

Effect of Bakofix[®] treatment prior to estrus synchronization on selected fertility parameters in Anatolian Merino ewes during the non-breeding season

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Summary

This study investigated the effect of Bakofix[®] (a supplement consisting of vitamin E, copper, zinc, manganese, cobalt, selenium, and calcium) on the reproductive performance of Anatolian Merino sheep. A total of 80 clinically healthy sheep were used in the study. They were synchronized with a progesterone-containing sponge outside the breeding season and divided into three groups. The first group (G1, n = 25) was administered Bakofix[®] treatment 7 days before estrus synchronization, the second group (G2, n = 25) was administered Bakofix[®] treatment when estrus synchronization began, and the third group served as the control (G3, n = 30). G1 showed a higher estrus response, conception rate, and pregnancy rate compared to the other groups. Particularly remarkable was the 84% pregnancy rate obtained in G1. The fecundity and litter size in G1 were 2.4 and 2.8, respectively, which were higher than those in the other groups. The multiple birth rate in G1 was significantly higher than that in G3, and Bakofix[®] treatment seven days before synchronization had a positive effect on the multiple birth rate, with a statistically significant difference between the two groups ($P < 0.05$). Although the multiple birth rate was high in G1, the birth weights of the lambs were not negatively affected. In conclusion, vitamin, mineral, and trace element supplementation can be applied to increase the reproductive performance of Anatolian Merino ewes, especially outside the breeding season and before estrus synchronization, which can lead to increased economic gain.

Keywords: estrus stimulation, fertility, reproductive performance, sheep, small ruminant, trace element

Synchronization of estrus in sheep is a technique employed to regulate reproductive cycles. Progesterone or progesterone analogs can be used for estrus synchronization in sheep outside of the breeding season. Progesterone is a hormone naturally secreted during the estrus cycle that suppresses ovulation. Provision of exogenous progesterone regulates this mechanism, leading to synchronized estrus in all ewes upon the completion of the progesterone treatment. Progesterone and equine chorionic gonadotropin (eCG) are two hormones frequently employed in estrus synchronization protocols for sheep. eCG, in contrast, promotes follicular growth and maturation, ultimately inducing estrus in sheep. Progesterone and eCG treatment during the anestrus period can trigger follicular development and ovulation, resulting in the synchronization of estrus within a group of ewes (1, 5, 6, 17).

Sheep farming is commonly practiced in many regions, with a focus on grazing-based systems. In such systems, the quality of the pasture is critical for preventing the occurrence of vitamin and mineral deficiencies in sheep. However, it is possible for sheep to develop deficiencies in these essential nutrients. Outside the breeding season, the use of estrus synchronization protocols can result in reduced fertility rates and economic losses. Insufficient or imbalanced nutrition in ruminants can lead to an increase in puberty age, anestrus, ovulation delay, and embryonic deaths. Certain vitamins and minerals play vital roles in fertility as well as the early and late stages of embryonic development. They also act as buffering mechanisms against reproductive stress in sheep. For example, vitamins A, D, and E, as well as minerals, such as zinc and selenium, are essential for reproductive processes.

These nutrients are involved in follicular development, ovulation, fertilization, and embryo development (2, 7, 24, 26). Deficiencies in vitamins and minerals may lead to oxidative stress, which can cause DNA damage, impair embryo development, and reduce reproductive performance. To prevent such negative outcomes, it is crucial for sheep farmers to provide balanced and adequate nutrition to their animals. They must use appropriate supplements and feeds to ensure that sheep receive the necessary nutrients, especially during critical reproductive stages. Adequate nutrition with appropriate vitamin and mineral supplementation is crucial for enhancing the reproductive performance of sheep, reducing economic losses, and ensuring the sustainability of sheep farming (2, 7, 8, 24, 26).

Micro minerals play a crucial role in scavenging and stabilizing free radicals when oxidative stress is high. They can also contribute to the structure of hormones in endocrine activities, and their deficiencies can impact reproduction and hormone production. Low plasma concentrations of certain minerals can negatively affect embryonic development, postpartum period, and fertility (7, 14, 26, 28). Therefore, vitamin and mineral combinations can be used in sheep prior to estrus synchronization to increase fertility parameters (2, 10, 16).

The Anatolian Merino sheep, which is a crossbreed of 80% German Mutton Merino and 20% Akkaraman, is raised in the Central Anatolia region for both meat and wool production because of its high productivity. The breed has adapted to the harsh climate conditions and rugged geography of the region thanks to its crossbreeding with Akkaraman sheep. This sheep has become very important for the local people and is spread across many provinces in Central Anatolia (11, 12). Although there have been several studies on the production traits of the Anatolian Merino sheep, such as meat, wool, and growth traits, only a few studies have been conducted on estrus synchronization (3, 9, 25). Furthermore, no studies have been found that investigate the effects of support treatments to improve fertility parameters during estrus synchronization outside the breeding season. Therefore, this study was conducted to investigate the effect of treatment with Bakofix® (vitamin E, copper, zinc, manganese, cobalt, selenium, and calcium) (Aldo İlaç, Türkiye) prior to estrus synchronization with progesterone-containing sponges on certain fertility parameters in Anatolian Merino sheep during the non-breeding season. This may be the first study conducted on this subject in Anatolian Merino sheep.

Material and methods

Ethical approval. The study was conducted with the approval of the Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2022-112) and permission from the Ministry of Agriculture and Forestry of the Republic of Türkiye.

Location. The estrus synchronization treatment was conducted in a commercial sheep farm located in the Sandıklı district of Afyonkarahisar province, Türkiye. Sandıklı is located at 38°27'53.7"N latitude and 30°16'22.7"E longitude, at an altitude of 1095 meters above sea level.

Animal and feeds. A total of 80 clinically healthy Anatolian Merino ewes aged 2-4 years were used. Ewes weighing between 60 and 70 kg and having a body condition score of 2.5-3.0 (1 = extremely emaciated, 5 = obese) were included in the study. A total of 16 fertile Anatolian Merino rams were selected for estrus detection and mating.

Sheep were grazed during the day and brought back to the barn in the evening. Upon returning from pasture in the evening, they were fed with a ration consisting of ground barley, ground corn, wheat bran, and dry oat hay. During the study, sheep were fed according to the same feeding criteria to avoid any negative effects of dietary changes on their performance.

Estrus synchronization protocols. Sheep were synchronized with a progesterone-containing sponge, eCG, and d-cloprostenol outside the breeding season. The sheep were divided into three groups, balanced in age and weight, and the first two groups were given Bakofix® (Tab. 1) treatment before synchronization.

Tab. 1. Contents of Bakofix®

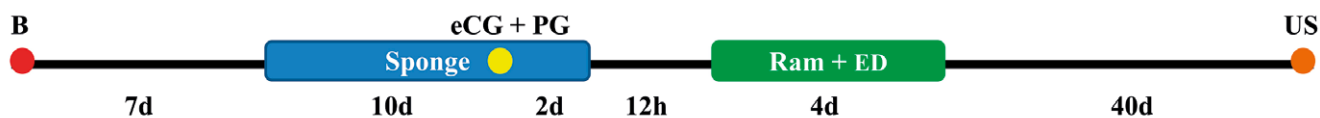
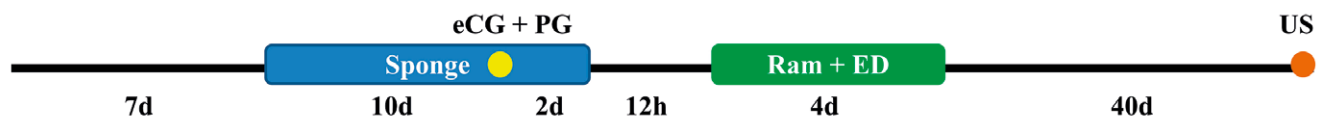
Contents	Amount*
Vitamin	
Vitamin E (Alpha tocopherol acetate)	25 mg
Mineral	
Calcium (Calcium iodate hexahydrate)	0.375 mg
Trace Elements	
Copper (Copper gluconate)	2 mg
Manganese (Manganese citrate)	2.5 mg
Zinc (Zinc gluconate)	3.8 mg
Selenium (Sodium selenite)	0.27 mg
Cobalt (Cobalt propionate)	2.6 mg

Explanation: * the above amounts indicate the contents in 1 mL of Bakofix®

Group 1 (G1, n = 25): Bakofix® (2 mL) was administered, intramuscularly 7 days before estrus synchronization. For the purpose of estrus synchronization, a progesterone-containing sponge (60 mg, Medroxyprogesterone acetate, Esponjavit®, Hipra, Türkiye) was inserted into the vagina for 12 days, and on the 10th day, eCG (500 IU, Oviser®, Hipra, Türkiye) and d-cloprostenol (0.075 mg, Dalmazin®, Fatro, Türkiye) were injected intramuscularly (Fig. 1).

Group 2 (G2, n = 25): Following the administration of Bakofix®, progesterone-containing sponges were inserted into the vagina for 12 days starting on the day of estrus synchronization, and on the 10th day, eCG and d-cloprostenol were injected (Fig. 1).

Group 3 (G3, n = 30): After the administration of physiological saline (2 mL, intramuscularly), progesterone-containing sponges were inserted into the vagina for 12 days to synchronize estrus, and eCG and d-cloprostenol were injected intramuscularly on the 10th day (Fig. 1).

Group 1 (Bakofix® injection 7 days before estrus synchronization, n = 25)**Group 2 (Bakofix® injection when starting estrus synchronization, n = 25)****Group 3 (n = 30)****Fig. 1. Schematic view of Bakofix® treatment time and estrus synchronization protocols**

Explanations: eCG – equine chorionic gonadotropin; B – Bakofix®; PG – d-cloprostenol; d – day; h – hour; US – transrectal ultrasonography; ED – estrus detection

Estrus detection and mating. After removing sponges, the ewes and rams were kept in a partition for four days with a female to male ratio of 5 : 1. Estrus signs were observed four times a day for four days. The sheep exhibiting mounting and standing behavior were separated from the flock.

Pregnancy diagnosis. Pregnancy diagnosis was performed by transrectal ultrasonography with a 7.5 MHz linear probe (WELLD WED-3000V®, Shenzhen WELLD Medical Electronics, China) 40 ± 3 days after mating. The transrectal ultrasonography was performed in the standing position, and pregnancy was diagnosed when the embryo was visualized.

Fertility parameters. To determine the effect of Bakofix® treatment (in G1 and G2) on reproductive efficiency, estrus response, conception rate, pregnancy rate, lambing rate, twinning, triplet, and quadruplet birth rates, fecundity, litter size, and survival rate were determined using formulas from our previous study (18). The lambs were monitored for 60 days, and the survival rate was calculated. Additionally, twin, triplet, and quadruplet birth rates were combined to calculate the multiple birth rate.

Statistical analysis. In order to assess the differences in fertility parameters between the groups, a chi-squared test or Fisher's exact test was performed. Multiple comparisons of birth weight between groups were evaluated with One-Way Analysis of Variance (ANOVA) and pairwise comparisons with the Tukey honestly significant difference test. All analyses were conducted with the GraphPad Prism® software (Version 8.0, GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant at a P-value of less than 0.05.

Results and discussion

Administration of Bakofix® had a positive effect on estrus response in Anatolian Merino ewes prior to estrus synchronization (Fig. 2). G1 had a higher estrus response compared to the other groups, but no statisti-

cally significant difference was observed ($P > 0.05$). The conception rate and pregnancy rate in G1 were also higher, as in the estrus rate. Notably, the 84% pregnancy rate obtained from G1 was remarkable (Fig. 2).

No problems were encountered during the pregnancy of Anatolian Merino ewes, such as late embryonic death/fetal loss or abortion, and deliveries were successful. There was no statistical difference between the groups in both fecundity and litter size ($P > 0.05$). The singleton rate in G3 was statistically higher than in G1 ($P < 0.05$, Fig. 2). The twinning rate was similar for all groups and was not affected by treatment, but the triplet and quadruplet rates were higher in G1 compared to the other groups. Furthermore, the multiple birth rate was significantly higher for G1 than it was for G3, and Bakofix® treatment seven days before synchronization positively affected the multiple birth rate, with a statistically significant difference between the two groups ($P < 0.05$, Fig. 2).

The lamb survival rate (Fig. 2) and sex ratio (Fig. 3) were not statistically different among the groups ($P > 0.05$). The birth weight obtained in G1 was statistically higher than in both G2 ($P < 0.001$) and G3 ($P < 0.05$). Interestingly, although the multiple birth rate was higher in G1, it did not negatively affect lamb birth weights (Fig. 3).

Seasonal polyestrous behavior is common among many sheep breeds, meaning that they do not exhibit estrus outside of the breeding season. However, in small ruminants, achieving pregnancy and lamb production during the non-breeding season can lead to additional economic benefits. In sheep, eCG plays a vital role in inducing estrus and follicular development outside of the breeding season (1, 4, 15, 17). In our study, the use of eCG with progesterone-containing sponges during the non-breeding season in Anatolian

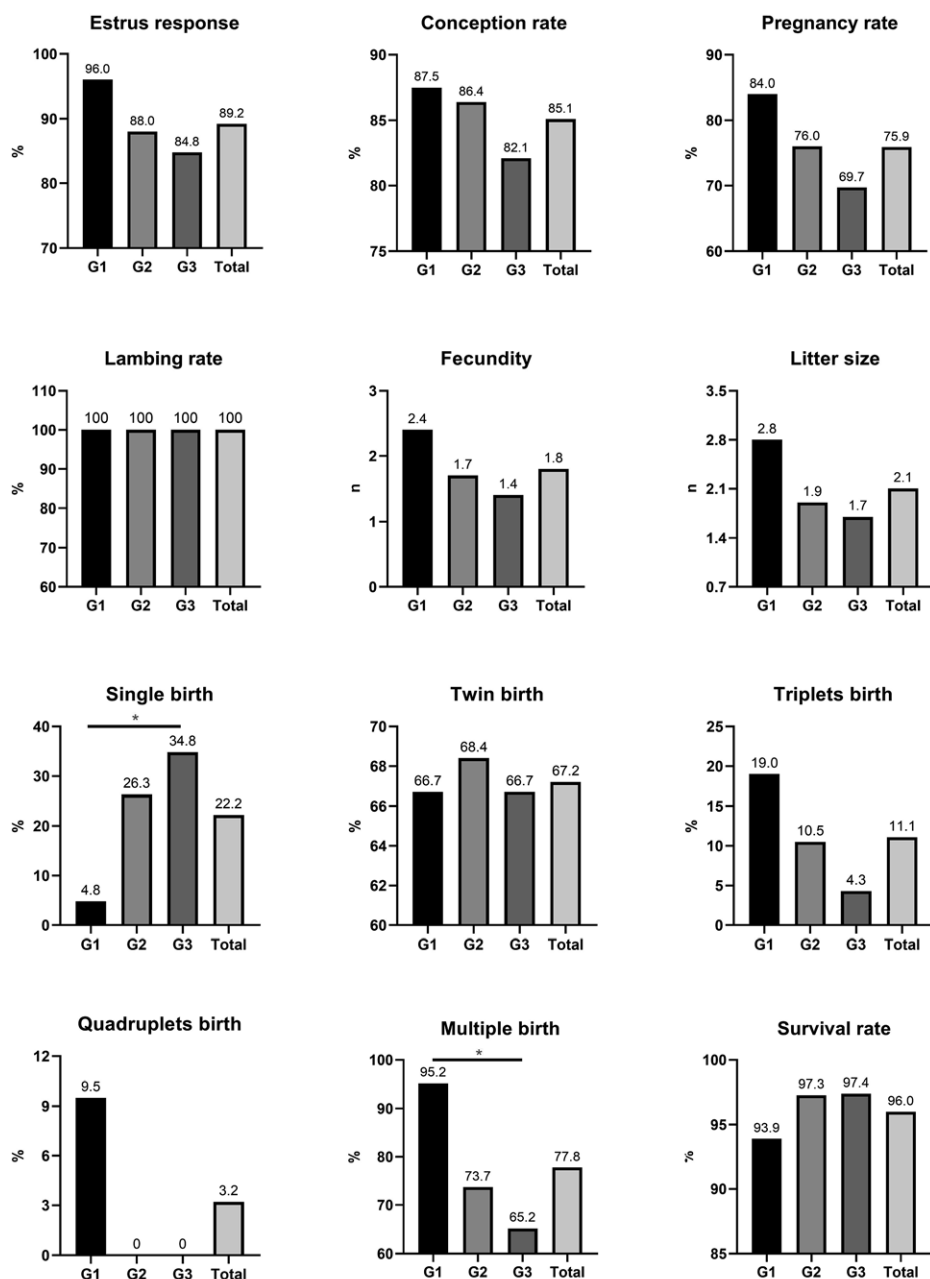


Fig. 2. Selected fertility parameters for different groups

Explanations: G1 – Bakofix[®] treatment seven days before estrus synchronization; G2 – Bakofix[®] treatment on the day of initiation of estrus synchronization; G3 – no Bakofix[®] treatment; * P < 0.05

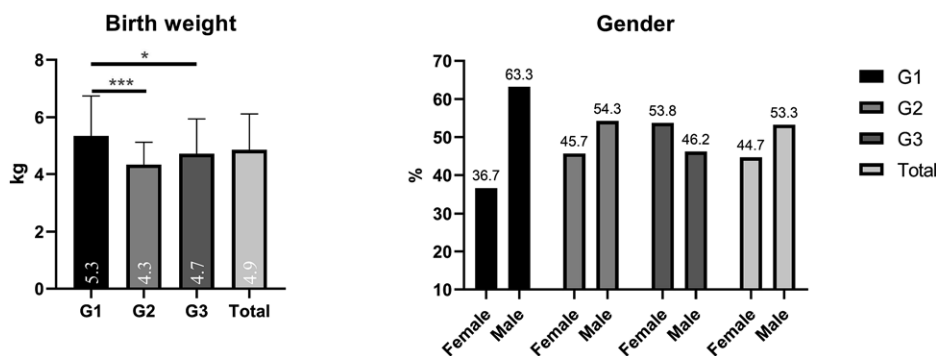


Fig. 3. Lamb birth weight and sex distribution for different groups

Explanations: G1 – Bakofix[®] treatment seven days before estrus synchronization; G2 – Bakofix[®] treatment on the day of initiation of estrus synchronization; G3 – no Bakofix[®] treatment; * P < 0.05, *** P < 0.001

Merino sheep resulted in excellent estrus induction, and this success was reflected in other fertility parameters, such as pregnancy rates. In addition, the effects of certain trace minerals and vitamin supplements on reproductive parameters in sheep during or before estrus synchronization have also been a subject of interest. Again, in our study, all ewes were exposed to all rams at the same time in the same pen. In this way, the possible male effect that may occur in the groups was also minimized. Many studies have shown that the additional administration of vitamins, minerals, and trace elements can have positive effects on fertility parameters and improve lamb performance (2, 16, 24).

For female reproductive health, both selenium and vitamin E are important minerals. Selenium plays a critical role as a component of antioxidant enzymes in preventing oxidative damage caused by free radicals, which can have negative effects on reproductive health by damaging female reproductive organs and ovaries. Additionally, selenium has an important role in DNA synthesis and repair, and selenium deficiency can lead to DNA damage during the division and proliferation of ovarian cells, negatively affecting female reproductive health. Selenium can also be effective in regulating female reproductive hormones and has an effect on thyroid hormones. Vitamin E also reduces oxidative stress caused by free radicals through its antioxidant properties, preventing damage to female reproductive organs. It contributes to female reproductive functions, reducing oxidative base damage in the ovarian epithelium due to ovulation, and is necessary for the healthy growth of the placenta and fetus. Vitamin E can also help regulate female reproductive hormones. In this context, supplementation with vitamin E and selenium can increase fertility rates in ewes (18-20, 22, 28). A study conducted on Karacabey

Merino sheep showed that selenium or selenium + vitamin E treatment did not have a significant effect on the pregnancy rate, but led to an increase in fecundity (13). Similarly, in another study on sheep, Vitamin E or Vitamin E + selenium treatment was found to have a positive effect on estrus response, pregnancy rate, and fecundity (2, 23). During our study, the sheep were fed primarily by grazing in the pasture, but the quality of the grass in terms of its nutritional content cannot always be guaranteed. Measuring the serum concentration of vitamins and trace elements in sheep can be costly for farmers, and as a result, they may be reluctant to have such measurements taken. Therefore, to ensure proper nutrition during estrus synchronization, small ruminants may require vitamin, mineral, and trace element supplementation. Unfortunately, the use of pasture, temperature, and intravaginal sponges during estrus synchronization can be stressful for the animals and can alter their oxidative status, leading to oxidative stress during reproductive events. To address this issue, we administered Bakofix[®], containing vitamins E and Se, one week before synchronization. Although this numerically increased both estrus response and pregnancy rate, there was no statistical difference between the groups. However, an increase in the multiple birth rate was observed.

Zinc and copper are important minerals for female reproductive health (16). Zinc plays a critical role in the division and growth of female reproductive cells. It also contributes to hormonal regulation during ovulation, fertilization, implantation, and pregnancy. Even a mild zinc deficiency can result in reduced lambing rates, lamb survival, and birth weight. (14, 27, 28). Copper, on the other hand, plays a role in many mechanisms of the female reproductive system, including the structure of many enzymes, estrogen metabolism, and neurotransmitter synthesis. In sheep, copper deficiency can lead to infertility and abortion, negatively affecting reproductive functions (27). Additionally, zinc and copper, along with other minerals and vitamins, interact to contribute to female reproductive health (21, 27). In our previous study, the use of a vitamin and mineral mixture containing zinc and copper during estrus synchronization had a positive effect on pregnancy rate, multiple birth rate, and litter size in Pırlak ewes (16). In our study, the treatment with Bakofix[®], containing copper and zinc, prior to estrus synchronization numerically increased fertility and litter size, but there was no statistically significant difference between the groups. The increased multiple birth rate in G1 can be attributed to the positive effects of zinc on ovulation and implantation, leading to both an increase in the number of lambs obtained and greater economic gain. However, the most important point to note here is that the multiple birth rate in the G1 group was statistically significantly higher than in the other groups. Therefore, it can be suggested that Bakofix[®] treatment one week before progesterone treatment may stimu-

late multiple birth rates. Interestingly, lamb weights in G1 were not lower than those in the other groups; in fact, they were higher. The ewes in G1 may have experienced a healthier implantation process, embryonic development, and pregnancy, but it is difficult to explain the mechanism with the available information and data.

Manganese plays a crucial role as a coenzyme in cholesterol synthesis, as well as in the synthesis of progesterone and estrogen, and is involved in corpus luteum formation. Manganese deficiency can result in sub-estrus, irregular estrus cycles, anovulation, embryonic death, and a low rate of successful pregnancies (14, 27). The absence of corpus luteum is one of the most critical problems in estrus synchronization outside of the breeding season, and treatment is required to induce estrus and promote the synthesis of necessary progesterone for pregnancy by stimulating corpus luteum formation (1, 17). Our use of Bakofix[®], a manganese-based supplement, yielded a positive numerical trend in pregnancy rates. However, this increase did not reach statistical significance. Embryonic death or fetal loss did not occur in our study, and lambing rates were similar across all groups.

Cobalt deficiency in farm animals can lead to decreased pregnancy rates and irregular estrus cycles (14). In early pregnancy, cobalt deficiency can reduce multiple birth rates and survival in lambs (27). In our study, the cobalt containing Bakofix[®] treatment administered 1 week before synchronization increased multiple birth rates in G1. However, the Bakofix[®] treatment immediately before the start of synchronization did not affect birth weight. The increased multiple birth rates in G1 cannot be solely attributed to cobalt supplementation; they could have been due to the combined effect of other vitamins and trace elements. Therefore, administration of supplementary drugs, such as Bakofix[®], at least one week before estrus synchronization may be more beneficial.

In conclusion, the nutrient quality of forage-based diets may be insufficient to meet vitamin and mineral requirements for optimal sheep health and reproductive performance, potentially jeopardizing their reproductive performance. To address this issue, we supplemented Anatolian Merino ewes with vitamins, minerals, and trace elements, particularly during and one week before estrus synchronization. This treatment, especially when applied one week before estrus synchronization, increased the multiple birth rate, resulting in an increased number of lambs and economic benefits. Administering the Bakofix[®] supplement one week before estrus synchronization appeared to be more beneficial than administering it immediately before the synchronization process. Although it did not make a significant difference, it also seemed to increase pregnancy rates. This was most evident when we looked at the birth weight of the lambs. Therefore, we believe that administering Bakofix[®] to ewes at least

one week before estrus synchronization can improve their fertility. Further studies are needed to verify its full effect on fertility.

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