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Effects of using hCG and Beta Carotene + Vitamin E Alone or in Combination on Fertility in Estrus-Synchronized Awassi Ewes

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ABSTRACT

This study was carried out in four different farms in Diyarbakır to determine the effects of beta carotene + vitamin E and HCG on fertility during the transition period (1.5-2 months before the breeding season). A total of 306 Awassi ewes were divided into four groups before the study. All groups were treated with a progesterone sponge for 12 days, PGF2α two days before sponge removal, 600 IU eCG on the day of sponge removal, and 150 IU hCG on the day of mating. The control group of ewes (n = 91) did not receive any additional treatment. The ewes in the vitamin group (n = 61) were treated twice with Beta (β) carotene + vitamin E once 7 days before sponge insertion and again on the day of mating. The ewes in the hCG group (n = 74) received 150 IU hCG on day 12 after mating. The ewes in the vitamin + hCG group (n = 80) received both β-carotene + vitamin E and hCG. Ewes showing signs of estrus were mated with rams of known fertility. Pregnancy was diagnosed by ultrasonography on day 30 after mating. Estrus, pregnancy, lambing, fecundity, and multiple birth rates were similar among the control, hCG, vitamin, and vitamin + hCG groups (P>0.05). It was concluded that treatment with vitamins (β-carotene + vitamin E), hCG, or their combination, in addition to estrus synchronization during the transition period, did not significantly improve fertility parameters in Awassi ewes.

Keywords: Awassi ewes; Beta carotene; hCG; Estrus synchronization; Progesterone

Östrus Senkronize İvesi Koyunlarında Beta Karoten + Vitamin E ve hCG 'nin Tek Başına veya Birlikte Kullanımının Fertilite Üzerine Etkileri

Süreç

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Teşekkür
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ÖZ

Bu çalışma, Diyarbakır'daki dört farklı çiftlikte, geçiş döneminde (aşım sezonundan 1,5-2 ay önce) beta karoten+ vitamin E ve Hcg'nin fertilite üzerindeki etkilerinin belirlenmesi amacıyla yürütüldü. Toplam 306 İvesi koyunu, çalışma öncesinde dört gruba ayrıldı. Tüm gruplara 12 gün boyunca progesteron süngeri, sünger çıkarılmadan iki gün önce PGF2α, sünger çıkarma gününde 600 IU eCG ve çiftleşme gününde 150 IU hCG uygulandı. Kontrol grubundaki koyunlara (n = 91) herhangi bir ek tedavi uygulanmadı. Vitamin grubundaki koyunlara (n = 61), sünger yerleştirilmeden 7 gün önce ve çiftleşme gününde olmak üzere iki kez Beta (β) karoten + E vitamini uygulandı. HCG grubundaki koyunlara (n = 74), çiftleşmeden sonraki 12. günde 150 IU hCG uygulandı. Vitamin + hCG grubundaki koyunlara (n = 80) hem β-karoten + E vitamini ve hem de hCG tedavisi uygulandı. Kızgınlık belirtileri gösteren koyunlar, bilinen fertiliteye sahip koçlarla çiftleştirildi. Gebelik teşhisi, çiftleşmeden sonraki 30. günde ultrasonografi ile yapıldı. Kontrol, hCG, vitamin ve vitamin + hCG grupları arasında kızgınlık, gebelik, kuzulama, döl verimi ve çoğul doğum oranları benzerdi (P>0.05). Sonuç olarak, İvesi koyunlarında geçiş döneminde östrus senkronizasyonuna ek olarak vitamin (β-karoten + E vitamini), hCG veya her ikisiyle birlikte yapılan uygulamaların fertilite parametrelerini önemli ölçüde iyileştirmediği sonucuna varıldı.

Anahtar Kelimeler: İvesi koyunu; Beta karoten; hCG; Östrus senkronizasyonu; Progesteron

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İntroduction

Embryonic loss during the pre-implantation stage is among the most significant factors restricting optimal reproductive efficiency in livestock. In ewes, 30% to 40% of fertilized ova are lost throughout the pre-implantation phase and the initial three weeks of gestation, while losses increase to as much as 70% to 80% within the first three weeks following insemination. (Bazer et al., 2014). However, it has been suggested that embryonic losses are preventable, as fertilization failure accounts for only 5% to 10% of losses in ewes (Khan et al., 2007). Insufficient luteal function is accepted as one of the important causes for embryonic mortality (de Brun et al., 2016). Therefore, various therapeutic applications have been used to improve luteal function in order to reduce embryonic losses. One of these therapeutic strategies is post-breeding progesterone (P4) application, which can be administered either as exogenous P4 therapy directly or as gonadotropin treatment to increase endogenous P4 levels. It has been suggested that treatment with Gonadotrophin Releasing Hormone (GnRH) or Human Chorionic Gonadotropin (hCG) on days 10, 11, 12, or 13 post-copulation can improve pregnancy rates by approximately 10% in ewes (Beck et al., 1994; Khan et al., 2009). However, quite contradictory results have been obtained from studies, and therefore new studies are needed.

Another approach to decreasing embryonic mortality in ewes involves the supplementation of specific minerals and vitamins known to enhance reproductive performance. The levels of certain vitamins and minerals in the body, such as β-carotene, vitamin E, and selenium, sourced from green grasses, play a vital role in fertility. If these substances are deficient, external supplements should be administered. (Yokuş et al., 2006). Beta carotene plays a crucial role in steroid hormone synthesis and ovulation, as it serves as the sole source of vitamin A in granulosa cells. Studies have shown that the corpus luteum (CL) contains high levels of β-carotene, which significantly influences luteal cell function alongside vitamin A. (McGrath et al., 2018). Rapaport et al. (1998) suggested that the ability of the corpus luteum to secrete progesterone (P4) is connected to the high levels of β-carotene in the ovaries. It has been suggested that supplementing β-carotene in animals such as cows, ewes, goats, and rabbits enhances their reproductive performance (Meza-Herrera et al., 2013; Özpınar et al., 1994). Özpınar et al. (1994) found that administering β-carotene injections every 20 days higher pregnancy rates, lamb production, and twinning rates following the first insemination in ewes.

Vitamin E, recognized as an essential nutrient for reproduction since 1922, is composed of tocopherols and tocotrienols. Research has shown that α-tocopherol safeguards cell membranes from oxidative harm by neutralizing lipid radicals generated during lipid peroxidation. (Traber &Atkinson, 2007). It also interferes with free radical intermediates and prevents oxidative reactions (Mohebbi-Fani et al., 2012). Vitamin E supplementation can prevent oxidative damage in the ovarian epithelium caused by ovulation stimulation in ewes (Murdoch & Martinchict, 2004). Previous studies on the impact of vitamin E on fertility have reported varying outcomes (Gabryszuk & Klewiec, 2002; Koyuncu et al., 2007; Yaprak et al., 2004). Various studies have been carried out to examine the effects of hCG treatment or the supplementation with different vitamins on breeding outcomes following mating in ewes. (Catalano et al., 2015; Kaya et al., 2013; Köse et al., 2013). As far as the authors are aware, no studies have examined the impact of combined administration of hCG, β-carotene, and vitamin E on reproduction in ewes.

The aim of this study was to combine two different strategies used to reduce embryonic losses and improve pregnancy rates in ewes. Therefore, the objective was to evaluate the impact of hCG, β-carotene, and vitamin E treatments, either individually or in combination, on fertility in estrus-synchronized Awassi ewes during the transition to the breeding season.

Material and Methods

Ethical statement

The procedures for this study were authorized by the Local Ethics Committee for Animal Experiments at the Dıcle University Health Sciences Application and Research Center (Document No. 25249).

Animals and experimental design

This study was conducted during transition period (1.5 to 2 months before season) at four different farms in Dıyarbakır province, located in south-eastern Türkiye. The farms were located close to each other, with the distance between the farthest farms was about 15 km, and the ewes grazed on pastures with similar habitat characteristics. The latitude, longitude, and altitude of this region were 37°55′01″N, 40°16′46″E, and 660 m, respectively.

The ewes were allowed to graze on natural pasture throughout the day, with water provided ad libitum. Only healthy ewes were included in the study; however, body condition scores were not evaluated. A total of 306 Awassi ewes, aged 2 to 4 years, average weight 45-50 kg were haphazardly divided into four groups; control, vitamin, hCG, hCG+vitamin. A standard estrus synchronization protocol was applied. For this purpose, intravaginal sponges containing 20 mg of flugestone acetate (Chronogest CR, MSD Animal Health) were inserted into the vaginas of all ewes and kept in place for 12 days. Two days before sponge removal, each ewe received 250 µg prostaglandin F2 alpha (PGF2α, Estrumate, MSD Animal Health) via the intramuscular (im) route. On the day of sponge removal, 600 IU of equine chorionic gonadotropin (eCG, Chronogest, MSD Animal Health) was administered IM. Additionally, 150 IU of human chorionic gonadotropin (hCG, Chorulon, MSD Animal Health) was administered IM on the day of mating. No additional treatment was given to the control group (n = 91). The treatment groups received the following: β-carotene + vitamin E (Ovostim, Provet) at a dose of 0.5 ml/10 kg was injected IM to the vitamin group (n = 61) 7 days before sponge insertion and again on the day of mating. The hCG group (n = 74) received 150 IU of hCG on day 12 post-mating. The vitamin + hCG group (n = 80) received both β-carotene + vitamin E and hCG.. Estrus signs were observed with the help of fertile rams aged 3 to 5 years over a period of four consecutive days, beginning on the day of sponge removal. After observation of the estrus, ewes were mated (ewe to ram ratio: 10:1). Pregnancy was diagnosed by ultrasonography on day 30 after mating. Reproductive performance was assessed using the following parameters: pregnancy rate (number of pregnant ewes/ number of ewes that showed estrus and were mated), lambing rate (number of ewes that lambed/ number of mated ewes), multiple birth rate (number of ewes with multiple births/number of ewes that lambed), and fecundity rate (number of lambs born/number of ewes that lambed). Pregnancy was diagnosed by ultrasonography on days 35 to 45 post-mating.

Statistical analyses

The results were presented as percentages, and the Chi-Square test was applied for group comparisons. Data analysis was performed using SPSS/PC software (Version 10.0; SPSS, Chicago, IL, USA), with a significance level of P<0.05 considered statistically significant.

Results and Discussion

The fertility results in ewes are presented in Table 1. The study found no significant differencesamong the groups in terms of estrus, pregnancy, lambing, multiple birth, and fecundity rates (P>0.05). The estrus rate were found as 96.7%, 98.4%,100% and 95.0% for the control, vitamin, hCG and hCG+ vitamin groups, respectively. Compared to the control group, hCG group exhibited numerically higher pregnancy and lambing rates but lower multiple birth and fecundity rates (P>0.05). Unexpectedly, multiple birth rate in the vitamin group was numerically lower than that in the control group (19.0% vs. 29.8%, P>0.05). The hCG + vitamin group showed slightly higher pregnancy (73.7% vs. 67.0%, P>0.05) and lambing rates (71.1% vs. 64.7%, P>0.05) compared to the control group.

Table 1. Comparison of fertility results among study groups.

Groups	Control n= 91	Vitamin n= 61	hCG n= 74	hCG + Vitamin n= 80	P
Estrus rate % (n/n)	96.7 (88/91)	98.4 (60/61)	100 (74/74)	95.0 (76/80)	>0.05
Pregnancy rate[a] % (n/n)	67.0 (59/88)	73.3 (44/60)	70.3 (52/74)	73.7 (56/76)	>0.05
Lambing rate[b] % (n/n)	64.7 (57/88)	70.0 (42/60)	68.9 (51/74)	71.1 (54/76)	>0.05
Multiple births rate[c] % (n/n)	29.8 (17/57)	19.0 (8/42)	21.6 (11/51)	27.8 (15/54)	>0.05
Fecundity rate[d] % (n/n)	131.6 (75/57)	121.4 51/42	121.6 (62/51)	131.5 (71/54)	>0.05

[a]: Number of ewes pregnant/all ewes mated
[b]: Number of ewes lambing/all ewes mated
[c]: Number of ewes giving multiple births/number of ewes lambing
[d]: Number of lambs born/ number of ewes lambing

The pregnancy rate in the control group was 67.0%. Compare to the control group, slight but non-significant increases were observed in the vitamin, hCG and HCG + vitamin groups, with pregnancy rates of 73.3%, 70.3%, and 73.7%, respectively. The lambing rates were also similar among the groups: 64.7% in the control group, 70.0% in the vitamin group, 68.9% in the hCG group and 71.1% in the hCG + vitamin group. Interestingly, the control group exhibited the highest multiple birth rate numerically. However, no significant differences were found in multiple birth (29.8%, 19.0%, 21.6%, and 27.8%) or fecundity rates (131.6%, 121.4%, 121.6%, and 131.5%) among the control, vitamin, hCG, and hCG+vitamin groups, respectively (P>0.05).

Studies have shown that hCG treatments, administered in various doses and regimens (single or multiple injections) during late diestrus in parous ewes, can positively impact fertility (Cam & Kuran, 2004; Kittok et al., 1983; Moeini et al., 2013; Nephew et al., 1994; Rostami et al., 2017), although some exceptions exist (Khan et al., 2007). Administering multiple injections of hCG (100 IU) on days 11, 12, and 13 post-mating has been demonstrated to elevate plasma P4 levels and increase conception rates (58% vs. 29%) in lactating anoestrus ewes (Kittok et al., 1983). Likewise, Nephew et al. (1994) suggest that a single injection of hCG (100 IU) on day 11.5 increased length of blastocysts and IFN-τ concentrations, and tended to improve pregnancy rates (84% vs. 95%) in ewes mated during spontaneous estrus. In a study conducted on ewes mated at spontaneous estrus, (Cam & Kuran, 2004) found that a 150 IU hCG treatment on the 12th day post-mating significantly improved the non-return rate, lambing rate, and twinning rate. Rostami et al. (2017) reported that treatment with 400 IU hCG on day 11 post-mating significantly increased plasma P4 concentration, conception rate, and litter size outside the breeding season.

However, in our study, no positive effect of hCG treatment administered on the 12th day after mating was observed on fertility parameters. The diverse results obtained in different studies may be due to factors such as breeding season, breed, nutritional and physiological status, and management systems (Moeini et al., 2013). It has been hypothesized that these differences may also be due to variations in the synchronization protocols used (Cam & Kuran, 2004). Since we routinely applied hCG injection at the time of mating in ewes with induced estrus in our field practice, we also administered hCG to all groups at the time of mating in this study. Mirzaei et al. (2014) reported that hCG treatment given two days after the removal of the P4 sponge significantly increased the pregnancy rate (86.4% vs. 60.0%). Similarly, it has been shown that hCG treatments at the time of mating or 12 days later resulted in similar pregnancy rates (50.0% and 47.9%, respectively), both significantly higher than the control group (35.2%) (Moeini et al., 2013). In our research, hCG treatment on the day of mating may positively affect ovulation and CL development. However, ovulation rates and P4 levels were not evaluated. Therefore, the potential positive effects of hCG treatments administered at the time of mating may have masked any differences in fertility outcomes between the hCG and control groups.

It is well known that vitamins play an essential role in the reproductive performance of animals. Natural antioxidants such as vitamin E, β-carotene, and vitamin C help counteract oxidative stress caused by various physiological and pathological conditions (Nayyar & Jindal, 2010). However, conflicting results have been reported regarding the effects of estrus synchronization combined with β-carotene and / or vitamin E supplementation on the reproductive performance of small ruminants. It has been reported that short-term β-carotene supplementation can improve ovarian function and P4 synthesis in goats (Arellano-Rodriguez et al., 2009). Kaçar et al. (2008) reported that out of season supplementation with testosterone antibody, β-carotene and vitamin E increased the incidence of multiple pregnancies in Tuj ewes. In contrast, Köse et al. (2013) reported that β-carotene injection (1 mg/kg) on the day of sponge removal in synchronized, out of season ewes did not positively affect pregnancy rate or lamb yield. While Koyuncu & Yerlikaya (2007) found that vitamin E supplementation improved estrus incidence and fertility in ewes, other studies (Gabryszuk & Klewiec, 2002; Yaprak et al., 2004) showed no such effect. More recently, Farahavar et al. (2020) reported that administering vitamin E and selenium supplements to ewes kept on pasture two weeks before CIDR insertion, at the time of insertion, and during CIDR removal did not improve fertility, prolificacy, or lambing rates. In a study conducted during the anestrus period in ewes, injection of vitamin E combined with β-carotene did not result in the expected increase in lamb yield, although it did increase the rate of multiple births (Kaçar et al., 2008). In the present study, the vitamin group exhibited a lower numerical rate of multiple births compared to the control group (19.0% vs. 29.8%; P>0.05). This outcome was unexpected, even though the difference was not statistically significant. Ewes treated with β-carotene and vitamin E showed a numerical increase in pregnancy rates compared to the control group; however, this difference was not statistically significant.

Lack of a significant positive contribution from β-carotene + vitamin E supplementation to reproductive performance in our study suggests that the ewes may have already obtained these substances from pasture in sufficient quantities. Supporting this view, Beytut et al. (2005) reported that ewes in the Kars province and surrounding areas had the lowest plasma vitamin A levels during winter and fall, while the highest plasma β-carotene levels were observed in spring and summer. Similarly, Afshari et al. (2008) found that serum vitamin A and β-carotene levels in Ghezel ewes in Iran were significantly lower in winter compared to summer. The findings of the present study did not support the hypothesis that co-administration of hCG with β-carotene and vitamin E would improve fertility. Özmen et al. (2022) reported that incorporating β-carotene, vitamin E, and hCG into the estrus synchronization protocol outside the breeding season had no significant effect on the reproductive performance of Awassi ewe lambs. However, they observed that administering hCG 12 days after mating enhanced pregnancy, lambing, and fertility rates, resulting in an economic benefit. In contrast to our study, their investigation specifically focused on ewe lambs. The absence of differences between the β-carotene + vitamin E group and the control group in our study may be due to all ewes receiving adequate levels of vitamins and minerals from the pasture. Considering the region and season in which the study was conducted, it is likely that all ewes had been grazing on high-quality pasture for the past 2-3 months. Therefore, conducting similar studies during periods of poor pasture quality or in winter may provide more insight into the potential benefits of vitamin supplementation.

The study concluded that β-carotene + vitamin E treatment combined with estrus synchronization in ewes during the transition season and hCG administration on the 12th day post-mating, or a combination of these two strategies did not significantly improve fertility parameters.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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