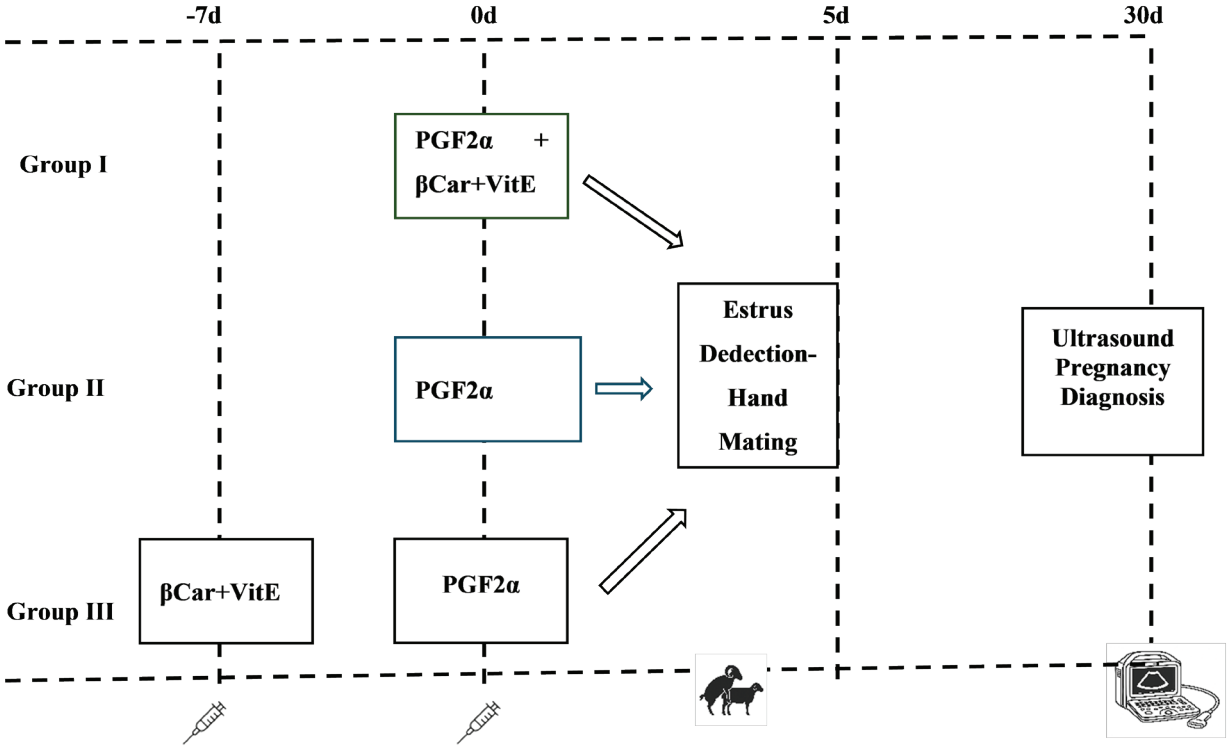

Figure 1. Treatment protocols in ewes.

Statistical analysis

All the statistical analyses were performed by using SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Chi-square test was used for the evaluation of reproductive parameters and Z-Test Bonferroni method was used for the comparison of differences between groups. One-Way Anova test was used to evaluate the onset of estrus, and Duncan test was used to compare the difference between the groups. Conception rate and litter size were analyzed using binomial logistic regression. Treatment group, body weight, and age were included as predictors, with Group 2 serving as the control. Because classical logistic regression showed quasi-complete separation, penalized regression with bootstrap resampling (500 iterations) was applied to obtain stable odds ratios (OR) and 95% confidence intervals (CI). The statistical results were considered significant if the p-value was < 0.05 in the calculated data.

RESULTS

In the presented study, reproductive parameters such as estrus rate, the onset of estrus, conception rate, twinning rate and litter size were evaluated (Table 1). While the onset of estrus was statistically significant in Group 1 ($p<0.05$), there was no difference between the groups in other parameters ($P>0.05$).

Logistic regression including body weight and age (Table 2)(Table 3) indicated no significant differences among treatment groups in conception rate or litter size. Compared with the reference (Group 2), both Group 1 and Group 3 showed similar odds. Body weight tended to increase the likelihood of conception (OR ≈ 1.20 per kg), but this effect was not significant. Age showed no relevant association with reproductive outcomes.

DİSCUSSİON

Prostaglandin F2α is widely used for estrus synchronization in sheep (Abecia et al., 2011). PGF2α-based

Table 1. Reproductive parameters

REPRODUCTİVE PARAMETERS	GROUP 1 *(n=46)*	GROUP 2 *(n=39)*	GROUP 3 *(n=33)*
ESTRUS RATE (%)	52.2[a] *(24/46)*	59.0[a] *(23/39)*	57.6[a] *(19/33)*
THE ONSET OF ESTRUS (Hours±standard error)	(34.0±2.47)[b]	(45.9±3.67)[a]	(41.7±3.60)[ab]
CONCEPTİON RATE (%)	91.7[a] *(22/24)*	87.0[a] *(20/23)*	84.2[a] *(16/19)*
TWİNNİNG RATE (%)	36.4[a] *(8/22)*	30.0[a] *(6/20)*	25.0[a] *(4/16)*
LİTTER SİZE	1.36 *(30/22)*	1.30 *(28/20)*	1.25 *(20/16)*

abc: Different letters in the same row are statistically different ($p<0.05$)

Table 2. Logistic regression analysis of conception rate with age and body weight as covariates (reference = Group 2; bootstrap 95% CI)

CONCEPTION RATE			
VARIABLE	**BETA**	**OR**	**95% CI (OR)**
INTERCEPT	0.003	1.003	1.00–1.00
GROUP 1 VS GROUP 2	0.001	1.001	1.00–1.00
GROUP 3 VS GROUP 2	0.001	1.001	1.00–1.00
BODY WEIGHT (KG)	0.187	1.205	1.20–1.21
AGE (YEARS)	0.009	1.01	1.01–1.01

OR: Odds Ratio; CI: Confidence Interval. Bootstrap confidence intervals (500 resamples) were calculated due to quasi-complete separation in classical models($p>0.05$).

Table 3. Logistic regression analysis of litter size with age and body weight as covariates (reference = Group 2; bootstrap 95% CI)

LITTER SIZE			
VARIABLE	**BETA**	**OR**	**95% CI (OR)**
INTERCEPT	-1.149	0.317	0.16–0.62
GROUP 1 VS GROUP 2	-0.11	0.896	0.45–1.91
GROUP 3 VS GROUP 2	-0.563	0.569	0.26–1.32
BODY WEIGHT (KG)	0.01	1.01	0.98–1.03
AGE (YEARS)	-0.033	0.967	0.60–1.42

OR: Odds Ratio; CI: Confidence Interval. Bootstrap confidence intervals (500 resamples) were calculated due to quasi-complete separation in classical models($p>0.05$).

protocols differ depending on the management capabilities of the breeders. A single dose of PGF2α injection is the most practical protocol for herd reproductive management. (Acritopoulou et al., 1977). In the present study, No significant differences were observed among the groups in terms of estrus rates (Table 1). However, the estrus onset in Group 1 was found to be significantly shorter than in Group 2 and Group 3 ($p < 0.05$). Acritopoulou (1980) reported that a single PGF2α injection administered to ewes during the breeding season induced luteal regression in 66% of the ewes and subsequently triggered estrus at 37.7 ± 1.6 hours. The administration of a second PGF2α dose 9 days after the initial treatment significantly improved estrus synchronization, resulting in 95% of ewes exhibiting estrus within 72 hours of the final injection. In a study conducted on Menz ewes and their crossbreeds, a single dose of PGF2α induced estrus in 55% to 65% of the ewes, with onset times of 34.14 ± 1.33 hours and 57.08 ± 2.71 hours, respectively (Zeleke Mekuriaw et al., 2016). In another study, PGF2α administration on days 8 to 11 of the estrous cycle (Estrus=0 day) resulted in an 84% estrus response, with onset occurring at 46.3 ± 1.32 hours post-injection. When the same ewes received two PGF2α injections 10 days apart, similar results were observed, with an 88% estrus rate and onset at 51.6 ± 2.4 hours (Das et al., 1999). In the present study regardless of the treatment, the estrus rate was very low. In ewes, the onset time of estrus following a single, randomly timed PGF2α injection during the breeding season varies depending on the stage of the estrous cycle at the time of administration (Deaver et al., 1986). This is because PGF2α is only effective when the corpus luteum (CL) is functional and responsive, which typically occurs between days 5 and 14 of the estrous cycle. If the injection is given outside this window, such as during the early luteal phase or after luteolysis has already begun, the response to PGF2α may be delayed or absent, leading to considerable variation in estrus onset times among treated animals. Additionaly, vitamins with antioxidant properties are known to have a positive effect on the occurrence of ovulation and the induction of estrus, likely due to their roles in pulsatile LH release and the rupture of the follicular wall during ovulation (Meza-Herrera et al., 2013). Therefore, it is suggested that these vitamins may have influenced the estrus onset time in groups.

In sheep, the onset of sexual cycles with the breeding season, the application of synchronization protocols, and factors such as environmental temperature contribute to increased oxidative stress in the metabolism (Agarwal et al., 2005; Kuru et al., 2016; Martinez-Ros et al., 2018). Oxidative stress can negatively affect fertility during ovulation, mating, and implantation periods (Al-Gubory et al., 2010). In the present study, no significant differences were observed among the groups in terms of conception rate, twinning rate, or litter size ($p > 0.05$) (Table 1). The results obtained are consistent with findings from some previous studies that Kıvrak et al. (2022) reported no significant improvement in conception rates (60% and 62%) in Kangal ewes during the breeding season when β-carotene and vitamin E were administered in addition to a double-dose PGF2α synchronization protocol. Similarly, in another study conducted on anestrous ewes, pre-synchronization injections of either β-carotene or vitamin E + selenium were found to have no significant effect on fertility parameters (Köse et al., 2013). Ataman et al. (2023) observed no statistically significant improvement in pregnancy rates (86.7% and 90%) in ewes synchronized with progesterone-containing

sponges and injected with β-carotene and vitamin E at the beginning and end of treatment. However, a notable increase in litter size (1.10 and 1.60) was reported. In a similar study, the addition of β-carotene + vitamin E, hCG, or both to a 12-day progesterone-based synchronization protocol resulted in pregnancy rates of 62.5%, 79.2%, and 77.8%, respectively, and corresponding lamb yields of 1.36, 1.28, and 1.25 ($p > 0.05$) (Özmen et al., 2022). For a more detailed examination of the findings, penalized bootstrap logistic regression analysis including age and body weight as covariates was applied (Table 2)(Table 3). This method was preferred to overcome the quasi-complete separation problem encountered in classical logistic regression and to obtain more reliable Odds Ratio (OR) estimates. The analysis showed that Groups 1 and 3 did not differ significantly from Group 2 in terms of conception rate and litter size. Body weight tended to increase the likelihood of conception ($OR \approx 1.20$), but this effect was not statistically significant. Age did not show a relevant effect on reproductive performance. It is suggested that the favorable nutritional and management conditions during the breeding season may have limited the effectiveness of the treatments on reproductive performance. Moreover, it is concluded that a single vitamin injection administered only at the onset of synchronization may be insufficient, and that combining antioxidant vitamins with gonadotropins within synchronization protocols could yield more effective results.

CONCLUSIONS

PGF2α-based synchronization protocols have both practical and economic significance for enhancing reproductive efficiency in sheep breeding. In this study, a single dose of PGF2α induced estrus in approximately two-thirds of the flock, thereby enabling controlled lambing within a defined period. The administration of β-Car+VitE was observed to significantly reduce the time to estrus onset; however, it did not elicit any notable changes in the other reproductive parameters in ewes synchronized with a single PGF2α injection. The effectiveness of antioxidant vitamin supplementation may vary depending on the physiological status of the ewes, husbandry-nutritional conditions, and the synchronization protocol used.

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest

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