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RESEARCH ARTICLE

The Effect of Vitamin E-Trace Mineral Treatments on Reproductive Performance in Morkaraman Sheep During the Breeding Season[1]

Semra KAYA [1] Gökhan KOÇAK [2] Murat Can DEMIR [1 (*)] Cihan KAÇAR [1]

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[1] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kafkas University, TR-36300 Kars - TÜRKİYE

[2] Iğdır University, Faculty of Applied Sciences, TR-76000 Iğdır - TÜRKİYE

ORCIDs: S.K. 0000-0002-7520-6631; G.K. 0000-0003-1917-9090; M.C.D. 0000-0002-3803-069X; C.K. 0000-0002-2642-697X

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Abstract: The aim of this study was to investigate the effect of vitamin E-trace mineral treatments before synchronization and $PGF_{2\alpha}$ injection one day before and on the day of sponge removal on reproductive parameters in Morkaraman ewes during the breeding season. Fifteen days before the synchronization, the sheep in Group I (n=11) and Group II (n=12) were given orally capsules containing a vitamin E-mineral combination. The sheep in Group III (n=10) and IV (n=14) weren't given. An intravaginal sponge was applied to all sheep to remain in the vagina for 6 days, and eCG was injected immediately in all groups after the sponge was removed. The sheep in Groups I and III were injected with $PGF_{2\alpha}$ only on the fifth day, and the sheep in Groups II and IV were injected with $PGF_{2\alpha}$ on both the fifth and sixth days. The highest rates of pregnancy (91.67%) were in Group II (P>0.05). The rate of multiple lambing was higher in Groups II (90.91%) and IV (87.5%) than in other groups (P>0.05). The lambing rate was lowest in Group III (60%, P>0.05). Embryonic mortality rates in the groups were found to be 14.29%, 9.09%, 20.00% and 12.50% in the Group I, Group II, Group III and Group IV, respectively. It was determined that litter size was 1.33, 2.3, 2.67, and 3.29 in the Group I, Group II, Group III and Group IV, respectively (P>0.05). It was determined that the average birth weight of lamb was highest in Group I (4.88±0.18 kg). It was determined that vitamin E-trace mineral treatments and double $PGF_{2\alpha}$ injection increased the pregnancy rate (P>0.05), especially double $PGF_{2\alpha}$ injection contributed to the formation of multiple pregnancies.

Keywords: *Double $PGF_{2\alpha}$ injections, Ewe, Trace minerals, Vitamin E, Reproductive performance*

Üreme Mevsimindeki Morkaraman Koyunlarında Vitamin E-İz Mineral Uygulamalarının Üreme Performansı Üzerine Etkisi

Öz: Sunulan çalışmada üreme mevsiminde bulunan Morkaraman koyunlarında senkronizasyondan önce Vitamin E ve mineral kombinasyonu içeren kapsüllerin ve sünger çıkarılmadan bir gün önce ve sünger çıkarıldığı gün $PGF_{2\alpha}$ enjeksiyonunun üreme parametreleri üzerine etkisinin araştırılması amaçlandı. Senkronizasyon protokolünden 15 gün önce Grup I ve Grup II'deki koyunlara vitamin E-mineral kombinasyonu içeren kapsüller ağızdan yutturuldu. Grup III ve Grup IV'teki koyunlara ise verilmedi. Tüm koyunlara 6 gün süre ile vaginada kalacak şekilde intravaginal süngerler yerleştirildi ve süngerin çıkarıldığı gün eCG enjeksiyonu yapıldı. Grup I ve III'teki koyunlara yalnızca beşinci gün, Grup II ve IV'teki koyunlara ise hem beşinci hem de altıncı gün $PGF_{2\alpha}$ enjeksiyonu yapıldı. Gebelik oranı (%91.67) en yüksek Grup II'deydi (P>0.05). Grup II (%90.91) ve Grup IV'te (%87.5) çoklu kuzulama oranı diğer gruplardan yüksekti (P>0.05). Kuzulama oranı en düşük Grup III'teydi (%60, P>0.05). Embriyonik ölüm oranları Grup I, II, III ve IV'te sırasıyla %14.29, %9.09, %20.00 ve %12.50 olduğu belirlendi. Doğum yapan koyun başına düşen kuzu sayısı gruplarda sırasıyla 1.33, 2.3, 2.67 ve 3.29 olduğu belirlendi. Ortalama doğan kuzu ağırlığının en yüksek Grup I'de olduğu saptandı (4.88±0.18 kg). Vitamin E ve mineral tedavisi ve çift $PGF_{2\alpha}$ enjeksiyonunun gebelik oranını artırdığı (P>0.05) tespit edildi, özellikle çift $PGF_{2\alpha}$ enjeksiyonunun çoklu gebelik oluşumuna katkı sağladığı belirlendi.

Anahtar sözcükler: *Çift $PGF_{2\alpha}$ enjeksiyonu, İz mineraller, Koyun, Üreme performansı, Vitamin E*

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(*) Corresponding author: Murat Can DEMİR

Phone: +90 535 475 52 52
E-mail: murat.can.demir@hotmail.com

Introduction

Reproduction is the most basic element for ensuring productivity in sheep [1]. Increasing the reproduction rate makes a great contribution to the economy by ensuring both the continuity of productivity and the increase in lamb production [2]. For many years, the breeding cycles of sheep have been interfered with artificially [3]. With these interventions, it is aimed to aggregate the estrus and birth time in a certain period, to provide uniformity in the offspring [4], and increase offspring productivity by stimulating multiple births [2]. They are the most basic methods used to stimulate reproduction and increase efficiency in various hormonal applications and changes in nutrition strategy. The use of $PGF_{2\alpha}$ to shorten the luteal phase during the breeding season or progestagen to artificially prolong the luteal phase is one of the most preferred methods [4-6].

The presence of various vitamins and minerals is essential for reproductive health in animals [7]. Vitamin E plays an important role in the management of oxidative stress. Oxidative stress compromises ovarian activity and follicular development. Vitamin E and selenium are synergistically effective [8]. Selenium is an important component of the enzyme glutathione peroxidase (GSH), which is involved in the detoxification of free radicals [1]. Graaf follicles and oocytes are reported to be highly sensitive to antioxidant status change, especially GSH [8] as the GSH level decreases, the rate of apoptosis in the antral follicle increases. Therefore, it is reported that embryonic and fetal deaths may be more likely in vitamin E and selenium deficiency [8]. It is known that trace elements such as copper, selenium, and cobalt are the most effective minerals for reproduction. These trace minerals affect the reproductive performance, as well as the survival of the embryo [9]. They are involved in the synthesis of many proteins and the activation of the enzyme system. Its deficiency affects the immune system during the recognition of pregnancy or implantation, changing embryonic growth. In farm animals, the incidence of premature embryonic death increases if they are deficient [9]. Sheep need cobalt more because they use a lot of propionic acid for gluconeogenesis, and they need sulfur-containing amino acids such as methionine for wool growth. It has been determined that subclinical cobalt deficiency causes a decrease in the ovulation rate in superovulated sheep [10].

The study is aimed to determine the effect of capsules containing Vitamin E-trace minerals 15 days before short-term (6 days) progestagen administration and double-dose $PGF_{2\alpha}$ injection on some reproductive parameters in Morkaraman sheep.

Material and Methods

Ethical Approval

The present study was approved by the Animal Research Ethics committee of the University of Kafkas (Ethics approval number: KAÜ-HADYEK, number 2021/157).

Animals

The study was performed during the breeding season of 2021 in Morkaraman sheep at the Iğdır University Application and Research Farm in Iğdır province, which is located at 39° north latitude and 44° east longitude. Forty-seven clinically healthy and non-lactating sheep in the breeding season were included in the study. The sheep were 2-4 years old, with a live weight of 55-65 kg. 4 rams, 2-3 years old, were used. 600 g of barley was given per sheep per day. Meadow grasses and water were offered ad libitum. In the last 1 month, close to birth, meadow grass, wheat straw, and clover straw were given *ad libitum,* and they were additionally fed with 500 g sheep milk feed.

Methods

The rams were separated from the herd 30 days before starting the synchronization protocol. 15 days before the synchronization in the breeding season, 2 capsules (Vit. E 500 IU, dicalcium phosphate 150 mg, sodium selenite 2.5 mg, copper sulfate 10 mg and cobalt sulfate 12.5 mg) containing vitamin and mineral combinations (Bakosel®, Ceva, Türkiye) were given orally for sheep in Groups I (n=11) and II (n=12). The sheep in Group III (n=10) and IV (n=14) were not given. An intravaginal sponge (20 mg flugeston acetate, Chronogest®, France) was applied to all sheep for 6 days. While sheep in Group I and III were injected with $PGF_{2\alpha}$ (1 mL, 5 mg, Dinoprost, Enzaprost®, France) on the fifth day (the day before removing the sponge), sheep in Group II and IV were injected with $PGF_{2\alpha}$ double, the day before removing the sponge and immediately after removing the sponge. After this, all sheep were given i.m. an injection of 600 IU of eCG (Chrono-gest/PMSG, Germany), and the four fertile rams joined the herd. After intravaginal sponges were removed, estrus was followed up 2 times a day (12 h intervals) for 30 min for 5 days. The dates and times of estrus were recorded. Estrus ewes were separated and mated naturally. Ewes were observed for mounting by the rams. They were tested for pregnancy detection on day 30 after mating using ultrasonography device (Hasvet model 838, HASVET, Türkiye) with 7.5 MHz linear array rectal transducer. The date of birth, multiple births, the sex of the offspring, and their body weight were recorded.

Blood was collected from the *v. jugularis* into serum tubes from all sheep on day 0 (the day the sponge was inserted), on day 6 (the day the sponge was removed), and on the

day of estrus. The collected blood was centrifuged at 3500 rpm for 10 min; the serum was removed, transferred to eppendorf tubes, and stored at -20°C until measurements were made.

Progesterone and estrogen analyses were performed quantitatively by direct chemiluminescence (CLIA) method using Siemens brand hormone determination device and the same brand ADVIA Centaur (Siemens®, Tarrytown, Newyork, USA) test analysis kits. The ADVIA Centaur test is a measurement technique with a sensitivity of 100% and a specificity of 95.5%. The sensitivity and test range for progesterone is 0.21-60 ng/mL, and for estrogen it is 11.8-3000 pg/mL.

The reproductive parameters were calculated from the findings obtained. These parameters are; The start time of estrus [removal of the sponge and observation of signs of estrus]

Estrus rate [Number of sheep showing estrus/Total number of sheep]x100

Pregnancy rate [Number of pregnant sheep/Total number of sheep]x100

Lambing rate [Number of lambing sheep/Number of pregnant sheep]x100

Fertility [Number of lambing sheep/Number of mating sheep]x100

Birth of a single offspring [Number of sheep lambing with a lamb/Number of sheep giving birth]x100

Multiple lambing [Number of sheep giving birth to two or more lambs/Number of sheep giving birth]x100

Number of lambs per mating sheep (fecundity) [Number of lambs born/Number of mating sheep]

Number of lambs per ewe giving birth (litter size) [Number of lambs born/number of sheep giving birth].

Statistical Analysis

Data analyzes were performed using the SPSS® (SPSS 18, IL, USA) program. Percentages between groups were compared using the Chi-square method. Normality tests of the data were analyzed with the Shapiro-Wilk test. One Way ANOVA and welch ANOVA were performed for the weights of the offspring. Tamhane's T2 Post hoc test was applied. The level of progesterone and estrogen was compared with the Mann-Whitney U test. The results were given as mean ± standard error (X±SE). P<0.05 was considered significant.

Results

It was found that the sponge fell only in one sheep in Group IV among the sheep that were applied intravaginal sponge. Slight vaginal inflammation was detected in all of the sheep included in the study when the sponge was removed. It was determined that the time between rams joined in the herd and onset of estrus symptoms varied between 18-83 h. Reproductive parameter results of the synchronization protocol performed during the season in Morkaraman sheep are presented in *Table 1*. The rate of estrus was found to be 90.91%, 100%, 80%, and 92.86% in Group I, Group II, Group III, and Group IV, respectively. It was determined that the highest rate of estrus and pregnancy was in Group II (P>0.05). The greatest number of embryonic mortality after pregnancy diagnosis was in Group III (20%, P>0.05). The highest rate of fecundity was in Group II. 6 of the offspring in Group IV (26.09%) were determined to have died after birth; all the others continued to live. It was determined that there was no statistically significant difference between the groups for these reproductive parameters (P>0.05). Pregnancy loss was determined to be 14.29% in Group I, 9.09% in Group II, 40% in group III, and 12.5% in Group IV.

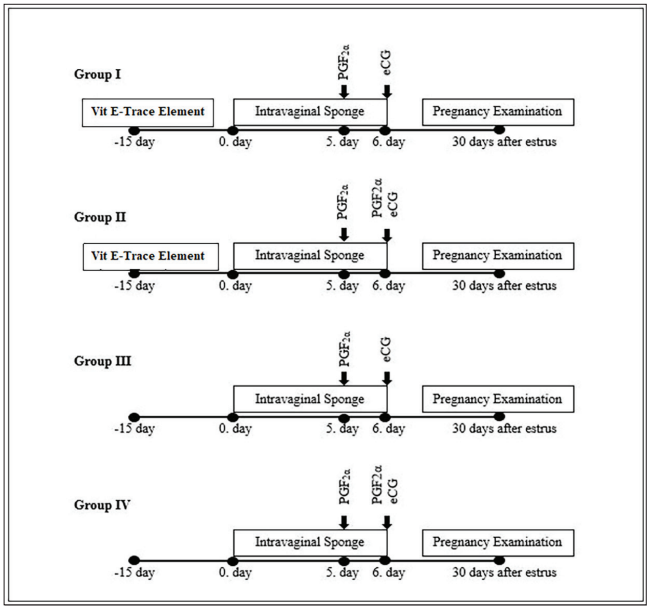

Fig 1. Application procedure in groups

It was determined that the highest rate of multiple births was in Group II and Group IV *(Table 2)*. The were 4 females and 4 males in Group I, 7 females and 16 males in Group II, 3 females and 2 males in Group III, 11 females and 12 males were born in Group IV.

The reproductive parameters obtained when the sheep were classified as Bakosel given and not given are presented in *Table 3*. It was determined that the rate of estrus, pregnancy, and lambing was higher in the group that received Bakosel than in the group that did not receive it (P>0.05). It was determined that the fecundity rate was higher in the Bakosel group (1.41) than in the non-Bakosel group (1.33). Litter size, on the other hand,

Table 1. *Reproductive parameters in groups*

Parameters	Group I	Group II	Group III	Group IV
Ewes joined (n)	11	12	10	14
Estrus rate (%)	10/11 (90.91)	12/12 (100)	8/10 (80)	13/14 (92.86)
Pregnancy rate (%)	7/11 (63.64)	11/12 (91.67)	5/10 (50)	8/14 (57.14)
Lambing rate (%)	6/7 (85.71)	10/11 (90.91)	3/5 (60)	7/8 (87.5)
Single lambing rate (%)	4/6 (66.67)	1/10 (10)	1/3 (33.33)	1/7 (14.29)
Multiple lambing rate (%)	2/6 (33.33)	9/10 (90)	2/3 (66.67)	6/7 (85.71)
Fecundity	8/10 (0.8)	23/12 (1.92)	5/8 (0.63)	23/13 (1.77)
Litter size	8/6 (1.33)	23/10 (2.3)	5/3 (1.67)	23/7 (3.29)
Survival rate (%)	100	100	100	73.91

Table 2. *Distribution of the number of offspring born in groups*

Parameters	Group I	Group II	Group III	Group IV
Number of lambs born	8	23	5	23
Single lambing rate	4/6 (%66.67)	1/10 (%10)	1/3 (%33.33)	1/7 (%14.29)
Twine lambing rate	2/6 (%33.33)	6/10 (%60)	2/3 (%66.67)	2/7 (%28.57)
Triple lambing rate	-	2/10 (%20)	-	1/7 (%14.29)
Quadruplet lambing rate	-	1/10 (%10)	-	1/7 (%14.29)
Quintuple lambing rate	-	-	-	1/7 (%14.29)
Six lambing rate	-	-	-	1/7 (%14.29)

was lower in the Bakosel group (P>0.05). Although there was a numerical difference in the reproductive parameters of these groups, this difference was not statistically significant.

The reproductive parameters obtained when the groups were classified as sheep with a single and double injection of $PGF_{2\alpha}$, was presented in *Table 4*. It was determined that the rate of estrus, pregnancy, and lambing were higher in the group that received a double injection of $PGF_{2\alpha}$ than in the group that received a single (P>0.05). It was found that the rate of multiple lambing was quite high in the group that underwent double $PGF_{2\alpha}$ (P>0.05). Fecundity and litter size was found to be higher in the double $PGF_{2\alpha}$ group (1.84 and 2.71, respectively) than in the other group (0.72 and 1.44, respectively), but this difference was not statistically significant.

Intravaginal sponge application is synchronized sheep (day 0), sponge removal (day 6), mean serum progesterone (P4) concentrations, and mean serum estradiol levels on the day of estrus are given in *Table 5*. It was determined that the progesterone level was >1 ng/mL in 76.60% (36/47) of sheep on the day of intravaginal sponge application. When the groups were compared in terms of progesterone levels, it was determined that there was a difference on day

Table 3. *Reproductive parameters in Bakosel given and not given sheep*

Parameters	Bakosel (+)	Bakosel (-)
Ewes joined (n)	23	24
Estrus rate (%)	22/23 (95.65)	21/24 (87.5)
Pregnancy rate (%)	18/23 (78.26)	13/24 (54.16)
Lambing rate (%)	16/18 (88.89)	10/13 (76.92)
Single lambing rate (%)	5/16 (31.35)	2/10 (20)
Multiple lambing rate (%)	11/16 (68.65)	8/10 (80)
Fecundity	31/22 (1.41)	28/21 (1.33)
Litter size	31/16 (1.98)	28/10 (2.8)
Mortality (lamb)	-	6

Table 4. The Reproductive parameters in sheep with a single injection of $PGF_{2\alpha}$ and double injection of $PGF_{2\alpha}$		
Parameters	**Single PGF**	**Double PGF**
Ewes joined (n)	21	26
Estrus rate (%)	18/21 (85.71)	25/26 (96.15)
Pregnancy rate (%)	12/21 (57.14)	19/26 (73.08)
Lambing rate (%)	9/13 (69.23)	17/22 (77.27)
Single lambing rate (%)	5/9 (55.56)	2/17 (11.76)
Multiple lambing rate (%)	4/9 (44.44)	15/17 (88.24)
Fecundity	13/18 (0.72)	46/25 (1.84)
Litter size	13/9 (1.44)	46/17 (2.71)

0 only between Group II and Group IV (P<0.05). There was no statistical difference in the level of progesterone and estrogen on the other measurement days. In 2 sheep in Group I, 1 sheep in Group II, 2 sheep in Group III, and 2 sheep in Group IV, the level of progesterone on the day of estrus were determined to be >1 ng/mL. It was determined that the level of progesterone on the day of estrus in the groups was between 0.61-0.92 ng/mL. The highest mean estrogen level was in Group I and Group III.

P=0.001 between Group III and Group IV. When the ewes were evaluated among themselves, a difference was found between Group I and Group IV and between Group III and Group IV. A statistical difference in rams was found between Group I and Group II, Group I and Group IV.

Table 5. Intravaginal sponge application (day 6), removal (day 0) and serum progesterone levels and serum estradiol levels on the day of estrus				
Groups	**Progesterone (ng/mL)**		**Estrus Day**	
	0. day	**6. day**	**Progesterone (ng/mL)**	**Estradiol (pg/mL)**
Group I (n=11)	3.83±0.71	1.38±0.45	0.92±0.22	56.29±7.97
Group II (n=12)	2.80±0.85*	1.67±0.51	0.69±0.16	48.19±3.54
Group III (n=10)	3.77±1.08	1.68±0.53	0.92±0.37	56.71±5.34
Group IV (n=14)	5.88±0.58*	1.72±0.46	0.61±0.18	54.09±8.03
· The difference between values with different letters in the same column is significant (P<0.05)				

Table 6. Average weight of offspring in groups			
Groups	**Average Lamb Weight (kg)**	**Average Female Lamb Weight (kg)**	**Average Male Lamb Weight (kg)**
Group I	4.74 ± 0.16^{a}	4.42 ± 0.28^{k}	4.88 ± 0.18^{x}
Group II	3.43 ± 0.16^{b}	3.36 ± 0.21^{kl}	3.47 ± 0.22^{y}
Group III	4.63 ± 0.23^{c}	4.72 ± 0.22^{mk}	4.41 ± 0.71^{xy}
Group IV	2.99 ± 0.30^{bd}	2.53 ± 0.40^{l}	3.34 ± 0.42^{yz}
a:d, k:m, x:z: The difference between values with different letters in the same column is significant, a:b:<0.001, a:bd: <0.001, b:c: 0.005, c:bd: 0.001, x:y: <0.001, x:z:0.03, k:l: 0.007, mk:l: 0.001			

The average weights of the lambs born are presented in *Table 6*. It was determined that the highest offspring weight was in Group I when the groups were compared in terms of weight. There was a statistical difference of P<0.001 between Group I-Group II and between Group I-Group IV, P=0.005 between Group II-Group III, and

Discussion

Since sheep bred in Turkey generally have low productivity, profitability decreases considerably [11]. Morkaraman sheep is a fat-tailed breed that is mainly bred in the east of Türkiye [11,12]. The factors that affect profitability the most are listed as fertility rate, litter size, and lamb's vitality [11]. The average weight of lambs in Morkaraman sheep is 3-4 kg; the lambing rate is 90%, the litter size is 1.13-1.28, the fertility rate is 0.92-1.07, and the twin lambing rate is 2% [13].

In recent years, short-term protocols have been preferred for the synchronization of breeding in sheep [2, 14-16]. These protocols use progestogen for 5 [17], 6 [2], 7 [14], and 9 days [11], which are generally performed by adding eCG and $PGF_{2\alpha}$ injection. It is reported that the fertility rate is higher in short-term protocols than in the long-term [18]. It is believed that low fertility in long-term protocols is due to the fact that the follicular wave in sheep is slower and stimulates the ovulation of the persistently dominant follicle. It is explained that pregnancy rates are higher since ovulation of newly growing follicles probably occurs in short-term progestogen treatment [18]. It was determined that the estrus rate was 100% in sheep treated with progestogen and $PGF_{2\alpha}$ analog for 5 days [17]. In the study conducted by Biehl et al [14], on the other hand, the rate of estrus was determined as 70.8% in sheep who were administered short-term (7 days, $PGF_{2\alpha}$, 300 IU PMSG) intravaginal progestogen. Najafi [19] determined the estrus rate as 80%. It has been reported that the pregnancy rate varies between 75-93.75% during the breeding season [14-16,19-21]. It was seen that the litter size ratio in sheep that received short-term progesterone supplementation and injected with $PGF_{2\alpha}$ and different doses of eCG varied between 1.24-2.20 [16-18,20,22]. Fertility rates have been reported to be between 75 and 87.5% [21]. In the present study, it was determined that the rate of estrus in the groups was between 80-100%. The pregnancy rates in the groups were 63.64%, 91.67%, 50%, and 57.14%, respectively. Although the multiple pregnancy rate was not statistically significant, it was determined that it was highest in Group II (90%). In all these studies, it is thought that the wide range in the values of the reproductive parameters is due to the breed of the animal, the synchronization protocol, the progesterone source used, the dose of $PGF_{2\alpha}$ and eCG, and the injection days.

It is known that due to the increasing number of animals and unconscious grazing in our country, the pastures are severely damaged, and the plant quality has decreased significantly [23]. There is insufficient protein intake in sheep grazing on low-quality pastures. Protein deficiency both reduces the absorption of trace elements and leads to a lack of amino acids necessary for the synthesis of antioxidant enzymes and, therefore to a deterioration in antioxidant capacity [24]. Vit E plays an important role in the management of oxidative stress [8]. Some trace elements, such as selenium [25,26] and copper, act as a structural element of many enzymes and hormones and act on fertility [25]. It is reported that a low copper level during estrus negatively affects the pregnancy rate [25]. In cobalt deficiency, it has been determined that ovulatory response decreases in superovulated sheep. In addition, it is reported that subclinical cobalt insufficiency affects both the pregnancy and conception rate and the survival and viability of the newborn [10]. In a study investigating the effect of Vit E and Selenium injection on reproductive performance in estrus synchronization, injections were made on the day the sponge was applied, the day the sponge was removed (600 IU PMSG), and 19 days after the sponge was removed. No difference was found between the injected group and the control group in terms of the lambing rate of twins or triplets. It was found that the pregnancy rate (86.8%) was higher in the vitamin-administered group than in the control group (63.9%) ($P<0.01$). It has been determined that vitamin-mineral administration reduces pregnancy loss. It has been determined that fecundity and prolificacy increase with vitamin administration, but this difference is not statistically significant [27]. According to the study conducted by Birdane et al.[28], it was found that the injection of vitamins A, D3, and E into sheep synchronized with progestagen and eCG increases the pregnancy rate. It was determined that the number of offspring per pregnant sheep (1.54) and the rate of multiple births (42.9%) increased in the vitamin-administered group compared to the control group (1.37 and 33.3%, respectively). However, no statistical difference was found in any of these values.

In the study conducted by Farahavar et al.[29], Vit E+Selenium injection was given to sheep 2 weeks before CIDR was administered (for 13 days), on the day CIDR was administered, and on the day CIDR was removed. There are 18 sheep in each group. In the first service period, the number of lambing sheep was 11 in the vitamin-administered group, 10 in the control group, and the number of lambs born was 13 and 11 in the groups, respectively. Single births were determined as 9 in both groups; twins or triplet births were determined as 2-to-1, male offspring as 7-to-8, and female offspring as 6-to-3. The fertility rate was determined as 61.11% to 55.56%, pregnancy loss as 38.88% to 44.44%, and productivity as 118 to 110%, respectively. According to Zonturlu et al.[7], it was found that bolus administration of trace amounts of vitamin E and minerals 14 days before synchronization increased the rate of multiple births. While the pregnancy rate and lambing rates were 75.4% and 75.4%, it was determined as 67.2% and 65.6% in the control group. The number of twin offspring was found to be significantly higher compared to the control group. In the study conducted by Musa et al.[30], Vit E injections were given to one group and Vit E and Selenium injections to the other group at 14-day intervals, while the third group received saline injections as the control group. CIDR is left in the body for 14 days. Estrus rate was 80%, 100%, and 100% in control, Vit E and Vit E + Selenium groups; the pregnancy rate was 75%, 80%, 80%, lambing rate was 66.6%, 100%, 100%, fecundity was 100%, 100%, 100% respectively. It is reported that Vit E and Selenium have a positive effect on fertility. Although there was no

statistically significant difference in the presented study, the rate of estrus (95.65%), pregnancy (78.26%), and lambing (88.89%) in the group given bolus containing vitamin E and minerals were compared to the group not given (87.5%, 54.16%, and 76.92% respectively) were determined to be higher. In addition, the fecundity rate was also higher in the vitamin-mineral group. The absence of a statistically significant difference between the groups may be due to the small number of animals. It is thought that this increase in fertility parameters may be due to the intracellular antioxidant properties of vitamin E and trace elements and their contribution to the protection of cell membranes from oxidative damage by cleansing reactive oxygen radicals. It is because reactive oxygen species play a role in folliculogenesis, oocyte maturation, ovulation, corpus luteum formation and regression, implantation, and fetal development [30]. In addition, it is thought that intravaginal sponge application may be one of the sources of oxidative stress in synchronized sheep and that Vit E + Selenium supplementation reduces oxidative stress [29] and thus contributes to reproductive performance.

Exogenous $PGF_{2\alpha}$ injection stimulates the production of utero ovarian $PGF_{2\alpha}$ [31]. In sheep, the corpus luteum responds to $PGF_{2\alpha}$ between days 4-14 of the cycle. It is explained that $PGF_{2\alpha}$ injection is needed at a dose of 6 mg [32] for a corpus luteum on days 2-4 of the cycle and 10 mg for a mature corpus luteum [33,34]. It is reported that the response rate increases as the dose of $PGF_{2\alpha}$ increases [35]. It has been reported that the maximum response in a single injection is at the dose of 20 mg [35], and it can be used in divided doses every 3 h [36], reducing the cost of medication [37]. It was determined that the progesterone level decreased within 24 h and the percentage of estrus within 1-5 days was 35% in sheep given $PGF_{2\alpha}$ injection at a dose of 5 mg. At doses of 15 mg and 20 mg, this ratio was found to be 70% and 95%, respectively [35]. Mekuriaw et al.[38] reported that the rate of estrus and the time of onset of estrus did not change depending on the dose, but the pregnancy rate was higher in the group that received a high dose of $PGF_{2\alpha}$ injection. In a study conducted by Dixon et al.[37], CIDR was applied intravaginally for 5 days. $PGF_{2\alpha}$ injections were given double (20 and 17 h before) a day before CIDR removal. During the first service period, the pregnancy rate was determined as 63.9% in the first group, the lambing rate was determined as 95%, the pregnancy rate was determined as 71%, and the lambing rate as 95% in sheep that received a double dose of $PGF_{2\alpha}$ injection 3-h intervals on the day the CIDR was removed. The pregnancy rate was found to be higher in cows who were administered the CIDR + cosynch protocol for 5 days; and in those who received $PGF_{2\alpha}$ injections every 8 h [39] and 12 h [40] compared to those treated with a single $PGF_{2\alpha}$ protocol [39]. It has been reported that luteolysis is not complete in a single injection of $PGF_{2\alpha}$ or that it is caused by less complete luteinization [39,40]. In the present study, by taking previous studies as an example [37,41,42], dinoprost was used at a dose of 5 mg for luteolysis. It was determined that the rate of estrus and pregnancy was higher in the group that received $PGF_{2\alpha}$ injections with 24 hours intervals compared to those who received a single dose of $PGF_{2\alpha}$. In addition, it was found that the rate of multiple lambing was higher in the group receiving a double dose than in the group receiving a single dose of $PGF_{2\alpha}$. It is believed that this increase may be because a double dose of $PGF_{2\alpha}$ fully provides luteolysis and acts on the follicular wave and ovulation.

It was reported that the average birth weight of Morkaraman lambs was 3.41±0.06 kg. It has been reported that there is a difference of 1.38 kg in favor of singles and 0.438 kg in favor of rams between rams and ewes [43]. In the presented study, it was determined that the average offspring weight ranged between 3.34 and 4.88 kg. It was determined that the average weight of the offspring was the least in Group II and Group IV. In these groups, lamb weights were determined to be lower due to the multiple pregnancies.

In the study, it was confirmed that the progesterone level was >1 ng/mL in 76.6% of sheep on the day of sponge application (mean 4.16±0.42 ng/mL); that is, the animals were in cyclicity. On the day the sponge was removed, the level of subluteal progesterone (1.61±0.24 ng/mL) was determined. A lower progesterone level on the day of removal is because chronogest, which contains synthetic progesterone, cannot be measured in the blood [22].

As a result, in this study, it was determined that Vit E-mineral therapy and double $PGF_{2\alpha}$ injection increased the reproductive performance numerically, but this difference was not statistically significant. In particular, it was determined that a double $PGF_{2\alpha}$ injection contributes to the formation of a complete luteolysis and increases fertility and productivity. In order to fully understand the effect of Vit E-mineral treatment and $PGF_{2\alpha}$ on reproductive performance, it is recommended that a more comprehensive study should be conducted by increasing the number of animals.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author (M.C. Demir) on reasonable request.

Ethical Approval

The present study was approved by the Animal Research Ethics committee of the University of Kafkas (Ethics approval number: KAÜ-HADYEK-2021/157).

Conflicts of Interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of paper.

Author Contributions

SK and CK planned the study, designed the experiments, and

helped manuscript writing; SK, CK, GK and MCD performed this study; GK and MCD collected blood samples and conducted laboratory process. SK analyzed the statistics data. All authors read and approved the final manuscript.

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