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Aims and Scope

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Amaç ve Kapsam

Turkish Veterinary Journal (Turk Vet J), Türkçe veya İngilizce olarak, temel bilimler, klinik ve klinik öncesi bilimler, zootekni, hayvan besleme ile gıda hijyeni ve teknolojisi alanlarında makaleleri (araştırma makalesi, kısa bildiri, editöre mektup, derleme ve vaka takdimi türlerinde) yayımlamayı amaçlar. Turkish Veterinary Journal (Turk Vet J), Sivas Cumhuriyet Üniversitesi, Veteriner Fakültesi tarafından yayımlanan, uluslararası, çift hakemli bilimsel bir dergidir. Dergiye, ulusal ya da uluslararası ilgi ve uygulama içiren ve yeni bilgiler barındıran makaleler sunulabilir. Yayınlanacak makalelerin, daha önceden yayımlanmamış ve araştırma sonuçlarına dayalı olması gerekmektedir (derleme makaleleri hariç). Turk Vet J açık erişim sağlamak üzere yılda üç defa online olarak yayınlanır. Dergi yönetiminin kararları doğrultusunda özel ya da ek sayılar yayımlanabilir. Turk Vet J Türkçe ve İngilizce yayınları kabul eder. Turk Vet J makale işlem ücreti (değerlendirme ücreti veya basım ücreti) ve makalelere erişim için herhangi bir ücret talep etmez.

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The Effect of Croton-Oil-Induced Experimental Hemorrhoid Model on Testicular Histology and Some Sperm Parameters

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ABSTRACT

It was aimed to investigate the effects of Croton oil obtained from the Croton Tiglium plant on testicular histology and sperm parameters in male rats in an experimental hemorrhoid model caused by rectal administration. Sixteen male Albino Wistar rats were used for the experimental study. The animals were randomly divided into 2 groups. No treatment was applied to the control group (n=8). In the Croton oil group (n:8), a hemorrhoid model was created by applying Croton oil into the rectum with the help of a swab. The rats were sacrificed after 5 days. After sacrifice, testicular tissue samples were fixed with 10% neutral buffered formalin and Bouin's fixative solution for histologic examination. Motility, concentration, and abnormal sperm rates were determined in sperm samples obtained by puncture of the cauda epididymis. Histologically, it was observed that the lumens of the capillaries between the seminiferous tubules were enlarged in the Croton oil treated group, but there was no change in spermatogenic cells, Sertoli cells, and Leydig cells. In sperm analysis, no statistically significant difference was found in motility rate, concentration, and abnormal sperm rates ($p>0.05$). It was determined that local venous inflammation caused by Croton oil in the rectal area may have partial effects on the venous structures in the testis, but did not cause any change in sperm parameters.

Keywords: Croton oil, Hemorroid model, Sperm, Testis.

Kroton-Yağıyla Oluşturulan Deneysel Hemoroid Modelinin Testis Histolojisi ve Bazı Sperm Parametreleri Üzerine Etkisi

Süreç

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ÖZ

Croton tiglium bitkisinden elde edilen kroton yağının rektal uygulama ile oluşturulan deneysel hemoroid modelinde erkek sıçanlarda testis histolojisi ve sperm parametreleri üzerine etkilerinin araştırılması amaçlanmıştır. Deneysel çalışma için 16 erkek Albino Wistar sıçan kullanıldı. Hayvanlar rastgele 2 gruba ayrılmıştır. Kontrol grubuna hiçbir tedavi uygulanmadı (n:8). Kroton yağı grubunda (n:8), kroton yağı rektum içine swap yardımıyla uygulanarak hemoroid modeli oluşturuldu. Sıçanlar 5 gün sonra sakrifiye edildi. Sakrifikasyondan sonra histolojik inceleme için testis doku örnekleri %10'luk nötral tamponlu formalin ve Bouin tespit solüsyonu ile tespit edildi. Kauda epididimis punksiyonu ile elde edilen sperm örneklerinde motilite, yoğunluk ve anormal sperm oranları belirlendi. Histolojik olarak, kroton yağı uygulanan grupta seminifer tübüller arasındaki kapillerlerin lümenlerinin genişlediği, ancak spermatojenik hücreler, Sertoli hücreleri ve Leydig hücrelerinde herhangi bir değişiklik olmadığı gözlendi. Sperm analizinde motilite oranı, yoğunluk ve anormal sperm oranlarında istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0.05$). Kroton yağının rektal bölgede neden olduğu lokal venöz inflamasyonun testisteki venöz yapılar üzerinde kısmi etkileri olabileceği, ancak sperm parametrelerinde herhangi bir değişikliğe neden olmadığı belirlendi.

Anahtar Kelimeler: Kroton yağı, Hemoroid model, Sperm, Testis.

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Introduction

Croton tiglium L. (Euphorbiaceae) is a plant species that grows widely in tropical and temperate climatic zones and has long been used in traditional medicine to treat various diseases (Antonio et al., 2007). Different parts of the plant, such as leaves, bark, roots, and seeds, are used in the treatment of many diseases. Croton tiglium is traditionally preferred for the treatment of gastrointestinal disorders, rheumatism, ulcers, headache, fever, parasitic infections, and snake envenomation (Antonio et al., 2007; Campos et al., 2002). Croton oil contains biologically active components such as phorbol esters, crotonic acid and various fatty acids (Wang et al., 2002).

Studies have revealed that C. tiglium extract inhibits cell growth in some cancer types by showing antiproliferative effects (Song et al., 2017). In addition, its ethanolic extract has been reported to have significant antifungal activity against dermatophyte fungi such as Trichophyton mentagrophytes, T. rubrum, and Epidermophyton floccosum (Tsai et al., 2004). Especially the seeds, due to the Croton oil they contain, show a strong laxative effect and also have toxic properties (Saputera et al., 2008). These properties of the plant support its pharmaceutical potential and provide a solid basis for scientific research.

Croton oil is widely used to induce hemorrhoids in experimental animal models. Croton oil triggers local inflammation, causing hyperemia, edema and hemorrhoidal-like swelling of the anal mucosa (Nallajerla & Ganta 2023). In recent years, a common connective tissue weakness and venous wall changes have been suggested between hemorrhoidal disease and systemic venous pathologies, especially varicocele (Pasqualotto et al., 2003; Taşkın et al., 2024). Hemorrhoids are characterized by abnormal dilatation and displacement of the anal venous plexus. Pathophysiologically, hemorrhoids have common features with other venous diseases such as varicocele (Yetkin & İleri, 2016; Sun et al., 2018). Venous insufficiency, inflammation, connective tissue weakness, and mechanical strain are thought to play a role in the pathophysiology of hemorrhoids. In the literature, it is still not fully clarified whether regional pathologies in the venous system affect distant organs. Especially, the effects of local venous inflammation on spermatogenesis and testicular microcirculation are unknown.

In the present study, we aimed to investigate the short-term effects of an experimental hemorrhoid model induced by Croton oil on testicular tissue histology and sperm parameters. This study is thought to contribute to the lack of knowledge about the distant organ effects of venous system pathologies.

Materials and Methods

Animal experiments were performed at Van Yüzüncü Yil University Experimental Medicine Application and Research Centre with the approval of the local ethics committee (Decision no: 2025/06-10 Date:29/05/2025). The rats were kept at a room temperature of 22-24 oC with 12 hours of light and 12 hours of dark, 55-60% humidity. They were fed ad libitum with pellet feed.

Experimental groups and induction of hemorrhoids

The experimental groups were formed with 8 male rats in each group, as a control and a hemorrhoid group. Rats in the hemorrhoid group were fasted overnight, and hemorrhoids were induced with Croton oil (Azeemuddin et al., 2014). Croton oil solution (a mixture of deionized water, pyridine, diethyl ether and 6% Croton oil in diethyl ether - ratios = 1: 4: 5: 10, respectively) was applied into the anal opening (rectoanal portion, 20 mm from anal opening) with a cotton swab. The swab was kept in the anal opening for 1 min and then observed. Edema formation was observed in the area in rats within 6-7 hours.

Experimentation and sample collection

After 5 days of follow-up, feeding, and care, the rats were anesthetized (xylazine/ketamine) on the 6th day, and the animals were euthanized (Sancak, 2023); tissue samples were taken. Testicular tissue samples were fixed with two different fixation solutions. Tissue samples fixed with 10% neutral buffered formalin and Bouin (Layton et al., 2019) were processed by routine histological method, and paraffin blocks were prepared (in Bouin fixation, washing was performed with 70% ethyl alcohol for 12 hours). Sections (6 µm) taken from the tissue blocks with a microtome (Leica RM-2135) were stained with the Crossman modification of Mallory triple stain (Erbaş et al., 2024). Spermatogenic cells and connective tissue, and their parts, were examined histologically. Photographs were taken under a microscope (Zeiss Lab A1- Axiocam).

Spermatological Analysis

Motility examination: Sperm sample was obtained by epididymis puncture immediately after the rats were sacrificed and placed on a slide on a heating table set at 38°C. The coverslip was closed at a 45° angle, and motility (in %) was determined by microscopy at X40 magnification.

Sperm concentration

After epididymal puncture, a 0.1 ml sperm sample was added to Eppendorf tubes containing 0.5 ml Hayem's solution. Sperm count per mL was calculated using a Thoma cell counting chamber.

Abnormal sperm rate

Semen obtained by epididymal puncture was transferred into Eppendorf tubes containing 0.5 ml Hancock solution. At least 400 sperm cells were examined at X40 magnification to determine the ratio.

Statistical Analysis

SPSS v.20 (Chicago, IL, USA) package program was used for statistical analysis. All data were expressed as mean ± standard deviation. Statistical analyses of the groups were statistically analyzed using One-Way ANOVA, followed by post hoc multiple comparisons (Tukey test) for comparative analysis between groups. $P<0.05$ was considered statistically significant.

Results and Discussion

Crotonic acid, crotonoleic acid, and several other fatty acids are toxic chemicals that restrict the potential therapeutic use of Croton tiglium. Its seeds contain this toxalbumin, which inhibits protein synthesis by disabling ribosomes, causing hemolysis and cell destruction. This is because the seed oil contains phorbol esters and crotonic acid along with fatty acids,

in addition to the presence of active phytoconstituents (Stirpe et al., 1976; Zhang et al., 2015).

The appearance of spermatogenic cells, Sertoli cells, and Leydig cells was normal in both fixation solutions and both experimental groups. Tubulus seminiferous contortus (TSC) lumens were filled with spermatozoa, and connective tissue structures were observed properly. Microscopic examinations showed that the lumens of the capillaries located close to the Leydig cells in the group with Croton oil were relatively enlarged (Figure 1).

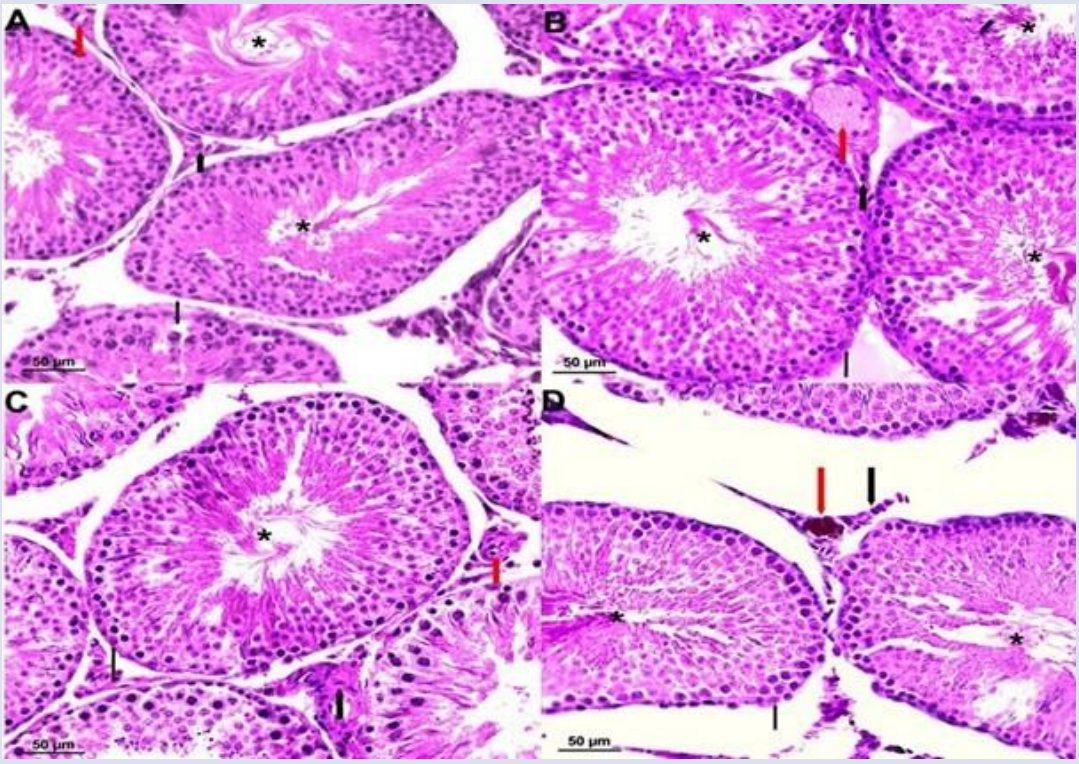

*Figure 1. A: Bouin-Control, B: Bouin-hemorrhoid, C: Bouin-Kontrol, D: Formol-hemorrhoid. *=TSC lumens, Red thick arrows = capillaries, Black thick arrow= Leydig cells, Black thin arrow= Sertoli cells.*

The overall structural integrity of the venous system may be affected by regional events such as anal inflammation. Studies have revealed the relationship between hemorrhoids and varicocele. It has been shown that venous hypertension and venous valve insufficiency play an important role in the pathogenesis of these diseases (Chen et al., 2022; Taşkın et al., 2024). In the present study, no differences were found in spermatogenic cells, Sertoli cells, and Leydig cells in the histopathologic examination of the testis in the group using Croton oil, but capillary vessels between the TSC were found to be enlarged. The observation of capillary dilatation between the TSCs suggests that Croton oil administered rectally also acts in the uro-genital region and causes vasodilation.

Studies examining the male reproductive system using Croton oil are limited in the literature. It is reported that Croton oil is used in traditional Chinese medicine and African countries to increase male reproductive parameters. In addition, Croton oil has toxic effects (Rampa et al., 2022; Junior et al., 2022; Isyaka et al., 2024; Sun et al., 2024). In the present study, no difference was observed in sperm motility, concentration, and abnormal sperm rates in the Croton oil group compared to the control group (Table 1). The difference between the studies examining the effects of Croton oil on the male reproductive system is thought to be due to the route of administration, duration of administration, and the use of different croton species (Croton membranaceus, Croton cajucara, Croton zambesicus) (Ofusori et al., 2007; Caneguim et al., 2011; Afriyie et al., 2014).

Table 1. Epididymal sperm analysis.

	Control	Croton oil	p
Motility (%)	80.55±5.87[a]	78.45±6.8[a]	>0.05
Concentration (x10^9)	2.04±0.09[a]	1.96±0.13[a]	>0.05
Abnormal (%)	16.54±1.98[a]	16.14±0.77[a]	>0.05

As a result of sperm analysis, no statistically significant difference was found between the control and Croton oil groups in terms of sperm motility, concentration, and abnormal sperm rates (p>0.05).

Conclusion

In an experimental hemorrhoid model induced with Croton oil, mild histopathological changes, such as lumens of the capillaries relatively enlarged in the testes. However, no significant inflammation or impaired spermatogenesis was observed. These

findings suggest that local inflammation in the anal region does not directly cause serious damage to the testicular tissue but may lead to mild changes in the systemic venous circulation. This study supports that local inflammatory processes may have effects on venous structures in distant organs, and future studies need to evaluate the long-term consequences of these effects and their possible reflections on fertility.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Investigation of Paraoxonase and Ceruloplasmin Concentrations in Hair Goats Grazing at Different Heights

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ABSTRACT

The aim of this study was to investigate the changes in serum paraoxonase and ceruloplasmin concentrations in hair goats grazed at different altitudes. A total of 80 hair goats from different regions of Adana were included in the study and divided into four groups. The first group grazed at an altitude of 10-99 meters above sea level, the second at 240-250 meters, the third at 750-800 meters and the fourth group was grazed at an altitude of 1200-1500 meters. Blood samples were collected from the jugular vein during the summer months and placed into red-capped tubes. Paraoxonase and ceruloplasmin levels were analyzed by spectrophotometric method. The results of paraoxonase in 240-250 meter (1113.91±387.54 U/mL) and 750-800 meter (974.27±295.20 U/mL) were found statistically higher than 10-99 meter group (651.72±253.84 U/mL; p<0.01). Ceruloplasmin levels were significantly different between the 10–99 meter (10.15±3.04 U/mL) and 750–800 meter (7.13±1.94 U/mL) groups, and also between the 240–250 meter (9.97±3.96 U/mL) and 750–800 meter (7.13±1.94 U/mL) groups (p<0.05). Studies have shown that antioxidant parameters of populations accustomed to living at high altitudes may be lower than those later moved to higher altitudes. In populations normally living close to sea level, relocation to high altitudes induces hypoxia, which activates the body's defense systems and leads to an increase in antioxidant enzymes. It is thought that the reason why the highest ceruloplasmin concentration was observed in hair goats grazed at 10-99 meters may be that the temperature is higher at those levels and heat stress increases the acute phase proteins and likewise heat stress causes to a decrease in paraoxonase enzyme activity.

Keywords: Altitude, antioxidant, hair goats

Farklı Yüksekliklerde Otlayan Kıl Keçilerinde Paraoksonaz ve Serüloplazmin Konsantrasyonlarının Araştırılması

Süreç

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Kabul: 25/06/2025

Teşekkür
Sonuçların bir kısmı, 23-25 Mayıs 2024 tarihlerinde Van-Türkiye'de düzenlenen "3. Uluslararası Veteriner Biyokimya ve Klinik Biyokimya Kongresi"nde sözlü bildiri olarak sunulmuştur.

ÖZ

Bu çalışmanın amacı, farklı rakımlarda otlatılan kıl keçilerinde serum Paraoksonaz ve Serüloplazmin konsantrasyonlarındaki değişiklikleri ortaya koymaktır. Çalışmaya Adana'nın farklı bölgelerinde yaşayan 80 kıl keçisi dâhil edilmiş ve bu keçiler dört gruba ayrılmıştır. Birinci grup deniz seviyesinden 10-99 metre yükseklikte, ikinci grup 240-250 metre yükseklikte, üçüncü grup 750-800 metre yükseklikte ve dördüncü grup ise 1200-1500 metre yükseklikte otlatılmıştır. Yaz aylarında jugular venden alınan kan örnekleri, kırmızı kapaklı tüplere alınmıştır. Paraoksonaz ve Serüloplazmin analizleri spektrofotometrik yöntemle gerçekleştirilmiştir. Paraoksonaz sonuçları, 240-250 metre (1113,91±387,54 U/mL) ve 750-800 metre (974,27±295,20 U/mL) gruplarında, 10-99 metre grubuna (651,72±253,84 U/mL; p<0,01**) göre istatistiksel olarak anlamlı şekilde yüksek bulunmuştur. Serüloplazmin sonuçlarında ise 10-99 metre (10,15±3,04 U/mL) ile 750-800 metre (7,13±1,94 U/mL) grubu arasında, ayrıca 240-250 metre (9,97±3.96 U/mL) ile 750-800 metre (7,13±1,94 U/mL) grubu arasında istatistiksel olarak anlamlı fark saptanmıştır (p<0,05*). Yapılan çalışmalar, yüksek rakımda yaşamaya alışık popülasyonlarda antioksidan parametrelerin, sonradan yüksek rakıma taşınanlara göre daha düşük olabileceğini göstermektedir. Normalde deniz seviyesine yakın yerlerde yaşayan ve sonradan yüksek rakımlara çıkan bireylerde, hipoksiye bağlı olarak vücut savunma sistemleri aktive olmakta ve buna bağlı olarak antioksidan enzim düzeylerinde artış beklenmektedir. 10-99 metre rakımda otlatılan kıl keçilerinde en yüksek serüloplazmin konsantrasyonunun gözlemlenmesinin, bu seviyelerde sıcaklığın daha yüksek olmasına ve buna bağlı olarak ısı stresinin akut faz proteinlerini artırmasına bağlanabileceği; benzer şekilde, ısı stresinin paraoksonaz enzim aktivitesinde azalmaya neden olduğu düşünülmektedir.

Anahtar Kelimeler: Antioksidan, Kıl Keçileri, Rakım

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Introduction

Goats are important farm animals in Turkey because of their easy care and feeding conditions. In Turkey, 80-90% of their nutrition is provided from natural pasture area, which is economic for farmers (Koyuncu and Taskin, 2016). Although Turkey is a country rich in natural pasture areas, it does not operate at full potential in terms of sheep and goat breeding (Kaymakci et al., 2004). Goats do not use only pasture areas, they prefer trees and shrubs for feeding too and this kind of feeding has a positive effect on biodiversity, which makes goats preferable for breeding (Yilmaz et al., 2012). The breed that forms the densest goat population in Turkey is the hair goat. Hair goats are combined breeds and their resistance to hard conditions is one of the most known characteristic for hair goats (Gunlu and Alasahan, 2010). Hair goats are mostly raised in Mediterrenean region and South east region of Turkey. Their population has increased in the last years and they can be raised mountainous villages and forested areas, which make them preferable (Gungor et al., 2021).

It is known that various conditions like nutrition, housing, age, sex, management, genetics, breed, temperature, humidity etc can change the biochemical profiles of farm animals. Even grazing can change the profile of goats (Mohammed et al., 2016). A study investigated the effect of heat and cold on heat adaptive and cold adaptive goat breeds. They found significant changes in hematological and biochemical profiles. So it is important to know the breeds and their adaptation on climate changes. High altitude in summer mostly results with decreased temperature and decreased oxygen consumption leads to higher hemoglobin and erythrocyte levels (Banerjee et al., 2015). Oxidative stress and hypoxia are related to each other and the cause of hypoxia in physiological conditions is seen in high altitudes. Increased oxidative stress depletes the antioxidant capacity of the body (Askew, 2002).

Ceruloplasmin is known for its anti- and pro-oxidant effect. Especially in chronic diseases it was found that ceruloplasmin levels in sick people increases (Demirpence et al., 2014). In a study ceruloplasmin and paraoxonase (PON) activity was found negatively related and in coronary artery diseases serum ceruloplasmin levels were increased (Gocmen et al., 2008). Both ceruloplasmin and PON have antioxidant effects but while ceruloplasmin is a positive acute phase protein (Fleck, 1989), PON is a negative acute phase protein (James and Deakin, 2004).

The aim of the study was to investigate the effect of altitude on serum PON and ceruloplasmin levels. For this purpose, 80 hair goats in different altitudes of Adana province were chosen and in summer during grazing in 0-99 m, 240-250 m, 750-800 m and 1200-1500 m heights blood samples were collected from hair goats for analysis.

Materials and Methods

The Ethical Statement of this study was approved by Cukurova University Local Ethics Committee of Ceyhan Faculty of Veterinary (Decision date and number: 12.12.2018; 1/11).

Animals and groups

Hair goats from the Adana province in Turkey, living in different regions of Adana, were included in the study. A total of 80 goats were enrolled and four groups were categorized according to the altitude. Table 1 presents the grouping of the goats, including the number of animals in each group, their sexes, and age ranges.

In summer, while grazing the goats in the pasture, the blood samples were collected from the jugular vein (V. jugularis) of each goat into red top tubes and serum samples were taken immediately by centrifugation at 3000 rpm for 10 minutes. Samples were stored at -80 ^{o}C until analysis.

Table 1. Groups and number of goats in every group.

Group Number	Group Name	Ages	Sex	n
1	10-99 m	2-5 years old	Female	23
2	240-250 m	2-5 years old	Female	21
3	750-800 m	2-5 years old	Female	18
4	1200-1500 m	2-5 years old	Female	18

Paraoxonase Analysis

PON activity was measured according to the method of Aviram and Rosenblat (2008). The working principle is based on the optic density at 412 nm of the yellow colored p-nitrophenol after the enzymatic hyrdrolisation of PON.

Ceruloplasmin Analysis

This analysis catalyzes the oxidation of p-phenylendiamin, which gives a purple color. The method of Ceron and Martinez-Subiela (2004) was used. In calculation Curzon and Vallet (1960)'s method was used.

Statistical Analysis

SPSS 21.00 version was used and after normality distribution with shapiro-wilk test one way ANOVA test was done and groups were compared according to the Levene's Test (Homogeneity of variances). In pairwise group comparison Bonferroni test was used and $p<0.05$ was accepted as statistically significant (Field, 2013). The positive or negative correlation between PON and ceruloplasmin was done with RStudio statistics 4.3.2 software.

Results and Discussions

The results for PON levels between groups were statistically significant ($p<0.01$) in 10-99 m (651.72±253.84 U/mL) and 240-250 m (1113.91±387.54 U/mL) and 10-99 m and 750-800 m (974.27±295.20 U/mL). In table 2 the results of PON are given.

Ceruloplasmin measurements gave statistically significance ($p<0.05$) between 10-99 m (10.15±3.04 U/mL) and 750-800 m groups; and 240-250 m (9.97±3.96 U/mL) and 750-800 m (7.13±1.94 U/mL) groups. In table 2 the ceruloplasmin results and group comparisons are given.

Table 2. The statistical differences and Me±SD values of PON and ceruloplasmin levels.

		N	Me±SD	Std. Error	95% Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
PON	**10-99 m**	23	651.72±253.84[a]	52.93	541.95	761.49	0.001
	240-250 m	21	1113.91±387.54[b]	84.57	937.51	1290.32	
	750-800 m	18	974.27±295.20[b]	69.58	827.46	1121.07	
	1200-1500 m	18	841.86±473.36[ab]	111.57	606.46	1077.25	
Cerulo-plasmin	**10-99 m**	23	10.15±3.04[a]	0.63	8.83	11.47	0.014
	240-250 m	21	9.97±3.96[a]	0.86	8.17	11.77	
	750-800 m	18	7.13±1.94[b]	0.46	6.16	8.09	
	1200-1500 m	18	8.88±3.22[ab]	0.76	7.28	10.48	

*Different letters in each column for PON and ceruloplasmin levels show the statistical difference.

Considering the negative acute phase protein effects of PON and the positive acute phase protein effects of ceruloplasmin, the highest ceruloplasmin and lowest PON values were observed in the same group. The correlation between PON and ceruloplasmin can be seen in figure 1. According to the correlation plots, a negative correlation was found between levels of PON and ceruloplasmin. The negative correlation was stronger for altitude and ceruloplasmin levels. Because of the negative correlation of PON and ceruloplasmin, altitude and PON shown a weak positive correlation.

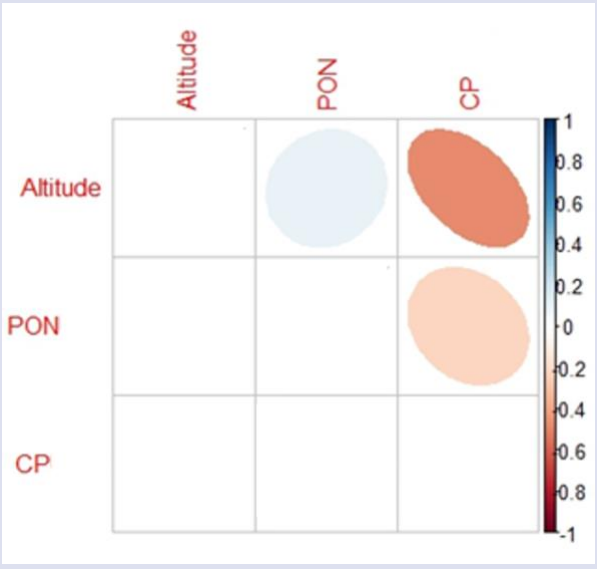

Figure 1. Pearson correlation matrix of altitude in PON and ceruloplasmin parameters of hair goats.

The study of Yildirim et al. (2008) investigated the effect of altitude on some acute phase proteins in 800 and 2600 meter and found that there was a significant increase in some positive acute phase proteins such as ceruloplasmin and alpha 1 antitripsin. Our study did not investigate higher altitudes, which is a limitation for this study. However, heat stress might explain high ceruloplasmin levels at the sea level. At 750-800 m ceruloplasmin concentration was lower than at 1200-1500 m but there was not a significant difference. So if the altitude could be higher maybe a significantly increased ceruloplasmin level could be seen because of elevated oxidative stress.

The physiologically healthy biochemical results of hair goats in Cukurova region, which is located in Adana province, were analysed according to altitude differences and it was found that the alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, triglyceride and glucose levels showed significant differences between 87 and 756 meters altitudes. Due to the study they found that the normal values of healthy hair goats can differ compared with other goat species (Er and Ok, 2020). This emphasizes that the species of goats are important to know because the results of biochemical analysis can change.

In a study done with humans, altitude was classified as low and moderate. According to the results the highest PON levels were found in 200-400 m heights and the lowest was seen in 0-200 m height (Cabrera de Leon et al., 2011). If compared with the current study with goats the results are similar, but in our study there was a statistically significant difference and in the study of Cabrera de Leon et al. (2011) there was not a significant difference between groups.

In a study conducted by Kurt et al. (2021) total oxidant capacity (TOC) of hair goats in Adana province was measured and the groups were categorized as 0-99 m, 100-500 m and higher than 500 meter. According to the results there was a significant difference between the groups and the highest total oxidant capacity (TOC) levels were measured in the >500 m group. Normally the closer to sea level the higher the heat stress but in this case the differences in oxygen pressure in high altitude could effect the reactive oxygen species and this could be the reason of high TOC levels (Bakonyi and Radak, 2004).

Kartal et al. (2021) investigated the effect of age on PON and ceruloplamin levels in hair goats from Adana. The results showed that there was not a significant difference between the groups in PON analysis but still the highest PON levels were found in 1.5-2 and 2.5-6 year old group. In ceruloplasmin levels, there was significant difference between 0-6 month and the last three groups.

After birth ceruloplasmin levels were higher and with age it slowly decreased. So working with 2-5 year old goats eliminate the effect of age on ceruloplasmin levels.

PON levels decrease at higher altitudes, which may result in lower HDL cholesterol and increased triglyceride levels. This could be a reason to be predisposed to atherosclerosis in people living at high altitudes. The study of Hirschler et al. (2018) investigated the PON levels in children living at high altitude (3700 meter) and low altitude (25 meter) and found that even the body mass index was significantly higher in lowlanders, HDL and PON activity were lower. In goats the highest altitude was 1500 meter so the differences could be because of a lower high altitude. But Hirschler et al. (2018) worked in October and November while the current study was studied in summer. Lower PON activity at the sea level could be the result of heat stress.

Two different heights (1432 and 3750 m) were selected and HDL, LDL, TC, TG and PON was measured. PON was higher in 1432 m height when the other parameters were higher in 3750 m height (Hirschler et al., 2019). So hypoxia results with high TG levels because of the hepatic lipid oxidation (Muratsubaki et al., 2003).

Living at high altitude is generally associated with a reduction in antioxidant profile as it is known. But a study conducted by Sinha et al. (2009a; 2009b) searched the adaptation in lowlanders when road to high altitude (4500 meter) and native highlanders. They found that there was a significant elevation in antioxidant enzymes in lowlanders after the trip to high altitudes. But in native highlanders antioxidant enzyme activity was lower than the lowlanders at the same altitude (4500 meter). Oxidative stress markers were found higher in lowlanders at high altitude compared with native highlanders. So an increase in oxidative stress will activate the defense system of the body and this could be the reason for the increase in antioxidant levels in lowlanders at high altitude (Halliwell et al., 1995).

A study investigated the effect of altitude on athletes. According to the study athletes blood samples were collected 24 h after exercise at the sea level and then athletes were ascended to high altitude (2400 m) for 4 weeks and 24 h after the last exercise second blood sample were collected. Results showed that MDA and GSH levels increased in high altitudes but SOD enzyme activity did not differ in this study (Belviranli et al., 2017). So elevated oxidative stress and antioxidant defense system can balance each other up to a certain exposure but the enzymatic antioxidant parameter (SOD) was not changed with altitude. This could be due to excessive hydrogen peroxide accumulation (Sinha et al., 2009c).

Conclusion

In conclusion, it can be stated that levels of serum PON and ceruloplasmin are related negatively and altitude has an effect on these parameters. But 1500 meter is mostly seen as moderate altitude so working in higher altitudes is necessary to see the differences better. But in this study the working area was limited to Adana province which is a region at the sea level, so the highest altitude there was 1500 meters height. The effect of altitude on biochemical parameters such as blood gases or antioxidant enzymes are mostly studied with humans and athletes. There are relatively few studies about farm animals. Goats are skilled climbers and this allows them to reach areas that many people/farm animals cannot reach. So this situation makes them important to study with goats at different altitudes to see how they are affected. There was no significant change in low (0-99 m) and high (1200-1500 m) altitudes. This may be attributed to the heat stress at sea level and adaptation to high altitudes. The significant difference in moderate levels in PON were higher than that at the sea level. This might again be explained by the heat stress because at 240-250 and 750-800 m heights humidity and heat is reduced and this leads to a decrease in oxidative stress too. On the other hand the significant difference in ceruloplasmin levels could be seen only in 750-800 m because of the same reason like in PON. To draw clearer conclusions, it could be better to know the copper and other positive acute phase protein values. Reference values of parameters determined in goats may vary depending on environment and breed. There are recommendations on creating a separate reference table of parameters for each region and breed in goats. Nonetheless, further research is needed for goats with different parameters and higher altitudes.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Effects of using hCG and Beta Carotene + Vitamin E Alone or in Combination on Fertility in Estrus-Synchronized Awassi Ewes

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ABSTRACT

This study was carried out in four different farms in Diyarbakır to determine the effects of beta carotene + vitamin E and HCG on fertility during the transition period (1.5-2 months before the breeding season). A total of 306 Awassi ewes were divided into four groups before the study. All groups were treated with a progesterone sponge for 12 days, PGF2α two days before sponge removal, 600 IU eCG on the day of sponge removal, and 150 IU hCG on the day of mating. The control group of ewes (n = 91) did not receive any additional treatment. The ewes in the vitamin group (n = 61) were treated twice with Beta (β) carotene + vitamin E once 7 days before sponge insertion and again on the day of mating. The ewes in the hCG group (n = 74) received 150 IU hCG on day 12 after mating. The ewes in the vitamin + hCG group (n = 80) received both β-carotene + vitamin E and hCG. Ewes showing signs of estrus were mated with rams of known fertility. Pregnancy was diagnosed by ultrasonography on day 30 after mating. Estrus, pregnancy, lambing, fecundity, and multiple birth rates were similar among the control, hCG, vitamin, and vitamin + hCG groups (P>0.05). It was concluded that treatment with vitamins (β-carotene + vitamin E), hCG, or their combination, in addition to estrus synchronization during the transition period, did not significantly improve fertility parameters in Awassi ewes.

Keywords: Awassi ewes; Beta carotene; hCG; Estrus synchronization; Progesterone

Östrus Senkronize İvesi Koyunlarında Beta Karoten + Vitamin E ve hCG 'nin Tek Başına veya Birlikte Kullanımının Fertilite Üzerine Etkileri

Süreç

Geliş: 28/04/2025
Kabul: 26/06/2025

Teşekkür
Bu çalışma, VII. Ulusal Hayvanlarda Üreme ve Suni Tohumlama Kongresi ile 32. Dünya Veteriner Hekimliği Kongresi'nde özet bildiri olarak sunulmuştur. Bu çalışma, T.C. Gıda, Tarım ve Hayvancılık Bakanlığı, Eğitim, Yayım ve Yayınlar Dairesi Başkanlığı tarafından desteklenmiştir (Proje numarası: 07.01.03.00).

ÖZ

Bu çalışma, Diyarbakır'daki dört farklı çiftlikte, geçiş döneminde (aşım sezonundan 1,5-2 ay önce) beta karoten+ vitamin E ve Hcg'nin fertilite üzerindeki etkilerinin belirlenmesi amacıyla yürütüldü. Toplam 306 İvesi koyunu, çalışma öncesinde dört gruba ayrıldı. Tüm gruplara 12 gün boyunca progesteron süngeri, sünger çıkarılmadan iki gün önce PGF2α, sünger çıkarma gününde 600 IU eCG ve çiftleşme gününde 150 IU hCG uygulandı. Kontrol grubundaki koyunlara (n = 91) herhangi bir ek tedavi uygulanmadı. Vitamin grubundaki koyunlara (n = 61), sünger yerleştirilmeden 7 gün önce ve çiftleşme gününde olmak üzere iki kez Beta (β) karoten + E vitamini uygulandı. HCG grubundaki koyunlara (n = 74), çiftleşmeden sonraki 12. günde 150 IU hCG uygulandı. Vitamin + hCG grubundaki koyunlara (n = 80) hem β-karoten + E vitamini ve hem de hCG tedavisi uygulandı. Kızgınlık belirtileri gösteren koyunlar, bilinen fertiliteye sahip koçlarla çiftleştirildi. Gebelik teşhisi, çiftleşmeden sonraki 30. günde ultrasonografi ile yapıldı. Kontrol, hCG, vitamin ve vitamin + hCG grupları arasında kızgınlık, gebelik, kuzulama, döl verimi ve çoğul doğum oranları benzerdi (P>0.05). Sonuç olarak, İvesi koyunlarında geçiş döneminde östrus senkronizasyonuna ek olarak vitamin (β-karoten + E vitamini), hCG veya her ikisiyle birlikte yapılan uygulamaların fertilite parametrelerini önemli ölçüde iyileştirmediği sonucuna varıldı.

Anahtar Kelimeler: İvesi koyunu; Beta karoten; hCG; Östrus senkronizasyonu; Progesteron

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İntroduction

Embryonic loss during the pre-implantation stage is among the most significant factors restricting optimal reproductive efficiency in livestock. In ewes, 30% to 40% of fertilized ova are lost throughout the pre-implantation phase and the initial three weeks of gestation, while losses increase to as much as 70% to 80% within the first three weeks following insemination. (Bazer et al., 2014). However, it has been suggested that embryonic losses are preventable, as fertilization failure accounts for only 5% to 10% of losses in ewes (Khan et al., 2007). Insufficient luteal function is accepted as one of the important causes for embryonic mortality (de Brun et al., 2016). Therefore, various therapeutic applications have been used to improve luteal function in order to reduce embryonic losses. One of these therapeutic strategies is post-breeding progesterone (P4) application, which can be administered either as exogenous P4 therapy directly or as gonadotropin treatment to increase endogenous P4 levels. It has been suggested that treatment with Gonadotrophin Releasing Hormone (GnRH) or Human Chorionic Gonadotropin (hCG) on days 10, 11, 12, or 13 post-copulation can improve pregnancy rates by approximately 10% in ewes (Beck et al., 1994; Khan et al., 2009). However, quite contradictory results have been obtained from studies, and therefore new studies are needed.

Another approach to decreasing embryonic mortality in ewes involves the supplementation of specific minerals and vitamins known to enhance reproductive performance. The levels of certain vitamins and minerals in the body, such as β-carotene, vitamin E, and selenium, sourced from green grasses, play a vital role in fertility. If these substances are deficient, external supplements should be administered. (Yokuş et al., 2006). Beta carotene plays a crucial role in steroid hormone synthesis and ovulation, as it serves as the sole source of vitamin A in granulosa cells. Studies have shown that the corpus luteum (CL) contains high levels of β-carotene, which significantly influences luteal cell function alongside vitamin A. (McGrath et al., 2018). Rapaport et al. (1998) suggested that the ability of the corpus luteum to secrete progesterone (P4) is connected to the high levels of β-carotene in the ovaries. It has been suggested that supplementing β-carotene in animals such as cows, ewes, goats, and rabbits enhances their reproductive performance (Meza-Herrera et al., 2013; Özpınar et al., 1994). Özpınar et al. (1994) found that administering β-carotene injections every 20 days higher pregnancy rates, lamb production, and twinning rates following the first insemination in ewes.

Vitamin E, recognized as an essential nutrient for reproduction since 1922, is composed of tocopherols and tocotrienols. Research has shown that α-tocopherol safeguards cell membranes from oxidative harm by neutralizing lipid radicals generated during lipid peroxidation. (Traber &Atkinson, 2007). It also interferes with free radical intermediates and prevents oxidative reactions (Mohebbi-Fani et al., 2012). Vitamin E supplementation can prevent oxidative damage in the ovarian epithelium caused by ovulation stimulation in ewes (Murdoch & Martinchict, 2004). Previous studies on the impact of vitamin E on fertility have reported varying outcomes (Gabryszuk & Klewiec, 2002; Koyuncu et al., 2007; Yaprak et al., 2004). Various studies have been carried out to examine the effects of hCG treatment or the supplementation with different vitamins on breeding outcomes following mating in ewes. (Catalano et al., 2015; Kaya et al., 2013; Köse et al., 2013). As far as the authors are aware, no studies have examined the impact of combined administration of hCG, β-carotene, and vitamin E on reproduction in ewes.

The aim of this study was to combine two different strategies used to reduce embryonic losses and improve pregnancy rates in ewes. Therefore, the objective was to evaluate the impact of hCG, β-carotene, and vitamin E treatments, either individually or in combination, on fertility in estrus-synchronized Awassi ewes during the transition to the breeding season.

Material and Methods

Ethical statement

The procedures for this study were authorized by the Local Ethics Committee for Animal Experiments at the Dıcle University Health Sciences Application and Research Center (Document No. 25249).

Animals and experimental design

This study was conducted during transition period (1.5 to 2 months before season) at four different farms in Dıyarbakır province, located in south-eastern Türkiye. The farms were located close to each other, with the distance between the farthest farms was about 15 km, and the ewes grazed on pastures with similar habitat characteristics. The latitude, longitude, and altitude of this region were 37°55’01”N, 40°16’46”E, and 660 m, respectively.

The ewes were allowed to graze on natural pasture throughout the day, with water provided ad libitum. Only healthy ewes were included in the study; however, body condition scores were not evaluated. A total of 306 Awassi ewes, aged 2 to 4 years, average weight 45-50 kg were haphazardly divided into four groups; control, vitamin, hCG, hCG+vitamin. A standard estrus synchronization protocol was applied. For this purpose, intravaginal sponges containing 20 mg of flugestone acetate (Chronogest CR, MSD Animal Health) were inserted into the vaginas of all ewes and kept in place for 12 days. Two days before sponge removal, each ewe received 250 µg prostaglandin F2 alpha (PGF2α, Estrumate, MSD Animal Health) via the intramuscular (im) route. On the day of sponge removal, 600 IU of equine chorionic gonadotropin (eCG, Chronogest, MSD Animal Health) was administered IM. Additionally, 150 IU of human chorionic gonadotropin (hCG, Chorulon,

MSD Animal Health) was administered IM on the day of mating. No additional treatment was given to the control group (n = 91). The treatment groups received the following: β-carotene + vitamin E (Ovostim, Provet) at a dose of 0.5 ml/10 kg was injected IM to the vitamin group (n = 61) 7 days before sponge insertion and again on the day of mating. The hCG group (n = 74) received 150 IU of hCG on day 12 post-mating. The vitamin + hCG group (n = 80) received both β-carotene + vitamin E and hCG.. Estrus signs were observed with the help of fertile rams aged 3 to 5 years over a period of four consecutive days, beginning on the day of sponge removal. After observation of the estrus, ewes were mated (ewe to ram ratio: 10:1). Pregnancy was diagnosed by ultrasonography on day 30 after mating. Reproductive performance was assessed using the following parameters: pregnancy rate (number of pregnant ewes/ number of ewes that showed estrus and were mated), lambing rate (number of ewes that lambed/ number of mated ewes), multiple birth rate (number of ewes with multiple births/number of ewes that lambed), and fecundity rate (number of lambs born/number of ewes that lambed). Pregnancy was diagnosed by ultrasonography on days 35 to 45 post-mating.

Statistical analyses

The results were presented as percentages, and the Chi-Square test was applied for group comparisons. Data analysis was performed using SPSS/PC software (Version 10.0; SPSS, Chicago, IL, USA), with a significance level of $P<0.05$ considered statistically significant.

Results and Discussion

The fertility results in ewes are presented in Table 1. The study found no significant differencesamong the groups in terms of estrus, pregnancy, lambing, multiple birth, and fecundity rates (P>0.05). The estrus rate were found as 96.7%, 98.4%,100% and 95.0% for the control, vitamin, hCG and hCG+ vitamin groups, respectively. Compared to the control group, hCG group exhibited numerically higher pregnancy and lambing rates but lower multiple birth and fecundity rates (P>0.05). Unexpectedly, multiple birth rate in the vitamin group was numerically lower than that in the control group (19.0% vs. 29.8%, P>0.05). The hCG + vitamin group showed slightly higher pregnancy (73.7% vs. 67.0%, P>0.05) and lambing rates (71.1% vs. 64.7%, P>0.05) compared to the control group.

Table 1. Comparison of fertility results among study groups.

Groups	Control n= 91	Vitamin n= 61	hCG n= 74	hCG + Vitamin n= 80	P
Estrus rate % (n/n)	96.7 (88/91)	98.4 (60/61)	100 (74/74)	95.0 (76/80)	>0.05
Pregnancy rate[a] % (n/n)	67.0 (59/88)	73.3 (44/60)	70.3 (52/74)	73.7 (56/76)	>0.05
Lambing rate[b] % (n/n)	64.7 (57/88)	70.0 (42/60)	68.9 (51/74)	71.1 (54/76)	>0.05
Multiple births rate[c] % (n/n)	29.8 (17/57)	19.0 (8/42)	21.6 (11/51)	27.8 (15/54)	>0.05
Fecundity rate[d] % (n/n)	131.6 (75/57)	121.4 51/42	121.6 (62/51)	131.5 (71/54)	>0.05

[a]: Number of ewes pregnant/all ewes mated
[b]: Number of ewes lambing/all ewes mated
[c]: Number of ewes giving multiple births/number of ewes lambing
[d]: Number of lambs born/ number of ewes lambing

The pregnancy rate in the control group was 67.0%. Compare to the control group, slight but non-significant increases were observed in the vitamin, hCG and HCG + vitamin groups, with pregnancy rates of 73.3%, 70.3%, and 73.7%, respectively. The lambing rates were also similar among the groups: 64.7% in the control group, 70.0% in the vitamin group, 68.9% in the hCG group and 71.1% in the hCG + vitamin group. Interestingly, the control group exhibited the highest multiple birth rate numerically. However, no significant differences were found in multiple birth (29.8%, 19.0%, 21.6%, and 27.8%) or fecundity rates (131.6%, 121.4%, 121.6%, and 131.5%) among the control, vitamin, hCG, and hCG+vitamin groups, respectively (P>0.05).

Studies have shown that hCG treatments, administered in various doses and regimens (single or multiple injections) during late diestrus in parous ewes, can positively impact fertility (Cam & Kuran, 2004; Kittok et al., 1983; Moeini et al., 2013; Nephew et al., 1994; Rostami et al., 2017), although some exceptions exist (Khan et al., 2007). Administering multiple injections of hCG (100 IU) on days 11, 12, and 13 post-mating has been demonstrated to elevate plasma P4 levels and increase conception rates (58% vs. 29%) in lactating anoestrus ewes (Kittok et al., 1983). Likewise, Nephew et al. (1994) suggest that a single injection of hCG (100 IU) on day 11.5 increased length of blastocysts and IFN-τ concentrations, and tended to improve pregnancy rates (84% vs. 95%) in ewes mated

during spontaneous estrus. In a study conducted on ewes mated at spontaneous estrus, (Cam & Kuran, 2004) found that a 150 IU hCG treatment on the 12th day post-mating significantly improved the non-return rate, lambing rate, and twinning rate. Rostami et al. (2017) reported that treatment with 400 IU hCG on day 11 post-mating significantly increased plasma P4 concentration, conception rate, and litter size outside the breeding season.

However, in our study, no positive effect of hCG treatment administered on the 12th day after mating was observed on fertility parameters. The diverse results obtained in different studies may be due to factors such as breeding season, breed, nutritional and physiological status, and management systems (Moeini et al., 2013). It has been hypothesized that these differences may also be due to variations in the synchronization protocols used (Cam & Kuran, 2004). Since we routinely applied hCG injection at the time of mating in ewes with induced estrus in our field practice, we also administered hCG to all groups at the time of mating in this study. Mirzaei et al. (2014) reported that hCG treatment given two days after the removal of the P4 sponge significantly increased the pregnancy rate (86.4% vs. 60.0%). Similarly, it has been shown that hCG treatments at the time of mating or 12 days later resulted in similar pregnancy rates (50.0% and 47.9%, respectively), both significantly higher than the control group (35.2%) (Moeini et al., 2013). In our research, hCG treatment on the day of mating may positively affect ovulation and CL development. However, ovulation rates and P4 levels were not evaluated. Therefore, the potential positive effects of hCG treatments administered at the time of mating may have masked any differences in fertility outcomes between the hCG and control groups.

It is well known that vitamins play an essential role in the reproductive performance of animals. Natural antioxidants such as vitamin E, β-carotene, and vitamin C help counteract oxidative stress caused by various physiological and pathological conditions (Nayyar & Jindal, 2010). However, conflicting results have been reported regarding the effects of estrus synchronization combined with β-carotene and / or vitamin E supplementation on the reproductive performance of small ruminants. It has been reported that short-term β-carotene supplementation can improve ovarian function and P4 synthesis in goats (Arellano-Rodriguez et al., 2009). Kaçar et al. (2008) reported that out of season supplementation with testosterone antibody, β-carotene and vitamin E increased the incidence of multiple pregnancies in Tuj ewes. In contrast, Köse et al. (2013) reported that β-carotene injection (1 mg/kg) on the day of sponge removal in synchronized, out of season ewes did not positively affect pregnancy rate or lamb yield. While Koyuncu & Yerlikaya (2007) found that vitamin E supplementation improved estrus incidence and fertility in ewes, other studies (Gabryszuk & Klewiec, 2002; Yaprak et al., 2004) showed no such effect. More recently, Farahavar et al. (2020) reported that administering vitamin E and selenium supplements to ewes kept on pasture two weeks before CIDR insertion, at the time of insertion, and during CIDR removal did not improve fertility, prolificacy, or lambing rates. In a study conducted during the anestrus period in ewes, injection of vitamin E combined with β-carotene did not result in the expected increase in lamb yield, although it did increase the rate of multiple births (Kaçar et al., 2008). In the present study, the vitamin group exhibited a lower numerical rate of multiple births compared to the control group (19.0% vs. 29.8%; $P>0.05$). This outcome was unexpected, even though the difference was not statistically significant. Ewes treated with β-carotene and vitamin E showed a numerical increase in pregnancy rates compared to the control group; however, this difference was not statistically significant.

Lack of a significant positive contribution from β-carotene + vitamin E supplementation to reproductive performance in our study suggests that the ewes may have already obtained these substances from pasture in sufficient quantities. Supporting this view, Beytut et al. (2005) reported that ewes in the Kars province and surrounding areas had the lowest plasma vitamin A levels during winter and fall, while the highest plasma β-carotene levels were observed in spring and summer. Similarly, Afshari et al. (2008) found that serum vitamin A and β-carotene levels in Ghezel ewes in Iran were significantly lower in winter compared to summer. The findings of the present study did not support the hypothesis that co-administration of hCG with β-carotene and vitamin E would improve fertility. Özmen et al. (2022) reported that incorporating β-carotene, vitamin E, and hCG into the estrus synchronization protocol outside the breeding season had no significant effect on the reproductive performance of Awassi ewe lambs. However, they observed that administering hCG 12 days after mating enhanced pregnancy, lambing, and fertility rates, resulting in an economic benefit. In contrast to our study, their investigation specifically focused on ewe lambs. The absence of differences between the β-carotene + vitamin E group and the control group in our study may be due to all ewes receiving adequate levels of vitamins and minerals from the pasture. Considering the region and season in which the study was conducted, it is likely that all ewes had been grazing on high-quality pasture for the past 2-3 months. Therefore, conducting similar studies during periods of poor pasture quality or in winter may provide more insight into the potential benefits of vitamin supplementation.

The study concluded that β-carotene + vitamin E treatment combined with estrus synchronization in ewes during the transition season and hCG administration on the 12th day post-mating, or a combination of these two strategies did not significantly improve fertility parameters.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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Anatomical Features of the Auditory Tube in Domestic Mammals

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ABSTRACT

The eustachian tube (Tuba auditiva) is an anatomical structure that plays a critical role in the respiratory system and hearing health in domestic mammals, extending between the middle ear and nasopharynx. It balances the middle ear pressure with the external environment and ensures that hearing functions are performed properly. The anatomical structure and physiological functions of the tuba auditiva vary among animal species. The air sac (diverticulum tuba auditiva), especially found in horses (equide), is connected to the tuba auditiva. Since this sac is in close contact with cranial nerves and large vessels due to its neighborhood, infection conditions and pathologies can cause serious systemic effects. Therefore, the structure has anatomical importance in terms of the diseases of the diverticulum tuba auditiva. Apart from horses, the structural features of the tuba auditiva are also important in terms of its relationship with hearing health and the respiratory system in ruminants, carnivores and pigs. In this review, a detailed evaluation of the tuba auditiva in terms of its anatomy, histology, and physiology is presented, and its structural and functional properties, pathological conditions, and anatomical importance in different animal species are discussed. Its importance on studies conducted from past to present is discussed and the current literature in this field is summarized. Considering the anatomical importance of the tuba auditiva and its role in the auditory system, it is concluded that research on this structure should continue in the presence of scientific and technological developments.

Keywords: Anatomy, diverticulum, physiology, guttural pouch, eustachian tube, auditory tube

Evcil Memeli Hayvanlarda Tuba Auditiva'nın Anatomik Özellikleri

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ÖZ

Eustachi borusu (Tuba auditiva), evcil memeli hayvanlarda orta kulak ile nasopharynx arasında uzanan solunum sistemi ve işitme sağlığında kritik rol oynayan anatomik bir yapıdır. Orta kulak basıncını dış ortamla dengeleyerek işitme fonksiyonlarının düzgün bir şekilde gerçekleşmesini sağlar. Hayvan türleri arasında tuba auditiva'nın anatomik yapısı ve fizyolojik işlevleri bazı farklılıklar göstermektedir. Özellikle at gibi tek tırnaklılarda (Equide) bulunan hava kesesi (diverticulum tuba auditiva) tuba auditiva ile bağlantılıdır. Bu kese, komşulukları itibariyle kranial sinirler ve büyük damarlarla yakın temas halinde olduğundan, kesenin patolojik durumlarında ciddi sistemik etkiler yaratabilir. Bu yüzden yapı diverticulum tuba auditiva'nın hastalıkları açısından anatomik öneme sahiptir. Atlar dışında ruminantlar, carnivorlar ve domuzlarda da tuba auditiva'nın yapısal özellikleri işitme sağlığı ve solunum sistemi ile olan ilişkisi bağlamında da önem taşımaktadır. Bu derlemede, tuba auditiva'nın anatomisi, histolojisi, fizyolojisi açısından detaylı bir değerlendirmesini sunarak, farklı hayvan türlerindeki yapısal ve işlevsel özellikleri, patolojik durumları ve anatomik önemi ele alınmıştır. Geçmişten günümüze yapılmış çalışmalar üzerindeki önemi ele alınmış olup bu alandaki güncel literatürü özetlemektedir. Tuba auditiva'nın anatomik olarak önemi ve işitme sistemindeki rolü göz önüne alındığında, bu yapı üzerine yapılan araştırmaların bilimsel ve teknolojik gelişmelerin eşliğinde devam etmesi gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Anatomi, diverticulum, fizyoloji, hava kesesi, östaki borusu, tuba auditiva.

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Giriş

Tuba auditiva orta kulak boşluğunu (cavum tympani) ile yutağın pars nasalis pharyngis bölümüne bağlayan dar yarık şeklinde bir tüptür (Standring et al., 2005). İlk olarak 1562'de Bartolomeus Eustachius tarafından bulunmuştur (Marchese-Ragona et al., 2014). Kas yapıları 1704'te Antonio Valsalva tarafından daha ayrıntılı olarak tanımlanmıştır. Tuba auditiva'nın görüntülenmesi ilk olarak yirminci yüzyılın sonlarında fiberoptik endoskopinin ortaya çıkmasıyla ortaya konulmuştur (Sadé & Ar, 1997). Tuba auditiva'lar nasopharynx'in lateral duvarının üst kısmına, köpekte 10 mm, kedide 4 mm uzunluğundaki küçük mukoza yastıklarının hemen rostral'ine açılır (Evans & De Lahunta, 2012). Tuba auditiva'nın yarık benzeri açıklıkları (ostium pharyngeum tuba auditiva), nasopharynx duvarında, yumuşak damağın orta noktası seviyesinde, çift taraflı olarak yerleşmiştir (Nickel et al., 1986). Tuba auditiva; cavum tympani'ye yakın bölümü kemikten oluşan pars ossea tuba auditiva ile pharynx'e yakın bölümü kıkırdaktan oluşan pars cartilaginea tuba auditiva olmak üzere iki kısımdan oluşur. Tuba auditiva'nın giriş kısmı cavum tympani'de bulunurken, nasopharynx'te yarık benzeri bir açıklık yaparak sonlanır (König & Liebich,

2020). İnsanda tuba auditiva yere göre 45 derecelik bir açıda bulunur (Standring et al., 2005). Tuba auditiva'nın kemik ve kıkırdak kısımları birbirleriyle yaklaşık 160 derecelik bir açı oluşturur (König & Liebich, 2020). Tuba auditiva'nın yaşla birlikte uzadığı, kemik ve kıkırdak bölüm arasındaki açının küçük yaşlarda daha keskin olduğu tespit edilmiştir (König & Liebich, 2020).

Pars Ossea Tuba Auditiva

Tuba auditiva'nın kemik bölümünü oluşturan kısımdır. Tuba auditiva'nın orta kulağa yakın kısmını şekillendirir. Dış ve iç iki ucu vardır. Dış ucu orta kulak boşluğuna ostium tympanicum tuba auditiva denilen bir delik ile açılır. Diğer ucu ise pars cartilaginea tuba auditiva ile birleşir (König & Liebich, 2020).

Pars Cartilaginea Tuba Auditiva

Tuba auditiva'nın kıkırdak bölümünü oluşturan ve pharynx'e yakın olan kısmıdır (König & Liebich, 2020). Bir ucu kemik bölümün (pars ossea tuba auditiva) ön ucuna fibröz doku aracılığı ile yapışmıştır. (Sadé & Ar, 1997). Kıkırdak bölüm, lamina (cartilaginis) medialis ve lamina (cartilaginis) lateralis denilen iki yaprak görünümündedir (König & Liebich, 2020). Kıkırdağın bulunmadığı küçük bölüm ise lamina membranacea denilen fibröz bir zardan meydana gelmiştir. (Nickel et al., 1986). Kıkırdak bölümün diğer ucu ostium pharyngeum tuba auditiva ile pars nasalis pharyngis'e açılır. Bu deliğin arka duvarı diğer kesimlere nazaran daha kabarıktır, dolayısıyla torus tubarius'u oluşturur (König & Liebich, 2020).

Torus levatorius: Nasopharynx'in yan duvarında, tuba auditiva'nın açıklığının altında, m. levator veli palatini tarafından meydana getirilen çıkıntıdır (Standring et al., 2005).

Tuba auditiva tüm seyri boyunca aynı genişliği göstermez. Başlangıç ve sonlanma yerleri en geniş bölümleri olduğu halde kemik kısım ile kıkırdak kısmın birleştiği yer borunun en dar kısmını oluşturur ki buraya isthmus tuba auditivae denir (König & Liebich, 2020). Tuba auditiva'nın çapı istmus'ta 0,6 ila 3,0 mm ve nasopharynx girişin de 20,0 ila 30,0 mm arasında değiştiği bildirilmiştir (Sadé & Ar, 1997).

Tuba Auditiva'nın Kasları

Tuba auditiva'nın pharynx tarafındaki deliğinin konstriktoru m. levator veli palatini'dir. Musculus tensor veli palatini yutak girişine yaklaşık 12 ila 20 mm derinlikte bir bağlantı sağlar ve dinamik kapak benzeri bir bölge oluşturur (Standring et al., 2005). Musculus levator veli palatininin daralması, çevresindeki diğer kaslarla uyum içinde koordineli bir dizi başlatır, bu da tuba auditiva'nın kapakçık benzeri açılıp kapanmasına yol açar. Bu işlem, yarım saniyeden daha kısa sürer. Bu kapağın anormal kapanmasının tuba auditiva'nın disfonksiyonunun altında yatan faktör olduğuna inanılmaktadır (Sadé & Ar, 1997). Bu kas m.tensor veli palatini'nin iç tarafında bulunur. Processus muscularis'ten başlangıç alır.Yumuşak damak içinde yayılarak orta çizgiye kadar gelir. Yumuşak damağı yukarı kaldırma fonksiyonuna sahiptir (Standring et al., 2005). Tuba auditiva'nın pharynx tarafındaki deliğinin dilatatoru m .tensor veli palatini'dir. Tuba auditiva'nın lateral duvarını destekleyen ve ortası kabarık m.tensor veli palatini, iki yandan basık, ortası kabarık processus muscularis'ten orjin alarak ventrocranial bir seyirle yelpaze şeklinde aponeurosis ile palatum molle'ye bağlanır. Öne ve aşağıya yönelir (Evans & De Lahunta, 2012; König & Liebich, 2020). Yelpaze tarzında yayıldığı aponeurosis yumuşak damakta sonlanır. Dış yanda m. pterygoideus medialis'e içten de m. levator veli palatini'ye komşudur (Standring et al., 2005). Musculus tensor veli palatini ve m. levator veli palatini kasılarak ostium pharyngeum tuba auditiva'yı açık tutar (Sadé & Ar, 1997). Tek taraflı çalıştığı zaman yumuşak damağı kasıldığı tarafa çeker. İki taraflı çalıştığı zaman da yumuşak damağı gerer (Evans & De Lahunta, 2012). Orta kulakta bulunan ve basıncı algılayan sinirlerin negatif basınca bağlı olarak uyarılmasıyla oluşan yutkunma refleksi sırasında kasılan kaslar kapalı pozisyonda duran tuba auditiva'yı açarlar (Sadé & Ar, 1997). Musculus tensor veli palatini, n. mandibularis (n. trigeminus'un bir dalı) tarafından innerve edilir (König & Liebich, 2020). Nervus mandibularis n. trigeminus'un üç dalından en kalın olanıdır. Sensitif ve motor liflerden oluşmuş bir sinirdir. Sensitif lifler alt çene eklemi, temporal bölge derisi dahil kulak kepçesi, kulak zarı, dış kulak yolu, alt çene diş ve diş etleri, alt dudak, yanak ve dilin ön yarımının duyusunu alır. Motor lifler ise çiğneme kasları ile m. tensor veli palatini ve m. tensor tympani'ye gider. Nervus mandibularis yukarıdan aşağıya doğru 7 dal verir. Bunlardan n. pterygoideus medialis, m. tensor veli palatini'de dağılan n. musculi tensoris veli palatini adındaki dallara ayrılır. Nervus glossopharyngeus: motor, parasempatik ve sensitif liflerden oluşmuştur. Orta kulak ile dilin caudal 1/3 lük kesiminin duyusunu alır. Motor iplikleri ile pharynx'in dilastasyonunu sağlayan yumuşak damak kaslarını uyarır. Atlarda n. hypoglossus ile birlikte diverticulum tuba auditiva'nın medialinde seyreder. Bu seyirlerinde n. hypoglossus, n. glossopharyngeus ile ortak kılıfla sarılıdır. Sinir a. carotis interna'yı çaprazlayarak radix lingua'ya ulaşır (Evans & De Lahunta, 2012; König & Liebich, 2020; Standring et al., 2005).

Histolojik Özellikleri

Tuba auditiva dıştan içe doğru mukoza, kıkırdak ve kas tabakasından oluşur, özellikle kıkırdak bölümünde glandulae tubariae denilen müköz bezler vardır. Tubal membranın korozyondan korunmasında, savunma mekanizmalarında ve hızlı basınç dengelemesinin kolaylaştırılmasında yer alan fosfolipid bileşiklerinin varlığı tespit edilmiştir. Kemik bölüme yakın olan kıkırdak bölümü hiyalin kıkırdaktır. Pharynx'e doğru elastik iplik sayısında bir artış görülür. Tuba auditiva'nın nasofarenkse bakan ucunda silyalı yalancı çok katlı silindirik epitel, cavum tympani'ye bakan ucunda ise silyalı basit silindirik epitel ile döşelidir. Nasopharynx'e komşu kısımlarda silyalı hücreler ve goblet hücreleri sayıca fazla miktardadır. Çalışmalar, equidaede diverticulum tuba auditiva'nın mukozasının yabancı madde temizleme yeteneği sağladığını, ancak yeteneğin epitelyumun farklı bölgeleri arasında değiştiğini göstermiştir (Evans & De Lahunta, 2012; König & Liebich, 2020; Sadé & Ar, 1997; Standring et al., 2005).

Fizyolojik Olarak Görevi

Genellikle tuba auditiva kapalıdır; yutma, esneme, hapşırma gibi hareketler sırasında açılır ve böylece orta kulak ve atmosferik basınçların eşitlenmesini sağlar. Tuba auditiva, membrana tympani'nin her iki tarafındaki (orta kulak ve yutak) atmosferik basıncı dengeleme görevini üstlenir. Basınç dengesiz olabilir (örneğin bir asansörde hareket halindeyken) bu ani değişikliğin düzeltilmesi işlemi, kulaklarda hafif bir çınlama sesine neden olabilir (Sadé & Ar, 1997; Standring et al., 2005).

Tuba Auditiva Fonksiyonları

Tuba auditiva'nın bilinen üç fonksiyonu vardır; havalandırma: Orta kulak boşluğunun atmosferik basınçla dengelenmesini sağlar. Drenaj: Orta kulakta üretilen normal veya patolojik sıvıların nasopharynx'e boşaltılmasını sağlar. Koruma: Orta kulağın, nasopharynx'teki basınçtan ve patolojik akıntılardan korunmasını sağlar. Tuba auditiva'nın drenaj görevi de çok önemlidir. Orta kulak boşluğunda birikebilen normal veya patolojik, transudat, eksudat vs. gibi sıvıların ve yabancı cisimlerin boşaltılmasını sağlar. Yutkunma ve esneme sırasında m. tensor veli palatini ve m. levator veli palatini kısa bir süre açılır. Kulak zarının ideal titreşimini sağlayabilmesi normal gerginlikte olmasına yani her iki tarafında hava basıncının dengede olmasına bağlıdır. Fonksiyonel özelliklerine bakıldığında nasopharynx, tuba auditiva kanalları vasıtasıyla orta kulakla iletişim kurar. Küçük kas demetleri, deliklerden pharynx duvarına doğru yayılırlar ve deliği genişletmek için bir mekanizma oluştururlar. Böylece hava, yutaktan orta kulağa, orta kulaktan da yutağa geçer ve kulak zarının her iki tarafındaki basınç dengelenmiş olur (Evans & De Lahunta, 2012; König & Liebich, 2020; Reece et al., 2015; Sadé & Ar, 1997).

Tuba auditiva'nın sürekli açık olması nispeten daha az görülen bir problemdir. Bu durum tuba auditiva'nın yapısal olarak geniş olması ya da hızla kilo verme sonucu çevresindeki yağ dokularının azalması gibi nedenlerle izlenebilir (Møller, 2012; Musiek et al., 2020).

Sürekli açık olması salgıların ve enfeksiyon etkenlerinin pharynx'ten orta kulağa geçmesine bağlı şikayetler yanında, kulakta basınç hissi, nefes alıp verme sırasında kulağa hava girip çıkmasının yarattığı rahatsızlık ve otofoni olarak adlandırdığımız kişinin kendi sesini duyması gibi şikayetlere neden olur (Bluestone, 2005). Daha sık karşılaştığımız tıkanma şeklindeki fonksiyon bozukluklarında ise; orta kulak basıncının dış ortam basıncıyla ideal şekilde eşitlenememesi sonucunda tıkanıklığın ciddiyetine göre sürekli mevcut olan ya da özellikle dış ortam basıncında belirgin değişikliğe neden olan hızlı irtifa değişimleridir (Seibert & Danner, 2006). Uçuş ya da su altı dalışı gibi durumlarda ortaya çıkan kulakta dolgunluk hissi, kulak çınlaması, kulak tıkanıklığı, işitme azlığı gibi şikayetler izlenir (Møller, 2012; Musiek et al., 2020).

Türler Arasındaki Farklılıklar

Tuba auditiva arkadan orta kulağı önden de pharynx'i örten mukozanın devamı olan bir mukoza ile döşenmiştir. Bu mukoza ruminant, sus ve carnivorlarda boru şeklindedir (König & Liebich, 2020). Equidae'de ise ventrale doğru bir genişleme yapar, dolayısıyla bu türe özgü olan ve diverticulum tuba auditiva denilen hava kesesini oluşturur. Tuba auditiva'nın kıkırdak bölümünden şekillenmiştir (Baptiste, 1997).

Diverticulum Tuba Auditive

Tek tırnaklılarda karakteristik bir anatomik yapı olarak tuba auditiva'nın kıkırdağının ventral yarığı boyunca kanalı döşeyen mukoza katmanının dışarı doğru genişlemesi ile oluşmuş diverticulum tuba auditiva (bir çift hava kesesi) bulunur. Yaklaşık 500 ml kapasiteye sahip olan keseler, dorsalde kafatası tabanı ile atlas, ventral'de ise oesophagus'un başlangıcı ile pharynx arasındaki boşluğu doldurur (Nickel et al., 1986). İki kesenin dorsal kısımları, medial'de mm. rectus capitis aracılığı ile birbirinden ayrılırken, ventral kısımlar birleşir. Sadece ince bir medial bölme ile ayrılırlar. ((Nickel et al., 1986). Os stylohyoideum, keseyi tam olmayan medial ve lateral iki kompartmana ayırır. Bu kemik keseyi daha büyük olan recessus medialis ve daha küçük olan recessus lateralis olmak üzerine iki bölmeye ayırmıştır (König & Liebich, 2020). Çok sayıda önemli oluşum keselerle yakın anatomik ilişki içerisindedir. Bunlardan a. carotis externa, kesenin lateral kompartmanının duvarındaki kıvrım içerisinde seyreder. (Oto & Hazıroglu, 2011). Arteria carotis communis medialis kesenin caudal kenarı yakınında a. carotis externa, a. occipitalis ve a. carotis interna kafatasının tabanına doğru kesenin caudal duvarı boyunca ilerler (König & Liebich, 2020). Nervus vagus bu damara paralel olarak seyreder (Budras et al., 2012). Kafatası boşluğuna n. carotis interna olarak devam eden ganglion cervicale craniale, n. vagus'un ön tarafında, yaklaşık 2 cm uzunluğunda ve iğ şeklinde yerleşmiştir (König & Liebich, 2020). A. Carotis externa ve devamı niteliğindeki a. maxillaris kesenin lateral bölümünün duvarı boyunca seyreder. Kesenin lümeninden her iki atardamarın nabzı da hissedilebilir (König & Liebich, 2020). Özellikle aspergillozis başta olmak üzere kese ile ilgili hastalıklar, yakınında bulunan bu atardamarlara zarar verecek ciddi burun kanaması ile karakterize ölümcül kanamalara yol açabilir (Rush & Mair, 2008). Bu tür hastalıklar sinirleride etkileyebilir. Nervus hypoglossus hatalı yapılan endoskobik muayene sonucu felç olabilir. Nervus hypoglossus diverticulum tuba auditiva'nın enfeksiyon veya kalıtımsal hastalıklarında zarar görür. Dilin felci ile karakterize klinik tablo ortaya çıkar (Borges & Watanabe, 2011). Kese duvarının müköz salgısı, normalde baş aşağı indirildiğinde kesenin en sarkık bölümü olan rostral parçasında bulunan ostium pharyngeum tuba auditiva vasıtasıyla pharynx'e direne edilir. Yutkunduğunda bağlantı açılır, otlanma sırasında normal olarak drenaj uyarılır. Delik tıkanır veya sekresyon herhangi bir sebepten dolayı birikirse kese genişler ve çoğunlukla çene arkasında palpe edilebilir durumda, belirgin bir şişkinlik görülür. Diverticulum tuba auditiva'nın, yalancı çok katlı prizmatik epitelle örtülü olan lamina propria'sında düz kas telleri, elastik ve kollagen iplikler, seröz ve müköz bezler ile lenf folikülleri vardır. Submukoza, çok sayıda kan ve lenf

damarları ile sinir telleri içerir. Kesenin en dışında ise adventitiae katmanı bulunur (Ghazi & Mobini, 2013; Manglai et al., 2000).

Viborg Üçgeni

Diverticulum tuba auditiva kesesine dışarıdan Viborg Üçgeni vasıtasıyla ulaşılır. Bu üçgenin ramus mandibula'nın arka kenarı, vena maxillaris externa ve m. sternomandibularis tendonunun oluşturduğu üçgendir. Kese sadece, kendisini aşağıya doğru çeken irin gibi patolojik bir sıvı ile dolduğu zaman bu üçgen ile sınırı çizilen bölgeye iner. Patolojik sıvının dışarıya akıtılması için, m. occipitohyoideus seviyesinde ve mandibula ile atlas kemiğinin kanadı arasında bulunan fossa retromandibularis'in dorsal bölümünden ikinci bir ensizyon yapılmalıdır. Atta boynun üst bölgesindeki anatomik yapıların ayrımı ultrason ile sağlanabilir (König & Liebich, 2020).

Diverticulum Tuba Auditiva Fonksiyonu

Diverticulum tuba auditiva'nın en önemli işlevi başın spesifik ağırlığının azaltılmasıdır. Son zamanlarda yapılan deneysel araştırmalar, keselerin, beyine giden kanın soğutulması mekanizmasında da etkili olduğunu göstermiştir. Bu çalışmalar, keselerin oldukça ince duvarı ile arteria carotis interna'nın ekstracranial bölümü arasındaki yoğun temasın bağlantısına vurgu yapmaktadır. Damarın bu şekilde kese duvarı üzerindeki seyrinin, beyine giden kanı soğutmak için bir potansiyel sağladığı, bu bölgede damar üzerine monte edilen sıcaklık sensörlerinin kullanıldığı denemelerle ortaya çıkarılmıştır. On beş dakika yoğun egzersiz yaptırılan hayvanda, a. carotis interna'nın distal ucunda ki sıcaklıkta belirgin bir düşüş, (yaklaşık 2°C) gözlenirken, dinlenme durumundaki atlarda kan sıcaklığında bölgesel farklılıklar görülmemiştir. Fiziksel aktivitenin vücut sıcaklığını yükselteceği ve sonuçta etkili önlemler yoksa beyin işlevini tehlikeye atabilecek seviyeye gelebileceği bir gerçektir. Kesenin güçlü bir şekilde ventilasyonu ile a. carotis interna'dan geçen kandan, kese içindeki havaya ısı transferi kolaylaştırılır (Baptiste, 1997; König & Liebich, 2020).

Diverticulum Tuba Auditiva Hastalıkları

Atta oldukça yaygındır. Bunlar: empiyem, mikosizi, ve taylarda görülen hava kesesi tympanisi gibi hastalıklardır. Enfeksiyon komşu diğer yapılara da yayılarak daha ileri komplikasyonlara neden olabilir. Hava kesesinin tanı ve tedavisindeki zorluk, hava kesesinin karmaşık anatomisinde ve kafatasındaki diğer önemli yapılarla olan yakın ilişkisinde yatmaktadır. Yakın temasta olan etkilenen yapılara güvenli cerrahi erişimde zorlukta önemlidir.

Eksudat kontamine olabilir, kanal boyunca veya lymphocentrum retropharyngeum'da da enfeksiyon gelişebilir. Kesenin mikotik enfeksiyonları da oluşur. Parotis bölgesinin ağrılı şişkinliği, kafanın anormal duruşu, burun akıntısı gibi göze çarpan etkilenen hayvanlarda, doğrudan keseyle ilişkili yapıların kalıtımından kaynaklanan çesitli spesifik anormallikler de görülebilir. Sık görülenler arasında orta kulak iltihaplanması (tuba auditiva boyunca enfeksiyonun yayılması) n. glossopharyngeus ve n. vagus tutulumunu takiben yutma güçlüğü, n. vagus tutulumunu takiben laryngeal hemipleji (kornaj), Horner sendromu olarak bilinen n. sympathicus ile ilişkili: Burun tkanıklığı, üst göz kapağının sarkması, pupilla daralması, terleme ile kafa ve boyun bölgesinin etkilenen tarafının derisindeki sıcaklık artışıdır. Arteria carotis externa nispeten daha iyi korunur. Nervus facialis tutulum belirtileri de nadirdir. Arteria carotis interna erozyonuna bağlı burun kanaması (epistaksis), atta oldukça yaygındır. Bunlar: empiyem, mikosiz, ve taylarda görülen hava kesesi tympanisi gibi hastalıklardır. Enfeksiyon komşu diğer yapılara da yayılarak daha ileri komplikasyonlara neden olabilir. Hava kesesinin tanı ve tedavisindeki zorluk, hava kesesinin karmaşık anatomisinde ve kafatasındaki diğer önemli yapılarla olan yakın ilişkisinde yatmaktadır. Yakın temasta olan etkilenen yapılara güvenli cerrahi erişimde zorlukta önemlidir (Hance, 1992; Lane, 1990).

Hava kesesi tympanisi

Tympani, sağlıklı bir tayda, bir miktar sıvı birikimi olsun veya olmasın, hava keselerinin hava ile tek taraflı veya çift taraflı şişmesidir. Muhtemel nedenler arasında, hava ve sıvıyı kese içinde tutan tek yönlü valf görevi gören bir mukozal flep (plica salpingopharyngea), üst solunum yolu enfeksiyonundan kaynaklanan iltihaplanma, inatçı öksürük ve kas disfonksiyonu yer alır (Sparks et al., 2009).

Nasopharyngeal tıkanıklık

Salpingofaringeal kıvrımdaki anatomik veya fonksiyonel bir kusurun neden olduğu bir veya her iki hava kesesinden hava çıkışının bozulmasıyla oluşan bir hastalıktır. Tuba auditiva'nın tıkanıklık şeklindeki fonksiyon bozukluklarına neden olan farklı etkenler vardır. Bunlar içerisinde en sık gördüklerimiz borunun içerisini döşeyen mukozada; üst solunum yolu enfeksiyonları, alerjik reaksiyonlar, burun ve sinüs enfeksiyonlarına bağlı olarak oluşan ödem, kalınlaşma sonucu tüp lümeninin daralması, tüp komşuluğunda yapılan cerrahi işlemler sırasında oluşan hasarlar ya da özellikle geniz bölgesindeki tümör ve kitlelerin neden olduğu baskı, Bununla birlikte tıkanıklıklar bir neden olmadan anatomik ve yapısal özelliklere bağlı olarak da oluşabilir (Hsu & Suh, 2018).

Empiyem

Hava keselerin empiyemi, bir veya her iki hava kesesinde purulent materyalin birikmesidir. Üst solunum yolu enfeksiyonları (özellikle Streptococcus equi 'nin neden olduğu enfeksiyonlar). Daha az yaygın nedenler; tahriş edici ilaçların infüzyonu, stylohyoid kemiğin kırılması, faringeal orifisin konjenital veya edinilmiş stenozu, nazogastrik tüp ile faringeal perforasyondur (Judy et al., 1999).

Hava kesesi mikozisi

Bariz bir yaş, cinsiyet, cins veya coğrafi yatkınlık olmaksızın hava kesesinin çatısını etkiler. Nedeni bilinmemektedir ve farklı mantarlar izole edilmiştir, ancak Aspergillus (Emericella) fumigatus en yaygın izolattır (Karagözoğlu et al., 2016).

Conflict of Interest

The authors declared that there is no conflict of interest.

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The Biotechnological Potential of Baculoviruses: From Insect Viruses to Biotechnology Workhorse

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Review Article

History

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ABSTRACT

Baculoviruses are a significant group of arthropod viruses widely recognized for their potential as biological control agents against pests in agriculture and forestry. The Baculoviridae is a vast family of viruses that primarily infect various species within the Arthropoda phylum, particularly insects. Baculoviruses are widely used not only as biopesticides in agricultural applications but also as efficient tools for recombinant protein production. The Baculovirus Expression Vector System (BEVS) is particularly effective for expressing complex or difficult-to-produce proteins in mammalian cells. Owing to its high expression capacity and post-translational modification capabilities, BEVS has been successfully employed in various biotechnological fields, including vaccine development, therapeutic protein production, and the synthesis of enzymes and antibodies.
In this review, the BEVS technique, one of the significant areas of use of Baculoviruses, is discussed along with its advantages and practical applications.

Keywords: Baculovirus, BEVS, recombinant protein, vaccine

Baculovirüslerin Biyoteknolojik Potansiyeli: Insekt Viruslerinden Çok Yönlü Biyoteknolojik Araçlara

Süreç

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ÖZ

Baculovirüsler, tarım ve ormancılıkta pestisitlere karşı biyolojik kontrol ajanları olarak potansiyelleri iyi bilinen artropodların önemli bir virüs grubudur. Baculoviridae, özellikle insektler olmak üzere, başlıca Arthropoda phylum şubesindeki farklı türleri enfekte eden geniş bir virüs ailesidir. Baculovirüsler, yalnızca tarımsal uygulamalarda biyopestisitler olarak değil, aynı zamanda rekombinant protein üretimi için etkili araçlar olarak da yaygın olarak kullanılmaktadır. Baculovirüs Ekspresyon Vektör Sistemi'nin (BEVS), memeli hücrelerinde kompleks veya üretilmesi zor proteinleri eksprese etmede özellikle etkili olduğu gösterilmiştir. BEVS, yüksek ekspresyon kapasitesi ve translasyon sonrası modifikasyon yetenekleri nedeniyle aşı geliştirme, terapötik protein üretimi, enzim ve antikor sentezi dahil olmak üzere çeşitli biyoteknolojik alanlarda başarıyla kullanılmıştır.
Bu derlemede, baculovirüslerin başlıca kullanım alanlarından biri olan BEVS tekniği, sağladığı avantajlar ve çeşitli pratik uygulamaları birlikte ele alınmaktadır

Anahtar Kelimeler: Baculovirus, BEVS, rekombinant protein, aşı

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Introduction

Baculoviruses are arthropod-specific viruses in the family Baculoviridae and infect more than 600 host species (Chambers et al., 2018). There are more than 500 species of baculoviruses in nature, all of which are restricted to a host range limited to invertebrates (Felberbaum, 2015). They are regarded as effective biological control agents for managing pest populations in agricultural and forestry sectors (Grzywacz, 2017). Baculovirus is an enveloped virus characterized by a double-stranded, circular DNA genome (Luckow, 1993). The length of the baculovirus rod-shaped nucleocapsid ranges from 200 to 400 nm, with a diameter of approximately 36 nm. The genome size varies between 80 and 180 kb (Van-Oers, 2011; Chambers et al., 2018). According to phylogenetic analyses, baculoviruses are divided into four genera: Alphabaculovirus, Betabaculovirus, Deltabaculovirus, and Gammabaculovirus. Alphabaculovirus includes all specific lepidopteran nucleopolyhedroviruses, single nucleocapsid type (SNPV) and multiple nucleocapsid type (MNPV). Betabaculovirus includes members of the lepidopteran-specific Granulovirus genus, Deltabaculovirus includes Diptera-specific baculoviruses, and Gammabaculovirus includes Hymenopteran-specific NPVs (Herniou & Jehle, 2007; Miele et al., 2011; Kelly et al., 2016). To date, 103 complete genome sequence data of at least 101 baculovirus species and isolates have been reported and are publicly available in GenBank (GenBank, 2024).

The majority baculoviruses have a minimal host range and typically target a single insect species (Clem & Passarelli, 2013). The replication cycle of this virus occurs in two distinct phases, resulting to the formation of two

morphologically different types: budded virus (BV) and occlusion-derived virus (ODV) (Thiem & Cheng, 2009). These two virions differ in the origin and constituents of their envelope structures and their functions in the life cycle. In both forms, the viral DNA associates with multiple copies of the small, positively charged protein p6.9, which neutralizes the DNA's negative charge. This nucleoprotein complex is further stabilized by structural proteins that assemble into the nucleocapsid (Haase et al., 2013). Primary infection and horizontal transmission of baculoviruses in host larvae are initiated orally through ODV. The second stage, secondary infection, which enables the spread of disease between tissues is initiated by virions known as the BV form (Chambers et al., 2018). The preferred virion form in cell culture for BEVS applications is the BV form, which has approximately 1800-fold higher infectivity than ODV (Possee et al., 2010) (Figure 1). Baculovirus vectors frequently used as vectors in studies are Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and Bombyx mori nucleopolyhedrovirus (BmNPV). Although AcMNPV and BmNPV are genetically close viruses with approximately 90% amino acid sequence similarity in their ORFs, AcMNPV has a broader host range, distinguishing these two viruses from each other. AcMNPV, which forms the basis of baculovirus expression systems and is widely used in biotechnological applications, was identified as the first fully sequenced baculovirus prototype with a double-stranded circular DNA genome of 134,894 bp and 154 ORFs (Kang et al., 1999; Kato et al., 2016).

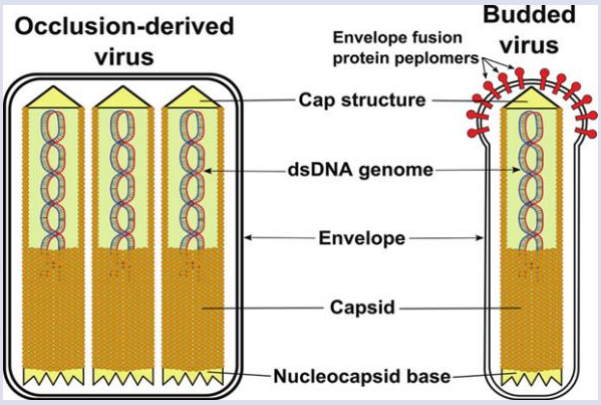

Figure 1. Extracellular Baculovirus virions: BV (budded virus) and ODV (occluded virus) (Harrison et al., 2018)

Baculoviruses are regarded as valuable vectors for producing foreign proteins due to their capacity to generate large amounts of polyhedrin. Polyhedrin is one of the essential genes that promotes viral production in vectors used to express foreign proteins. Recombinant baculoviruses have been engineered to express foreign genes under the control of the polyhedrin promoter, facilitating high-yield protein production (Yu et al., 2012). The ability of these viruses to produce polyhedrin has led to the notion that they can be utilized for the large-scale production of other proteins of significant interest to the research community. The discovery that baculoviruses do not require the polyhedrin protein for replication in insect cells supported the idea that recombinant baculoviruses could be produced by replacing the polyhedrin-encoding gene of these viruses with a targeted foreign gene. This approach enabled the production of independent recombinant baculoviruses that expressed the target proteins with high efficiency by integrating the foreign gene instead of the polyhedrin gene. The theoretical framework was subsequently implemented through the development of the first recombinant baculoviruses, generated via homologous recombination between the polyhedrin locus of the AcMNPV genome and transfer plasmids carrying a foreign gene under the regulatory control of the polyhedrin promoter (Jarvis, 2009; Rychlowska et al., 2011; Hong et al., 2022; Hong et al., 2023).

Baculovirus expression vector system (BEVS)

Baculovirus expression vector system (BEVS) is a system that utilizes the insect cell-specific infection ability of baculoviruses (Felberbaum, 2015). The basis of BEVS is to replace non-essential regions in the baculovirus genome with a foreign gene of interest, allowing the recombinant baculovirus to serve as a delivery vector and to transfer the target gene into insect host cells for subsequent replication and protein expression (Hong et al., 2023). The first study describing a baculovirus vector in insect cells was conducted by Smith et al. (1983) to produce recombinant human β-interferon. Since its establishment, BEVS has been widely utilized to produce numerous recombinant proteins, including those used in commercial vaccine development (Hong et al., 2022).

Baculoviruses possess two gene products, polyhedrin and p10, which are not essential for the continued production of viral particles in cell culture. Therefore, a foreign gene encoding the desired protein can be inserted into the coding regions of the genes corresponding to these products (Maeda et al., 1993). The classical BEVS is defined as a recombinant baculovirus engineered to carry a foreign nucleic acid sequence, typically a complementary DNA (mostly cDNA), under the transcriptional regulation of the strong polyhedrin promoter, enabling high-level expression of the target protein (Jarvis, 2009).

BEVS comprises three fundamental elements: a plasmid vector containing the target gene, a baculovirus genome (in the form of a bacmid or linear DNA), and an insect cell line (Hitchman et al., 2011). The BEVS process starts by creating a recombinant baculovirus containing the interest gene. This gene is cloned into a transfer plasmid under the control of a strong promoter, such as polyhedrin or p10, both of which are known to promote high levels of protein expression in insect cells (Felberbaum, 2015; Martínez-Solís et al., 2016).

The many commercially available BEVS can be classified into two main groups based on the mechanism of recombinant virus production. Commercially available BEVS are generally classified into two main categories based on the mechanism of recombinant virus generation.

The first category utilizes transposon-mediated technology, enabling recombinant bacmids to be produced. In this system, the gene of interest is initially cloned into a transfer vector, which is subsequently introduced into an Escherichia coli (E. coli)-based bacmid. Integration of the target gene into the bacmid genome occurs via a site-specific transposition process, facilitating the generation of a recombinant baculoviral genome suitable for transfection into insect cells (Wang et al., 2024). One commercially developed system that implements this method is the Bac-to-Bac™ system, provided by Invitrogen Inc. This method enables the integration of a target gene (GOI) into bacmid DNA using a transfer plasmid through site-specific transposition under the control of a specific promoter. This process occurs in E. coli DH10Bac cells and facilitates the transformation of bacmid DNA into a baculovirus expression vector. These cells include the bacmid and a helper plasmid, which provides the Tn7 transposase enzyme. After transposition, the recombinant bacmid is isolated and transfected into insect cells to generate recombinant baculovirus for expression (Pidre et al., 2023). The baculovirus genome and the transfer vector, which carries the gene of interest, are simultaneously transfected into insect cells. In vivo homologous recombination occurs within the host cells, creating a recombinant virus (Adeniyi and Lua, 2020).

Advantages and Disadvantages of Baculoviral Expression Vector System

The baculovirus expression vector system is a highly efficient and versatile with many advantages for protein expression (Bruder & Aucoin, 2022). BEVS provides several advantages over other widely utilized expression systems (Sandro & Benchaouir, 2019). In general, it is a safe, easy, and effective eukaryotic expression system for the production of recombinant proteins. Protein expression facilitated by the BEVS is considerably more cost-effective than expression in mammalian cell systems. It has many advantages, including high levels of protein expression, expression of large proteins, simultaneous expression of multiple genes, etc. Proteins expressed with BEVS are generally correctly folded and biologically active (Fath-Goodin et al., 2006; Adeniyi & Lua, 2020; Tang et al., 2020).

The baculovirus expression system facilitates the possible production of large amounts of recombinant proteins because it uses a baculovirus capable of high-titer replication in insect cells cultured in suspension without the need for a helper virus. Due to the viral genome being large and has a flexible capsid structure, it can accommodate significant foreign genes and large DNA fragments, allowing for the packaging and expression of polyprotein-encoding DNA. A definitive upper limit for inserting foreign DNA into the baculovirus genome has not been established. Baculoviruses are considered safe for vertebrates, as they are non-infectious and have been demonstrated to be inactive in mammalian cells. Therefore, proteins produced by BEVS can be used for functional studies, vaccine preparations, or diagnostic purposes. It also provides an advantage over other systems expressing oncogenes or toxic proteins (Murphy & Piwnica-Worms, 2001; Haase et al., 2013; Mishra, 2020).

Due to their optimal replication at temperatures between 25°C and 30°C, baculoviruses represent an advantageous expression system for producing proteins from ectothermic organisms that require lower temperatures to maintain biological activity. Therefore, this platform has been critical in developing protein or vaccine platforms against Arbovirus infections. As a result of the efficient replication of arboviruses in insect cells, the arboviral proteins produced in baculovirus-infected insect cells typically exhibit correct folding and glycosylation patterns, which are biologically active (Metz & Pijlman, 2011). Baculoviruses naturally infect arthropods and cannot replicate in vertebrates, plants, or microorganisms. Nonetheless, they can enter certain vertebrate cells under specific conditions and deliver their genetic material. Because of this, the BEVS has become a valuable tool for studying gene expression and protein function in vertebrate cells (Rychlowska et al., 2011).

Additionally, the BEVS is capable of simultaneously expressing multiple heterologous proteins. For example, the MultiBac system, derived from the BEVS platform, is suitable for expressing various proteins at the same time, allowing the formation of specific multi-subunit protein complexes. HR-Bac is another MultiBac-based system that simplifies expression screening and high-yield protein production applications (Sari et al., 2016; Hong et al., 2023). BEVS can carry out various post-translational modifications, such as phosphorylation, different types of glycosylation (N-linked and O-linked), ubiquitination, acetylation, and proteolytic processing. These modifications help understand protein functions and are especially useful for structural biology and crystallography studies (Fraser, 1992; Mishra, 2020; Hong et al., 2023).

Despite its advantages, using baculoviruses in gene therapy presents certain limitations. It can trigger immune responses characterized by the release of inflammatory cytokines and chemokines and activation of the complement system. These immune reactions may cause unwanted effects and contribute to the degradation of the viral genome, particularly when baculoviruses are used for therapeutic purposes other than vaccination (Schaly et al., 2021).

Compared to other viral vectors, baculoviral vectors differ in how long the introduced genetic material remains inside the host cell's nucleus. While the DNA delivered by retroviral, lentiviral, and adenoviral vectors can stay in the nucleus for extended periods either by integrating into the host genome or by existing as episomal DNA, baculoviral DNA has been observed to persist in mammalian cell nuclei for only 24 to 48 hours (Tjia et al., 1983). In addition, it has been demonstrated that baculoviral DNA is degraded over time due to a decrease in the transgene

copy number and mRNA transcription level in baculovirus-transduced cells (Hu, 2006).

Similar to other enveloped viruses, baculoviruses are highly fragile. As a result, they are sensitive to mechanical shear forces, contributing to their relatively low stability. Ultracentrifugation is usually used for baculovirus purification; however, this process can damage the viral envelope and significantly decrease infectivity (Pidre et al., 2023).

Application of the Baculoviral Expression Vector Systems

Viral Vaccinology

The baculovirus expression system has become essential for synthesizing structurally complex eukaryotic proteins. Its first notable application involved successfully expressing recombinant human interferon-beta (IFN-β) in insect cell cultures. The successful expression of IFN-β marked the initial establishment of the baculovirus–insect cell protein expression system. Soon after, other recombinant proteins such as E. coli β-galactosidase, human c-Myc, and human interleukin-2 (IL-2) were also expressed at high levels (Murphy & Piwnica-Worms 2001).

The use of baculovirus as a vector for vaccination was first described by Aoki et al. (1999). The study demonstrated that recombinant baculovirus carrying the pseudorabies viral gB gene induced specific antibody responses in mice. It has subsequently been widely used in many recombinant vaccine development platforms. This system has been used to investigate capsid formation (VLP) of many viruses, including rotavirus, picornavirus, orbivirus, calicivirus, papillomavirus, herpesvirus, and parvoviruses (Ju et al., 2011). In the study conducted by Li et al. (2016), the N and G protein genes of Rift Valley fever virus (RVFV) were inserted into the pFastBacDual baculovirus expression vector under the control of the pP10 and pPH promoters. The resulting RVFV VLPs were concluded to provide a foundation for developing future VLP-based RVFV vaccines. In the other study by Dai et al. (2018), the Zika virus (ZIKV) premembrane (prM) and envelope (E) proteins were co-expressed in insect cells. ZIKV VLPs were efficiently and rapidly produced in large quantities using this system. The findings demonstrated that these VLPs exhibited strong immunogenicity in immunized mice by eliciting high virus-neutralizing antibodies, ZIKV-specific IgG responses, and robust memory T cell activation. Yang et al. (2022) reported that H5N6 VLPs, a highly pathogenic avian influenza (HPAI) strain, induced higher neutralizing antibody titers as well as higher levels of IL-2, IL-4, IL-5, IFN-γ, and TNF, suggesting that H5N6 VLPs may be a potential vaccine candidate for broad-spectrum H5Nx avian influenza vaccines. In a study producing Getah virus (GETV) VLPs in insect cells using BEVS (Miao et al., 2024), it was reported that adjuvant-free vaccination with GETV VLP protected wild-type C57/BL6 mice against GETV viremia and arthritic disease. In a study establishing a VLP-based vaccine platform for the recently pandemic SARS-CoV-2 virus (Nguyen et al., 2024), envelope and membrane proteins of the SARS-CoV-2 Wuhan strain were expressed by the recombinant baculovirus BacMam. In a mouse trial, two intramuscular immunizations of the VLP BacMam elicited specific antibodies in sera and bronchoalveolar lavage fluids. In recent years, various virus-like particle (VLP)-based vaccine studies have been conducted, including HIV-1 Gag VLPs (Puente-Massaguer et al., 2020), Simian virus 40 (SV40) VLPs (Saika et al., 2020), Bovine viral diarrhea virus (BVDV) E2 and Erns VLPs (Wang et al., 2021), human papillomavirus (HPV) L1 protein (Razavi-Nikoo et al., 2023), Porcine deltacoronavirus (PDCoV) VLPs (Liu et al., 2023), novel goose parvovirus (NGPV) VP2 protein (Zhang et al., 2024), Rabbit hemorrhagic disease virus (RHDV) VP60 gene (Hu et al., 2025), and Feline panleukopenia (FPL) VP2 protein (Feng et al., 2025).

In vaccine development, BEVS is one of the most commonly used systems for producing the recombinant proteins needed for different types of vaccines. In the study conducted by Skoberne et al. (2013), the herpes simplex virus (HSV) glycoprotein D2-expressing subunit vaccine elicited humoral immune responses in immunized mice and induced $CD4^+$ and $CD8^+$ T cells characterized by multiple cytokine secretion and cytolytic antigen-specific T cell responses. Yin et al. (2013) demonstrated that the rabies virus's nucleoprotein (N) gene can be highly expressed using a silkworm-baculovirus expression system. The N antigen vaccine is a promising approach for preventing the rabies virus. Respiratory syncytial virus (RSV) fusion glycoprotein (F) was modified and expressed as an antigen in BEVS by Blanco et al (2014). Ge et al., 2016, reported that recombinant baculovirus vaccines expressing Newcastle disease virus (NDV) F or HN genes induced a strong cellular and humoral response in chickens. In the study by Hu et al. (2019), a baculovirus-based vaccine was developed to express the hemagglutinin (HA) protein of highly pathogenic avian influenza A (H7N9). The recombinant baculovirus stably expressed the HA protein in insect cells, and immunization with the candidate vaccine effectively reduced both viral shedding and viral replication in chickens. The study by Zhang et al., 2023 explored the development of recombinant vaccine candidates for Crimean-Congo hemorrhagic fever virus (CCHFV) using the BEVS. They designed and constructed three vaccine candidates that encoded the Gn glycoprotein and the nucleocapsid protein (Np) of CCHFV. Experimental results reported that all three recombinant baculoviruses exhibited significant humoral immunity in BALB/c mice. In a study conducted by Caillava et al. (2024), Chikungunya virus CHIKV E1 and E2 envelope proteins expressed on the surface of budded baculovirus virions induced IgG antibodies, neutralizing antibodies, and a specific IFN-γ CD8 + T cell response in C57BL/6 mice. In a comparative analysis between a baculoviral vector vaccine (AcHERV-gE-gB) encoding varicella-zoster virus (VZV) gE and gB glycoproteins and a live attenuated vaccine strain, vOka (Lee et al., 2024), in a mouse model, AcHERV-gE-gB elicited similar or higher levels of IgG, IgG2a and neutralizing antibodies than vOka.

Several studies have shown that baculoviruses exhibit potent adjuvant effects that boost immune responses in vaccination strategies. Blazevic et al., 2016 demonstrated that recombinant polymeric Rotavirus (RV) VP6 protein produced in a baculovirus-insect cell expression system acts as an adjuvant in combination with NoV VLPs and the vaccine candidate induced strong potential protective immune responses in BALB/c mice. According to Heinimäki et al. (2017), baculovirus enhanced the adaptive immune response to monomeric ovalbumin and oligomeric norovirus VLPs, indicating its potential as an effective adjuvant in vaccine development.

The BEVS has facilitated the FDA approval of eight vaccines for human use, including Cervarix™ for the prevention of cervical cancer, Flublok® and Flublok Quadrivalent® for influenza, and the COVID-19 vaccines NVX-CoV2373 and Weikexin, as well as five additional vaccines approved for veterinary purposes. The FDA approved Cervarix, the first therapeutic protein produced in insect cells using baculoviruses as expression vectors 2009. Cervarix is a virus-like particle (VLP) vaccine formulated with the L1 capsid proteins of HPV types 16 and 18 and protects against human papillomavirus (HPV). Flublok, developed against influenza in 2013, was also approved by the FDA. Flublok is a recombinant hemagglutinin (rHA) vaccine developed using the BEVS, derived from three influenza virus strains. Vaccines against SARS-CoV-2, recently synthesized using BEVS, are also based on 3 different VLP-based technologies. In addition to these vaccines, many BEVS-derived vaccines are in clinical trials against Norovirus, Parvovirus B19, human respiratory syncytial virus (RSV) and Ebola (EBOV) viruses. Not only has the BEVS been used in human vaccines, but it has also significantly contributed to veterinary medicine, enabling the development of two protective vaccines against classical swine fever virus and three vaccines targeting porcine circovirus type 2 (Sokolenko et al., 2012; Hong et al., 2023).

Pesticide

Historically, a primary focus of baculovirus-based product development has been controlling Heliothis/Helicoverpa species in cotton, as these insects have long represented the most significant pests of this globally important agricultural commodity. The first baculovirus used commercially was a nucleopolyhedrovirus (NPV) obtained from Helicoverpa zea, and it was developed for use in cotton farming. It became a promising alternative for pest control, mainly due to the growing resistance of cotton pests to synthetic pyrethroids (Cory & Bishop, 1997; Grzywacz, 2017). Similarly, the gypsy moth virus (Lymantria dispar nucleopolyhedrovirus) is considered a key natural agent in regulating gypsy moth populations. In the same way, baculoviruses infecting the Douglas fir tussock moth (Orgyia pseudotsugata) have also been important in regulating this insect's population (Rohrmann, 2019).

According to the OECD's 2023 report (OECD, 2023), approximately 60 baculovirus-based pesticides have been marketed to control pest insects worldwide.

Gene Therapy

With the advancement of baculovirus research, it has been demonstrated that recombinant baculoviruses can enter not only insect host cells but also a variety of mammalian cells through the envelope glycoprotein GP64, and are capable of expressing foreign genes under the control of mammalian promoters without any viral replication (Ono et al., 2018).

Baculoviruses are considered promising gene transfer vectors for vertebrate cells, primarily due to the low activity of their promoters in mammalian cells and the high level of biosafety conferred by their non-pathogenic, budding viral forms (Liu et al., 2017).

Baculovirus has been investigated as a potential cancer therapy vector due to its ability to suppress tumor growth (Ono et al., 2018). It has been used to deliver diphtheria toxin A for targeting malignant glioma cells (Wang et al., 2006) and to express herpes simplex virus thymidine kinase (HSVtk), inducing cell death in glioblastoma cells in the presence of ganciclovir (Balani et al., 2009). Espíritu-Ramírez et al. (2018) demonstrated that gene therapy using a baculovirus vector carrying the glutamine synthetase (Bac-GS) gene effectively reduced ammonia levels in a rat model of acute hyperammonemia. In a study (Garcia Fallit et al., 2023) investigating the potential of baculoviral vectors for gene therapy targeting brain cancer, the findings indicated that baculoviruses could serve as effective vehicles for delivering therapeutic transgenes to brain cells, highlighting their potential applicability in both degenerative and neoplastic brain disorders.

Baculovirus vectors have been emerged as effective vaccine carriers in mice and non-human primates by stimulating both humoral and cellular immune responses through antigen expression or surface display of peptides fused to Baculovirus envelope proteins. This approach is being evaluated as a potential strategy for preventing and treating human and animal infectious diseases such as malaria, influenza and rabies (Kwang et al., 2016).

Conclusion

Initially used for pesticide purposes, Baculoviruses have become powerful tools in modern biotechnology. Thanks to their biological properties, such as high biosafety profile and large gene splicing capacity, they have been successfully applied in various fields such as recombinant protein production, vaccine development, and gene therapy. BEVS is a strong and versatile platform tool for producing complex eukaryotic proteins with the appropriate post-translational modifications. Despite some limitations, recent improvements continue to increase the usefulness of baculovirus-based technologies. With further development, baculoviruses will become even more important in research and therapeutic contexts, providing a safe, scalable, and efficient solution for future biotechnological applications.

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A Case of Mature Orbital Teratoma in a Simmental Breed Calf

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Case Report

History

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ABSTRACT

Teratoma is defined as a congenital neoplasm containing elements from all three germinal cell layers (ectoderm, mesoderm, endoderm) and classified as mature, immature, and malignant according to their cell structure. Mature dermoid cysts, which are among teratomas, are benign tumors that represent the simplest form of teratoma. However, to date, detailed research was not found on the ocular dermoid cysts in calves. No similar case was found in the literature review, and we believe that this study will make significant contributions to the literature.

Keywords: Calf, Dermoid cyst, Mature, Orbital, Teratoma, Tumor

Simental Irkı Bir Buzağıda Olgun Orbital Teratom Olgusu

Süreç

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ÖZ

Teratom, her üç germinal hücre katmanından (ektoderm, mezoderm, endoderm) elemanlar içeren ve hücre yapılarına göre matür, immatür ve malign olarak sınıflandırılan konjenital bir neoplazm olarak tanımlanır. Teratomlar arasında yer alan matür dermoid kistler, teratomun en basit formunu temsil eden iyi huylu tümörlerdir. Ancak bugüne kadar buzağılarda oküler dermoid kistlerle ilgili detaylı bir araştırmaya rastlanmamıştır. Literatür taramalarında benzer bir vakaya rastlanılmamış olup, yapmış olduğumuz bu çalışmanın literatüre önemli katkıları olacağı kanaatindeyiz.

Anahtar Kelimeler: Buzağı, Dermoid kist, Olgun, Orbital, Teratom, Tümör

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Introduction

Congenital teratomas are rare, usually benign neoplasms originating from cells that make up the three germinal cell layers (Chaudhry et al., 2021). Teratomas are classified according to their cellular structure as mature, immature, and malignant, with mature teratomas being the most common. Dermoid cysts, which are included in mature teratomas, are benign tumors and represent the simplest form of teratoma (Ahmed and Lotfollahzadeh, 2022). Dermoid cysts can often be found on the eyelids, conjunctiva, nictitating membrane, cornea, or the entire eye (Brudenall et al., 2008). These tumoral formations, which can be unilateral or bilateral, may form only in the lateral angle of the eye and in a very narrow area that does not affect vision much, or they may form in a widespread manner that covers the entire eye (Kajal et al., 2024). Orbital dermoids cause chronic epiphora, keratitis, lacrimation and keratoconjunctivitis by constantly irritating the eye and surrounding tissues (Adıyeke et., 2014). Dermoid cysts usually contain hair, keratin, and sebum, and these formations cause progressive enlargement of the tumor structure, making the cyst more clinically obvious (Barkyoumb and Leipold, 1984). Depending on the extent and location of orbital dermoid cysts and available equipment, excision of the tumoral structure is performed either surgically or by cryotherapy, hyperthermia, radiofrequency, or a combination of these methods (Sarrafzadeh-Rezaei et al., 2007). Dermoids have been reported in dogs and cattle, calves, cats, and rarely in other domestic animals such as horses, donkeys, buffaloes, goats, sheep, camels, and pigs, wild animals such as white-tailed deer, red deer, lions, and antelopes, and birds. However, there is no detailed research and long-term study on the incidence of ocular dermoid cysts in

calves (Sarangom et al., 2016). The aim of this case report was to describe the clinical and pathological findings of mature orbital teratoma in a newborn calf.

Materyal ve Yöntem

Case Report

This case is a 6-day-old male Simmental calf that was brought to the Veterinary Clinic of the Kafkas University Faculty of Veterinary Medicine with a complaint of a soft mass in the left eye. During the dpatient's owner, it was determined that the mass, localized on the left eye conjunctiva and lower eyelid, was a 5 cm long, hairy and skin-like tissue extending out of the eye (Figure 1). A detailed eye examination revealed that the mass was not associated with the cornea and that the eye had the ability to see. After obtaining consent from the animal owner, preparations were made for total surgical removal of the mass. The area was shaved and cleaned for surgery, which was performed under sedation and local infiltration anesthesia. The calf was sedated with xylazine HCl (0.2 mg/kg, IM, Rompun®, Bayer) and placed in the lateral decubitus position on the operating table. Local infiltration anesthesia was achieved by injection of lidocaine HCl (10 ml. Vilcain®, Vilsan) at the base of the third eyelid and subconjunctivally for regional anesthesia. Asepsis and antisepsis of the region were provided and the eye and its surroundings were limited with a sterile drape. A circular incision was made with a scalpel in the area where the mass was connected to the lower eyelid and conjunctiva, and the boundaries of the mass to be excised were determined. The mass was released with blunt dissection, and the blood vessels responsible for the nutrition of the mass were ligated with 4.0 absorbable suture material and the mass was completely removed. The region was closed with a simple continuous suture technique using the same suture material. The operation was terminated after the eye and its surroundings were washed with sterile 0.9% isotonic solution. In the postoperative period, tobramycin (3 mg/mL TOBRASED® 0.3% eye drops) eye drops were added to the treatment protocol three times a day to prevent infections that may be caused by pathogenic agents and to ensure rapid recovery. After the effects of sedation disappeared in the postoperative period, the patient was discharged after a clinical examination. Considering that the incision area was limited to the lower eyelid conjunctiva and was not directly connected to the external environment, no dressing was applied in the postoperative period. For prophylaxis purposes, it was recommended that the patient be kept separate from his peers and hospitalized in a clean environment.

Figure 1. A Hairy mass extending down the conjunctiva and lower eyelid in the left eye (arrow).

Histopathological Method

Tissue samples were excised and sent to our department for histopathologic examination and fixed in 10% buffered formaldehyde solution for microscopic examination. After routine tissue processing, paraffin blocks were prepared. Sections of 5 µm thickness were cut from the prepared paraffin blocks for hematoxylin and eosin (H&E) staining. The sections were examined under a light microscope (Olympus Bx53) and photographed.

Histopathologic Results

Histopathologic examination of the tissue sample revealed that the tissue had the normal histological structure of skin and consisted of epidermis, dermis, and subcutis. The epidermal layer was observed to be lined with multilayered flat keratinized epithelial cells. Just below the epidermis, there was a dermis layer composed of elastic fibers. In the dermis layer, blood vessels and well-developed hair follicles, sweat and sebaceous glands were observed. In addition, cartilage and muscle tissue formation were found in the subcutaneous layer of the tissue (Figure 2).

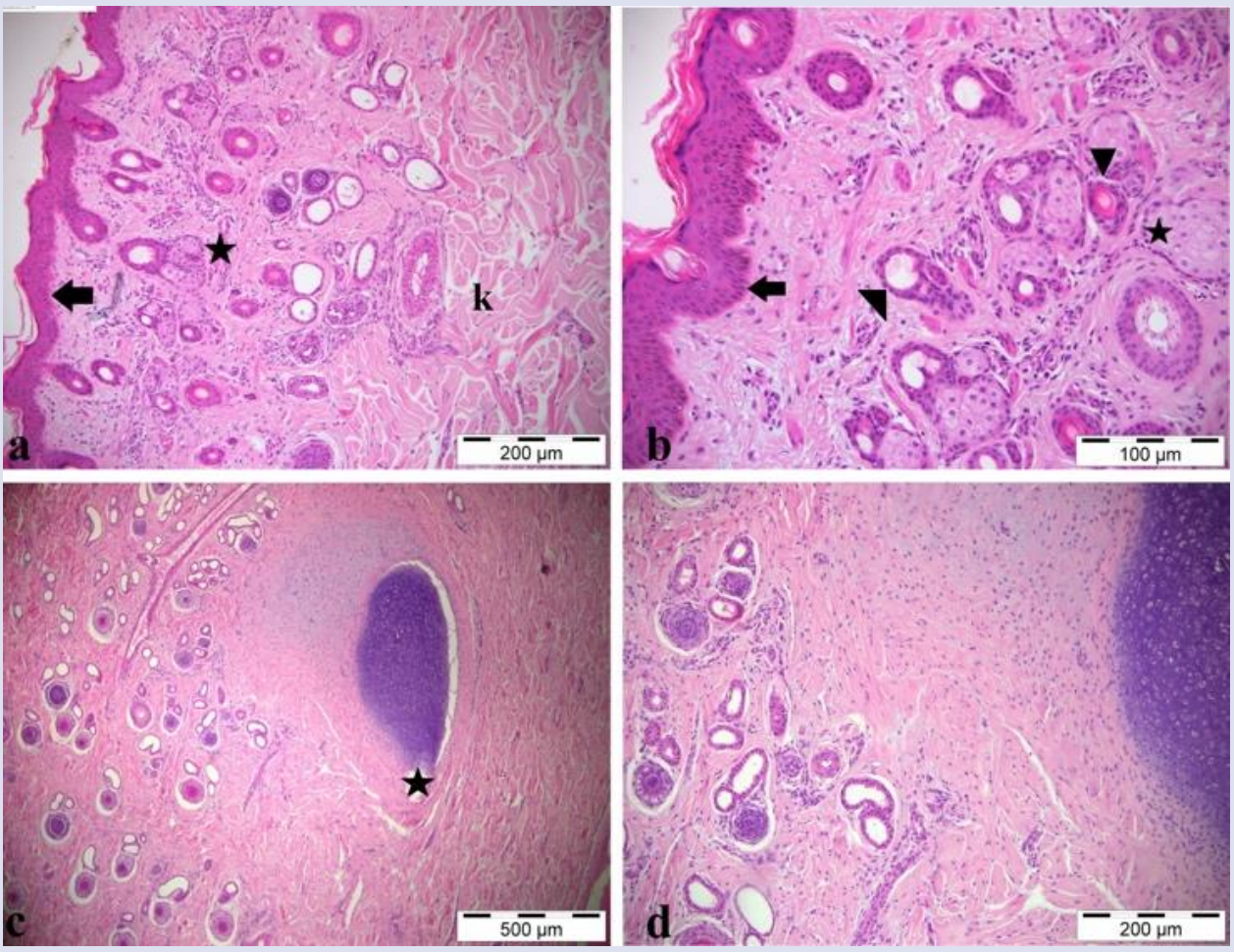

Figure 2. a) Orbital mature teratoma showing normal epidermal (arrow), dermal (asterisk), and muscular layers (k), hematoxylin and eosin (H&E), 200 µm.b) High magnification of epidermal (arrow) and dermal structures, including hair follicle (arrowhead), sweat glands (triangle), and sebaceous glands (asterisk), H&E, 200 µm.c) Cartilage structure formation in the subcutis (asterisk), H&E, 500 µm.d) Cartilage structure and skin attachment structures, H&E, 100 µm.

Discussion

Teratomas account for approximately 6-10% of pediatric tumors in humans and are most commonly found in the testes, ovaries, or retroperitoneum. Congenital orbital teratomas are extremely rare and are among the tumors that require urgent attention (Tsoutsanis and Charonis, 2021). Dermoid cysts, also known as mature cystic teratomas, are rare congenital or acquired tumor-like developmental anomalies. The developmental mechanisms involved in the pathogenesis of ocular dermoids are not fully understood. The most likely mechanism is mesenchymal metaplasia (mainly of neural crest origin) leading to abnormal differentiation of the surface ectoderm. The resulting dermoid is composed of ectodermal elements (keratinized epithelium, hair, sebaceous and apocrine glands) and mesenchymal elements (fibrous tissue, fat and cartilage) combined in varying proportions (Alam and Rahman, 2012, Oryan et al., 2012). There is a breed predisposition for ocular cystic dermoids, and they have been reported in Burmese cats, Dachshunds, Dalmatians, Doberman pinschers, Golden retrievers, German shepherds, Saint Bernards, and Quarterhorses. In cattle, ocular dermoids are not very common, with an estimated prevalence of 0.002%-0.4%. Ocular dermoids have been reported in almost all cattle breeds except Hereford, although with

low prevalence. These tumoral structures may be single or multiple, firm to fluctuant, well circumscribed, smooth and round, and usually the overlying skin is normal (Adıyeke et al., 2017, Brudenall et al., 2008). The material of this study was a 6-day-old Simmental calf. Ocular dermoids are very rare in calves and very few studies were found in the literatüre research. Ocular dermoids in domestic species can develop in a variety of locations, including the eyelids, conjunctiva (bulbar and palpebral), cornea, and nictitating membrane, and can occur unilaterally or in both eyes. In a study conducted in calves in 2019, Badanes and Ledbetter reported that cases of ocular dermoid cysts were more common in the left eye at the level of the caudus (Badanes and Ledbetter, 2019). Sarrafzadeh-Rezaei et al. reported in 2007 that in the left eye of a 2-day-old newborn female river buffalo (Bubalus bubalis) calf, a soft, fluctuant, fluid-filled, nontender, hyperemic ocular dermoid cyst was found in the inferior palpebral conjunctiva completely covering the orbit (Sarrafzadeh-Rezaei et al., 2007). In 2008, Brudenall et al. reported that dermoid cyst formation was observed in a 5-day-old Angus × Hereford female calf causing moderate blepharospasm, ulceration, and discharge in both eyes (Brudenall et al., 2008). In our case, the lesion was unilateral, localized to the left eye, and had a hard structure. Sarrafzadeh-Rezaei et al., 2007 reported that the microscopic mass consisted of epidermis, dermis, and skin appendages, some of the sebaceous glands had both intact and disintegrating sebum-containing secretory cells, and some parts had cystic structures (Sarrafzadeh-Rezaei et al., 2007). Brudenall et al. In 2008, in a case of dermoid cyst examined histopathologically, it was reported that the mass had normal skin histology and consisted of hair follicles, sebaceous and sweat glands, and a small number of inflammatory cells (Brudenall et al., 2008). Similar to Sarrafzadeh-Rezaei et al. (2007) and Brudenall et al. (2008), we observed from histopathologic examination that the mass we obtained had a normal skin structure and consisted of epidermis and dermis. The epidermis was lined with multilayered squamous keratinized epithelial cells, and the dermis contained well-developed, numerous hair follicles, sebaceous and sweat glands, and cartilaginous tissue formation. Considering that the mass contained both ectodermal and mesenchymal elements, its location and the time of its formation.

This study contributes to the literature on a very rare tumor type in ruminants by presenting a case of teratoma in a calf and constitutes an important reference in the veterinary field for the diagnosis, treatment and prognosis of similar cases. Intraoperative evaluation revealed that the mass had limited connection with the lower eyelid conjunctiva, was poor in terms of nerve tissue and blood vessels, and was not associated with the cornea, and the operative intervention was completed without any complications. Since the incision area had a contact surface with the cornea, it is possible for a corneal lesion to form in the postoperative period. In order to prevent this situation, absorbable suture material was used and care was taken to ensure that the knot was buried between the wound lips. In addition, since the patient is a farm animal, recommendations were made to the patient's owner to provide optimal environmental conditions for the patient, taking into account the risk of postoperative contamination. In addition, it was recommended to keep her away from her peers during this period and to use antibiotic eye drops. Thus, possible infections were prevented and ideal wound healing was achieved within the desired period.

Conflict of Interest

The authors declared that there is no conflict of interest.

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