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Evaluation of vitamin D status at different stages of pregnancy in Gray Shirazi ewes

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Abstract

The aim of this research was to examine the serum vitamin D status in Gray Shirazi ewes during different reproductive stages, including mating, pregnancy, and early lactation. A total of 60 healthy ewes were sampled at three stages. The first stage was during mating, the second stage was in the late pregnancy, and the third stage was after delivery. The blood samples were analyzed for 25-hydroxy vitamin D levels. At delivery, multiple births, weight of lambs, and stillbirth rates were recorded. The results revealed that the mean serum vitamin D levels were significantly higher in the third stage of sampling (beginning of lactation) compared to the first stage (mating) and the second stage (pregnancy) (P=0.014 and P=0.017). However, there was no significant difference in vitamin D levels among the non-pregnant (the ewes that did not become pregnant after mating), singleton, and twin groups at any of the sampling stages. Vitamin D levels were highest at the beginning of lactation in gray shirazi ewes. Additionally, serum vitamin D was slightly decreased in pregnant ewes as the number of lambing increased. In total, the findings suggest that maintaining sufficient vitamin D levels may play a role in reproductive health in sheep, as there appears to be a correlation between vitamin D levels and fertility.

Key words: Vitamin D, Ewe, Pregnancy, Gray Shirazi breed

Introduction

Vitamin D as a micronutriant maintains skeletal and bone health by regulating calcium and phosphorus absorption, bone formation, and mineral circulation (Holick, 2007). However, recent research has shown that the association of this vitamin deficiency with numerous medical conditions affects various parts of the body other than the skeletal system. Autoimmune diseases, hypertension, and cancer are some of the conditions that have been linked to low levels of vitamin D (Lappe et al, 2007; Zhang and Naughton, 2010). Vitamin D also affects reproductive function, and its insufficiency throughout pregnancy has been linked to lower success in reproduction and a higher possibility of fetal abnormalities, such as failure to gain normal weight, low birth weight, or reduced head circumference (Lerchbaum and

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Obermayer-Pietsch, 2012; Cleal et al, 2017; Gernand et al, 2013; Chen et al, 2017; Mumford et al, 2018). It was also demonstrated that vitamin D significantly influences redox balance and energy profile and serves as a valuable predictor for subclinical pregnancy toxemia in pregnant sheep (Nisar et al, 2024). In addition, Kobeisy et al, (2021) reported an improvement in reproductive performance of ewes and their lamb survival following vitamin D administration at late pregnancy of ewes. Furthermore, application of vitamin D contributed to the increase in the yield of offspring obtained from the synchronization protocol in Romanov sheep in breeding season (Kaya and Kocak, 2022). The fetus depends entirely on the mother's vitamin D. 25-Hydroxyvitamin D passes through the placenta and is converted into $1,25(OH)_2D$ by the kidneys of the fetus. In addition, the placenta also synthesizes $1,25(OH)_2D$ in order to regulate its metabolism. The significance of vitamin D in pregnancy has been thoroughly established in terms of maintaining the balance of calcium in the mother's body and supporting the development of fetal bone formation. Nonetheless, there is ongoing discussion regarding the impact of mother vitamin D status on pregnancy outcomes, fetal development, and the long-term health of newborns (Larqué et al, 2018). In a study performed by Dittmer et al, (2020), a positive relationship was recorded between serum concentration of 25(OH)D of ewes during mating and the pregnancy rate in a breeding farm in New Zealand (Dittmer et al, 2020). in addition, a recent study on Scottish blackface sheep showed that vitamin D level before mating had a positive correlation with the lamb weight in single and twin pregnancies (Zhou et al, 2019).

Considering the importance of breeding native breeds of Iran, it seems necessary to identify the physiological indicators of these breeds in order to detect possible disorders and improve breeding methods and increase production efficiency. Although Iranian sheep are bred for meat production, some breeds are known for their special traits, such as the skin breeds (Karakul, Zandi and Gray Shirazi) (Karimi et al, 2015).

One of the most well-known skin sheep breeds in Iran is Gray Shirazi which is raised in Fars province. Compared to other breeds, these sheep are more compatible with the weather and climate of the region and have a lower cost of maintenance. In the herds of Gray sheep in Fars province, two lambing per year are applied in ewes that have suitable body scores. Synchronization and artificial insemination are also effective in increasing reproductive efficiency. Furthermore, feeding ewes with concentrate, two weeks before mating (flushing), is being performed in order to increase fertility (Rahayej Torfi et al, 2022; Karimi et al, 2015).

Considering the importance of vitamin D in the pregnancy period and fetal development and the lack of sufficient information about the normal values and changes of this nutrient in different stages of sheep reproduction, this study aims to monitor the status of vitamin D in Gray Shirazi ewes during mating, pregnancy and the beginning of lactation and its comparison in singleton and multiple pregnancies.

Materials and Methods

A total of 60 healthy primiparous and multiparous Gray Shirazi ewes were randomly selected for this study. The study was performed from September 2021 to April 2022. The animals raised at the research station of Aliabad Kamin, affiliated to Fars Agricultural and Natural Resources Research and Training Center, in the north of Fars province. They were fed manually, which included barley, straw and alfalfa. Mineral and vitamin supplements were also administrated once a week at the rate of 1% of ration. In addition, lick bricks and bicarbonate were freely provided to these sheep (to prevent possible acidosis

and mineral deficiencies). Shearing was done once a year, in May. The body condition score (BCS) of all the studied ewes was recorded according to Russell et al. (1969). All the ewes had ear tags to ensure accurate data recording. All ewes were synchronized using the ram effect along with a dose of PGF2α injection. If no estrus was observed, a second dose of PGF2α was injected approximately 8 days later. Neck bands were applied for easy identification of the studied ewes in the flock.

Sampling was performed in 3 stages, starting in the breeding season (September and October). The first stage was performed at mating, the second stage, approximately in 2 to 4 final weeks of gestation (pregnancy was diagnosed by ultrasonography), and the last stage, 1 to 2 weeks post parturition. Blood samples were collected from the jugular vein in plain tubes (without anticoagulant) and serum separated by centrifugation. Serum samples were subsequently kept at -20°C till laboratory assessments.

During parturition, ewes were observed for the frequency of multiple births, in addition to stillbirth and lamb weight, and the data were recorded.

Level of 25-Hydroxy Vitamin D was assessed in serum using Monobind ELISA kit, USA (Cat. No: 6734-96). The test was a sequential competitive enzyme immunoassay and was done according to the manufacturer's instructions.

Statistical analysis of data was done by SPSS 26 software. To compare the data between different stages of pregnancy, repeated Measures ANOVA and LSD supplementary test were used. Also, in order to compare between different groups in each stage, One-way ANOVA and LSD post-Hoc test were applied. The comparison between ewes with males or female offspring was done using independent Samples T-test. Meanwhile, the correlation between different variables was checked by calculating the Spearman correlation coefficient. Data were displayed as mean ± standard error, and $P < 0.05$ was assumed as significant difference.

Results

Comparison of vitamin D levels in various sampling stages in ewes

The data obtained from examining the quantity of vitamin D in the serum of the ewes of this study showed that all the investigated animals were within the normal range in all sampling stages.

The results of the comparison of vitamin D values in different stages of sampling are shown in Table 1. According to the results, mean vitamin D level in the last sampling stage (beginning of lactation) was increased significantly compared to the first stage of sampling (mating) ($P=0.014$) and the second stage of sampling (pregnancy) ($P=0.017$).

Table 1: Serum vitamin D concentration in different sampling stages as mean ± SE

Sampling stage	Vitamin D (ng/ml)
1st Stage (mating)	96.06±10.04[a]
2nd Stage (pregnancy)	95.53±9.71[a]
3rd Stage (early lactation)	134.25±11.54[b]

* Different lower-case letters represent significant difference between stages.

Comparison of vitamin D concentration in various sampling stages based on pregnancy status in ewes

Comparison of ewes in different stages of sampling in three groups of non-pregnant (the ewes that did not become pregnant after mating), singleton and twin groups revealed no significant difference in terms of vitamin D level ($P>0.05$) (Table 2).

Comparison of vitamin D levels in various sampling stages based on parity in ewes

According to Table 3, there was not any significant difference in vitamin D levels in different stages of sampling based on parity (recent pregnancy plus the number of previous lambing) ($P>0.05$).

Table 2: Serum vitamin D concentration in different sampling stages and various pregnancy status as mean ± SE.

Sampling stage	Pregnancy status	Vitamin D (ng/ml)
1st Stage (mating)	Non-pregnant	90.24±13.74
	Singleton	77.75±12.76
	Twin	134.31±30.90
2nd Stage (pregnancy)	Non-pregnant	90.46±12.88
	Singleton	104.32±16.18
	Twin	102.15±29.34
3rd Stage (early lactation)	Non-pregnant	115.73±14.55
	Singleton	152.64±19.21
	Twin	119.03±28.63

* No statistically significant difference was observed between groups.

Table 3. Serum vitamin D concentration in different sampling stages and parity as mean ± SE

Sampling stage	Parity	Vitamin D (ng/ml)
1st Stage (mating)	1 (n= 5)	105.81±32.60
	2 (n= 17)	107.86±17.03
	3 (n=23)	73.83±12.80
	4 (n=15)	95.07±24.68
2nd Stage (pregnancy)	1 (n= 5)	108.04±41.18
	2 (n= 17)	83.37±17.64
	3 (n=23)	102.37±14.95
	4 (n=15)	100.97±21.10
3rd Stage (early lactation)	1 (n= 5)	99.89±27.93
	2 (n= 17)	112.11±18.71
	3 (n=23)	141.52±19.64
	4 (n=15)	144.75±24.62

* No statistically significant difference was observed between groups.

Comparison of vitamin D levels in various sampling stages in ewes based on the gender of lambs

The comparison between the ewe groups in terms of the gender of the lambs showed that there were not any significant differences in vitamin D levels in any of the sampling stages between ewes with male and female offspring (P>0.05) (Table 4).

Table 4: Serum vitamin D concentration in different sampling stages and the gender of lambs as mean ± SE

Sampling stage	Gender of lamb	Vitamin D (ng/ml)
1st Stage (mating)	Male (n= 26)	87.97±21.28
	Female (n= 23)	85.40±14.41
2nd Stage (pregnancy)	Male (n= 26)	94.12±17.52
	Female (n= 23)	114.90±18.93
3rd Stage (early lactation)	Male (n= 26)	178.67±21.96
	Female (n= 23)	135.53±23.32

* No statistically significant difference was observed between groups.

Comparison of vitamin D levels in various sampling stages based on body condition score (BCS) of ewes

Table 5, which contains the results of the comparison of vitamin D concentrations based on the body condition score (BCS) of ewes in different stages of sampling as mean ± standard error, indicates that there was no significant difference (P<0.05).

Table 5: Serum vitamin D concentration in different sampling stages and ewe body condition scores as mean ± SE.

Sampling stage	Ewe BCS	Vitamin D (ng/ml)
1st Stage (mating)	2.5 (n= 9)	116.64±22.36
	3 (n= 13)	77.68±16.78
	3.5 (n= 22)	92.94±17.45
	4 (n= 16)	89.46±20.50
2nd Stage (pregnancy)	2.5 (n= 9)	61.20±17.12
	3 (n= 13)	128.06±26.73
	3.5 (n= 22)	120.18±16.95
	4 (n= 16)	72.39±11.09
3rd Stage (early lactation)	2.5 (n= 9)	91.28±33.71
	3 (n= 13)	122.15±23.54
	3.5 (n= 22)	128.61±21.41
	4 (n= 16)	152.01±17.98

* No statistically significant difference was observed between groups.

Correlation of vitamin D values and body condition score (BCS) of ewes in various sampling stages

There was not any significant correlation between vitamin D levels and body condition score (BCS) of ewes in any of the sampling stages (P>0.05) (Table 6).

Table 6: Spearman correlation coefficient of vitamin D status and body condition score of ewes in different stages of sampling

Sampling stage	Correlation	Sig. (2-tailed)
1st Stage (mating)	- 0.073	0.587
2nd Stage (pregnancy)	0.076	0.586
3rd Stage (early lactation)	0.026	0.846

Correlation of vitamin D values and weight of lambs in various sampling stages in ewes

There was no significant correlation between the amounts of vitamin D and the weight of lambs (Table 7) in any of the sampling stages (P>0.05).

Table 7: Spearman correlation coefficient of vitamin D status and lamb weight in different stages of sampling

Sampling stage	Correlation	Sig. (2-tailed)
1st Stage (mating)	- 0.023	0.894
2nd Stage (pregnancy)	0.084	0.637
3rd Stage (early lactation)	0.276	0.121

Discussion

Sheep breeding is an important industry in many countries, including Iran, and improving production efficiency is a priority for breeders. Nutritional and environmental conditions play a crucial role in various stages of sheep reproduction. However, there is limited research on micronutrients such as vitamin D and its effect on fetal development and pregnancy, particularly in native Iranian breeds such as the Gray Shirazi (Ruiz-Larrañaga et al, 2020). The aim of this study was investigate the levels of vitamin D at different stages of pregnancy in ewes of the Gray Shirazi breed.

The finding that vitamin D levels were highest at the third stage (beginning of lactation) in gray shirazi ewes is consistent with previous research on the influence of reproduction and lactation on vitamin D status in animals. Lactation is known to increase the demand for calcium, which is regulated by vitamin D, and thus may trigger an increase in vitamin D levels. Additionally, considering the storage of vitamin D in body fat, lipolysis due to the negative energy balance before and during delivery, may play a role in the increase of vitamin D post-parturition. Similar results have also been reported in humans, where vitamin D levels were realized to be lower during pregnancy but increased significantly postpartum during lactation (Holick and Chen, 2008).

The observed increase in vitamin D levels during lactation in gray shirazi ewes could be affected by numerous issues including diet, sunlight exposure, and breed characteristics. Former studies have shown that breed differences can affect vitamin D metabolism and status (Zhou et al, 2019), and additional study is necessary to explore the potential role of breed in vitamin D status in sheep.

Previous studies also demonstrated a significant effect of fetal development and pregnancy on the regulation of vitamin D levels (Goyal et al, 2016; Paulson and Langman, 1990). Additionally, a study conducted on Scottish sheep revealed a positive association between annual reproductive efficiency and vitamin D level, which was defined as the total of Lambs that stay alive up to one year of age (Handel et al, 2016).

Concerning the effects of pregnancy on the status of vitamin D in ewes, Goyal et al. (2016) found that both pregnancy and hypoxia positively affect vitamin D status, which is attributed to changes in the expression of vitamin D receptors in the kidney, lung, and placenta. Furthermore,

administration of an AD_3E vitamin mixture to pregnant ewes led to improvements in antioxidant status in leukocytes and reductions in negative energy balance during the transition period (Sucupira et al, 2019).

In this research, Vitamin D levels were compared across three pregnancy statuses: non-pregnant, singleton, and twin. Correlation results showed that during the later stages of pregnancy and early lactation, non-pregnant sheep had lower vitamin D levels than pregnant sheep (singleton and twin), although these variations were not statistically significant. These findings suggest that there may be a correlation between vitamin D levels and fertility in sheep and they are consistent with previous research indicating that lower vitamin D levels may be associated with decreased fertility in human and other species. For instance, a research conducted by Lerchbaum et al, (2012) found that women with depleted vitamin D status were more likely to be infertile in comparison with women with normal levels of this micronutrient. Similarly, Merino et al, (2019) stated that male rats fed a diet with deficiency in vitamin D showed reduced sperm count and motility. Overall, while the specific mechanisms linking vitamin D and fertility are not yet fully understood, the findings of this study, along with previous research, suggest that maintaining sufficient vitamin D concentrations may be important for reproductive health in sheep and other species.

In the present study, vitamin D levels were compared in pregnant ewes based on the number of births. The ewes were allocated into four groups based on their parity: primiparous (one birth), biparous (two births), triparous (three births), and quadriparous (four or more births). Although no significant difference was detected among the groups, mean vitamin D concentration in the primiparous ewes was higher than the other groups at both stages of sampling (during mating and pregnancy). It is possible that as the number of pregnancies and births increases, the body reserves of ewes gradually decrease, leading to lower vitamin D status in later pregnancies. Nevertheless, all the studied sheep had normal concentrations of vitamin D, and none of the examined samples showed a deficiency in this micronutrient. The outcomes of this study propose that there might be a slight decrease in vitamin D levels in pregnant ewes as the number of births increases. This could be due to the depletion of body reserves over time. However, additional investigation is needed to verify these conclusions and to consider whether vitamin D supplementation can help maintain adequate vitamin D levels in multiple-pregnancy ewes.

In the present study, no significant association was observed between the serum vitamin D of ewes and the weight of lambs at birth. This finding suggests that there was no relation between maternal vitamin D status and fetal growth in the studied sheep. Still, it is essential to note that this is a primary study and further research is needed to fully realize the potential role of vitamin D in fetal development.

Vitamin D is critical for macromineral balance, bone health, immune function, and many other physiological processes (Holick, 2007). In pregnancy, vitamin D deficit is associated with a greater risk of low birth weight, neonatal hypocalcemia, stillbirth, and neonatal mortality (Weinert and Silveiro, 2015). While this study did not find a significant correlation between maternal vitamin D levels and lamb birth weight, previous studies have reported conflicting results. For example, positive correlations between maternal vitamin D status and fetal growth in sheep were described by some researchers (Zhang and Naughton, 2010), while others have found no association (Symonds et al, 2009). It is important to note that there are several factors that can influence fetal growth, including genetics, maternal nutrition, stress, and environmental factors (Bazer et

al, 2015). Therefore, it is possible that other factors may have masked any potential role of maternal vitamin D level on fetal development in this study. In all, this study highlights the need for further research for an improved understanding of the potential role of vitamin D in fetal development in sheep. While the current findings suggest that there may not be a significant association between maternal vitamin D status and lamb birth weight, it is important to consider the limitations of the study and the complex interplay of factors that can influence fetal growth.

In conclusion, the findings suggest that there may be a relationship between vitamin D levels and fertility in sheep and that maintaining sufficient vitamin D levels may be important for reproductive health in sheep. Additionally, the study indicates that there may be a slight decrease in vitamin D levels in pregnant ewes as the number of births increases. Finally, the study did not find a significant correlation between maternal vitamin D levels and lamb birth weight, highlighting the need for further research to better understand the potential role of vitamin D in fetal development in sheep. In general, identifying the normal amounts and changes of this micronutrient in relation to the physiological and reproductive processes of sheep is crucial in investigating and diagnosing possible nutritional deficiencies and interpreting pathological changes in livestock. These findings suggest that monitoring serum vitamin D levels in ewes during reproduction can aid in improving sheep breeding and management practices.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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بررسی وضعیت ویتامین D در مراحل مختلف آبستنی در میش کبوده شیراز

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چکیده

هدف از این تحقیق بررسی وضعیت سرمی ویتامین D در میش‌های کبوده شیراز در مراحل مختلف تولیدمثلی از جمله جفت‌گیری، آبستنی و اوایل شیردهی بود. از تعداد ۶۰ رأس میش سالم در سه مرحله نمونه‌برداری شد. مرحله اول در زمان جفت‌گیری، مرحله دوم در اواخر بارداری و مرحله سوم بعد از زایمان بود. نمونه‌ها از نظر سطوح ۲۵ هیدروکسی ویتامین D مورد بررسی قرار گرفتند. در هنگام زایمان، چند قلوزایی، وزن بره‌ها و میزان سقط ثبت شد. نتایج نشان داد که میانگین سطوح سرمی ویتامین D در مرحله سوم نمونه‌برداری (ابتدای شیردهی) نسبت به مرحله اول (جفت‌گیری) و مرحله دوم (آبستنی) به طور معنی‌داری بیش‌تر بود، با این حال، تفاوت معنی‌داری در سطوح ویتامین D در بین گروه‌های غیر آبستن، تک قلو و دوقلو در هیچ یک از مراحل نمونه‌گیری مشاهده نشد. سطح ویتامین D در میش‌های کبوده شیراز در ابتدای شیردهی بالاتر بود. علاوه بر این، با افزایش تعداد زایش، ویتامین D سرم در میش‌های آبستن اندکی کاهش یافت. در مجموع، یافته‌ها نشان می‌دهد که حفظ سطح کافی ویتامین D ممکن است در سلامت باروری در گوسفند نقش داشته باشد، زیرا به نظر می‌رسد بین سطوح ویتامین D و باروری همبستگی وجود دارد.

کلمات کلیدی: ویتامین D، میش، آبستنی، نژاد کبوده شیراز

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