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Serum retinol, alpha-tocopherol, cholecalciferol, and some mineral levels in ruminants with congenital digestive and urogenital system anomalies[1]

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ABSTRACT.- Karasu A., Gençcelep M., Kayıkcı C. & Kuşcu Y. 2024. **Serum retinol, alpha-tocopherol, cholecalciferol, and some mineral levels in ruminants with congenital digestive and urogenital system anomalies**. *Pesquisa Veterinária Brasileira 44:e07535, 2024*. Department of Surgery, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, Türkiye. E-mail: abdullahkarasu@hotmail.com

This study aimed to investigate the relationship between serum vitamin and mineral levels and congenital defects in digestive and urogenital system anomalies in calves, lambs, and kids. The study material consisted of 13 calves, 15 lambs, 10 kids clinically and radiologically diagnosed with congenital digestive and urogenital system anomalies and 10 newborn clinically healthy calves, 10 lambs, and 10 kids. Congenital defects were diagnosed by clinical and radiological examination. Blood samples were collected from all animals, and sera were extracted for biochemical analysis. Vitamins A, D, and E, calcium, phosphorus, sodium, potassium, chlorine, magnesium, copper, iron, zinc, selenium, and manganese levels were measured in serum samples. Penile urethral diverticulum in kids, atresia ani, atresia ani with vaginal fistula in lambs, and atresia ani and atresia coli defects in calves were determined. Copper levels were higher, and zinc levels were lower in kids with penile urethral diverticulum compared to the control group. Vitamin A levels were lower in lambs with digestive system anomalies compared to the control group. Meanwhile, copper levels were higher in lambs with digestive system anomalies. Vitamin A and D levels were lower in calves with digestive system anomalies compared to the control group. There was no difference in the levels of the other parameters compared to the control group. In conclusion, insufficient serum vitamin A levels may play a role in the etiopathogenesis of congenital intestinal atresia in calves and lambs. Therefore, we believe that parenteral vitamin A administration to the mother, especially in the last trimester of pregnancy in regions with continental climates and poor green vegetation, would be beneficial. Further research should be conducted to determine the role of vitamin A in the etiopathogenesis of congenital atresia ani and coli.

INDEX TERMS: Congenital, anomaly, ADE vit, mineral, ruminant.

Resumo.- [Retinol sérico, alfa-tocoferol, colecalciferol e alguns níveis de minerais em ruminantes com anomalias congênitas do sistema digestivo e urogenital.] Este estudo teve como objetivo investigar a relação entre os níveis séricos de vitaminas e minerais e defeitos congênitos com envolvimento do sistema digestivo e urogenital em bezerros, cordeiros e cabritos. O material de estudo foi constituído por 13 bezerros, 15 cordeiros e 10 cabritos clinicamente e radiologicamente diagnosticados com anomalias congênitas do sistema digestivo e urogenital, e 10 bezerros, 10 cordeiros e 10 cabritos recém-nascidos clinicamente saudáveis. Defeitos congênitos foram diagnosticados por exame clínico e radiológico. Amostras de sangue foram coletadas de todos os animais e os soros foram extraídos para análise bioquímica. Os níveis de vitaminas A, D e E, cálcio, fósforo, sódio, potássio, cloro, magnésio, cobre, ferro, zinco, selênio e manganês foram determinados nas amostras de soro. Divertículo uretral peniano em cabritos, atresia anal, atresia anal com fístula vaginal em cordeiros e defeitos de atresia anal e atresia coli em bezerros foram determinados. Os níveis de cobre foram mais altos e os níveis de zinco foram mais baixos em cabritos com divertículo uretral peniano em comparação com o grupo de controle. Os níveis de vitamina A foram mais baixos em cordeiros com anomalias do sistema digestivo em comparação com o grupo de controle. Enquanto isso, os níveis de

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cobre foram mais altos em cordeiros com anomalias do sistema digestivo. Os níveis de vitaminas A e D foram mais baixos em bezerros com anomalias do sistema digestivo em comparação com o grupo de controle. Não houve diferença nos níveis dos outros parâmetros em comparação com o grupo de controle. Em conclusão, níveis insuficientes de vitamina A sérica podem desempenhar um papel na etiopatogênese da atresia intestinal congênita em bezerros e cordeiros. Portanto, acreditamos que a administração parenteral de vitamina A à mãe, especialmente no último trimestre da gravidez em regiões com clima continental e vegetação verde escassa, seria benéfica. Mais pesquisas devem ser conduzidas para determinar o papel da vitamina A na etiopatogênese da atresia ani e coli congênita.

TERMOS DE INDEXAÇÃO: Congênito, anomalia, vitamina ADE, mineral, ruminante.

INTRODUCTION

Congenital anomalies are a range of structural, functional, or metabolic disorders that occur during the development of the embryo or fetus (Su et al. 2023). Congenital anomalies, important in veterinary medicine, constitute 11.50% of surgical diseases. It is difficult to determine the number and types of patients with congenital anomalies because they are not usually referred for treatment. The incidence of anomalies varies according to the species and the environment in which the animals live, depending on various factors (Doğan & Şındak 2013). Although rare, they are considered one of the leading causes of neonatal morbidity and mortality and cause significant economic loss (Samad 2021). Congenital anomalies occur in all animal species. However, they are more common in calves, lambs, and kids (Doğan & Şındak 2013). They occur mostly in the musculoskeletal and digestive systems and less frequently in the urogenital, ocular, and other organ systems (Aksoy et al. 2006). It is reported that anorectal anomalies are the most common digestive system anomalies (Aslan et al. 2009).

The causes of congenital anomalies in ruminants have not been definitively identified. It has been suggested that these anomalies may develop at different stages of embryogenesis or fetal development due to genetic factors, infectious agents, drugs, toxic substances, various plants, mineral and vitamin (A, D, E) deficiencies, and hormonal and environmental factors, or combinations of these factors (Newman et al. 1999). Vitamins are necessary for a normal functioning metabolism and maintaining a healthy state; they cannot be synthesized in the body or are synthesized in insufficient amounts and must be ingested in small amounts from the environment in the form of food. The importance of vitamins in physiological events stems from the fact that the metabolites formed from some of them in the body function as coenzymes and cofactors in cells (Kayaalp 2002).

Trace elements have important roles in the continuity of life, growth, development, and production activities and in fulfilling many other vital functions of living organisms. Deficiency or excess of these trace elements disrupts physiological functions at the cellular level and directly or indirectly predisposes animals to the development of various metabolic and infectious diseases (Okatan et al. 2008). A regular and adequate intake of vitamins and trace elements, along with basic nutrients and building materials, such as proteins, fats, carbohydrates, amino acids, and mineral salts, is necessary for the organism to perform and maintain its functions in a healthy way (Işıkyıldız & Altıntaş 1994). Vitamins and trace elements play a role in embryonic and fetal development and ensure and maintain pregnancy. Some vitamin and trace element deficiencies significantly impair fetal development (Mahan & Vallet 1997, Blair 2020).

Given the need to investigate the contribution of vitamins and trace elements in the development of congenital malformations (Kocylowski et al. 2019, Polat 2022, Nakamura et al. 2023), this study aimed to evaluate some vitamins and minerals in the blood serum of ruminants with congenital digestive and urogenital anomalies. Also, it aimed to contribute to the etiology of the disease with scientific data and to develop prophylaxis and treatment options in light of our findings.

MATERIALS AND METHODS

Ethical approval. This study was approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (2016/02).

This study consisted of 38 animals diagnosed clinically and radiologically with congenital digestive and urogenital anomalies, including 13 calves, 15 lambs, and 10 kids of different breeds, ages, and sexes, as well as 30 healthy controls, including 10 clinically healthy newborn calves, 10 lambs, and 10 kids, obtained from Van region.

Anamnesis. Information like the mother's breeding method, whether the mother was supplemented with vitamins and minerals during pregnancy, the mother's nutrition, the number of offspring, and whether there were similar disorders in previous offspring were obtained from the animal owners.

Clinical and radiographic examination. Routine physical and clinical examinations, such as general condition, heart, and respiratory frequency, were performed after anamnesis. Radiographic examinations of the abdominal and pelvic regions were performed in latero-lateral (L/L) and ventro-dorsal (V/D) positions following clinical examination methods, including inspection, auscultation, palpation, and percussion. A colonoscopy was performed in necessary cases to diagnose anomalies.

Laboratory analysis. Blood samples collected from the vena jugularis of both congenital anomalies (38 animals) and healthy animals (30 animals) were placed in gel biochemistry tubes. Blood samples were transferred to biochemistry tubes and centrifuged at 3,000rpm for 10 minutes to remove serum. Sera were transferred to Eppendorf tubes and stored at -20°C until the analysis day. Serum retinol, α-tocopherol, cholecalciferol, calcium, phosphorus, sodium, potassium, chlorine, magnesium, copper, iron, zinc, selenium, and manganese levels were analyzed.

Statistical analysis. Statistical evaluation of the data was performed using the SPSS statistical package program. An independent t-test was used to determine the significance of the differences between the groups. If $p<0.05$, the difference was statistically significant.

RESULTS

Anamnesis

The distribution of calves with anomalies according to breeds was as follows: 10 were Simmental, two were Montafon, and one was Indigenous. Seven were male, five were female, and one was intersex. Age ranged from 1-8 days old. Among the diseases of the digestive and urogenital systems, six calves had atresia coli, five had atresia ani, and two had atresia ani with rectovaginal fistula. All the lambs with anomalies were the Akkaraman breed. The distribution of lambs by

sex was nine males and six females. In terms of digestive and urogenital system diseases, 10 lambs had atresia ani, and five lambs had atresia ani with rectovaginal fistula. The goats were distributed with anomalies according to breed, with four colored Mohair and six Sanen. The age range of all male kids was between 4-15 days old. All kids had penile urethral diverticula. In the anamnesis, the owners reported that the calves suckled their mothers after birth and appeared healthy. However, in the following days, they noticed a loss of appetite, inability to defecate, and/or closed anus. They said the calves had difficulty urinating and observed swelling in the preputium area. None of the mothers were given vitamin and mineral supplements during the gestation period, and they did not observe any defects in their previous offspring. Sheep and goats were conceived by natural mating, and cows were conceived by artificial insemination.

Clinical and radiographic results

In the clinical examination of the animals with digestive system anomalies, there was a decrease in bowel sounds, abdominal tension, and straining in all cases. In cases of atresia ani, the anus was closed. Moreover, with atresia ani, the diagnosis was made by the formation of a bulge due to the pressure of the rectum on the anal region during straining or when pressure was applied to the abdominal region. In animals with atresia ani with rectovaginal fistula, the clinical findings were similar to the clinical findings of the other cases, with some differences; fecal residues were observed in the vagina and vulva lips. Varying amounts of feces exited the vulva due to straining or after abdominal pressure. When the vagina was opened with a speculum in these animals who were placed in the supine position, the presence of a fistula on the dorsal wall was detected. In cases with atresia coli, the intestines were filled with gas, peristalsis was increased, and mucus was observed during rectal palpation. Some animals who presented late to our clinic had tachycardia and varying degrees of dehydration. Although the anus was open, abdominal distension, straining, and depression were observed. In addition, some mucus was coming out from the anus with straining. In goats with penile urethral diverticulum, the swelling was observed in the pre-scrotal region, and urination normally occurred when this swelling was pressurized. In cases of atresia ani and atresia ani with rectovaginal fistula, the last part of the rectum filled with meconium and gas was identified by direct abdominal radiography in the L/L position. The clinical diagnoses were confirmed in this way (Fig.1). In addition, the position of the fistula was determined by contrast radiography in cases of atresia ani with rectovaginal fistula. In cases of atresia coli, direct antegrade and indirect retrograde radiographs showed no intestinal passage.

Laboratory results

Blood-serum retinol, α-tocopherol, cholecalciferol, calcium, phosphorus, sodium, potassium, chlorine, magnesium, copper, iron, zinc, selenium, and manganese levels for animals with congenital anomalies and healthy controls are presented in Table 1.

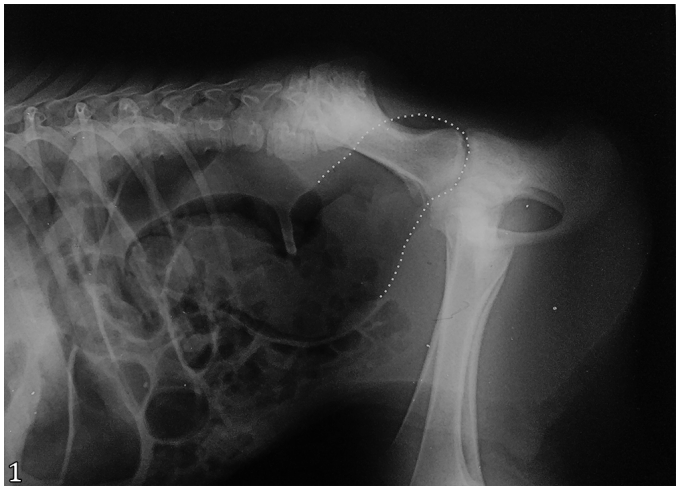

Fig.1. Atresia ani in a calf in the latero-lateral (L/L) position. White dots indicate the border of the blind end of the rectum.

Table 1. Blood-serum retinol, α-tocopherol, cholecalciferol, calcium, phosphorus, sodium, potassium, chlorine, magnesium, copper, iron, zinc, selenium and manganese levels of animals with congenital anomalies and healthy control group animals

Parameter	Control group calves (n = 10)	Study group calves (n = 13)	Control group lambs (n = 10)	Study group lambs (n = 15)	Control group kids (n = 10)	Study group kids (n = 10)
Vit A (µg/dl)	30.28±8.28	10.66±4.56*	36.88±7.51	12.21±3.98*	27.38±4.21	30.34±6.06
Vit D (ng/ml)	25.56±5.86	20.24±4.55**	27.70±4.39	25.86±5.72	21.55±0.24	21.75±0.43
Vit E (mg/l)	2.10±0.90	1.61±0.91	0.40±0.15	0.47±0.21	24.04±5.13	21.48±12.77
Zn (µg/ml)	1.61±0.26	1.49±0.35	1.47±0.17	1.40±0.32	1.71±0.19	1.39±0.23*
Cu (µg/dl)	58.86±2.63	61.79±5.14	54.57±7.75	66.56±7.77**	51.63±4.49	58.92±7.22*
Fe (µg/dl)	61.00±17.07	54.23±16.89	178.60±41.95	163.42±67.63	146.60±81.32	202.00±71.78
Ca (mg/dl)	10.15±0.87	9.29±2.93	12.94±2.87	10.46±1.68	11.18±1.25	11.32±2.77
Mg (mg/dl)	1.78±0.19	1.96±0.26	2.22±0.49	2.07±0.72	2.14±0.21	2.30±0.55
Se (ng/ml)	84.28±16.96	94.47±44.52	130.59±44.17	131.44±45.28	90.55±29.76	123.11±55.26
Mn (ng/ml)	2.19±1.47	4.31±3.32	1.30±0.75	1.19±0.64	1.13±0.80	1.68±1.17
P (mg/dl)	8.25±2.25	7.82±3.75	6.25±1.54	6.52±1.27	8.37±0.40	8.86±0.65
Na (mEq/l))	135.42±3.87	138.23±4.55	140.01±8.77	143.28±7.65	140.27±1.85	142.44±1.24
K (mEq/l))	6.56±1.25	6.03±0.95	5.51±1.22	5.81±1.03	6.04±0.56	6.23±0.65
Cl (mEq/l)	92.62±7.49	101±10.85	98.16±7.81	95.28±8.11	101.13±0.95	103.35±1.67

* $p<0.001$, ** $p<0.05$.

DISCUSSION

Congenital anomalies are structural and functional defects that develop during intrauterine life and are present in one or more organs at birth (Ortega-Pacheco et al. 2020). These anomalies can range from minor anatomical defects to semi-fatal or fatal diseases, depending on the degree of malformation (Uzar et al. 2020). Congenital anomalies can result in significant economic losses due to reduced fertility, increased perinatal and neonatal losses, and, consequently, a reduction in the number of offspring obtained (Karaman et al. 2013). Although the prevalence of congenital anomalies in farm animals is low, many are born with congenital defects yearly (Williams 2010).

Most studies investigating the incidence of congenital anomalies have been conducted either as regional surveys or in animals admitted to clinics. In these studies, congenital anomalies were found between 1.71% (Hossain et al. 2016) and 2.96% (Oğurtan et al. 1997) in newborn ruminants. We believe these different rates of anomalies in ruminants vary according to geographical region, dietary level, and environment (Sonfada et al. 2010). Many studies have reported the musculoskeletal, ocular, and digestive systems in calves (Özaydın et al. 1995, Oğurtan et al. 1997, Sonfada et al. 2010, İşler et al. 2016), the digestive system in lambs (Özaydın et al. 1995, Aksoy et al. 2006, İşler et al. 2016), and the urinary system in kids as the most common system anomalies (Özaydın et al. 1995, Aksoy et al. 2006, Doğan & Şındak 2013, İşler et al. 2016). In our study, digestive system anomalies were observed in 2.87% and urinary system anomalies in 1.02% of newborn ruminants admitted to our clinic during the spring season for three years. Anorectal anomalies and atresia coli were the most common digestive system anomalies in calves and lambs, while penile urethral diverticulum anomaly was the most common urinary system anomaly in kids.

Different results were reported when congenital anomalies in calves were evaluated in terms of breed and sex. While some researchers reported that there was no significant difference between congenital anomalies in calves and their breeds (Özaydın et al. 1995), many studies reported that congenital anomalies in calves were mostly seen in Holstein (İşler et al. 2016), Simmental (Aksoy et al. 2006), and Montafon (Kaya et al. 2011) breeds. The present study observed congenital anomalies mostly in Simmental calves, Akkaraman lambs, and Sanen kids. The high incidence of congenital anomalies in these breeds in our study may be related to the fact that they are common breeds in the region. In some studies, although anomalies are related to the breed, they are also found in non-susceptible breeds, which may be related to some regional environmental and pathological factors (Göksel & Sarıtaş 2016). It is thought that when a new breed is reared in a region, an increase in congenital anomalies may occur due to environmental factors (Aksoy et al. 2006). Many studies investigating the sex distribution of congenital anomalies in ruminants have reported that congenital anomalies are more common in males (Özaydın et al. 1995, Oğurtan et al. 1997, Aksoy et al. 2006, Göksel & Sarıtaş 2016, İşler et al. 2016). Similarly, in the present study, congenital anomalies observed in calves, lambs, and kids were predominantly in males. A discussion of gender and the causes of the anomalies has not yet been put forward by the authors (Özaydın et al. 1995, Oğurtan et al. 1997, Aksoy et al. 2006, Göksel & Sarıtaş 2016, İşler et al. 2016).

In the first three weeks of pregnancy, if congenital malformations arise, the embryo dies, or the regulatory mechanism in the embryo prevents damage and allows the embryo to continue its life. Between three and eight weeks (e.g., the oogenesis period), the embryo is vulnerable to abnormal development. Structural malformations are inevitable in the affected embryo during this period. After eight weeks of gestation, major structural anomalies are unlikely to occur. During this period, many organs have completely developed (Sinowatz 2010). We think the malformations observed in our study resulted from the oogenesis period in the uterus. Studies have shown that only 30% to 35% of human congenital malformations have an identifiable etiology. The unknown causes of most human congenital malformations are reported to be multifactorial interactions between genetic and environmental factors, potentially representing complex host-pathogen-environment interactions that occur in disease pathogenesis. Similar complexities are inevitable in studying animal congenital disorders (Windsor 2019). In contrast to the situation in humans, reliable data on the etiology of congenital defects in domestic animals are scarce (Sinowatz 2010).

In the literature, it has been suggested that the etiology of congenital anomalies in ruminants may result mainly from genetic, chromosomal, infectious, or environmental factors or a combination of these (Dennis & Leipold 1986, McGeady et al. 2017). Among environmental factors, nutritional deficiencies (Sinowatz 2010, McGeady et al. 2017), vitamin deficiency (e.g., A, D, E, folic acid) (Dennis & Leipold 1986), consumption of toxic plants, exposure to environmental pollutants or harmful physical factors play an important role (Sinowatz 2010, McGeady et al. 2017). Maternal nutrition plays a critical role in fetal growth and development. Despite considerable effort in the last 30 years to define the nutrient requirements of animals, malnutrition during pregnancy remains a major problem for many animals, such as cattle, pigs, and sheep, worldwide (Wu et al. 2004). Nutritional deficiencies are a known cause of congenital defects in many animal species (McGeady et al. 2017). Studies have suggested that vitamin deficiencies, including A, D, E, and folic acid, and trace elements, such as Cu, Zn, Mg, and Se, may lead to congenital malformations. In many human studies, maternal concentrations of some vitamins and trace elements were significantly lower in mothers of infants with congenital anomalies compared to mothers who gave birth to healthy babies (Sarmah et al. 2016).

Zinc deficiency during pregnancy is teratogenic in many species, such as rats, mice, sheep, and chickens. Offspring of rats fed a zinc- or copper-deficient diet during pregnancy developed congenital anomalies affecting almost every organ and system. Zinc or copper deficiencies are considered effective in developing anomalies, causing changes in cellular redox balance, migration of neural crest cells, expression of key regulatory genes, tissue oxidative stress and inappropriate cell death (Ovayolu et al. 2020). Studies investigating trace element levels in serum (Kocylowski et al. 2019) and amniotic fluid in humans suggested that low trace element levels may be associated with congenital malformations (Kocylowski et al. 2019, Ovayolu et al. 2020).

In our study, although serum copper values (66.56±7.77µg/dl) were statistically higher (p<0.05) in lambs with digestive system anomalies compared to the control group (54.57±7.75µg/dl), we found that this value was between the reference values (Page et al. 2018) for lambs (Table 1). In addition, when the interaction between copper and low serum vitamin A levels was examined, copper deficiency or excess did not affect vitamin A metabolism (Dulin et al. 1992). High levels of any mineral substance or trace element, such as calcium, phosphate, iron, zinc, copper, and manganese, may cause deviations in the availability of other elements (Davis 1972). Regarding Zn and Cu, Zn and Fe, and Fe and Mn, it has been reported that adequate or high levels of one nutrient and critical or inadequate dietary levels of the other nutrient can lead to an antagonistic interaction (Graham et al. 1994). In addition, the absorption of Zn and Cu from the gastrointestinal tract is mediated by carriers (e.g., transferrin and metallothionein), and there is competition between these two elements because they share the same binding sites in the carrier (Davis 1972). The main negative interaction affecting Zn absorption is excess copper in the diet (Price et al. 1987), and high levels of Cu in the diet and liver can lead to suppression of Zn levels (Davis 1972). In our study, serum copper (59.83±6.58µg/dl) was significantly higher (p<0.05), and serum zinc (1.44±0.25µg/dl) was significantly lower (p<0.01). However, these values were within the reference values (Haenlein & Anke 2011) for goats (Table1). However, the low Cu levels at high Zn levels may be explained by the antagonistic effect of high Cu on Zn (Davis 1972, Price et al. 1987). The decrease in Zn levels in various tissues is suggested to be primarily due to the negative effect of high Cu levels on the absorption and transport of Zn through the intestinal mucosa (Davis 1972). Vitamins are important in physiological processes because some of them act as coenzymes and cofactors in the cells of metabolites formed. Active metabolites consisting of vitamin A and D precursors control the basic events in target cells through their steroid-like effects on the expression of certain genes via specific receptors (Kayaalp 2002).

Vitamin A and its natural metabolites and synthetic derivatives are called retinoids. Vitamin A is not biologically active on its own but is oxidized in tissue to retinaldehyde and then to retinoic acid (Blomhoff & Blomhoff 2006). Retinoic acid (all-trans retinoic acid) is the most active metabolite of vitamin A. Retinoic acid is critical in regulating various biological functions, such as gene expression, cell differentiation and proliferation of epithelial cells, and embryonic development (Sasaki et al. 2011).

Excessive vitamin A during pregnancy was first shown to be teratogenic in 1953. Since then, many studies have confirmed the teratogenic effect of vitamin A and its metabolites (Holson et al. 1999). Both clinical and experimental studies have reported that excess and deficiency in vitamin A during pregnancy are teratogenic to the developing fetus and cause congenital malformations in humans and animals (Pitera et al. 2001, Freytag et al. 2003).

Vitamin A exerts its effect by binding retinoic acid to nuclear retinoid receptors, which then bind to specific DNA promoters and control the transcription of specific genes. Retinoic acid can either initiate or repress the expression of genes important for embryonic development. In several studies, retinoid deficiency (Freytag et al. 2003) or excess (Pitera et al. 2001, Freytag et al. 2003) has been shown to impair retinoid-mediated signal transduction in pregnant experimental animals (Freytag et al. 2003, Guo et al. 2005), causing laterality defects in vertebrate embryos (Freytag et al. 2003) and a disruption in normal morphogenesis, particularly in the caudal region of the hindgut (Guo et al. 2005, Blomhoff & Blomhoff 2006). Studies have reported that retinoic acid inhibits cecal bud formation, has a direct effect on intestinal morphogenesis and innervation, and retinoic acid signaling is involved in regulating genes involved in intestinal morphogenesis (Pitera et al. 2001).

Vitamin A deficiency is primarily caused by a lack of vitamin A or its precursor carotene in the diet. Additionally, vitamin A or beta-carotene deficiency can occur at the tissue level due to interference with digestion, absorption, or metabolism. The diagnosis of vitamin A deficiency is based on clinical findings and serum vitamin A levels (Sarmah et al. 2016).

The normal value of serum vitamin A in calves is reportedly 25-35µg/dL, and a decrease in this value below 20µg/dL is considered a vitamin A deficiency (Millemann et al. 2007). In calves, the average blood plasma vitamin A value was 25.35µg/dL between the third day and 22nd day (Hidiroglou & Markham 1996). Herdt & Stowe (1991) found that average blood plasma vitamin A values in calves between the first day and the 29th day were 20.00-27.5mcg/dl. Below 15µg/dL was considered insufficiency (Herdt & Stowe 1991). In the literature, mean serum vitamin A was found to be 13.6µg/dL in calves with ocular deformity, including amaurosis, which is considered hypovitaminosis A (Millemann et al. 2007).

There is no consensus in the literature on reference ranges for vitamin A in sheep (Panousis et al. 2007). Normal serum vitamin A levels in sheep vary between 19.6-47.7µg/dL; a decrease below 20µg/dL is considered a vitamin A deficiency (Rooke et al. 2008). In a different study, plasma vitamin A levels in sheep below 17.1µg/dL were considered insufficient (Webb Jr. et al. 1971). In an experimental study, the serum vitamin A levels of lambs fed a diet containing adequate levels of vitamin A and a diet devoid of vitamin A were found to be 37µg/dL and 15µg/dL, respectively, on the 40th day of the study (Bruns & Webb Jr. 1990). Another study reported that xerophthalmia developed in lambs with serum vitamin A levels below 13µg/dL, and neurological disorders and death occurred in lambs with serum vitamin A levels of 7µg/dL and below (Ghanem & Farid 1982).

In this study, serum vitamin A values were 10.66±4.56µg/dL in calves with congenital atresia ani and atresia coli anomalies and 30.28±8.28µg/dL in healthy control calves (p<0.001). In our study, serum vitamin A levels were 12.21±3.98µg/dL in lambs with congenital atresia ani and atresia ani with rectovaginal fistula anomalies and 36.88±7.51µg/dL in healthy control lambs (p<0.001) (Table 1). Given that the serum vitamin A levels observed in calves and lambs with anomalies were lower compared to the control group and compatible with vitamin A deficiency levels reported in the literature, we expect the anomalies found in the calves and lambs may be due to vitamin A deficiency. In addition, in a study conducted in humans, serum vitamin A concentration in newborns with congenital anorectal malformations was statistically significantly lower (11.2±3.92µg/dL) compared to the control group (15.12±2.8µg/dL) (Wang et al. 2019). Similarly, in our study, serum vitamin A levels in calves and

lambs with intestinal atresia were lower than in healthy animals (Table 1).

In a study investigating the effect of maternal vitamin A deficiency on the embryological development of anorectal malformations and the enteric nervous system in rats, it was suggested that vitamin A deficiency might cause anorectal malformations by impairing the development of the enteric nervous system. The same study suggested that maternal vitamin A deficiency causes teratogenicity in rat offspring (Huang & Zheng 2011). Further, atresia ani may occur in animals with congenital vitamin A deficiency (Brown et al. 2007). In the literature, vitamin A deficiency during pregnancy has been associated with an increased risk of congenital anorectal malformations in many species, and vitamin A deficiency has been reported to be related to inappropriate development of the enteric nervous system (Uzal et al. 2016). In our study, we think intestinal atresia in calves and lambs may have been affected by vitamin A deficiency by impairing the development and morphogenesis of the intestinal nervous system (Guo et al. 2005, Huang & Zheng 2011, Sasaki et al. 2011).

In ruminants, beta-carotene from green plants is synthesized into vitamin A in the intestinal epithelium or liver and stored in the liver (Nielsen et al. 1966). Vitamin A deficiency occurs primarily due to a deficiency of green forages in the ration, insufficient vitamin A supplementation in the feed, and prolonged feed storage under inappropriate conditions. A secondary cause is inadequate intestinal absorption or disruptions in vitamin A synthesis (Kopcha 1987). It has been reported that vitamin A deficiency occurs frequently in regions with dry summers and continental climates (Sarmah et al. 2016). We think that continental climate, poor green vegetation, inadequate feed storage under appropriate conditions, and faulty feeding practices in animal husbandry may play a role in developing vitamin A deficiency in our region.

Vitamin D has many bodily functions, such as bone development, regulation of glucose homeostasis, and aiding in the anti-inflammatory response. Maternal vitamin D levels, especially before and during pregnancy, play a critical role in the future life of the fetus. Decreased or increased maternal vitamin D concentration causes intrauterine developmental disorders and changes in genetic and epigenetic mechanisms in the fetus. Therefore, optimal placental vitamin D concentrations are important for maintaining a healthy pregnancy and ensuring fetal development (Küçükcankurtaran & Caferoğlu 2021). In our study, although vitamin D levels (20.24±4.55ng/ml) were statistically lower (p<0.05) in calves with atresia ani and atresia coli anomalies compared to the control group (25.56±5.86ng/ml) (Table 1), the values were within the normal limits (20-50ng/ml) (Nelson et al. 2016).

CONCLUSION

In light of the anamnesis obtained in our study, it was understood that no specific teratogen and drug administration was performed. The etiology of the congenital anomalies observed could not be clearly defined. Various genetic and environmental factors are thought to be responsible for congenital anomalies in ruminants. We found high copper levels in lambs and kids and low zinc levels in kids were within normal reference values. However, vitamin A deficiency in calves and lambs with congenital atresia may be an important environmental factor in atresia formation. Therefore, we believe that vitamin A administration to the mother during pregnancy, especially in the last trimester, would benefit regions with continental climates and poor green vegetation. We also believe that more studies should be conducted to evaluate the role of vitamin A in the etiopathogenesis of congenital atresia ani and atresia coli.

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REFERENCES

Aksoy Ö., Kılıç E., Öztürk S., Özaydın İ., Kurt B. & Baran V. 2006. Congenital anomalies encountered in calves, lambs and kids 1996-2005 (262 cases). Kafkas Üniv. Vet. Fak. Derg. 12(2):147-154.

Aslan L., Karasu A., Gençcelep M., Bakır B. & Alkan İ. 2009. Evaluation of cases with congenital anorectal anomalies in ruminants. YYU Vet. Fak. Derg. 20(1):31-36.

Blair P. 2020. The genetics of prenatal diagnosis, c.1950-1990: the case of Malcolm Ferguson-Smith. PhD Thesis, University of Glasgow, Glasgow. 365p.

Blomhoff R. & Blomhoff H.K. 2006. Overview of retinoid metabolism and function. J. Neurobiol. 66(7):606-630. <https://dx.doi.org/10.1002/neu.20242> <PMid:16688755>

Brown C.C., Baker D.C. & Barker I.K. 2007. Alimentary system: intestine, p.60-110. In: Maxie M.G. (Ed.), Jubb, Kennedy and Palmer's Pathology of Domestic Animals. Vol.1. 5th ed. Elsevier Saunders, Edinburgh.

Bruns N.J. & Webb Jr. K.E. 1990. Vitamin A deficiency: serum cortisol and humoral immunity in lambs. J. Anim. Sci. 68(2):454-459. <https://dx.doi.org/10.2527/1990.682454x> <PMid:2312433>

Davis G.K. 1972. Competition among mineral elements relating to absorption by animals. Ann. N. Y. Acad. Sci. 199(1):62-69. <https://dx.doi.org/10.1111/j.1749-6632.1972.tb46443.x>

Dennis S.M. & Leipold H.W. 1986. Congenital and inherited defects in sheep, p.864-868. In: Morrow D.A. (Ed.), Current Therapy in Theriogenology. 2nd ed. W.B. Saunders, Philadelphia.

Doğan H. & Şındak N. 2013. The incidence of anomalies in calves, lambs and goat kids in the district of Nizip and its villages and determination of some biochemical parameters in these cases. Harran Üniv. Vet. Fak. Derg. 2(2):61-66.

Dulin A.M., Bieri J.G & Smith Jr. J.C. 1992. Copper deficiency fails to affect vitamin A status. Nutr. Res. 12(11):1365-1372. <https://dx.doi.org/10.1016/S0271-5317(05)80535-4>

Freytag T.L., Liu S.M., Rogers Q.R. & Morris J.G. 2003. Teratogenic effects of chronic ingestion of high levels of vitamin A in cats. J. Anim. Physiol. Anim. Nutr. 87(1/2):42-51. <https://dx.doi.org/10.1046/j.1439-0396.2003.00400.x> <PMid:14511148>

Ghanem Y.S. & Farid M.F.A. 1982. Vitamin A deficiency and supplementation in desert sheep. 1. Deficiency symptoms, plasma concentrations and body growth. World Rev. Anim. Prod. 18(2):69-73.

Göksel B.A. & Sarıtaş Z.K. 2016. Clinical and operative approach of intestinal atresia in calves. Kocatepe Vet. J. 9(3):200-210. <https://dx.doi.org/10.5578/kvj.27977>

Graham T.W., Thurmond M.C., Mohr F.C., Holmberg C.A., Anderson M.L & Keen C.L. 1994. Relationships between maternal and fetal liver copper, iron, manganese, and zinc concentrations and fetal development in California Holstein dairy cows. J. Vet. Diagn. Invest. 6(1):77-87. <https://dx.doi.org/10.1177/104063879400600114> <PMid:8011786>

Guo J., Wang L.Y., Zhang Z.B. & Bai Y. 2005. Anorectal malformation and accompanied malformations in rat fetuses induced by retinoic acid. Chinese J. Birth Health Heredity 13(1):93-95.

Haenlein G.F.W. & Anke M. 2011. Mineral and trace element research in goats: a review. Small Rumin. Res. 95(1):2-19. <https://dx.doi.org/10.1016/j.smallrumres.2010.11.007>

Herdt T.H. & Stowe H.D. 1991. Fat-soluble vitamin nutrition for dairy cattle. Vet. Clin. N. Am., Food Anim. Pract. 7(2):392-415. <https://dx.doi.org/10.1016/S0749-0720(15)30796-9> <PMid:1893278>

Hidiroglou M. & Markham F. 1996. Effect of oral supplements of vitamin A on the plasma retinol levels in calves and their immunological unresponsiveness. Reprod. Nutr. Dev. 36(5):467-472. <https://dx.doi.org/10.1051/rnd:19960502> <PMid:8987098>

Holson R.R., Adams J. & Ferguson S.A. 1999. Gestational stage-specific effects of retinoic acid exposure in the rat. Neurotoxicol. Teratol. 21(4):393-402. <https://dx.doi.org/10.1016/S0892-0362(99)00007-0> <PMid:10440483>

Hossain M., Hasan M. & Bhuiyan M.J.U. 2016. Prevalence of clinical diseases of cattle of moulvibazar district in Bangladesh. Int. J. Nat. Sci. 6(2):54-61.

Huang Y. & Zheng S. 2011. The effect of vitamin A deficiency during pregnancy on anorectal malformations. J. Pediatr. Surg. 46(7):1400-1405. <https://dx.doi.org/10.1016/j.jpedsurg.2011.02.042> <PMid:21763842>

Işıkyıldız A. & Altıntaş A. 1994. Bakarkör buzağı ve danalarda serum ve karaciğer iz element (Zn, Cu, Mn) düzeyleri. A. Üniv. Vet. Fak. Derg. 41(3/4):477-488. <https://dx.doi.org/10.1501/Vetfak_0000000349>

İşler C.T., Altuğ M.E., Gönenci R. & Aytekin İ. 2016. 2005-2009 yılları arasında Bolu bölgesinde buzağılarda tespit edilen anomali olgularının değerlendirilmesi. Harran Üniv. Vet. Fak. Derg. 5(2):100-104.

Karaman M., Özen H. & Dağ S. 2013. Buzağılarda konjenital defektler. Turkiye Klinikleri J. Vet. Sci. 4(1):113-116.

Kaya M., Okumuş Z., Doğan E., Çetin E.M. & Yanmaz L.E. 2011. Erzurum yöresindeki buzağılarda doğmasal anomalilerin görülme sıklığı ve sağaltım oranları. F. Üniv. Sağ. Bil. Vet. Derg. 25(2):83-93.

Kayaalp S.O. 2002. Rasyonel Tedavi Yönünden Tıbbi Farmakoloji. 11th ed. Hacettepe-Taş Kitapçılık, Ankara. 1726p.

Kocylowski R., Grzesiak M., Gaj Z., Lorenc W., Bakinowska E., Barałkiewicz D., von Kaisenberg C.S., Lamers Y. & Suliburska J. 2019. Associations between the level of trace elements and minerals and folate in maternal serum and amniotic fluid and congenital abnormalities. Nutrients 11(2):328. <https://dx.doi.org/10.3390/nu11020328> <PMid:30717440>

Kopcha M. 1987. Nutritional and metabolic diseases involving the nervous system. Vet. Clin. N. Am., Food Anim. Pract. 3(1):119-135. <https://dx.doi.org/10.1016/S0749-0720(15)31184-1> <PMid:3552147>

Küçükcankurtaran S. & Caferoğlu Z. 2021. D Vitamininin Maternal ve Fetal Sağlık Üzerine Etkisi: Fetal Programlama, Genetik ve Epigenetik Mekanizmalar. CBU-SBED 8(4):709-714. <https://dx.doi.org/10.34087/cbusbed.929505>

Mahan D.C. & Vallet J.L. 1997. Vitamin and mineral transfer during fetal development and the early postnatal period in pigs. J. Anim. Sci. 75(10):2731-2738. <https://dx.doi.org/10.2527/1997.75102731x> <PMid:9331877>

McGeady T.A., Quinn P.J., FitzPatrick E.S., Ryan M.T., Kilroy D. & Lonergan P. 2017. Veterinary Embryology. 2nd ed. John Wiley and Sons, Oxford, p.73-80.

Millemann Y., Benoit-Valiergue H., Bonnin J.-P., Fontaine J.-J. & Maillard R. 2007. Ocular and cardiac malformations associated with maternalhypovitaminosis A in cattle. Vet. Rec. 160(13):441-443. <https://dx.doi.org/10.1136/vr.160.13.441> <PMid:17400904>

Nakamura Y., Kobayashi S., Cho K., Itoh S., Miyashita C., Yamaguchi T., Iwata H., Tamura N., Saijo Y., Ito Y., Seto Y., Honjo R., Ando A., Furuse Y., Manabe A., Kishi R. & JECS Group. 2023. Prenatal metal concentrations and physical abnormalities in the Japan environment and children's study. Pediatr. Res. 95(7):1875-1882. <https://dx.doi.org/10.1038/s41390-023-02851-4> <PMid:37857850>

Nelson C.D., Lippolis J.D., Reinhardt T.A., Sacco R.E., Powell J.L., Drewnoski M.E., O'Neil M., Beitz D.C. & Weiss W.P. 2016. Vitamin D status of dairy cattle: Outcomes of current practices in the dairy industry. J. Dairy Sci. 99(12):10150-10160. <https://dx.doi.org/10.3168/jds.2016-11727> <PMid:27743666>

Newman S.J., Bailey T.L., Jones J.C., DiGrassie W.A. & Whittier W.D. 1999. Multiple congenital anomalies in a calf. J. Vet. Diagn. Invest. 11(4):368-371. <https://dx.doi.org/10.1177/104063879901100414> <PMid:10424656>

Nielsen S.W., Mills J.H.L., Woelfel C.G. & Eaton H.D. 1966. The pathology of marginal vitamin A deficiency in calves. Res. Vet. Sci. 7(2):143-154. <https://dx.doi.org/10.1016/S0034-5288(18)34693-9>

Oğurtan Z., Alkan F. & Koç Y. 1997. Ruminantlarda kongenital anomaliler. Türk. Vet. Hek. Derg. 9(4):24-28.

Okatan A.G., Çam Y. & Leblebici Z. 2008. Kayseri yöresinde dil oynatma hastaliği olan siğirlarda bazi iz elementlerin serum düzeylerinin değerlendirilmesi. Sağlık Bilimleri Dergisi 17(1):16-22.

Ortega-Pacheco A., Lezama-García M.A., Colín-Flores R., Jiménez-Coello M., Acevedo-Arcique C. & Gutiérrez-Blanco E. 2020. Presence of congenital anomalies in three dog litters. Reprod. Domest. Anim. 55(5):652-655. <https://dx.doi.org/10.1111/rda.13652> <PMid:32003081>

Ovayolu A., Ovayolu G., Karaman E., Yuce T., Ozek M.A. & Turksoy VA. 2020. Amniotic fluid levels of selected trace elements and heavy metals in pregnancies complicated with neural tube defects. Congenital Anomalies, Kyoto, 60(5):136-141. <https://dx.doi.org/10.1111/cga.12363> <PMid:31743503>

Özaydın İ., Kılıç E., Okumuş Z. & Cihan M. 1995. 1992-1995 yılları arasında Kafkas Üniversitesi veteriner fakültesi kliniklerine getirilen buzağılarda doğmasal anomali olguları. Vet. Cer. Derg. 1(2):22-25.

Page C.M., Murphy T.W., Van Emon M.L., Bowman J.G.P., Wyffels S.A. & Stewartt W.C. 2018. Blood serum mineral element concentrations of weaned Montana ram lambs and their relationship with water quality characteristics. Professional Anim. Scient. 34(5):410-420. <https://dx.doi.org/10.15232/pas.2018-01747>

Panousis N., Giadinis N., Roubies N., Fytianou A., Kalaitzakis E., Pourliotis K., Polizopoulou Z. & Karatzias H. 2007. Selenium, vitamin E and vitamin A status in dairy sheep reared under different feeding systems in Greece. J. Vet. Med. A 54(3):123-127. <https://dx.doi.org/10.1111/j.1439-0442.2007.00907.x> <PMid:17381674>

Pitera J.E, Smith V.V., Woolf A.S. & Milla P.J. 2001. Embryonic gut anomalies in a mouse model of retinoic acid-induced caudal regression syndrome: delayed gut looping, rudimentary cecum, and anorectal anomalies. Am. J. Pathol. 159(6):2321-2329. <https://dx.doi.org/10.1016/S0002-9440(10)63082-9> <PMid:11733381>

Polat E. 2022. Descriptive study of congenital anomalies encountered in ruminants in Elazig region of Turkey. FAVE Secc. Cienc. Vet. 21:e0001. <https://dx.doi.org/10.14409/favecv.2022.1.e0001>

Price J., Will A.M., Paschaleris G. & Chesters J.K. 1987. Identification of thiomolybdates in digesta and plasma from sheep after administration of 99Mo-labelled compounds into the rumen. Br. J. Nutr. 58(1):127-138. <https://dx.doi.org/10.1079/BJN19870076> <PMid:3620434>

Rooke J.A., Dwyer C.M. & Ashworth C.J. 2008. The potential for improving physiological, behavioural and immunological responses in the neonatal lamb by trace element and vitamin supplementation of the ewe. Animal 2(4):514-524. <https://dx.doi.org/10.1017/S1751731107001255> <PMid:22443565>

Samad M.A. 2021. Systematic review of congenital anomalies in calves and kids reported during the period from 1975 to 2021 in Bangladesh. J. Vet. Med. One Health Res. 3(2):129-153. <https://dx.doi.org/10.36111/jvmohr.2021.3(2).0029>

Sarmah S., Muralidharan P. & Marrs J.A. 2016. Common congenital anomalies: Environmental causes and prevention with folic acid containing multivitamins. Birth Defects Res. C, Embryo Today 108(3):274-286. <https://dx.doi.org/10.1002/bdrc.21138> <PMid:27718306>

Sasaki Y., Iwai N., Kimura O., Ono S., Tsuda T. & Deguchi E. 2011. Establishment of a rescue program for anorectal malformations induced by retinoic acid in mice. J. Pediatr. Surg. 46(7):396-1399. <https://dx.doi.org/10.1016/j.jpedsurg.2010.10.011> <PMid:21763841>

Sinowatz F. 2010. Teratology, p.339-382. In: Hyttel P., Sinowatz F., Vejlsted M. & Betteridge K. (Eds), Essentials of Domestic Animal Embryology. Saunders Elsevier, Philadelphia.

Sonfada M.L., Sivachelvan M.N., Haruna Y., Wiam I.M. & Yahaya A. 2010. Incidence of congenital malformations in ruminants in Northeastern Region of Nigeria. Int. J. Anim. Vet. Adv. 2(1):1-4.

Su J., Gao S., Yan R., Liu R., Su S., Nie X., Liu X., Zhang E., Xie S., Liu J., Zhang Y., Yue W., Yin C. & Peng X. 2023. Is the tradeoff between folic acid or/and multivitamin supplementation against birth defects in early pregnancy reconsidered? evidence based on a Chinese birth cohort study. Nutrients 15(2):279. <https://dx.doi.org/10.3390/nu15020279> <PMid:36678149>

Uzal F.A., Plattner B.L. & Hostetter J.M. 2016. Alimentary system, p.1-257. In: Maxie M.G. (Ed.), Jubb, Kennedy and Palmer's Pathology of Domestic Animals. Vol.2. 6th ed. Elsevier, St Louis. <https://dx.doi.org/10.1016/B978-0-7020-5318-4.00007-3>

Uzar T., Szczerbal I., Serwanska-Leja K., Nowacka-Woszuk J., Gogulski M., Bugaj S., Switonski M. & Komosa M. 2020. Congenital malformations in a Holstein-Fresian calf with a unique mosaic karyotype: A case report. Animals 10(9):1615. <https://dx.doi.org/10.3390/ani10091615> <PMid:32927643>

Wang Z., Wang Q., Gu C., Zhang J. & Wang Y. 2019. Abnormal serum vitamin A levels and retinoic acid receptor α expression patterns in children with anorectal malformation. Pediatr. Surg. Int. 35(8):903-910. <https://dx.doi.org/10.1007/s00383-019-04495-0> <PMid:31190129>

Webb Jr. K.E., Mitchell Jr. G.E. & Little C.O. 1971. Plasma and urinary components in vitamin A-deficient ewes. J. Anim. Sci. 32(1):157-160. <https://dx.doi.org/10.2527/jas1971.321157x> <PMid:5546876>

Williams D.L. 2010. Congenital abnormalities in production animals. Vet. Clin. N. Am., Food Anim. Pract. 26(3):477-486. <https://dx.doi.org/10.1016/j.cvfa.2010.09.001> <PMid:21056796>

Windsor P. 2019. Abnormalities of development and pregnancy, p.168-194. In: Noakes D.E., Parkinson T.J. & England G.C.W. (Eds), Veterinary Reproduction and Obstetrics. 10th ed. Elsevier, St Louis. <https://dx.doi.org/10.1016/C2014-0-04782-X>

Wu G., Bazer F.W., Cudd T.A., Meininger C.J. & Spencer T.E. 2004. Maternal nutrition and fetal development. J. Nutr. 134(9):2169-2172. <https://dx.doi.org/10.1093/jn/134.9.2169> <PMid:15333699>