

Oestrus synchronisation with progesterone-containing sponge and equine chorionic gonadotropin in Pirlak ewes during the non-breeding season: can Toryum improve fertility parameters?

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Received: May 1, 2020 Accepted: October 20, 2020

Abstract

Introduction: The aim of the study was to determine the effect of the vitamins, omega-3 polyunsaturated fatty acid and minerals in the supplement Toryum administered before and during oestrus synchronisation on some fertility parameters of ewes during the non-breeding season. **Material and Methods:** The experimental animals were clinically healthy Pirlak ewes, 55–75 days postpartum, aged 2–4 years and weighing 40–50 kg. A sponge was inserted into the vagina for 10 d (G1, n = 30; G2, n = 30) or 14 d (G3, n = 30; G4, n = 30) for oestrus synchronisation, and on the day of removal, 400 IU equine chorionic gonadotropin was injected. Toryum soft capsules were administered individually (1 capsule/ewe p.o.) to G1 and G3 ewes seven days before the sponge was inserted and on the day it was removed. Oestrus detection was started 12 h after sponge removal. Pregnancy was diagnosed by transrectal ultrasonography on the 30th day after mating. **Results:** The pregnancy rate was statistically different between G1 and G4 ($P < 0.05$). The onset of oestrus was statistically different ($P < 0.001$) between the 10-d groups (G1 and G2) and the 14-d groups (G3 and G4). The litter size and oestrus, conception, lambing, multiple birth, and survival rates were not significantly different between the groups ($P > 0.05$). **Conclusion:** Toryum administered to Pirlak ewes during progesterone-based oestrus synchronisation protocols during the non-breeding season may increase pregnancy rates. The relationship between Toryum and fertility parameters in ewes would be better understood by comprehensive studies.

Keywords: ewes, oestrus synchronisation, progesterone-containing sponge, Toryum, vitamins.

Introduction

The Pirlak, which is a hybrid of Daglic × Kıvrırcık, is a local sheep breed in Kütahya, Afyonkarahisar, Uşak, Manisa, Isparta and Burdur provinces in Turkey. Female Pirlaks are known to be resistant to poor environmental conditions and diseases (2). Pirlak ewes produce meat, milk and wool. The coat colour in this breed is usually white with black spots around the mouth and eyes and on the ears, and ewes are generally hornless.

Many sheep breeds are seasonally polyoestrous, and their sexual activities start when daily hours of sunlight decrease (11). In Turkey, ovine sexual activity generally starts in the autumn when daylight hours become shorter, and many sheep breeds do not show oestrus outside this season (17). There is not much difference in the follicle-stimulating hormone (FSH) concentration in ewes during the non-breeding and breeding seasons. However, the peak luteinising hormone (LH) level is much lower during the non-

breeding season than in the breeding season. The progesterone concentration in particular is at almost undetectable levels during anoestrus, and is significantly different over the two seasons. During the anoestrous period, both ovaries and the LH pulse production system are inactive (11).

Fertility may be increased in small ruminants with hormone administration. Progesterone suppresses GnRH and LH secretion in sheep and goats with negative feedback. After the progesterone concentration decreases rapidly, the LH wave level increases significantly, and the dominant follicle ovulates. Due to these effects in sheep and goats, progesterone is used in oestrus synchronisation protocols. Intravaginal sponges containing medroxyprogesterone acetate (MAP) and fluorogestone acetate are frequently used for oestrus synchronisation in ewes. Again, to obtain high oestrus rates and ovulation during the non-breeding season, equine chorionic gonadotropin (eCG) injections are given in addition to 11–14 days intravaginal insertion of progesterone-impregnated sponges, and oestrus starts in 24 to 48 h (1, 17). The gonadotropin has simultaneous FSH- and LH-like effects. For the progesterone treatment to be effective, endogenous gonadotropins need to be augmented by exogenous FSH provided by eCG administration, and in this way oestrous activity is induced during the non-breeding season (1). Equine chorionic gonadotropin is required for induction of ovulation in anoestrous sheep and precise synchronisation of ovulation before artificial insemination and embryo transfer (10). It should be kept in mind that oestrus synchronisation may be affected by factors such as nutrition, body condition score, lactation, age, temperature, and light (11, 17).

Deficient or imbalanced nutrition in ruminants may be contributive to reproductive problems such as delayed puberty, anoestrus, delayed ovulation, low pregnancy rate, and high loss rates of embryos and foetuses (26). In ewes, some trace minerals and vitamins may play an important role in fertility and affect reproductive performance (22). Some vitamins (like A and E) are integral to biological processes such as fertility and embryonic development. These vitamins can increase reproductive performance in ewes, reduce the oxidative stress caused by mating and pregnancy, and sustain fertility (12). Inadequate intake of trace elements may impair reproductive performance (19). Microminerals are involved in both the intracellular mechanism detoxifying them of free radicals and the stabilisation of secondary molecules. Additionally, microminerals affect endocrine activities because they are material for the structure of hormones, and changes in the plasma levels of these minerals may alter reproduction and production of hormones. The improper concentration of microminerals may negatively affect embryonic development and the postpartum process and lead to impaired fertility (15, 29).

The purpose of this study was to determine the effects of oral administration of Toryum, a supplement containing vitamins A, D₃, E, and K₃, omega 3, sodium,

potassium, calcium, magnesium, copper, iron, manganese, zinc, phosphorus, iodine, and selenium, on some fertility parameters in Pirlak ewes whose oestrus is synchronised by 10-d or 14-d placement of progesterone-containing sponges during the non-breeding season.

Material and Methods

Location, animals, and feeds. The study was carried out in March–April at a commercial sheep farm located in the Bolvadin district of the Afyonkarahisar province in Turkey. Bolvadin is at the coordinates of 38°42'40"N and 31°02'55"E and an altitude of 1016 m above sea level.

A total of 120 2–4-year-old clinically healthy Pirlak ewes 55–75 days postpartum were used. Ewes were selected with a weight of 40–50 kg and a body condition score of 2.5–3.0 (on a scale of 1, signifying extremely emaciated, to 5, signifying obese). Twenty fertile Pirlak rams were used for oestrus detection and mating.

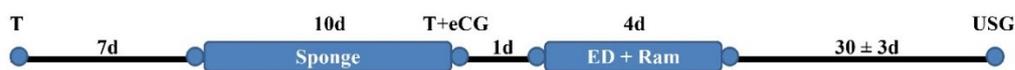
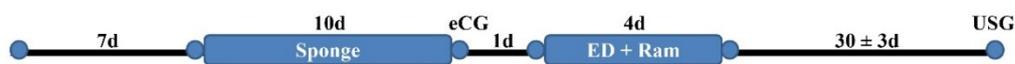
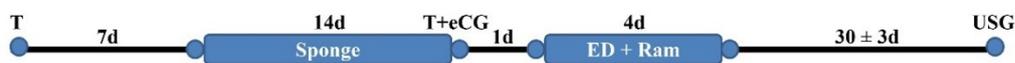
The ewes were fed hay, alfalfa hay, and barley throughout the synchronisation period. In pregnancy, they were additionally fed a concentrated feed at 450 g/ewe/day of 14% crude protein providing 2,700 kcal/kg metabolizable energy, which is described in Table 1. The animals were maintained under the same conditions for the whole study. During the synchronisation protocols, the ewes were penned on their farm, but after the treatment, the ewes were grazed in the pasture during the day and kept on the farm at night. Water was given *ad libitum* at all times.

Table 1. Ingredients and composition of concentrate mixture fed to ewes

| Items | % |
|-----------------------------|-------|
| Barley | 28.00 |
| Wheat bran | 16.65 |
| Corn | 40.00 |
| Cottonseed meal (38 CP) | 6.00 |
| Soybean meal (48 CP) | 5.28 |
| Marble powder | 2.77 |
| Salt | 1.00 |
| Vitamin and mineral premix* | 0.30 |

CP – crude protein; * – contents of vitamin and mineral premix in 1 kg: 100 g of Na, 33 mg of I, 7 mg of Ca, 27 mg of Se, 3,000 mg of Fe, 2,660 mg of Mn, 167 mg of Cu, 10,000 mg of α -tocopherol, 300,000 IU of cholecalciferol, and 3,000,000 IU of retinol

Oestrus synchronisation protocols. The ewes were synchronised with progesterone-containing sponges and eCG hormone during the non-breeding season. The sheep were divided into four groups ensuring the age, body weight, and condition score of the groups were balanced. Two groups were given Toryum soft capsules (Mira İlaç, Turkey) orally before and during synchronisation, which supplied the compounds and elements shown in Table 2.

Group 1 (Toryum + 10-d Progesterone + eCG, n=30)**Group 2 (10-d Progesterone + eCG, n=30)****Group 3 (Toryum + 14-d Progesterone + eCG, n=30)****Group 4 (14-d Progesterone + eCG, n=30)****Fig. 1.** Schematic view of synchronisation

eCG – equine chorionic gonadotropin; T – Toryum soft capsule; d – day; USG – transrectal ultrasonography; ED – oestrus detection

Table 2. Contents of Toryum soft capsules in 1 kg

| Vitamins | |
|--|--------------|
| Vitamin A | 5,000,000 IU |
| Vitamin D ₃ | 1,200,000 IU |
| Vitamin E | 50,000 mg |
| Vitamin K ₃ | 600 mg |
| Fatty acid | |
| Eicosapentaenoic Acid (EPA, Omega 3) | 43,450 mg |
| Docosahexaenoic Acid (DHA, Omega 3) | 29,000 mg |
| Minerals | |
| Sodium | 500 mg |
| Potassium | 500 mg |
| Calcium | 1,600 mg |
| Magnesium | 800 mg |
| Trace elements | |
| Copper (CuSO ₄) | 2,000 mg |
| Iron (FeCO ₃) | 2,000 mg |
| Manganese (MnSO ₄) | 2,000 mg |
| Zinc (ZnO) | 2,000 mg |
| Phosphorus (H ₃ PO ₄) | 800 mg |
| Iodine (NaI) | 100 mg |
| Selenium (Na ₂ SeO ₃) | 1,500 mg |

In group 1 (G1, n = 30) Toryum soft capsules were administered individually (one capsule/ewe p.o.) with an applicator seven days before sponge insertion and on the day of sponge removal (two capsules per sheep in total). A progesterone-containing sponge (Esponjavit, 60 mg MAP, Hipra, Turkey) was inserted into the vagina on day 0. The sponge was removed from the vagina on day 10 and 400 IU of eCG (Oviser, Hipra) was injected intramuscularly (Fig. 1).

In group 2 (G2, n = 30) a progesterone-containing sponge was inserted into the vagina on day 0. The sponge was removed from the vagina on day 10 and 400 IU of eCG was injected intramuscularly (Fig. 1).

In group 3 (G3, n = 30), as for group G1, Toryum soft capsules were administered individually (one capsule/ewe p.o.) with an applicator seven days before sponge insertion and on the day of sponge removal (two capsules per sheep in total). A progesterone-containing

sponge was inserted into the vagina on day 0. The sponge was removed from the vagina on day 14 and 400 IU of eCG was injected intramuscularly (Fig. 1).

In group 4 (G4, n = 30), like in group G2, a progesterone-containing sponge was inserted into the vagina on day 0. The sponge was removed from the vagina on day 14 and 400 IU of eCG was injected intramuscularly (Fig. 1).

Oestrus detection and mating. The rams were randomly divided into groups, and 12 h after removal of the sponge, the sheep were placed with rams for four days at a 6:1 female:male ratio.

Oestrus signs were monitored by observing the ewes four times a day for at least 20 min over a four-day period. The ewes showing mounting and standing behaviour were removed from the herd.

Pregnancy diagnosis. Pregnancy was confirmed 30 ± 3 days after mating, with a 7.5 MHz linear probe (WED-3000V, Shenzhen WELLD Medical Electronics, China) by transrectal ultrasonography, performed in a standing position. The ewe was accepted as pregnant when an embryo was observed.

Fertility parameters. Fertility parameters such as rates of oestrus onset, oestrus, pregnancy, conception, lambing, twin and triplet births, litter size, and survival in sheep were determined according to the formulae reported by Kuru *et al.* (16). Lambs were monitored for 30 days after birth and deaths among them during this period were recorded against the ewes which were their mothers, and the survival rate was determined. The multiple birth rate was calculated by combining the twin and triplet birth rates.

Statistical analysis. The time of oestrus onset (mean ± SEM) was analysed by one-way ANOVA, and the differences among the groups were determined by *post-hoc* Games–Howell test. To determine the intergroup differences in the other fertility parameters, a chi-squared test or Fisher's exact test was conducted. SPSS version 18.0, (SPSS, USA) software was used in all analyses. The differences between the groups were accepted as significant at P < 0.05.

Table 3. Changes in fertility parameters in the groups

| Fertility parameters | G1 (n = 30) | G2 (n = 30) | G3 (n = 30) | G4 (n = 30) | P value |
|---------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|---------|
| Oestrus onset (h) [‡] | 37.3 ± 0.8 ^a | 36.5 ± 0.9 ^a | 31.9 ± 1.4 ^b | 30.1 ± 1.3 ^b | < 0.001 |
| Oestrus rate (%) | 100 (30/30) | 96.7 (29/30) | 100 (30/30) | 93.3 (28/30) | NS |
| Pregnancy rate (%) [#] | 66.7 (20/30)* | 46.7 (14/30) | 50 (15/30) | 40 (12/30)* | < 0.05 |
| Conception rate (%) | 66.7 (20/30) | 48.3 (14/29) | 50 (15/30) | 42.9 (12/28) | NS |
| Lambing rate (%) | 100 (20/20) | 100 (14/14) | 93.3 (14/15) | 100 (12/12) | NS |
| Multiple birth rate (%) | 50 (10/20) | 14.3 (2/14) | 42.9 (6/14) | 25 (3/12) | NS |
| Litter size (n) | 1.55 | 1.14 | 1.43 | 1.25 | NS |
| Survival rate (%) | 87.1 (27/31) | 87.5 (14/16) | 95.0 (19/20) | 86.67 (13/15) | NS |

G1 – Toryum, 10-d progesterone-containing sponge, eCG, Toryum; G2 – 10-d progesterone-containing sponge, eCG; G3 – Toryum, 14-d progesterone-containing sponge, eCG, Toryum; G4 – 14-d progesterone-containing sponge, eCG; ^{a,b} – difference between values in the same row with different letters is significant; * – significant difference between G1 and G4; eCG – equine chorionic gonadotropin; [‡] – oestrus onset hour is given as mean ± SEM; [#] – pregnancy rate on the 30th d after mating; NS – non-significant

Table 4. Type of birth and gender rates in groups

| Parameters | | G1 (n = 30) | G2 (n = 30) | G3 (n = 30) | G4 (n = 30) | P value |
|----------------|----------|--------------|--------------|-------------|-------------|---------|
| Birth type (%) | Single | 50 (10/20) | 85.7 (12/14) | 57.1 (8/14) | 75 (9/12) | NS |
| | Twin | 45 (9/20) | 14.3 (2/14) | 42.9 (6/14) | 25 (3/12) | NS |
| | Triplets | 5 (1/20) | 0 (0/14) | 0 (0/14) | 0 (0/12) | - |
| Gender (%) | Female | 51.6 (16/31) | 37.5 (6/16) | 45 (9/20) | 40 (6/15) | NS |
| | Male | 48.4 (15/31) | 62.5 (10/16) | 55 (11/20) | 60 (9/15) | NS |

G1 – Toryum, 10-d progesterone-containing sponge, eCG, Toryum; G2 – 10-d progesterone-containing sponge, eCG; G3 – Toryum, 14-d progesterone-containing sponge, eCG, Toryum; G4 – 14-d progesterone-containing sponge, eCG; eCG – equine chorionic gonadotropin; NS – non-significant

Results

During the study, no premature births or stillbirths occurred. Additionally, none of the intravaginally inserted sponges became displaced during the study. One (1/14) ewe that was determined as pregnant in G3 did not give birth, and this was recorded as a pregnancy loss.

The oestrus onset time after removal of the sponge was significantly different between the groups ($P < 0.001$), the oestrus symptoms of G1 and G2 starting later than those of G3 and G4. Oestrus was detected by a mean time of 36–37 h in G1 and G2 and 30–32 h in G3 and G4 (Table 3). Thirty days after mating, the pregnancy rates in the groups were 66.7%, 46.7%, 50% and 40% in G1, G2, G3, and G4, respectively. The pregnancy rates of the G1 and G3 groups, which were administered Toryum soft capsules, were numerically higher than those of the G2 and G4 groups. However, there was only a statistically significant difference ($P < 0.05$) between the G1 and G4 rates (Table 3).

There was no significant difference in the groups in terms of the oestrus, conception, lambing, multiple birth, or survival rates, nor in litter size ($P > 0.05$). The oestrus rate was generally high in the groups. All sheep (100%) in G1 and G3 showed oestrus, and the numerically highest conception rates were also in these two groups. One ewe in G3 did not give birth despite having been determined as pregnant, while all ewes diagnosed pregnant in the other groups (100%) gave birth. The multiple birth rates of G1 and G3 (50% and 42.9%, respectively) were numerically higher than those of the

other groups. The mean litter size in the groups was between 1.14 and 1.55. In the 30-day period after birth, four lambs in G1, two in G2, one in G3 and three in G4 died (Table 3).

There were higher twin birth rates in G1 and G3 (45% and 42.9%, respectively) in comparison to the other two groups (14.3% and 25%, respectively), but there was no statistically significant difference ($P > 0.05$). One ewe in G1 gave birth to triplets (Table 4). The gender distributions of the lambs in the groups were not significantly different ($P > 0.05$) (Table 4).

Discussion

As many sheep breeds are sexually inactive during the non-breeding season, their oestrus can be stimulated by exogenous hormone manipulation. In this process, mostly progestogens are preferred, and progestogen treatment creates a dioestrus effect during the non-breeding season as if there is the corpus luteum (1, 10, 17). Nevertheless, eCG needs to be added to this treatment, because it provides higher pregnancy rates (5). The combination of a norgestomet implant with eCG in ewes during the non-breeding season increased reproductive performance (9). Moreover, some trace minerals and vitamins can improve reproductive parameters in ewes notably and enhance the performance of lambs (4, 20, 22). No information about the administration of Toryum having been found, this study sought to investigate if the soft capsules of this supplement could affect fertility parameters before and

during synchronisation of induced oestrus in Pirlak ewes with progesterone-containing sponges and eCG treatment during the non-breeding season.

After synchronisation with progestogens during the non-breeding season, the onset of oestrus occurred after 22.5–35.1 h in Kivircik ewes (6) and 32.1–35.1 h in Awassi ewes (7). Segureña ewes were synchronised with controlled internal drug release (CIDR) and eCG treatment for 5, 6, 7, and 14 days. The oestrus and pregnancy rates were similar among the groups, while oestrus onset came in a shorter time in 14-d progesterone treatment ewes (21). In our previous study in Pirlak ewes, oestrus started later in the 11-d progesterone group than in the 14-d group (16). In this study, the oestrus onset was 30.1–37.3 h later and was consistent with the results of many studies. The oestrus onset time was shorter after 14-d progesterone treatment than 10-d ($P < 0.001$). Likewise, the oestrus onset time was influenced by sponge treatment days (10-d or 14-d).

In studies on synchronisation during the non-breeding season using progestogens, the oestrus rates were reported as 77.8%–88.9% in Kivircik ewes (6) and 96.8%–97.8% in Pirlak ewes (3). In our previous study on Pirlak ewes, the oestrus rate was between 92% and 100% (16). In this study, the oestrus rate was 93%–100% in Pirlak ewes treated with progesterone-containing sponges for different durations (10-d or 14-d) and eCG. If long-term intravaginal sponge treatments are to be performed, the 10-d progesterone protocol may be preferred.

Oestrus synchronisation using progestogens yielded pregnancy rates of 37.5%–44% in Pirlak ewes (16), 56%–84% in Merino ewes (23), and 76% in Kivircik ewes (6). In Pirlak ewes with intravaginal progesterone-containing sponge and eCG injection treatment, conception rates between 41% and 49.5% were determined (3). In our study, the pregnancy rate was between 40% and 66.7% and the conception rate was between 42.9% and 66.7%. The pregnancy rate we determined was generally compatible with those reported in previous studies, but it was lower than the pregnancy rate in Kivircik ewes, and this may have been influenced by the breed factor. The pregnancy rate in the Pirlak ewes was not affected by different lengths of progesterone-containing sponge treatment (10-d or 14-d).

In our previous study on Pirlak ewes, the lambing rate was in the range of 95%–100%. The twin birth rate was 11.11%–22.75%, and the mean litter size was in the range of 1.11–1.23 (16). In this study, the lambing rate was 93.3% to 100%, and the mean litter size was in the range of 1.25–1.55. These findings were in agreement with a previous report (3). After synchronisation with progesterone in Pirlak ewes (16), the survival rate of the lambs was between 91.7% and 100%. In this study, the survival rate varied from 80% to 95% in the groups. The survival rate in this study was lower than those reported in previous studies and may have been due to season, care and feeding differences.

After 12-d progesterone-containing sponge and eCG treatment in Malpura ewes, minerals and antioxidants (cobalt, chromium, zinc, selenium, and vitamin E) were added to the feed of one group and this group and a group with unaugmented feed were subjected to heat stress. While the oestrus rate did not vary between the treatment and control groups, the oestrus duration was longer on controls and supplemented heat-stressed ewes compared to ewes under heat stress without feed enrichment (25). In Awassi ewes, oral vitamin E and trace minerals (Se, Ca, Cu, and Co) given in addition to 14-d progesterone and eCG treatment did not change the gestation length or the rates of oestrus, total pregnancy, or lambing, while it increased the number of multiple births and litter size (30). Again, in Awassi ewes, feed supplementation containing multiple nutrients such as vitamins, minerals, amino acids, and sorbitol affected animal health and reproductive performance positively, as well as positively affecting embryo survival, litter size, and birth weight (13). The supplement treatment given in this study did not significantly change the oestrus, lambing, or survival rates. However, we determined oestrus in all ewes (100%) in the groups that were given Toryum. Oestrus onset time was not affected by Toryum, while days of sponge placement (10-d or 14-d) did have effect. The pregnancy rate was the fertility parameter most affected by supplementation, and in the treated groups it increased. The more pronounced effect was the combination of the 10-d progesterone treatment and Toryum, because the highest pregnancy rate was achieved. The multiple birth rate and litter size also increased, but the difference was not statistically significant.

Se and vitamin E have antioxidant properties. In Se deficiency, suboestrus, early embryonic mortality, and low fertility rates may be observed (26, 29). Likewise, vitamin E prevented ovulation-induced oxidative base damage in the ovary epithelium (20). In Tuj ewes, vitamins A and E and testosterone antibody in addition to oestrus synchronisation protocols increased reproductive performance (12). During oestrus synchronisation, inserting the progesterone-containing sponge into the vagina may lead to vaginitis and oxidative stress (18). To mitigate this oxidative damage, vitamin E and Se may be injected (4). Awassi ewes were synchronised with 12-d progesterone and eCG treatment during the non-breeding season, and additionally, vitamin E and Se injections were given to one group in the experiment. In the ewes to which vitamin E and Se injections were given, the first service pregnancy rate was higher than those that were not given injections (93.3% and 64%, respectively). No positive effect on other fertility parameters was determined (4). In Mehraban ewes, vitamin E and Se injection in addition to 13-d progesterone and eCG treatment had no effect on fertility (8). In our previous study on Pirlak ewes, likewise no effect on fertility parameters was seen from Se injection in addition to progesterone and eCG

treatment (16). In this study, Toryum affected the pregnancy rate positively, while it did not create a statistically significant difference in the other fertility parameters.

The Toryum soft capsules that were used contain vitamin E and Se, in addition to vitamins A, and D₃, omega 3, minerals (Na, K, Ca, Mg), and trace elements (Cu, Fe, Mn, Zn, P, I). These vitamins, minerals, and trace elements in particular may have shown a positive effect on the fertility parameters. As support for this idea, in Red Karaman ewes that were synchronised in anoestrus, the Cu and Zn concentration decreased during mating, and this influenced the pregnancy rate negatively (28). In ruminants, while anoestrus, delayed oestrus, reduced pregnancy rate, embryo death, and stillbirth may occur in Cu deficiency (27), in the case of decreased serum Zn concentration, pregnancy and oestrus rates decrease (15, 29). This is because Zn has an important role in implantation in ewes (29). Additionally, Mn takes a part in the functioning of the corpus luteum and production of sex hormones (27), and reduced pregnancy rate, infertility and abortion may occur in its deficiency (15). Iodine deficiency may also result in early embryo death, abortion, delayed puberty, irregular sexual activity, and fertilisation problems (15). Iodine supplementation may increase the twin birth rate in ewes (24). In Karakaya ewes, addition of omega-3 polyunsaturated fatty acids into feeds during 14-d progesterone and eCG treatment increased fertility (14). Toryum is a complex supplement drug that contains the vitamins, minerals, trace elements, and omega-3 fatty acids reported in the aforementioned studies. It caused an increase in some fertility parameters (litter size, multiple birth rate, and oestrus rate), but we could not determine statistically significant differences. The most important augmentative effect of Toryum was on the pregnancy rate. Due to increases in it, this supplement will provide higher lamb production, and economic gain will be greater.

In conclusion, according to the fertility parameters obtained for Pirlak ewes, the 10-d progesterone treatment may be preferred over that of 14 d. Before and during oestrus synchronisation during the non-breeding season, treatment with Toryum will contribute positively to fertility parameters and increase lamb production. More comprehensive studies on the effect of this treatment on fertility parameters in oestrus synchronisation protocols (especially in short-term progesterone treatments) should be conducted in ewes.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The authors declare that they did not have any funding source or grant to support their research work.

Animal Rights Statement: This study was carried out after obtaining approval from the Kafkas University's Ethics Committee for Animal Experiments (KAÜ-HADYEK) and permission from the Turkish Ministry of Agriculture and Forestry.

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