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Effects of Multivitamin, Vitamin E/Selenium and Prostaglandin Injections on Reproductive Performance in Kıvırcık Ewes

ABSTRACT

Objective: This study investigated the effectiveness of the single-dose treatment of three different commercial products on reproductive performance in Kıvırcık ewes during the transition to the breeding season.

Material and Methods: Ninety-eight Kıvırcık ewes aged 3-4 years and weighed from 50 to 55 kg have been used in this study. The ewes used in the study were randomly divided into four different (PGF2α, Vit.E/Se, multivitamin and control) groups. All these injections were applied to all groups one week before the ram-mating process.

Results: The difference between the control group and the treatment groups was statistically significant ($P < 0.01$); however, the difference between PGF2α and Vit.E/Se, and multivitamin treatments was insignificant ($P > 0.05$) in terms of multiple-birth rate. The highest value in terms of litter size was found in the PGF2α treatment group.

Conclusion: In the study, all three treatments were more successful than the control group in inducing estrus and pregnancy in Kıvırcık ewes during the transition from anoestrus period to the breeding season and all ewes healthily gave birth.

Keywords: Sheep, reproduction, PGF2α, vitamin E/Selenium, vitamin A, vitamin D3, vitamin B

Multivitamin, Vitamin E/Selenyum ve Prostaglandin Enjeksiyonlarının Kıvırcık Koyunlarının Üreme Performansı Üzerine Etkileri

ÖZ

Amaç: Bu çalışmada üreme mevsimine geçişte Kıvırcık ırkı koyunlarda döl verim performansı üzerine üç farklı ticari ürünün tek doz uygulanma etkinliği araştırılmıştır.

Materyal ve Metot: Bu çalışmada 3-4 yaşlarında ve 50 ila 55 kg ağırlığında 98 Kıvırcık koyunu kullanılmıştır. Çalışmada kullanılan koyunlar rastgele dört farklı (PGF2α, Vit.E/Se, multivitamin ve kontrol) gruba ayrılmıştır. Tüm bu enjeksiyonlar koç katım sürecinden bir hafta önce tüm gruplara uygulanmıştır.

Bulgular: Kontrol grubu ile uygulama grupları arasındaki fark istatistiksel olarak anlamlıydı ($P < 0.01$); ancak PGF2α ile Vit.E/Se ve multivitamin uygulamaları arasındaki fark çoğuz doğum oranı açısından önemsizdi ($P > 0.05$). Yavru büyüklüğü açısından en yüksek değer PGF2α grubunda bulunmuştur.

Sonuç: Çalışmada, anöstrus döneminden üreme mevsimine geçişte Kıvırcık koyunlarında östrus ve gebeliği teşvik etmede her üç uygulama da kontrol grubundan daha başarılı olmuş ve tüm koyunlar sağlıklı bir şekilde doğum yapmıştır.

Anahtar Kelime: Koyun, üreme, PGF2α, vitamin E/Selenyum, vitamin A, vitamin D3, vitamin B

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INTRODUCTION

Various synchronization protocols are implemented in the off-breeding season, aiming to generate the estrus response, ensure pregnancy, and improve fertility since ewes develop estrus seasonally (Abecia et al. 2012). Applying progesterone-containing vaginal equipment in conjunction with an pregnant mare serum gonadotropin injection is a typical synchronization method among ewes (Zohara et al. 2014; Swelum et al. 2015). However, some public concerns such as animal health and welfare, food safety, and environmental impact have recently brought forward reconsidering the use of the progestogen-based protocols of vaginal apparatus used for this purpose (Gonzalez-Bulnes et al. 2020; Ozis Altincekic et al. 2021). At this point, prostaglandin (PGF2α) use becomes more popular due to its cheaper and an environmental pollutant than progestogen intravaginal apparatuses (Fierro et al. 2013). Additionally, since prostaglandins are metabolized rapidly in the lung, they leave no residue in the body (Davis et al. 1980). Therefore, using PGF2α or its analogs among sheep is an alternative synchronization method.

Vitamins are natural compounds with unique properties allowing animals to function optimally. It is well established that vitamin treatments alleviate the harmful impacts of the existing conditions in animals and improve animal productivity (Marai et al. 2008). Animals cannot produce fat-soluble vitamins within their bodies; thus, A, D3, and E vitamins should be outsourced regularly to meet their physiological requirements and sustain high-performance standards (Hafez 2012). In addition to their impacts on growth and physiological processes, vitamins and minerals play a critical role in the reproductive performances of animals (Gabryszuk and Klewiec 2002). According to literature, vitamins A and E have specific functions on ovarian and uterine activities; concurrently, they are indispensable for the body's antioxidant mechanism defending animals against diverse stressors such as the mating season and high ambient temperature (Chauhan et al. 2014). Vitamin D3 plays a critical role in follicle development, oxidative stress, and the generation of steroid hormones, also responsible for the hemostasis of calcium (Ca) and phosphorus (P), which plays a role in fetal development (Yao et al. 2017). B group vitamins in ewes are essential for sustaining animal welfare, ensuring healthy lambing, and functioning efficient reproduction and immune system (Vijayalakshmy et al. 2018). Vitamin C supplement in ewes improves fertility and contributes to lambs gaining live weight (Haliloğlu and Serpek 2000). Selenium (Se) and vitamin E supplements play an essential role in ewes' reproduction and affect the performances of the ewes and lambs equally (Rooke et al. 2008). Injection of Se supplement two weeks before ram-mating with insufficient Se initial status raised their serum Se levels (Ramírez-Bribiesca et al. 2004). The Se supplement is also a component of selenoprotein involved in metabolism, immunity, and other bodily processes (Hefnawy and Tórtora-Pérez 2010). As a result, studies suggested that vitamin E or Se supplements lowered the oxidative stress generated during estrus synchronization practices; consequently, the fertility rate improved conceivably (Sönmez et al. 2009; Kuru et al. 2016). Recent studies also defined the Se supplement as the most critical factor in enhancing animal health and welfare (Surai 2006). However, vitamin treatment in previous studies was applied to ewes with synchronized estrus during the removal of the sponge (Köse et al. 2013), right before the insertion of the sponge into the vagina (Kuru et al. 2017), or while inserting, removing and after the removal of the sponge (Awawdeh et al. 2019).

The objective of this study is to evaluate the effects of PGF2α, Vit.E/Se, or multivitamin injections on fertility and lamb performance in Kıvırcık sheep. For this purpose, pregnancy rate, lambing rate, multiple rate, fecundity, litter size, survival rate, pregnancy prolificacy and total prolificacy were determined as reproductive parameters.

MATERIAL and METHODS

Location

This study was performed at the Application and Research Farm of the Agricultural Faculty, Uludag University in Bursa, a Province in the northwestern region of Turkey. The farm was located at the humid lowland tropics at an altitude of 100 m above sea level, and at longitude 29 °E and latitude 40 °N.

Animals and management

Ninety-eight Kıvırcık ewes aged 3-4 years and weighed from 50 to 55 kg have been used in this study. Before mating, all the ewes grazed the rangeland during the day and were kept in a shelter at night. The ewes were grazed on a pasture consisting of a mixture of common vetch (Vicia sativa L), Hungarian vetch (Vicia

pannonica L), alfalfa (Medicago sativa) and sainfoin (Onobrychis sativa). Minerals salt lick and clean drinking water provided ad libitum. To meet their increased nutrient needs in late pregnancy, the ewes were given 300-400 g from concentrate feed prepared on the farm per ewe that was in addition to ad libitum access to pasture according to the seasonal conditions (Table 1). The metabolizable energy content of the supplement was calculated according to NRC (2007). In Bursa-raised Kıvırcık eves, the estrus season begins in September and lasts through the end of January. As a result, injections and the ram-mating processes were executed in 25th of August; accordingly, this period was designated as the beginning of the breeding season.

The birth process ended in the February-March season. In addition to grazing, approximately 300 to 400 g of concentrate feed was provided per ewes to meet their growing nutritional needs during the early stages of pregnancy. After measuring their birth weights, the lambs received colostrum and had their ear identity numbers immediately in the first postnatal hour. The ewes and their lambs were kept together for about two weeks postnatal, and the lambs received no supplementary feeding. After two weeks, while lambs stayed in the sheep pen when ewes were grazing during the daylight, they were allowed to circle in their mothers in the evening and switched to a feeding system called creep-feeding. When the lambs were three months old, they were weaned and separated from their mothers.

Table 1. Ingredients and chemical composition of diet for Kıvırcık ewes
Tablo 1. Kıvırcık koyunları için diyetin içeriği ve kimyasal bileşimi

Ingredients	Content, %	Chemical Composition	Content, %
Barley	46,0	Dry matter, %	91,30
Corn	25,6	Organic matter, %	84,83
Sunflower meal	26,0	Crude protein, %	15,11
Limestone	1,2	Crude fat, %	3,14
Salt	1,0	Crude ash, %	6,47
Mineral-vitamin premix*	0,2	Metabolic energy, kcal/kg DM	2712

***150 mg ZnSO47H2O, 80 mg, MnSO4H O, 200 mg MgO, 5 mg CoSO47H2O, 1 mg KIO3, 4000, IU vitamin A, 1000 IU vitamin D ve 20 IU vitamin E**

Study design

All ewes were clinically normal with a healthy appearance. The ewes used in the study were randomly divided into four different groups. The first group (n=26) was treated by 12.5 mg/ewe PGF2α injection (Dinolytic, Zoetis). Doses of 12.5 mg of dinoprost (PGF2α)/animal (equivalent to 1ml of medicament). In the second group (n=26), however, ewes were injected with 5 ml/ewe of Vitamin E/Selenium solution (Vit.E/Se) (Yeldif, Ceva) intramuscularly. The third group (n=26) had an injection of a 5 ml/ewe multivitamin solution (Vitaflash, Aksu) intramuscularly as stated in the prospectus. All these injections were applied to all groups one week before the ram-mating process. The fourth group (n=20) contained the ewes for the control group (Table 2). After 24 hours of the treatment, six rams joined the herd as part of the free ram-mating, and they remained there for 34 days (two ram-mating cycles). Rams that were 4 years old and had a condition score of 3 were used in mating.

Table 2. Treatment groups
Tablo 2. Uygulama grupları

Groups	Treatments	Number of ewes (head)
I*	PGF2α (1 ml/ewe)	26
II**	Vit.E/Se (5 ml/ewe)	26
III**	Multivitamin (5 ml/ewe)	26
IV	Control	20

***: 12.5 mg dinoproston tromethamine in 1 ml**
****: 1 mg sodium selenate, 60 mg Vitamin E, 40 mg Vitamin B1 in 1 ml**
*****: 50.000IU vit-A (palmitate), 25.000IU vit-D3, 4mg vit-E (acetate), 2.5mg vit-B1, 2mg vit-B2, 12.5 mg vit-B3, 1.25mg vit-B6, 30mcg vit-B12, 2mg vit-C, and 3mg D-panthenol in 1 ml**

Data collection

Ram-mating and lamb birth dates, as well as lamb birth and postpartum records, were routinely documented. Descriptive values of reproductive parameters were calculated as follows (Kaymakçı 2006).

•*Pregnancy rate (%): Number of pregnant ewes/number of ewes in the group × 100*

•*Lambing rate (%): Number of ewes giving birth/number of pregnant ewes × 100*

•*Multiple rate (%): Number of ewes giving birth to multiple/ number of pregnant ewes × 100*

•*Fecundity (head): Number of lambs born/total ewes mated*

•*Litter size (head): Total number of lambs/number of ewes giving birth*

•*Survival rate (%): Number of living lambs/number of lambs born × 100*

•*Pregnancy prolificacy, kg: Total weight of lambs live-born from every 100 ram-mated ewes.*

•*Total prolificacy, kg: Total weight of lambs obtained from every 100 ram-mated ewes at weaning time.*

Data Analyses

A Chi-square test was used to determine the effects of application methods on reproductive parameters (pregnancy rate, multiple birth rate and survival rate) in ewes (Minitab 19.0).

RESULTS and DISCUSSION

The use of prostaglandins for estrus synchronization in ewes has become more popular with its low cost, ease of application, and ability to minimize vaginal deformations during insertion and removal of vaginal apparatus (Ozis Altincekic and Koyuncu 2017; Adnane et al. 2018). Application of the PGF2α during the anoestrus period aims to induce estrus, initiating a new follicular phase with the disappearance of the corpus luteum and, therefore, releasing progesterone (Kaymakçı 2006). Some literature, such as Doğan and Nur (2006), Ataman et al. (2006), and Yadi et al. (2011), achieved a pregnancy rate of 57.1%, 84.6%, and 70% with the PGF2α injection in anoestrus ewes, respectively. Demiral et al. (2014) reported a 72.1% lambing rate with the PGF2α injection in ewes, indicating that the PGF2α treatment eventuated a more effective response, specifically in the anoestrus periods of ewes previously given birth. Yadi et al. (2011) discovered a 42% multiple-birth rate via PGF2α treatment, stating that the PGF2α was more effective than other synchronization approaches, primarily during the anoestrus season. Ozis Altincekic and Koyuncu (2017) reported comparable and reasonable results with the PGF2α injection in anoestrus ewes regarding fertility criteria with traditional synchronization methods. Similarly, PGF2α treatment in this study led to a 42% pregnancy rate in ewes within the initial few days of the ram-mating; furthermore, 33% more of the ewes also became pregnant during the first ram-mating cycle. Accordingly, PGF2α injection may successfully stimulate the estrus during the transition from anoestrus to the breeding season. Titi et al. (2010), Mirzaei et al. (2017), and Dursun (2019) reported that the PGF2α-based protocol increased estrus rate, lambing rate, and litter size during anoestrus season, boosting the profitability of sheep herd ($P<0.01$). On the other hand, Ayaseh et al. (2021) reported that PGF2α treatment during the anoestrus season appeared to raise the lambing rate in the groups; however, the difference was insignificant in terms of multiple-birth rates, litter size, and BW when compared to the control group ($P>0.05$). This study, however, found that while PGF2α injection performed similarly to vitamin treatments for pregnancy rate and lambing rate, it had significantly higher performance than the control group, outperforming both the other application groups and the control group in terms of multiple rates, fecundity and litter size ($P<0.01$). PGF2α may induce ovulation through a mechanism independent of luteolysis, i.e., by increasing the sensitivity of the pituitary to GnRH, thus increasing LH pulsatility (Randel et al. 1996). Therefore, the possibility that the administered Dinoprost may have acted as a stimulus for cyclicity induction should not be excluded.

Figure 1. The effects of different practices on pregnancy rate, twinning rate and survival rate of lambs in the period before the ram-mating process in ewes
Şekil1. Koyunlarda koç katım öncesi dönemde farklı uygulamaların gebelik oranı, ikizlik oranı ve kuzuların yaşama gücü oranına etkileri

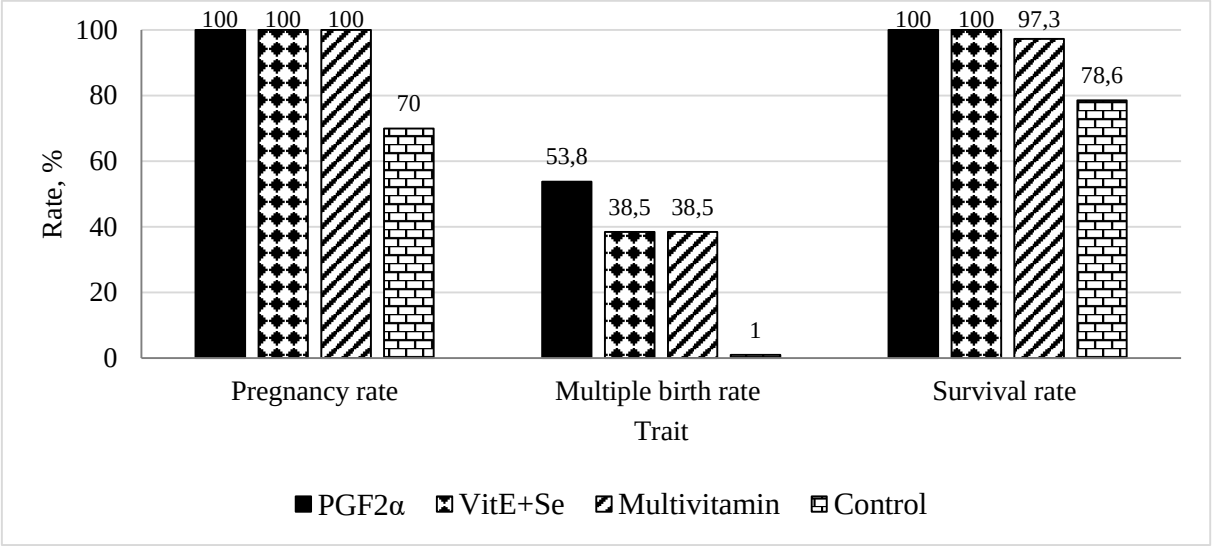

Table 3 illustrates the findings acquired on fertility parameters among the experimental groups in the study. Accordingly, while no multiple births were observed in the control group, multiple births were observed in the PGF2α, Vit.E/Se and multivitamin treatment groups. The difference between the control group and the treatment groups was statistically significant (P<0.01); however, the difference between PGF2α and Vit.E/Se and multivitamin treatments was insignificant (P>0.05) in terms of multiple-birth rate. The highest value in terms of litter size was found in the PGF2α treatment group with 1.54, followed by the multivitamin (1.42), Vit.E/Se (1.38), and control (1.00) groups. The viability (survivability) rate of the lambs until the weaning time was considerably lower (78.6%) in the control group compared to the other groups, where it was 97.3% in the multivitamin group and 100% in the PGF2α and Vit.E/Se groups. While the vitamin E/Se groups had the highest pregnancy prolificacy, the multivitamin group had the highest total prolificacy.

Table 3. Fertility parameters in Kıvırcık ewes
Tablo 3. Kıvırcık koyunlarında döl verim parametreleri

	Treatments					
Parameters	**PGF2α (1ml/ewe)**	**Vit.E/Se (5 ml/ewe)**	**Multivitamin (5 ml/ewe)**	**Control**	**P-value**	**χ^2**
Pregnancy rate (%)	100.0 [a]	100.0 [a]	100.0 [a]	70.0 [b]	0.00	24.9
Lambing rate (%)	100.0	100.0	100.0	100.0		
Multiple birth rate (%)	53.8 [a]	38.5 [a]	38.5 [a]	0 [b]	0.01	11.4
Fecundity (head)	1.54	1.38	1.42	0.70		
Litter size (head)	1.54	1.38	1.42	1.00		
Survival rate (%)	100.0 [a]	100.0 [a]	97.3 [a]	78.6 [b]	0.00	17.8
Pregnancy prolificacy, kg:	442.5	464.7	428.2	391.33		
Total prolificacy, kg:	2338.3	2379.5	2452.7	2.010		

Studies indicate that Vit.E/Se application facilitates the steroid formation process by acting as an antioxidant before mating and triggers folliculogenesis in the ovaries by stimulating the secretion of gonadotropin from the anterior pituitary gland (Ullah et al. 2020). Thus, Vit.E/Se promotes neutrophil function and enhances uterine health by stimulating ovarian activity. Vit.E/Se also augments fertility by diminishing mortality in the early embryonic period (Amin et al. 2016). Hemingway (2003) reported that Vit.E/Se supplement before mating improved the pregnancy rate among cattle and sheep by reducing early embryonic mortality. Consequently, this study found that Vit.E/Se treatment resulted in pregnancy among 34% of the ewes in the first days of ram-mating, 54% during the first ram-mating cycle, and 12% in the second cycle. Therefore, it is safe to state that Vit.E/Se treatment is a successful stimulus in estrus and that it is synchronized to a large extent in ewes in the transition period. Koyuncu and Yerlikaya (2007) reported that Vit.E/Se injection boosted the fecundity in

Karacabey Merino sheep. Similarly, Munoz et al. (2008) emphasized that the Se element is critical for successful fertilization, implantation, and fetal development. Musa et al. (2018) also noted that injection of Vit.E/Se in Yankasa ewes significantly enhanced reproductive performance by increasing pregnancy, lambing, and fertility rates. The findings of this study, such as all ram-mated ewes got pregnant, gave birth successfully, had the highest pregnancy prolificacy rate in the group receiving Vit.E/Se injection, and all newborn lambs reached the age of weaning healthy, supported the results of the studies described above. Although the multiple-birth rate in the Vit.E/Se and multivitamin groups were similar, it was substantially higher than the control group ($P<0.01$). It could be due to the potential effect of selenium on antral follicles, granulosa cells, and oocytes by regulating the activity of glutathione peroxidases and the ability of Vitamin E to avert oxidative base damage in sheep ovarian epithelium and enhance the uterine environment by preserving the functional integrity of the membranes (Murdoch and Martinchict 2004).Awawdeh et al. (2019) also indicated that while Vit.E/Se injection in Awassi sheep reduced pregnancy losses from 44.8% to 24.3% ($P = 0.08$), it yielded no substantial effect on multiple-birth rate ($P>0.20$). Gabryszuk and Klewiec (2002) reported that Vit.E/Se treatment before ram mating had no impact on estrus rate, fertility and fecundity in Polish Merino ewes compared to the control group ($P>0.05$). Such differences between study results can be caused by several variables such as number of dosages, methods of administration (feed or injection), duration of administration (before and/or after ram mating) and frequency of administration.

Hashem et al. (2016) reported that vitamin A and C supplements before ram-mating increased the number of ovulatory follicles and estradiol (E2) synthesis. Similarly, Abdelrahman and Al-Karablieh (2002) discovered that vitamin AD3E injected in two dosages, 2-3 weeks before and two months after the ram-mating process, increased the fertility rate (82.1%) and lambing rate (86.7%) of Awassi sheep in comparison to the control group (74.0% and 77.9%, respectively). Additionally, these scientists found no statistically significant difference between the treatment and control groups for lambs' birth weights and weaning weights ($P>0.05$). In their analysis of the effects of AD3E vitamins on fertility in Pırlak sheep with synchronized estrus, Birdane and Avdatek (2020) found that the vitamin-treated group had a pregnancy rate of 87.5% and a litter size of 1.54%, compared to the control group's 75% and 1.37%, respectively; however, these increments in rates were statistically insignificant ($P>0.05$). In contrast to the rates observed in both studies, the multivitamin group yielded higher pregnancy and lambing rates in the current study. Besides, multivitamin treatment resulted in pregnancy among 88% of the ewes in the initial days of the ram-mating process. In this context, it is conceivable to argue that the multivitamin is more effective in stimulating and synchronizing estrus in the transitional period than the PGF2α and Vit.E/Se. It is also worth mentioning that despite being comparable to the Vit.E/Se treatment group for pregnancy, lambing, and multiple birth rates, the multivitamin-treated group had significantly higher rates than the control group ($P<0.01$). Vitamins A, C, and E act as antioxidant agents; improving follicle quality or raising the number of healthy follicles may have contributed to such an outcome (Kamiloğlu et al. 2006; Nayyar and Jindal 2010). It may also be related to the fact that vitamin AD3E injection improves animal performance by accelerating sex hormone levels, simply evoking sexual behavior (Al-Asadi et al. 2020). As another issue, the multivitamin group yielded better litter size and fecundity success than both Vit.E/Se and control groups. Whaley et al. (1997) reported that the vitamin A treatment did not reproduce the number of CLs; instead, the larger litter size may be attributable to vitamin A's advancing effect on the number of oocytes and other mechanisms such as improved embryonic survival. Jindal et al. (1996) discovered that vitamin A treatment before mating elevated serum progesterone concentrations and improved early embryonic survival; consequently, it yielded an increment in litter size. Hashem et al. (2016) reported that vitamin A-driven progesterone production during early pregnancy increased fecundity and lambing rate, boosted embryo survival and/or quality, supported the maturation and function of oviducts, uterus, and placenta, and facilitated blastocyst development. Fisher and MacPherson (1991) reported that vitamin B12 supplements played a critical role in embryonic development in ewes, and lambs born from these ewes were highly active, began milking their mothers earlier, and had lower morbidity and mortality rates. Vitamin B2 deficiency, on the other hand, led to a slowing of adolescent growth, and anomalies such as premature birth, stillbirth, and neonatal losses in pregnant animals (Frank et al. 1984). Therefore, higher results for litter size, fecundity, and total prolificacy in the multivitamin group than in the Vit.E/Se group is attributable to the effects of vitamins A and B.

CONCLUSIONS

In conclusion, all ewes in the treatment groups-PGF2α, Vit.E/Se, and multivitamins-got pregnant and delivered healthily. All the injections positively affected ewes' reproductive performance at the beginning of the breeding season. Since the cycles of the sheep were unknown at the time of these injections, it can be assumed that the 34-day relationship of the ewes with the rams and thus the ram effect may have contributed to the occurrence of estrus and the high pregnancy rates. Therefore, such injections can be part of an effective program to improve reproductive performance in sheep breeding by replacing the traditional use of progestagen-containing apparatus + eCG as low-cost, safe and practical applications that provide benefits in terms of animal health and welfare. In addition, these findings will help further explore the frequency, timing, and amount of vitamin injection that may improve the reproductive performance of the ewe. In future studies evaluating the effects of various vitamin levels on reproductive performance, reproductive hormone levels should also be taken into account.

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Author contributions: All authors contributed equally to the preparation of the article.

Competing interests.: There is no conflict of interest between the authors in this study

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