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Research Article

Effect of selenium, vitamin E, and β-carotene administration on fertility of synchronized Awassi ewes during non-breeding season

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ABSTRACT

The aim of this study was to evaluate the effect of selenium (Se), β-carotene, and vitamin E administration on fertility of Awassi ewes synchronized in non-breeding season. The study included 80 multiparous Awassi ewes ranging in age from 2 to 6 years. Intravaginal sponges containing flugestone acetate were inserted and left in for 9 days to allow estrus synchronization. Ewes were divided into two groups at random (group I: study group; group II: control group). In group I (n=40), while the drug containing Se and vitamin E was only administered on the day of sponge insertion, another drug containing β-carotene and Vitamin E was administered three times (sponge insertion, withdrawal, and on day 18 after mating) during estrus synchronization. The control group (group II, n=40) received no supplementary injections. When the sponges were removed, 500 IU PMSG and 0.075 mg d-cloprostenol were administered intramuscularly. The rams joined the herd for an hour twice a day 24 hours after the removal of sponges. Mating occurred after the detection of estrous in ewes. Estrus rates and estrus onset timings of group I and group II were 81.08% and 45.30±1.71 h, and 80.55 % and 43.94±1.72 h, respectively. The conception rates, pregnancy rates, kidding rates, and litter size in group I and group II were 66.66% - 72.41%, 54.05% - 58.33%, 100% - 100%, and 135% - 138%, respectively. There were no statistical differences ($p>0.05$) between the groups in terms of fertility traits. In conclusion, administering Se, β-carotene, and vitamin E at estrus synchronization protocols during the non-breeding season has no positive effect on Awassi sheep fertility traits. However, measuring blood levels of Se, β-carotene, and vitamin E before beginning the treatment may be beneficial in identifying the optimal effect of this treatment.

INTRODUCTION

In ovine breeds that normally deliver once a year, inducing reproductive activity during the non-breeding season allows lambing twice a year or lambing three times in two years. Furthermore, by increasing milk and meat production, these products can be marketed in non-breeding season. In non-breeding season, the most effective methods for stimulating ovarian functions in sheep are progesterone, GnRH, melatonin, and, in addition to these, PMSG or LH effective hormone applications (Gordon, 1997; Wildeus, 2000). A meta-analysis study conducted in Turkey discovered that pregnancy rates were lower in progesterone + PMSG based estrus synchronization protocols (59.36 %) used in non-breeding season compared to the reproductive period (90.37%). The deep anestrus in animals, diminished hormonal effects, and low ovarian activity are the causes of the low pregnancy rates during the non-breeding season (Arikan et al., 2021). The ovarian follicle population in sheep is known to be highly sensitive to dietary intake, and it has been reported that dietary manipulations can increase both folliculogenesis and ovulation (Scaramuzzi et al., 2006). Farm animal reproductive performance, on the other hand, is determined by four major factors: genetics, environment, nutrition, and management. Because of their direct effects on reproduction and potential to mitigate the effects of other factors, nutritional factors are cited as the most important factors. Additionally, nutritional factors more than others are open to adjustment for successful outcomes (Smith and Akinbamijo, 2000). Many minerals and vitamins are required for optimum reproductive performance of animals. Plasma concentrations of these chemicals and reproductive performance are significantly correlated. Some trace element deficiencies, such as those of copper, cobalt, and selenium (Se), can prevent ovulation, cause embryo loss, and potentially lead to fetal mortality (Hostetler et al., 2003; Liu et al., 2014; Zonturlu et al., 2017). The use of intravaginal estrus synchronization devices like sponge and CIDR may also lead to an increase in oxidative stress (Kuru et al., 2016; Farahavar et al., 2020; Eşki et al., 2021). Oxidative stress impairs ovarian function and follicle growth. Vitamin E and beta-carotene are known essential nutrients in the management of oxidative stress (Hostetler et al., 2003; Liu et al., 2014; Zonturlu et al., 2017).

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Ewes must obtain all of their β-carotene needs from the feeds since they are unable to synthesize it. As a result, the season and the type of feed may affect the serum β-carotene levels of ewes (Weiss, 1998). Additionally, β-carotene is also an antioxidant. Similar to vitamin E, which is effective at higher oxygen concentrations, β-carotene scavenges superoxide radicals and neutralizes free peroxide radicals in tissues thus synergizing the antioxidant activity (Arechiga et al., 1998). A lack of β-carotene results in sub-estrous, delayed ovulation, decreased pregnancy rates, an underdeveloped and small-diameter corpus luteum (CL), and decreased progesterone synthesis throughout the cycle and the first trimester of pregnancy, which increases the risk of embryonic death (Ayaşan and Karakozak, 2010). It has been reported that β-carotene has a positive effect on fertility when incorporated into feed or administered parenterally. β-carotene affects fertility either indirectly via vitamin A conversion (Ayaşan and Karakozak, 2010), or directly (Trojacanec et al., 2012).

Vitamin E, also known as the anti-sterility vitamin, is essential for all animal species including humans. It saturates the peroxides and hydroperoxides that disrupt the structure of intracellular membranes thus preventing the peroxide radical formation (Putnam and Comben, 1987; Kott et al., 1998). Vitamin E supplementation can improve ovulation rates and the number of offspring in ewes by playing an important role in oocyte maturation and quality, fertilization, and early embryonic development (Kott et al., 1998).

Se is an essential component of organisms' antioxidant defense system. It contributes to the building of the endogenous antioxidant defense enzyme glutathione peroxidase (GSH-Px) and participates in the catabolism of peroxidase that takes place during lipid peroxidation. Se has biological activity in growth and fertility along with vitamin E, and it also acts as an antioxidant to prevent and repair cellular damage (Hostetler et al., 2003). A study reported that Se contributes to the growth of granulosa cells, the production of estrogen, and the production of prostaglandins (Wichtel et al., 1996). Se also protects follicles from oxidative stress that occurs during follicle growth, maturation, and dominance (Ceko et al., 2014).

Sheep breeding in Turkey is typically spread in a traditional pasture-based manner. According to reports, both types of grass grown in winter and dried in summer for winter feeding have less β-carotene, vitamin E, and Se, each of which has been linked to a variety of effects on reproductive processes. For this reason, it is recommended to add these nutrients during periods of deficiency, especially in pasture-based agriculture, and it is stated that when combined with estrus synchronization, the yield obtained and the economic gain due to this will increase even more (Beytut et al., 2005; Köse et al., 2013). Numerous studies have used these supplements to treat estrus in sheep, goats, and cows. However, its use under local conditions needs to be proven. The purpose of this study was to determine how Se, β-carotene, and vitamin E supplements affected the fertility of synchronized Awassi sheep in non-breeding season.

MATERIALS and METHODS

In the second half of March 2021, the study was conducted on 80 Awassi ewe from a sheep farm in Hatay aged between 2 and 6 years. The ewes weighed 45-60 kg, had lambed once or more, and were in their second month postpartum at least. Study site was situated in Yayladağı, Hatay in the Eastern Mediterranean region of Türkiye (latitude: 35º 90' N; longitude: 36º 06' E). The average ambient temperature during the study period was 17.5°C during the day and 10.8°C at night, with an average day and night length of 13 and 11 hours, respectively.

Standard management and feeding procedures of the holding were applied to the study animals. The ewes, which were not lactating at the time of the trial, were given daily access to 150 g of a concentrate mix comprising 13% crude protein and 2650 kcal/kg energy per animal while grazing on the pasture between the hours of 08:00–18:00 (Table 1).

Table 1. Content of the concentrated feed mix

Raw Materials	%
Corn	33
Barley	30
Coarse wheat bran	11
Dried pitted olive pulp	8
Sunflower meal	14,15
Marble dust	2,6
Salt	1
Vit-Min. premix	0,25

A treatment using intravaginal cylindrical polyurethane sponges impregnated with 20 mg of cronolone (flugestone acetate) (Chronogest CR®, Intervet, Turkey) was administered to each of the 80 ewes that were a part of the study and were not in breeding season. Then, using the random sample technique, the ewes were divided into two equal groups (group I: study group; group II: control group). In Group I, while the medication containing Se and vitamin E (1 mg/1 ml of sodium selenite and 60 mg/ml of vitamin E, Yelvit®, Teknovet, Turkey) was administered only on the day the sponges were inserted intravaginally, another medication containing β-carotene and vitamin E (15 mg/ml of β-carotene and 20 mg of dl-α-tocopherol acetate equivalent to 18.22 mg/ml of vitamin E, Dalmavital®, Vetaş, Turkey) was administered at the same dose three times (sponge insertion, withdrawal, and on day 18 following mating) during estrus synchronization (Figure 1). The control group (Group II, n=40) did not receive any supplementary injection (Figure 1). The sponges were left in place for 9 days in both groups. In addition, we injected 0.075 mg of d-cloprostenol (Senkrodin®, Vetaş, Turkey) and 500 IU of PMSG (Chronogest/PMSG, 6000 IU, Intervet, Istanbul, Turkey) intramuscularly when removing the sponges. Twenty-four hours after the sponges were removed, the rams joined the herd for an hour twice a day. The ewes that successfully mated after the estrous detection were separated from the herd and put in a different compartment.

parameters that were tested:

Estrus onset time: The period from sponge withdrawal to acceptance of mating (hours)

Estrus rate = (Number of ewes in estrus ÷ Number of ewes treated for estrus synchronization) × 100

Pregnancy rate = (Number of pregnant ewes ÷ Number of ewes in the group) × 100

Conception rate = (Number of pregnant ewes ÷ Number of naturally mated ewes) × 100

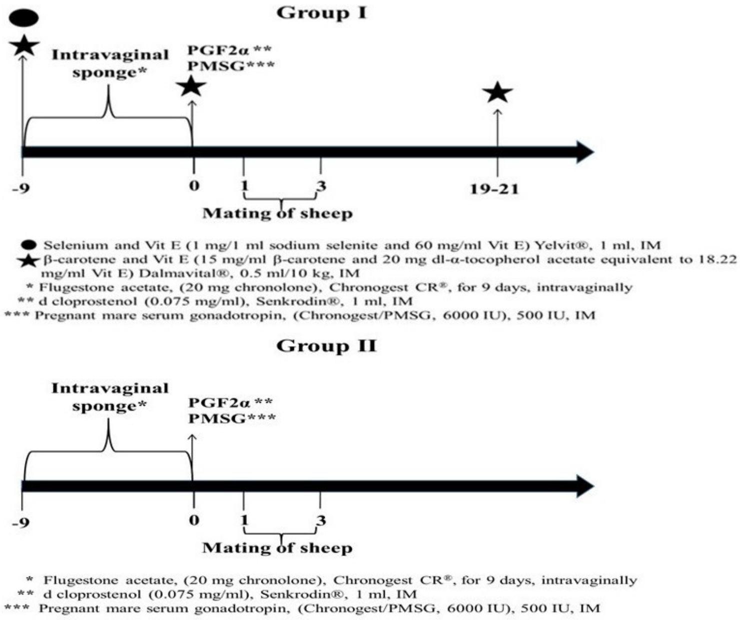

Figure 1. Applications in Group I and Group II

A 6-8 MHz probe real-time ultrasound instrument (Falco, Pie Medical, Netherlands) was used to detect transabdominal pregnancy 50 days after the mating. The existence of fetuses, fetal secretions, placentomes, and a fetal heartbeat were all considered positive evidence of pregnancy.

The SPSS 22.0 software program and the chi-square test were used to statistically examine fertility indicators. A significance level of $p < 0.05$ was used for all statistical analyses.

The following formulas were used to calculate the fertility

Kidding rate = (Number of ewes that have lambed ÷ Number of pregnant ewes) × 100

Litter size = (Number of lambs born ÷ Number of ewes that have lambed) × 100

RESULTS

In the current study, two ewes in group I and three ewes in group II did not retain intravaginal sponges. Additionally,

Table 2. Fertility parameters of Group I and Group II

	Onset of estrus (h)	Estrus rate (%)	Conception rate (%)	Pregnancy rate (%)	Kidding rate (%)	Litter size (%)
Group I	45.30±1.71	81.08 (30/37)	66.66 (20/30)	54.05 (20/37)	100 (20/20)	135 (27/20)
Group II	43.94±1.72	80.55 (29/36)	72.47 (21/29)	58.33 (21/36)	100 (21/21)	138 (29/21)
P	-	-	-	-	-	-

No significant difference between treatment groups (P>0.05).

one ewe from each group was diagnosed with laminitis. These animals were removed from the study. During the sponge treatment, no ewes displayed any symptoms of estrus. Estrus began 24 hours following the removal of the sponges and concluded 60 hours later. After sponge withdrawal in group I, the percentage of ewes in standing estrus was 6.66 % at 24–25 h, 26.66 % at 36–37 h, 53.33 % at 48–49 h, and 13.33 % at 60–61 h. On the other hand, these rates for group II were 6.89% at 24–25 h, 34.48% at 36–37 h, 48.27% at 48–49 h, and 10.34% at 60–61 h. There was no significant difference in fertility traits between the groups (Table 2, p>0.05).

DISCUSSION

The pregnancy rates in groups I and II in the current study were found to be 54.05% and 58.33 %, respectively (Table 2, p>0.05). According to Karagiannidis et al. (2001), progesterone administration to sheep during non-breeding season resulted in fertilization rates of 22% to 70%. According to Gordon (1997), progesterone-treated ewes had pregnancy rates between 70% and 80%. The conception rates for groups I and II in the current study were found to be 66.66 % and 72.41 %, respectively. The fact that the current investigation was carried in non-breeding season may be the cause of the results being comparable to those previously reported by Gordon (1997). The animals used in this study were non-lactating ewes. Moss et al. (1980) suggested that increased serum prolactin concentrations in lactating ewes cause a decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, lowering the conception rate significantly. The pregnancy and conception rates found in the current study show that ovarian activity was adequately induced during the non-breeding season in non-lactating ewes.

Short-term consumption of β-carotene-supplemented rations increases ovulation rates in goats (Arellano-Rodriguez et al., 2007). Furthermore, prolonged β-carotene supplementation increases pregnancy rates, and long-term supplementation is required to increase the tissue concentration of the β-carotene molecule (Arechiga et al., 1998; De Ondarza et al., 2009). According to Ay et al. (2012), high β-carotene concentrations increase antioxidant activity, creating a favorable uterine environment for implantation and embryo development, and increasing pregnancy rates. Despite a body of research showing that β-carotene supplementation improves fertility (Haliloğlu et al., 2002; Arellano-Rodriguez et al., 2007; De Ondarza et al., 2009), some studies show that it has no effect (Wang et al., 1987; Arechiga et al., 1998; Çelik et al., 2009; Trojacanec et al., 2012). Arechiga et al. (1998) suggested that the failure of β-carotene injections to increase pregnancy rates could be attributed to embryos being resistant to antioxidants during the early developmental stages. These researchers also reported that only long-term dietary supplementation with β-carotene could increase β-carotene levels in the bovine oviduct and uterus. Similar to previous studies (Wang et al., 1987; Arechiga et al., 1998; elik et al., 2009; Trojacanec et al., 2012), the current study found that the pregnancy rate of the β-carotene-supplemented group did not increase (Table2).

Gore and Lehloenya (2020) reported that 50 mg/kg β-carotene added to a 60-day feeding period had no effect on follicle number, follicle diameter, or CL diameter in Saanen goats synchronized with 11-day CIDR during the breeding season. The authors also found that β-carotene increased plasma progesterone concentration and glutathione peroxidase activity without affecting estradiol 17-β concentration. They attributed this finding to the fact that β-carotene did not affect estradiol 17-β and LH concentrations. These two hormones, particularly estradiol 17-β, have a key role in controlling estrous behavior. In this study, we found no positive or negative effects on pregnancy rates with vitamin E, Se, or β-carotene supplementation administered simultaneously with synchronization and continued in the following days. These vitamins and minerals have been reported to positively affect ovarian functions, conception rates, and the sexual cycle in various animal species in some of the studies mentioned above (Haliloğlu et al., 2002; Arellano-Rodriguez et al., 2007; De Ondarza et al., 2009). The presence of an anestrus phase in the ovine sexual cycle, defined by the absence of ovarian functions and the presence of sexual hormones at basal levels, is the main factor that defines treatment success. Furthermore, as Gore and Lehloenya (2020) have suggested, the success of treatment may be related to the fact that estradiol 17-β and LH concentrations are not affected.

Because vitamin E and Se have similar biological effects that are demonstrated together, and their deficiencies are associated with similar symptoms, it is suggested that they should be administered together (Hostetler et al., 2003; Mehdi and Dufrasne, 2016). In a previous study, Awawdeh et al. (2019) used transitional Awassi ewes that were synchronized for estrus with a 12-day treatment with FGA-impregnated intravaginal sponges to administer vitamin E and Se injections at doses of 13.6 mg/kg and 0.045 mg/kg, respectively, at the time of sponge insertion, withdrawal, and 19 days after the withdrawal. The rates of embryonic mortality and pregnancy were 24.3 % and 86.8%, respectively, in the treatment group and 44.8 % and 63.9 %, respectively, in the control group, based on progesterone testing on day 19 post-mating and pregnancy examination on day 40 post-mating. According to Liu et al. (2014), dry grass pastures in the Mediterranean region have insufficient vitamin E content, particularly during the summer and autumn. According to Koyuncu and Yerlikaya (2007), sheep fed grass on dry pastures and stubbles with low vitamin E levels are at a particularly high risk of deficiency. We found no statistically significant differences in fertility parameters between the groups in our study, which may be related to the geographic layout and climatic features of the study area. Because of the region's good pasture conditions, we believe there were no vitamin or mineral deficiencies.

Injections of vitamin E and Se during the breeding season enhanced estrous and pregnancy rates, offspring yields, and improved reproductive metrics in Merino sheep, according to a study by Koyuncu and Yerlikaya (2007). During the mating season, El-Shahat and Abdel Monem (2011) added various amounts of vitamin E and Se to the feed of Baladi sheep and saw a higher pregnancy rate in the experimental groups compared to the controls. There are studies that show supplementation with vitamin E and Se improves sheep fertility (Koyuncu and Yerlikaya, 2007; El-Shahat and Abdel Monem, 2011; Awawdeh vet al., 2019), but there are also reports that claim supplementation has no such beneficial effects (Sanchez et al., 2008; Farahavar et al., 2020). Farahavar et al. (2020) injected Mehraban sheep with 5 ml Ese (0.5 mg/ml sodium selenite and 50 IU DI-α-tocopherol) 2 weeks before CIDR insertion, during CIDR insertion, and during CIDR removal at synchronization during the breeding season. CIDR stayed for 13 days, and the authors discovered no differences in estrous, pregnancy, or twin births between the groups. Se treatment before the breeding season increased embryonic mortality in synchronized sheep, according to Sanchez et al. (2008); this may have a negative impact on fertility. We found no harmful effects of vitamin E or se supplementation on fertility in this study. Supplementing sheep with vitamins and minerals via hormones or injections (as performed here) prior to, during, and after mating shouldn't have any negative consequences on pregnancy or lambing rates.

Köse et al. (2013) investigated sheep in anestrus throughout a 10-day synchronization based on progesterone (20 mg FGA). On the day of sponge removal, pregnancy rates and lamb yields were 59.1% and 45.5% for the β-carotene group (1 mg/kg), 50.0% and 68.2% for the vitamin E + Se (200 mg DL-alpha tocopherol acetate+0.67 mg Se) group, and 64.3% and 57.1% for the control group. According to the authors, the applications had no positive effect on fertility parameters. They also mentioned that the injections were given just before the expected estrous period, so there was no time for vitamin E or Se to exert their biological effects; additionally, only one administration was given. Furthermore, the application occurred shortly before mating, which may have prevented the cellular antioxidant effects of vitamin E and Se on the Graafian follicle and the oocyte within it. The addition of β-carotene, vitamin E, and Se resulted in no increase in estrous or pregnancy rates in the current study. However, unlike Köse et al. (2013), we made the β-carotene and vitamin E injections during both sponge insertion and sponge removal before estrus, suggesting that good pasture conditions during the study period may affect the results.

Kuru et al. (2017) found that injecting barium selenite into anestrus Pırlak ewes on the day of intravaginal sponge insertion had no effect on fertility parameters. Furthermore, Yıldız et al. (2015) discovered that a single injection of vitamin E and Se to dairy cattle prior to Ovsynch protocol treatment increased progesterone levels but did not increase the pregnancy rate. According to these researchers, antioxidants may not always be sufficient in preventing embryonic death or completely counteracting the effects of free oxygen radicals. The present study does not provide any data on embryonic deaths as the study design was based on ultrasonographic examination alone and it was not possible to detect embryonic deaths. According to Sarıbay and Erdem (2007), post-mating embryonic deaths can be detected by ultrasonography if repeated examinations are performed at regular intervals.

Van Niekerk et al. (1996) discovered that Se administration following synchronization/gonadotropin treatment had a negative effect on both pregnancy rate and litter size, with the latter being 19% lower than the litter size of animals in natural estrus. This result was attributed to a significant interaction between Se and the synchronization treatment. According to Sanchez et al. (2008), synchronization/gonadotropin treatments increase Se toxicity and even cause a progressive increase in this toxic effect in ewes. Twin-pregnant ewes were found to be more severely affected by this increased toxicity, according to these authors. According to Scaramuzzi et al. (2006), dietary supplementation with Se and vitamin E raises blood urea levels, which lowers the uterine pH and ovulation rate and results in the release of prostaglandin F2α (PGF2α). This has a negative impact on the pregnancy rate. The ewes' first pregnancy examination was performed on day 50 post-mating in the current study. Due to the lack of pregnancy examinations and plasma progesterone measurements during the embryonic development period, the potential effects (positive or negative) of vitamin E and Se supplementation on embryonic deaths could not be investigated.

While some researchers have reported that vitamin E and/or Se supplementation improves fertility in ewes (Koyuncu and Yerlikaya, 2007; El-Shahat and Abdel Monem, 2011; Awawdeh et al., 2019), others have suggested that supplementation has no effect (Segerson et al., 1986; Köse et al., 2013; Farahavar et al., 2020), and (Scaramuzzi et al., 2006; Sanchez et al., 2008). Awawdeh et al. (2019) attributed varying results in different studies to differences in the administration dose, administration route (dietary supplementation or parenteral injection), and timing of administration (before and/or after mating) of the supplements used, as well as the animals' pre-supplementation initial vitamin E and Se levels. Segerson et al. (1986) suggested that protein, energy, calcium (Ca), magnesium (Mg), and phosphorus (P) levels in the diet could also be effective. According to Van Metre et al. (2001), when investigating the underlying causes of fertility problems in a flock, the effects of all nutrients on fertility should be considered, not just vitamin E and Se. There was no blood analysis performed in this study to determine the pre-supplementation Se, β-carotene, and vitamin E levels in ewes. As a result of the lack of data, the extent to which supplementation had an effect could not be demonstrated.

According to a study on vitamin E and Se supplementation in ewes treated for estrus synchronization, pre-treatment blood vitamin E and Se levels should be measured, and treatment should be adjusted accordingly (Segerson et al., 1986; Van Metre et al., 2001). In the present study, vitamin E and Se supplementation having not caused any alteration in the fertility parameters suggested that the pre-treatment initial vitamin E and Se levels of the ewes should have been determined by blood analyses and supplementary injections should have been performed according to the measured blood levels.

CONCLUSION

As a conclusion, it was found that progesterone-impregnated intravaginal sponges and vitamin E supplementation did not increase reproductive parameters at a synchronized estrus in sheep under range conditions during non-breeding season. More thorough studies examining seasonal nutritional conditions as well as blood analyses for Se, β-carotene, and vitamin E levels could establish the impact of supplementing with Se, β -carotene, and vitamin E on reproductive parameters in synchronization programs used in non-breeding seasons.

DECLARATIONS

Ethics Approval

This study was conducted pursuant to the 28/01/2021 dated and 2021/01-09 numbered approval of the Local Ethics Board for Animal Experiments of Hatay Mustafa Kemal University.

Conflict of Interest

Any conflict of interest exists not.

Consent for Publication

Not applicable

Author contribution

Idea, concept and design: Sarıbay MK,

Data collection and analysis: Özar E, Sertkol R, Sarıbay MK

Drafting of the manuscript: Sarıbay MK, Köse AM

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Data Availability

Data can be accessed from the author when needed.

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