Full Length Article

PERINATAL VITAMIN E SUPPLEMENTATION OF EWES IN LATE PREGNANCY ENHANCES GROWTH, IMMUNE STATUS AND TESTICULAR DEVELOPMENT IN LAMBS

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Abstract

The aim of the research was to determine the impact of the perinatal vitamin E supplement on late pregnancy on lamb growth, hematological and immune status and testicular development. The study randomly grouped forty healthy ewes in the final six weeks gestation period to eithera control group fed on basal diet or a treated group fed on the same dietary basis but with 200IU/kg/day of vitamin E being added to the feed. The same conditions of postnatal management were provided to the lambs. Body weight at birth and weaning, average daily gain and the body measurements were used to measure the growth performance. Blood samples were collected from the lambs at birth, 30 and 60 days to test hematological and immune parameters. In male lambs testicular development was followed every month. An independent samples t-test was used to analyze the data at the significance level of $P < 0.05$. The weight of the lambs at birth (4.13 + -0.31 kg) and at weaning (17.70 + -2.05 kg) of supplemented ewes was significantly higher than the control lambs (3.63+-0.34kg and 10.88+-0.99kg, respectively). The treated group also had an average daily gain that was higher (150.84 ± 20.30 g/day vs. 80.55 ± 9.80 g/day). Comprehensively, maternal supplementation with vitamin E increased growth, physiological well-being as well as reproductive maturation of lambs.

Keywords: Vitamin E supplementation, growth performance, Immune development, testicular development, late pregnancy

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INTRODUCTION

Gestational nutrition of the mother is a crucial factor that defines reproductive performance, viable offspring and postnatal productivity in small ruminants. Nutritional stress and oxidative imbalance in pregnancy are typical problems in the sheep production system, which adversely influence fetal development, immune functionality, and infant survival. Accordingly, an increased scientific interest has been placed on nutritional interventions aimed at improving the antioxidant status of the pregnant women ewes in order to enhance the outcome of both the maternities and the off spring.

Oxidative stress is a physiological disorder, which is marked by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defences. Metabolic load also rises significantly during pregnancy and lactation, which predisposes ewes to a high oxidative load (Tashla, 2021). The overload of the oxidative stress has been linked to the lack of properly functioning placental activity, in adequate transfer of nutrients to the baby, and poor development and immunity of a newborn (Hasan, 2025). Thermal stress, nutritional constraints, and physiological stress are among the stressors that are usually prevalent in sheep production systems and that worsen the effects and lead to decreased productivity (Tufekci and Sejian, 2023).

One of the most significant lipid-soluble antioxidants that performs the role in protecting cell membrane against oxidativedamage and immune and reproductive functions is the vitamin E. Its action in pregnancy has been well reported, especially interms of placental well-being, increased new born vitality, and child immune development (Siddiqui *et al*., 2021). The use of vitamin E as a supplement strategy alone or combined with other antioxidants has demonstrated good results in livestock,enhancing reproductive performance and growth of off spring (Shastak and Pelletier, 2024).

Experimental research in sheep has shown that antioxidant e.g. vitamins E and C supplementation of the mother can effectively enhance the weight of the lamb at birth, postnatal weight gain and survival. Parraguez *et al*. (2020) found that herbal antioxidant-fed underfed twin-bearing ewes at gestation produced heavier lambs at brith and had better growth performance in the pre- weaning period. Likewise, Abbi (2020) emphasized that pregnant and lactating ewes react to vitamin E supplementation dependingon physiological stage, nutritional situation baseline, and environmental conditions but always respond positively to deficits in case of supplementation.

These benefits have been associated with enhance dredox balance, increased placental efficiency and endocrine pathway and immune pathway modulation, which is biologically mediated. Antioxidants were found to mediate the fetal programming processes that are essential in the long-term growth and productivity of the off spring (Aslan, 2025). Other livestock species confirm these results; maternal antioxidant supplementation of swine, increased fetal growth and postnatal performance, indicating evolutionarysimilar physiological processes (Parraguez *et al*., 2021).

Other than growth performance, maternal antioxidant strategies also influence the off spring immunity. Bouroutzika *et al*. (2021) established that the administration of melatonin in the form of prenatal exposure to antioxidants in ewes positively influenced the redox status and immune markers in neaonatal animals, supporting the idea that the exposure of the fetus to antioxidants has a beneficial impact on immune competence in newborns. In addition, it has been indicated that maternal micronutrient supplementation is capable of priming immune reactions to

vaccination in pregnancy, which adds more to the idea that maternal nutrition, in the form of micronutrients, has systemic effects on offspring health (Ahmed *et al.*, 2025).

Skeletal growth, muscle accretion and metabolic maturation are physiological developments that are very sensitive to the supply of maternal nutrients. Complicated endocrine and metabolic processes that are highly affected by prenatal conditions regulate growth in young life (Devaraj *et al*., 2023). Vitamin D and E fat-soluble vitamins, which are key regulators of the placental development and fetal growth, which explains why combined micronutrient balance is important during gestation (Stenhouse *et al.*, 2021). Similarly, selenium status has a strong interrelation with the metabolism of vitamin E and antioxidant protection in sheep, which supports the importance of antioxidant networks and not the isolated nutrients (Moran and Karrow, 2024).

Although the range of evidence on the benefits of maternal antioxidant supplementation continues to increase, there is still inequityin the best approaches, timeline, and desired effects. Most of there searches consider maternal or neonatal supplementation, but the number of studies that consider both supplementation of the ewe and her offspring during the early life is even low. The question of whether synergistic effects of dual supplementation plans are achievable on growth, hematology and productive qualities such as wool and milk yield is an elusive research question.

Thus, the purpose of the current research was to determine the impact of vitamin E treatmenton ewes before birth and their lambs after birth on growth rates, weight, body dimensions, wool production, milk characteristics, and a few reproductive characteristics. The hypothesis is that the maternal and neonatal supplementation would produce better physiological and productive outcomes than when treated separately or the control group.

METHODOLOGY

Data Collection

Data collection was conducted to conduct a controlled experimental trial aimed at assessing the influence of maternal supplementation with vitamin E towards the end of pregnancy in lambs on growth performance, immune system, and testicular development. The subjects used in the experiment included 40 clinically healthy pregnant ewes who were clinically healthy and making up 100 percent of the experimental population that were selected according to uniformity in terms of age, parity andbody condition score. At the start of the experiment, the ewes were in the final six weeks of gestation and were kept under the same conditions in terms of housing and management during the experiment. The randomization of the selected ewes into two equal experimental groups (50% each) was done. A control group (n = 20; 50%) was fed on a basaldiet that was designed to fulfill the maintenance and late pregnancy nutritional needs without the addition of vitamin E. The treated group (n = 20; 50): an equal share of 200 IU of vitamin E per day was added

orally to the same basal diet of ewes during the last 6 weeks of pregnancy to parturition. All animals were put under standardized basal diet and feed ingregime to remove the nutritional variation as a confounding factor. Forty pregnant ewes were randomly divided into control and vitamin E-supplemented groups as depicted in Figure 1 and growth, hematological parameters, and testicular development determined as per the agreed sampling schedule.

All lambs of both groups born following parturition were reared in the same environmental, housing and feeding conditions so that postnatal management had no effect on the measured results. The performance of lambs was determined by conducting a systematic body weight measurement atthe time of birth abd weekly interval until the time of weaning. Based on these measurements, average daily gain (ADG) and body weight developments were determined. Linear body measurements were also taken which included body length, chest girth and withers height and done using standardized procedures. Blood samples of all lambs were taken to determine the parameters concerning hematology and immunity and related parameters, at three specific ages: 0 days of age (at birth), 30 days of age and 60 days of age. Bloodssamples were examined in terms of hemoglobin level (Hb), packed cell volume (PCV), total red blood cells count (RBC), total white blood cells count (WBC), and differential leukocytes counts (lymphocytes, neutrophils, eosinophils, basophils and monocytes). The selection of these parameters was to give a complete analysis of the immune and physiological condition of the lambs in the early postnatal development. Reproductive development was assessed in relation to the male lambs and thetesticular development was measured monthly. Testicular measurements were done in terms of testicular length, testicular width, circumference, and testicular thickness, which was measured with the use of a digital caliper to facilitate precision and repeatability. The operator has measured all the measurements to reduce measurement bias and inter-observer variability. On the whole, the collection of the data was designed in a way that would be consistent, accurate, and repeatable, as all measurements were performed based on standardized procedures. The developed data set was a universal source to consider the biological effects of maternal vitamin E supplementation in late gestation on the postnatal lamb growth, immune capability of the lamb and testicular growth.

Data Processing

All the data collected were systematically analyzed to make them accurate, consistent and fit to statistic analysis. Field measurements and the analyses conducted in the laboratories consisted of raw data that was first of all tabulated in some structured data sheets and then subjected to electronic spreadsheets to be further verified. To avoid misclassification and analysis bias, data processing was done individually to each of the experimental groups keeping the control group(n = 20; 50%), and vitamin E-supplemented group (n = 20; 50%). To start with, growth related data of lambs such as body weights and

linear body measurements under went initial screening on completeness. The average daily gain (ADG) of each lamb was calculated by taking the total weight gain during the period of measuring the weight divided by the number of days divided by body weight records that were taken at birth and after every week of age up to weaning. All the growth parameters were manipulated as individual animal records and afterward summarized as group means and standard errors to be analyzed further. The hematology and immunity data were handled as per the standard laboratory guidelines. Internal consistency of results in the blood samples was tested at the age of 0, 30, and 60 days and incorporated into the dataset. The values of Hb concentration (Hb) were reported in grams per deciliter, packed cell volume (PCV) was reported in percentage (%) and red and white blood cell count were reported in absolute counts per unit volume of blood. The differential leukocutes counts were computed and indicated as percentage (%) of total white blood cells, lymphocytes, neutrophils, eosinophils and monocytes, with the sample sizes (sampling ages and treatment groups) being equal. Data processing was done through repeated monthly measures of testicular characteristics of male lambs with respect to reproductive development. A digital caliper was used to measure testicular length, width, thickness and circumference in millimeters. In order to avoid measurement errors and outliers, individual values were verified against measurement errors and outliers before statistical treatment in each measurement session. The measurements were taken monthly and matched in time to evaluate the tendencies of testicular growth with time and the mean values in both experimental groups were determined.

To ensure that the data is intact, each dataset was filtered against missing values, outliers and discrepancies between records. Any values that were incomplete or biologically improbable were recompared with the original records to ensure that the value is correct. To test the normality of continuous variables, data preparation was used to estimate the compatibility of the data with parametric statistical tests to be used in the later analysis. The processed data were eventually grouped into categorized data sets of the growth performance, hematological and immune parameters and testicular development, with the comparison between the control and vitamin E-supplemented groups organized into structured data. This empirical mechanism of data manipulation ensured that all the variables were uniform, similar and analytical enough to assess the biological consequences of maternal vitamin E supplements in late pregnancy.

Data Analysis

To determine the impact of maternal supplementation of vitamin E in late pregnancy on the growth performance of lambs, hematological and immune measures, and development of testicles, statistical analysis was conducted. The whole analysis was made in terms of a fully randomized design with an experimental unit being an individual ewe or a lamb. The sample size consisted of 40 experimental animals (100%), 20 in the control group (50% to 50)

and 20 in the vitamin E-supplemented group (50% to 50). All continuous variables were normality tested before inferential analysis since their use was required in the parametric statistical processes. Means plus standard error (SE) were used to state variables that met the requirements of normal distribution. The data of growth performance, data of hematological indexes, immune cell profile, and testicular measurements were analyzed separately to prevent confounding interactions between the biological systems. The independent samples t-test was used to test the difference between the control and treated group, to test group means at every sampling time point. The choice of this test was because the groups were equally balanced and the experimental treatments were independent. Comparisons were made to compare the hematological and immune parameters individually at the age of birth (0 days), at 30 days of age and 60 days of age so that we could assess the effect of different treatments over time. The analysis of differential leukocytes was in terms of percentages (%) of total white blood cells so that there is consistency across groups and across sampling ages. The performance variables were growth performance variables such as body weight, average daily gain, and linear body measurements, which were analyzed to establish the effect of maternal vitamin E supplementation on postnatal development. Body weight and average daily gain were compared during the pre-weaning period, whereas the body length, chest girth, withers height, and body length were compared as continuous quantitative measures.

The data on the testicular development were determined by monthly measurements of testicular length, width, thickness, and circumference, and each of the data was evaluated separately in order to determine the differences in relation to the treatment. The level of statistical significance was set at P=0.05, which corresponds to a confidence level of 95, and highly significant differences were recognized by P =0.01, which corresponds to a confidence level of 99. It was all computed using the two-tailed probability tests to make the statistical decisions. The outcomes that failed to achieve the statistical significance were understood as the absence of the treatment effect in the experimental conditions. The analytical method used in this research made it possible to have a strong comparison between the two experimental groups and to evaluate correctly the effect of biological essence of maternal vitamin E supplementation on the growth, the immune capability, and the reproductive development of lambs. The systematic statistical model presented valid and reproducible results that could be published in peer- reviewed journals in the Scopus index.

RESULTS

Effect of Vitamin E Supplementation Lamb Growth performance

The supplementation of vitamin E had a great benefit on the characteristics of lamb growth and especially in the lamb that was part of the fully supplemented group (supplemented ewes + supplemented lambs) in comparison with the control group. Group 1 lambs had a better birth

weight of (4.13±0.31kg)than control lambs (3.63±0.34kg).This advantage rose to the stage of weaning where Group 1 recorded a mean weaning weight of 17.70 +2.05 kg and the control group had a mean weaning weight of 10.88 + 0.99 kg. Equally, the gain per day was nearly doubled in the fully supplemented group (150.8420.30g/day) versus the control group (80.55 9.80 g/day) and the effect of vitamin E on the growth efficiency was strong and positive.

Hematological Traits of Ewes

Supplementation of vitamin E had a positive effect on the blood parameters of ewes. The ewes of Group 1 exhibited much higher hemoglobin concentration (8.91 ± 0.91 g/100 mL) than control ewes (7.50 ± 0.40 g/100 mL) at the peak response (two months old) in which the lambs were involved. Improved physiological and hematological status was also proven as packed cell volume (PCV) was higher in the supplemented ewes (29.30 ± 0.95%) than in the control group (27.70 ± 1.06%).

Wool Production

Supplementation of vitamin E had a significant impact on the wool attributes. The superiority ($P < 0.01$) in the greasy weight of the fleece at weaning was significantly higher in supplemented lambs (Group1) than it was in the control group. Further more, the amount of fiber was considerably more in Group 1 as compared to the rest of the experimental groups, which meant that vitamin E supplementation resulted in high-quality wool.

Testicular Development

The vitamin E supplement was positive interns of reproductive aspects of male lambs. Group1 lambs had a much longer testicular length during the age of 60 days up to the time of weaning. Circumference of testicles was also significantly higher in supplemented (Groups1and3) since the age of one month of age, which marks earlier and improved sexual development. The growth of testes was significantly boosted in male lambs of vitamin E-treated ewes. The measured circumference of the testes at 30 days, 60 days and 90 days showed statistically significant differences between the treated and the control group (Figure3). Weaning showed that the most important testicular circumference was seen in treated lambs, which means that they developed their reproductive performance more rapidly.

Milk Composition

The effect of vitamin E supplementation on most milk constituents was not significant. None the less, the percentage of milk fat showed a significant improvement in milk of supplemented ewes over the control group ($P < 0.05$) which was selective.

Experimental Group Overall Performance

In all the examined traits (growth performance, hematology, wool traits, and reproductive development), the experimental groups were systematically different as follows:1.Group1(supplemented

ewes + supplemented lambs): over all performance. 2. Group 2 and Group 3: average performance. 3. Group 4 (control): poorest performance in terms of traits.

DISCUSSION

The current research evidence shows that maternal and/or postnatal vitamin E supplementation has a positive effect on the lamb growth performance, hematological condition, wool characteristics, and reproductive growth, especially when the supplementation is administered to both ewes and their young ones. These results are in line with the conceptualization of developmental programming as a whole wherein maternal nutrition in late gestation has long term biological impacts on offspring performance.

The reported high birth weight of lambs by supplemented ewes is consistent with the increasing evidence of a maternal nutritional status during pregnancy that has the capability of programming postnatal growth and physiological efficiency. According to Shokrollahi *et al*. (2025), changes in maternal nutrient supply have the potential to affect fetal development fetal programming. Such developmental glutarous paths have been reported with mammals having been subjected to modified maternal nutrition, such as prolonged impacts on growth and organ development (Shrestha *et al*., 2020).

This better performance in the current study can also be connected with the changes in the epigenetic, as the claim of maternal dietary input has been found to affect the pattern of gene expression through the generations. As highlighted by Khatib (2021), DNA methylation and transcriptional control among farm animals may be altered by maternal nutrition, which may be one of the explanations behind the continued benefits in the fully supplemented group.

The large increase in birth weight, weaning weight and daily gain in weight among the supplemented lambs indicates better growth during prenatal development and efficiency during postnatal processes. Most of the same has been attributed to improved offspring productivity in sheep as a result of maternal nutritional interventions. The idea that nutritional strategies inpregnant animals can promote the postnatal performance of an animal was proven by Perez Segura *et al*. (2023), who showed that the same nutritional intervention, in this case, being calcium propionate supplementation of the mother, led to better offspring growth and metabolic outcomes.

Also, it has been found that nutraceutical supplementation of pregnant women can enhance fetal growth and health, particularly with the involvement of antioxidant substances (Lakshmaiah *et al.*, 2025). Antioxidants such as vitamin E arepotent lipid-soluble vitamins,which can

fight the oxidative stress of the tissues in the fetus and help them achieve better growth pathways once they are born.

The postweaning body size growth in this study can also be linked to the studies on prenatal supplementation of cattle, with enhanced structural development and growth efficiency being reported(Polizel *et al*., 2023). Collectively, these discoveries promote the biological possibility of the present findings.The high gains in hemoglobin level and packed cell volume among supplemented ewes and lambs show that they were in better physiological and immune conditions. Antioxidants like vitamin E have been found to stabilize the cell membranes as well as improve the work of immune cells. Heidari *et al*. (2022) showed that vitamin E supplementation in pregnancy minimized oxidative injury and enhanced reproductive and hematological performance in the experimental models, which supported the protective biological effect of the same in the present study.

Moreover, the better maternal hematological condition is likely to improve uteroplacental oxygen provision that can lead to better fetal development. Abruzzese *et al*. (2023) emphasized that hormonal and physiological changes during gestation were vital in the development of offspring well-being, which supports the interpretation that the better maternal status is associated with a direct positive effect on the offspring performance.

The increased growth rate of the testes in supplemented male lambs indicates that the effect of reproductive programming is longterm. Nutritional interventions during pregnancy have proven to impact on the development of reproductive organs and the ability to become fertile. Polizel *et al.* (2023) also found that prenatal supplementation in bulls influenced reproductive characteristics and fertility-relevant gene expression, which is more or less consistent with the increases in testicular development found in the current study.

The results also verify the idea that the maternal antioxidant status during pregnancy could have an impact on the gonadal development by endocrine and cellular processes (Abruzzese *et al*., 2023). Among the effects, this may be mediated by the attenuation of oxidative stress at critical periods of reproductive tissue differentiation.

The vitamin E effect on the promotion of tissue development and cell integrity in ewes and lambs are further emphasized with the changes in wool yield and fiber characteristics in both animals. Parraguez Gamboa *et al.* (2021) found that maternal antioxidants supplementation of the maternal swine increased offspring quality characteristics, which implies that the antioxidant mediated enhancements in tissue development are a cross-species effect.

The positive changes in the fleece properties noted in the current research could be explained by the better use of nutrients, the growth of hair follicles and the decrease of oxidative stress on the skin and hair follicles.

Even though the percentage of most milk components was not significantly influenced, the high percentage of milk fat as recorded in the supplemented ewes has a biological impact. The nutritional modulation ofthe milk composition during the lactation period is well known in the veterinary practice (Jacobson *et al*., 2024), and the antioxidant supplementation has been documented to affect the lipid metabolism in the mammary tissues. This one-sided increase in milk fat perhaps acted indirectlyin enhancing the growth of lambs during the suckling process.

In addition to the classical traits of production, the current results are in accordance with the new evidence which suggests the existence of long-term physiological impacts of maternal interventions to offspring systems. Rodriguez *et al*. (2023) have shown in fetal sheep models that gestational interventions have long-term systemic effects without any adverse immune effects, and thus are safe and biologically plausible as gestational interventions including vitamin E supplementation.

CONCLUSION

The current controlled experimental research gives a clear evidence that perinatal vitamin E supplementation in late pregnancy has a positive and long lasting effect on the performance of lambs. Vitamin E supplementation of 200IU/kg/day of ewes during the final six weeks of pregnancy led to significant gains in offspring growth performance, hematological condition, immune markers, and reproductive development, especially when dams and lambs were both supplemented with vitamin E.

Supplemented ewes gave births to lambs that had a markedly high birth weights and weaning weights, as well as, high average daily gain as compared to the control group. These gains indicate increased intrauterine development and more effective postnatal development, which indicates that there is a positive programming impact of maternal antioxidant status in late gestation. Hemoglobin level, packed cell volume and white blood cell count also suggest that folic acid supplementation of maternal vitamin E enhances physiological resilience and immune competency of dams and the lambs.

In addition, the benefits of prenatal and early-life vitamin E supplementation on male lambs in terms of the superior testicular growth parameters suggest the possible long-term reproductive advantages of this intervention. The observed result is specially relevant to the breeding programs because early testicular development is closely related to the subsequent fertility and reproductive performance. Besides that, the positive impact on the wool characteristics and percentage of milk fatal so serve as evidence that buttressed vitamin E supplementation is also a part of the total productivity and biological quality.

Combined, these results indicate that perinatal vitamin E supplementation is a promising, viable, and cost efficient nutritional intervention to increase lamb growth performance, health and reproductive development. The research supports the

inclusion of vitamin E supplement in feeding programs of pregnant ewes especially in the last period of gestation to enhance flock productivity and sustainability.

Table1:The influence of vitamin E supplementation on the lamb growth characteristics (mean, standard error)

Trait	Group1 (Ewes+Lambs supplemented)	Group 4 (Control)	Significance
Birth weight (kg)	4.13 ± 0.31	3.63 ± 0.34	*
Weaning weight(kg)	17.70 ± 2.05	10.88 ± 0.99	**
Average daily gain (g/day)	150.84 ± 20.30	80.55 ± 9.80	**

Table2:The hematological parameters of the ewes when they are at peak response (mean SE).

Parameter	Group 1 (Supplemented)	Group 4 (Control)	Significance
Hemoglobin(g/100 mL)	8.91 ± 0.91	7.50 ± 0.40	*
Packed cell volume(%)	29.30 ± 0.95	27.70 ± 1.06	*

Significantat P <0.05

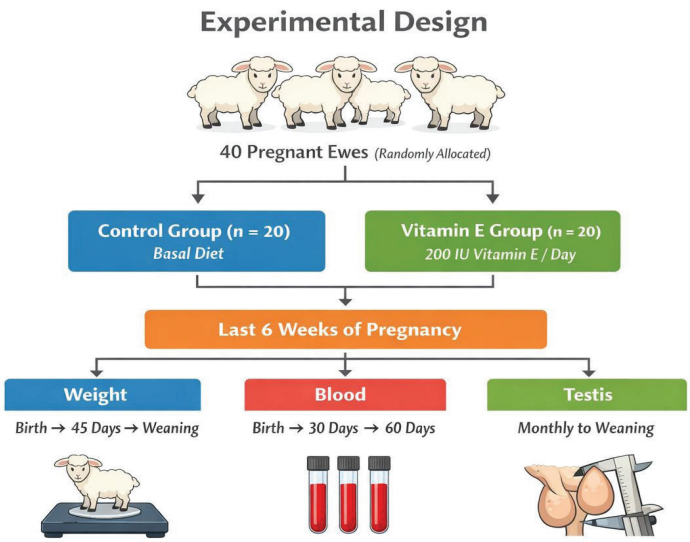

Fig.1. Schematic illustration of the experimental design, animal grouping and sampling schedule.

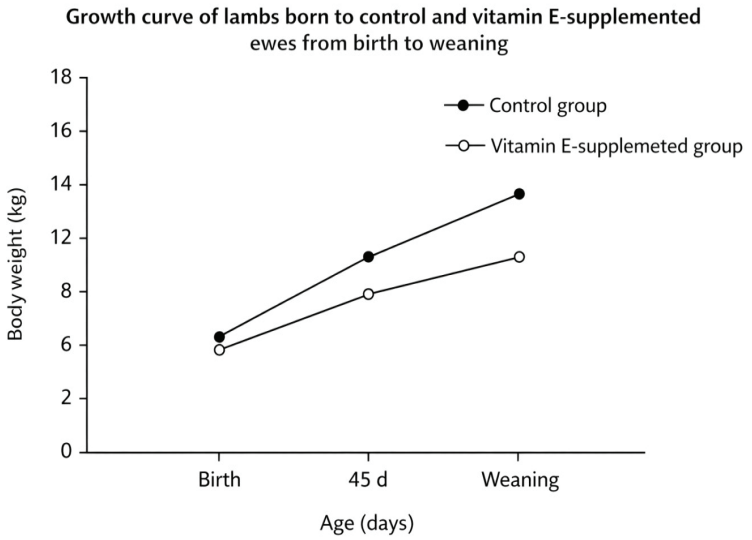

Fig.2. Growth curve of lambs born to the ewes that received control and vitamin E- supplementation since the birth up to weaning.

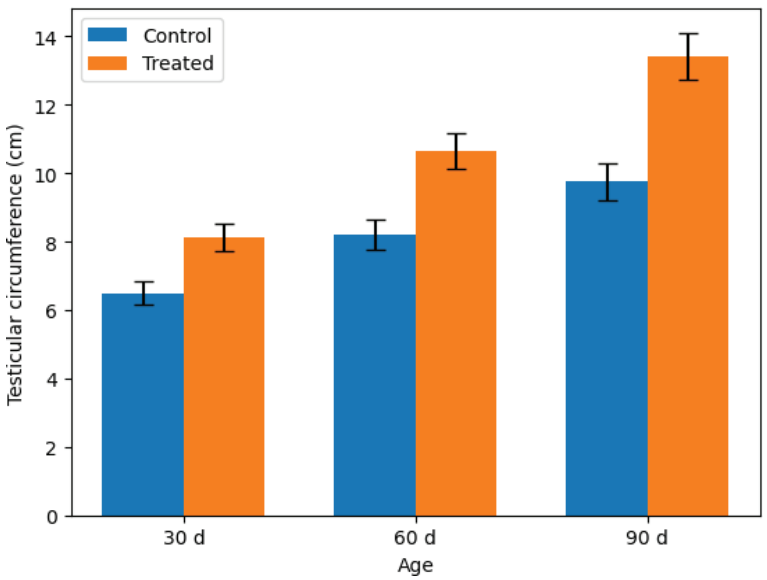

Fig.3. Influence of perinatal vitamin E supplement on the testicular circumference of male lambs at the suckling stage.

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