The Effect of Selenium, Vitamin E, Vitamin A and Vitamin D3 Applications on Fertility in Awassi Sheep with Estrus Synchronization During the Breeding Season

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A R T I C L E  I N F O
Research Article

The purpose of this study was to determine the effects of selenium, vitamin E, vitamin A, and vitamin D3 treatments on reproductive parameters in Awassi sheep fed solely dry grass and grain stubble during the breeding season with estrus synchronization. Seventy-five sheep were implanted with intravaginal sponges containing 20 mg of flugestone acetate for 9 days for estrus synchronization. On the day the sponges were inserted, the first group received an intramuscular injection of a supplement containing 200,000 IU of vitamin A, 30,000 IU of vitamin D3, and 20 mg of vitamin E, as well as a supplement containing 1 mg of sodium selenite and 60 mg of vitamin E. The second group was the control group, with no supplementary vitamin injected. On the day of sponge removal, the sheep received intramuscular injections of 500 IU FMSG and 250 mcg cloprostenol sodium in both groups. The sheep in the first group were given a second injection of the supplement on the same day that contained 20 mg of vitamin E, 30,000 IU of vitamin D3, and 200,000 IU of vitamin A. The sheep that showed signs of estrus were mated naturally after the estrus synchronization. Estrus rates were 86.11% and 85.29% in Group 1 and II, respectively (P=0.858). Although there was no significant difference in the reproductive characteristics between the groups (P>0.05), Group I had a greater pregnancy rate and litter size. During the breeding season, it is believed that the regular application of vitamin and mineral supplements on a program basis in sheep fed only dry pasture and grain stubble contributed to fertility.

Keywords:
Sheep
Synchronization
Selenium
Vitamin E
Vitamin A

A B S T R A C T

In sheep herds, estrus synchronization during the breeding season provides estrus, ovulation, and lambing simultaneously (Alaçam, 1993). Hormones (progestins, prostaglandins, pregnant mare serum gonadotropin, gonadotropin-releasing hormone) are frequently used for estrus synchronization in the breeding season (Alaçam, 1993; Alaçam, 1999; Wildeus, 2000). Vitamin and mineral supplementation is also used to increase fertility during synchronization. According to research, sheep bred in areas where dry grass and grain stubble are fed may benefit from the addition of vitamins A, E, D3, and selenium, especially during breeding season (Chew, 1993; Boland et al., 2005; Köyuncu and Yerlikaya, 2007; Köse et al., 2013; Yeşil and Sarıözkan, 2017). The synthesis of ovarian steroid hormones and the process of folliculogenesis are both require vitamin A (Bagavandoss and Midgley, 1988; Bozkurt et al., 1998; Halilolu et al., 2002; Pu et al., 2014). Animal products and vegetable oils contain vitamin A, while most grains other than corn do not. Consequently, ruminants may suffer from vitamin A deficiency. A lack of vitamin A can result in the formation of follicular and luteal cysts, inadequate CL development, and embryonic and fetal mortality from insufficient progesterone secretion. Furthermore, it is believed that conditions, such as anestrus, sub-estrus, and delayed ovulation can be occurred (Eberhardt et al., 1999; Bindari et al., 2013). It has been reported that the use of vitamin A supplements improves fertility parameters and reduces the prevalence of reproductive diseases (Bindari et al., 2013; Yeşil and Sarıözkan, 2017).

Although the primary function of vitamin D is to regulate calcium and phosphorus absorption and balance, bone mineralization, and neuromuscular functions (Hors et al., 2003), but it is also linked to fertility. The fact that the vitamin D receptor is expressed in granulosa cells suggests that vitamin D is essential for steroidogenesis. Furthermore, because it affects the gene expression of anti-mullerian hormone and follicle-stimulating hormone, it appears to be a crucial factor in follicle development (Muscoqur et al., 2017). Its deficiency can result in sub-estrus and structural abnormalities in the fetus (Yeşil and Sarıözkan, 2017; Turan, 2018). The active form of vitamin D, calcitriol (1,25(OH)2D3), is reported to increase the formation of follicular and luteal cysts, inadequate CL development, and embryonic and fetal mortality from insufficient progesterone secretion.
synthesis of progesterone by 13%, estradiol by 9%, and estrone by 21% (Muscogiu et al., 2017).

Vitamin E prevents the formation of free radicals that have detrimental effects on the structure of intracellular membranes by saturating peroxides and hydroperoxides (Infante, 1999). Disruption of ovarian function, vascular degeneration in the embryo, early embryonic death, fetal resorption, stillbirths, and fetal muscular dystrophy can all result from vitamin E deficiency in sheep (Braun et al., 1991; Kott et al., 1998; Kaçar et al., 2008).

The most important function of selenium is to participate in the structure of Glutathione peroxidase (GSH-Px), which protects the cell membrane from free radicals during lipid peroxidation (Hostetler et al., 2003; Mehdi and Dufrasne, 2016; Yeşil and Sariözkan, 2017). Selenium has been linked to the synthesis of estrogen and prostaglandins, as well as the proliferation of granulosa cells (Nebbia, 1982). Furthermore, it protects follicles from the damage caused by increased oxidative stress during folliculogenesis (Ceko et al., 2014). Selenium deficiency can cause low fertility, sub-estrus, increased stillbirth rate, low birth weight, growth retardation, and immune system deficiency (Humann-Ziehank, 2016).

It is stated that oxidative stress, which develops due to the increase in the level of free radicals in the organism, can lead to infertility (Arechiga et al., 1998; Agarwal and Allamaneni, 2004). Free radicals such as peroxides and hydroperoxides that form during oxidative stress cause damage to the structural integrity of the cell by oxidizing unsaturated fatty acids of mitochondrial, microsomal, and cell membrane phospholipids (Infante, 1999). It is suggested that estrus synchronization techniques can also raise the concentration of free radicals (Arechiga et al., 1998). It is stated that free radicals increasing in the preovulatory follicle fluid adversely affect the quality of the oocyte, fertilization rate, and embryo quality (Jowzik et al., 1999). On the other hand, according to reports, vitamin E can improve oocyte quality and maturation, decrease the concentration of free radicals in the follicular fluid, and have positive effect on fertilization and early embryonic development (Agarwal and Allamaneni, 2004; Agarwal et al., 2005; Ceko et al., 2014). Selenium assists in the binding plasma lipoproteins. Given the similarities of their effects and the similar symptoms associated with their deficiencies, simultaneous use is recommended, it is recommended that they be applied together (Hostetler et al., 2003; Mehdi and Dufrasne, 2016). Se and Vit E administrations in sheep are reported to improve fertility by preventing oxidative damage caused by progesterone used in estrus synchronization (Hostetler et al., 2003; Mehdi and Dufrasne, 2016; Kuru et al., 2017).

According to reports, the Mediterranean region’s pastures and grain stubbles are deficient in Se, vitamin E, and vitamin A in the summer and fall, which are thought to have a positive impact on reproductive processes. The study’s objective was to establish the effects of Se, vitamin E, vitamin A, and vitamin D3 treatments on reproductive parameters in Awassi sheep with estrus synchronization that were solely fed natural grass and grain stubble during the breeding season.

**Material and Methods**

The study was conducted at a private commercial sheep farm in the Hatay province during the breeding season (36°26’ North latitude, 36°56’ East longitude) at the beginning of July 2021. Animal material was 75 Awassi sheep aged 2–6 years who had given birth at least once and weighed 45–60 kg. The average ambient temperature was 31.6 °C during the day and 25.13 °C at night, and the average length of the day and night was 14 hours, 15 minutes, and 9 hours, 45 minutes, respectively. Feeding and management schedule of farm was followed during the study. Sheep were not in lactation, spent the day eating on pasture and grass stubble.

Sponges that contain 20 mg of flugestone acetate (Chronogest CR®, Intervet, Türkiye) were inserted into the vagina of all the sheep by speculum. After that, sheep were randomly divided into two groups: treatment (Group I, n=38) and control group (Group II, n=37). On the day of inserting the sponges to the sheep in the first group, 1 ml of selenium and Vit E mixture (1 mg/ml of sodium selenite and 60 mg/ml of Vit E, Yelvit®, Teknovet, Türkiye) and 1 ml/50 kg of Vit A, Vit D3 and Vit E (200,000 IU/ml Vit A, 30,000 IU/ml Vit D3 and 20 mg/ml Vitamin E containing Adevil®, Vilsan, Türkiye) mixture injected intramuscularly. The sheep in the second group didn’t received any treatment. Sponges were left in the vagina for 9 days in both groups, and during the removal of the sponges, 500 IU PMSG (Chronogest/PMSG, 6000 IU, Intervet, Istanbul, Türkiye) and 250 µg cloprostenol sodium (Minoprost®, Vilsan, Türkiye) were administered intramuscularly. The sheep in the treatment group received a second injection of vitamin A, vitamin D3, and vitamin E on the day of sponge removal (Figure 1).

**Figure 1. Applications in Group I and Group II**

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Discussion

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Results

24 hours a day after the sponge removal, the rams
joined the flock and spent an hour with the sheep twice
daily (morning and evening). The ewes that stood estrus
mated and were taken out of the herd and put in a separate
area. The sheep were examined for pregnancy 50 days after
mating using a 6-8 MHz probe real-time ultrasound device
(Falco, Pie Medical, Netherlands). The sheep were
considered as pregnant when the part of placenta, fetus, and
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examination.

The Stata 12/MP4 statistical package program was used
to conduct all statistical analyses for the study. All
variables that were received had their descriptive statistics
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offspring in each group was assessed using a Student's t-
test. Litter size was also represented as a "Percentage" for
the number of offspring in each group. The threshold for
statistical significance was set at P<0.05.

The following formulas were used to determine the
reproductive parameters evaluated in the study:

Estrus ratio: (Number of sheep stand estrus / number of
sheep in group) x 100

Pregnancy rate: (Number of pregnant sheep / Number
of sheep in the group) x 100

Conception rate: (Number of pregnant sheep / Number
of vaccinated sheep) x 100

Kidding rate: (Number of sheep lambing / Number of
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Litter size: (Number of lambs born / Number of sheep
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Results

Three sheep were removed from the study and not
counted in the evaluation: two sheep in Group I with fallen
sponges, one sheep in Group II with fallen sponge, and one
sheep with laminitis.

Estrus rates were 86.11% and 85.29%, conception rates
were 74.19% and 58.62%, pregnancy rates were 63.88% and
50%, kidding rates and litter size were 100% and
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The fact that free-grazing is the predominant method of
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Table 1. Fertility parameters of Group I and Group II

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<th>Conception rate (%), x/n</th>
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<th>Kidding rate (%), x/n</th>
<th>Litter size (%), x/n</th>
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<td>63.88 (23/36)</td>
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<td>126.08 (29/23)</td>
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<td>Group II</td>
<td>85.29 (29/34)</td>
<td>58.62 (17/29)</td>
<td>50</td>
<td>100</td>
<td>123.52 (21/17)</td>
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<td>P</td>
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Discussion

The fact that free-grazing is the predominant method of
sheep breeding in our country suggests that the mineral
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removal. They are also reported that vitamin E and selenium supplements applied at the beginning of the breeding season when sheep start to graze on dry pastures and stubbles have beneficial effects on increasing fertilization rates. Koyuncu and Yerlikaya (2007) found that the pregnancy rates were not different between the Se, Se+VitE and control groups in Merino sheep, but the Se group had the highest oestrus rate. According to Farahavar et al. (2020) pregnancy rate of ewes after administration of 0.5 mg/ml sodium selenite and 50 IU DI-α-tocopherol for 3 times during synchronization protocol (2 weeks before CIDR insertion, at the CIDR insertion, at the CIDR removal) was 61.11%, while it was 55.56% in the control group. Pregnancy rates in our study were 50% in the control group and 63.88% in the group that received vitamin E and selenium supplements. Despite a 13.88% rise in pregnancy rates, there was no statistically significant change (P>0.334) (Table 1). It has been reported that Mediterranean dry grass pastures are deficient in vitamin E during the summer and fall (Liu et al., 2014). On the other hand, Koyuncu and Yerlikaya (2007) stated that sheep grazing on dry pastures and grain stubble are at a significant risk of vitamin E deficiency. The number of animals used in the study, the application time (before and/or after breeding), the route of administration, the frequency, dose, or amount of administration, the environment and management style of the farms, ewe breed, the phase of cyclic activity, feeding, and most importantly, whether there was a deficiency in vitamin E and Se in sheep prior to the applications were factors reported by researchers (Köse et al., 2013; Awawdeh et al., 2019) as to why the pregnancy rates varied between studies. It is thought that the result obtained in our study may depend on many factors, especially the small sample size (or numbers). Ruminant fertility is directly impacted by vitamin A, which the organism naturally produces as retinol, retinal, and retinoic acid (Chew, 1993). Because of its antioxidant properties (Nayyar and Jindal, 2010) and support for the growth of embryos and fetuses as well as progesterone production, vitamin A has beneficial effects on fertility (Chew, 1993; Kolb and Seehawer, 1998). Furthermore, it has an impact on ovulation and folliculogenesis process (Bozkurt et al., 1998; Haliloglu et al., 2002; Brown et al., 2003; Hashem et al., 2016). Depending on the severity of the vitamin A deficiency, gametogenesis abnormalities and embryonic fatalities are known to occur. In addition, diseases like impairment of ovum’s structure, a lack of progesterone synthesis, reduced fertilization ability of ovum, infertility are also possible during vitamin A deficiency (Chew, 1993; Bozkurt et al., 1998; Eberhardt et al., 1999). In Jordan, where mostly natural pastures and stubble are used for sheep breeding, Harb (1994) states that the low nutritional content of these pastures from June to the end of September may have detrimental effects on fertility. Given that vitamin A is only available in green grass, it is reported that sheep fed on grain stubble and dry pastures of dry months for three to four months have reduced fertility because of vitamin A deficiency (Abdelrahman and Al-Karakibeh, 2002). The present study suggests that the nutritional quality of dry pasture and grain stubble in the breeding season may be low, as suggested by Harb (1994), because pregnancy rates were lower in the control group than in the treatment group after feeding sheep just pasture and grain stubble.

Vitamin D is crucial for absorption of calcium and phosphorus, mineralization of bones, and neuromuscular activity, but also for fertility. Vitamin D stimulates steroidogenesis in granulosa cells, and influences the gene expression FSH and increases the synthesis of progesterone and estrogen. Its deficiency has been linked to sub-estrus, according to reports (Muscoğur et al., 2017; Yeşil and Sarıözkan, 2017).

Although the roles played by vitamins and minerals in the body and the disorders caused by their deficiency are known separately, it is advised to be used simultaneously due to their interactions. Study of Birdane and Avdatek (2020) estrus rate, pregnancy rate, and litter size in the study group of sheep that were synchronized with FGA during the breeding season and administered a combination of vitamin A (300,000 IU), vitamin D3 (100,000 IU), and vitamin E (50 mg) intramuscularly were 92.5%, 87.5%, and 154%, respectively, compared to 90%, 75%, and 137% in the control group that received no treatment. In Awassi sheep synchronized with FGA outside of breeding season, Özor et al. (2022) founded pregnancy rates of 54.05% and 58.33%, respectively, in the group given Vitamin E, Se, and β-carotene and the control group. They emphasized that there was no vitamin/mineral deficiency because of the good quality pasture conditions in March, when the study was conducted. It is also reported in the same study that geographical characteristics and climatic conditions of breeding region may have effect on pregnancy rate be related to the. It has been reported that the possible reasons for the different results obtained in studies investigating the effects of vitamins A and E in sheep are variables such as vitamin dose, application frequency, form, timing, pasture and feed quality (Birdane and Avdatek, 2020). Harb (1994) underlines that vitamins A, D3, and E should be supplemented when dry pasture and stubble are used as sheep feed. Abdelrahman and Al-Karakibeh (2002) investigated the effects of vitamin A, D3, and E supplementation on fertility in Awassi sheep during the breeding season, with pregnancy rates of 74.1% and 82.1% in the control and treatment groups, respectively, and reported that the sheep had a 8% rise in the pregnancy rate with this application particularly when vitamin A deficiency was a significant issue. The reproductive parameters in the group that received vitamins A, D3, E, and Se in the current study did not differ statistically from the control group (Table 1). It would not be accurate to conclude that the supplements were entirely unsuccessful, as these vitamin and mineral levels in sheep were not evaluated before the study. Pregnancy rate increased by 13.88% even though there was no statistically difference (P>0.05) between the treatment group and the control group. It is believed that this increase would have substantial economic contributions for the breeder.

Since the number of offspring represents a significant economic output in the animal breeding, it has been established that the proportional increase in pregnancy rate and litter size cannot be disregarded. It was found that estrus synchronization in the sheep fed on dry pasture and grain stubble throughout the breeding season, where herd management is crucial, may benefit from the routine administration of vitamin and mineral supplements on a
program basis. However, in further studies to be conducted on the same subject, it is recommended to expand the sample size in order to reach concrete data.

Acknowledgements

This study was conducted pursuant to the 16/06/2021 dated and 2021/04-07 numbered approval of the Local Ethics Board for Animal Experiments of Hatay Mustafa Kemal University. The article was summarized from the master’s degree thesis of Mehmet EFE.

References


