ATATURK
UNIVERSITY
PUBLICATIONS

Veterinary Sciences *and* Practices

Formerly: Atatürk University Journal of Veterinary Sciences
Official journal of Atatürk University Veterinary Sciences

Volume 17 • Issue 3 • December 2022

EISSN 2822-3608
veterinarysciences-ataunipress.org

Veterinary Sciences and Practices

AVES

Founder
İbrahim Kara

General Manager
Ali Şahin

Publishing Directors
İrem Soysal
Gökhan Çimen

Editor
Bahar Albayrak

Publications Coordinators
Arzu Arı
Deniz Kaya
Irmak Berberoğlu
Alara Ergin
Hira Gizem Fidan
Vuslat Taş
İrem Özmen

Web Coordinators
Sinem Fehime Koz
Doğan Oruç

Finance Coordinator
Elif Yıldız Çelik

Contact
Publisher: Atatürk University
Address: Atatürk University, Yakutiye, Erzurum, Turkey

Publishing Service: AVES
Address: Büyükdere Cad., 105/9 34394 Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
E-mail: info@avesyayincilik.com
Webpage: www.avesyayincilik.com

Veterinary Sciences and Practices

AIMS AND SCOPE

Veterinary Sciences and Practices (Vet Sci Pract) is a scientific, open access, online-only periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is published triannually in April, August, and December. The publication languages of the journal are Turkish and English. The articles submitted in English will be given priority in evaluation.

Veterinary Sciences and Practices aims to contribute to the literature publishing mauscripts at the highest scientific level on all fields of veterinary medicine. The journal publishes original articles, invited reviews, case reports that are prepared in accordance with ethical guidelines.

The scope of the journal includes but not limited to; basic and clinical veterinary sciences, raising livestock, veterinary genetics, animal nutrition and nutritional diseases, zooneses, veterinary medicinal products and public health, and food hygiene, technology, exotic animal sicence and laboratory animal sicence.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of veterinary medicine.

Veterinary Sciences and Practices currently indexed in Scopus, DOAJ, EBSCO, CABI, China National Knowledge Infrastructure (CNKI) and TUBITAK ULAKBIM TR Index.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Open Access Statement

Veterinary Sciences and Practices is an open access publication, and the journal's publication model is based on Budapest Access Initiative (BOAI) declaration. All published content is available online, free of charge at https://veterinarysciences-ataunipress.org/. Veterinary Sciences and Practices is content is licensed under a Creative Commons Attribution-NonCommercial (CC BY-NC) 4.0 International License which permits third parties to share and adapt the content for non-commercial purposes by giving the appropriate credit to the original work.

You can find the current version of the Instructions to Authors at https://veterinarysciences-ataunipress.org/.

Editor-in-Chief: Mustafa Sinan AKTAŞ
Address: Atatürk University, Faculty of Veterinary Medicine, Department of Internal Medicine, Erzurum, Turkey
E-mail: vetdergisi@atauni.edu.tr

Publisher: Atatürk University
Address: Atatürk University, Yakutiye, Erzurum, Turkey

Publishing Service: AVES
Address: Büyükdere Cad., 105/9 34394 Şişli, İstanbul, Turkey
Phone: +90 212 217 17 00
E-mail: info@avesyayincilik.com
Webpage: www.avesyayincilik.com

Veterinary Sciences and Practices

AMAÇ VE KAPSAM

Veterinary Sciences and Practices; (Vet Sci Pract), bağımsız, tarafsız ve çift-kör hakemlik ilkelerine uygun olarak yayınlanan, bilimsel, açık erişimli, yalnızca çevrimiçi bir süreli yayındır. Dergi yılda üç kez Nisan, Ağustos ve Aralık aylarında yayımlanır. Derginin yayın dili Türkçe ve İngilizce'dir. İngilizce olarak gönderilen makalelere değerlendirmede öncelik verilecektir.

Veterinary Sciences and Practices; veteriner hekimliğin tüm alanlarında bilimsel olarak en üst düzeyde makaleler yayınlayarak literatüre katkı sağlamayı amaçlamaktadır. Dergi, etik kurallara uygun olarak hazırlanmış özgün makaleler, davetli derlemeler, olgu sunumları yayınlar.

Derginin kapsamı bunlarla sınırlı olmamak üzere; temel ve klinik veterinerlik bilimleri, hayvancılık, veteriner genetiği, hayvan besleme ve beslenme hastalıkları, zoonozlar, veteriner tıbbi ürünler ve halk sağlığı ile gıda hijyeni, teknoloji, egzotik hayvan bilimi ve laboratuvar hayvanı bilimidir.

Derginin hedef kitlesi, veteriner hekimliğin tüm disiplinlerinde çalışan ve ilgilenen uzmanlar ve profesyonellerdir.

Veterinary Sciences and Practices; Scopus, DOAJ, EBSCO, CABI, China National Knowledge Infrastructure (CNKI) ve TÜBİTAK ULAKBİM TR Dizin tarafından indekslenmektedir.

Derginin editöryel ve yayın süreçleri International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE) ve National Information Standards Organization (NISO) kılavuzlarına uygun olarak biçimlendirilmiştir. Veterinary Sciences and Practices editöryel ve yayın süreçleri, Akademik Yayıncılıkta Şeffaflık ve En İyi Uygulama (doaj.org/bestpractice) ilkelerine uygun olarak yürütülmektedir.

Veterinary Sciences and Practices yayınlanma modeli Budapeşte Açık Erişim Girişimi (BOAI) bildirgesine dayanan açık erişimli bilimsel bir dergidir. Derginin arşivine https://veterinarysciences-atauni press.org/ adresinden ücretsiz olarak erişilebilir. Veterinary Sciences and Practices'in içeriği, Creative Commons Alıntı-GayriTicari (CC-BY-NC) 4.0 Uluslararası Lisansı ile yayınlanmaktadır.

Yazarlara Bilgi'nin güncel versiyonuna https://veterinarysciences-atauni press.org/ adresinden ulaşabilirsiniz.

Baş Editör: Mustafa Sinan AKTAŞ
Adres: Atatürk Üniversitesi, Veteriner Fakültesi, İç Hastalıkları Anabilim Dalı, Erzurum, Türkiye
E-posta: vetdergisi@atauni.edu.tr

Yayıncı: Atatürk Üniversitesi
Adres: Atatürk Üniversitesi, Yakutiye, Erzurum, Türkiye

Yayınevi: AVES
Adres: Büyükdere Cad., 105/9 34394 Şişli, İstanbul, Türkiye
Tel: +90 212 217 17 00
E-posta: info@avesyayincilik.com
Web: www.avesyayincilik.com

Veterinary Sciences and Practices

CONTENTS / İÇİNDEKİLER

RESEARCH ARTICLES / ARAŞTIRMA MAKALELERİ

71 **Endocrinological and Metabolic Profile in Relation to Pregnancy at the First Insemination in Cows Housed Under Cold Conditions**
Soğuk Çevre Koşullarında Barındırılan İneklerde İlk Tohumlamada Gebelikle İlgili Endokrinolojik ve Metabolik Profil
Mehmet CENGİZ, Vefa TOHUMCU, Muhammad FURQAN ASGHAR CHACHER, Armağan HAYIRLI, Özgür KAYNAR, Mustafa İLERİTÜRK, Emre YILMAZ, Bulent BALLI, Armağan ÇOLAK

76 **Erzurum Bölgesinde Üretilen Ballarda Bazı Antibiyotik Kalıntılarının Belirlenmesi**
Determination of Some Antibiotic Residues in Honey Produced in Erzurum Region
Meryem AYDEMİR ATASEVER, Adil Talha YÜKSEL

81 **Investigation of the Effectiveness of Chromogenic Media in the Isolation of *Pasteurella multocida* and *Mannheimia haemolytica* from Calf Nasal Swab Samples**
Buzağı Nasal Svap Örneklerinden *Pasteurella multocida* ve *Mannheimia haemolytica* İzolasyonunda Kromojenik Besiyerinin Etkinliğinin Araştırılması
Osman Yaşar TEL, Songül ÖTKÜN, Ayfer Güllü YÜCETEPE, Oktay KESKİN

87 **Tavşanlarda Kronik Unilateral Kısmi Üreteral Obstrüksiyonun Diüretik Doppler Ultrasonografi ile Değerlendirilmesi**
Evaluation of Diuretic Renal Doppler Ultrasonography Findings in Chronic Unilateral Partial Ureteral Obstruction in Rabbits
Mahir KAYA, Ali BUMIN

91 **Systemic Effect of Metformin on Bone Healing at Bone Defect in Rabbits Using Radiographical and Serum Alkaline Phosphatase Assessment**
Kemik Defektli Tavşanlarda Metforminin Kemik İyileşmesi Üzerindeki Sistemik Etkisinin Radyografik ve Serum Alkalin Fosfataz Kullanılarak Değerlendirilmesi
Raad MAHMOOD HUSSEIN, Ghada ABDULRAHMAN TAQA

98 **Evaluation of CBC, Lipid Profile, Oxidative Stress Biomarkers, Total Thiol, Native Thiol, and Disulfide Levels in Dogs Diagnosed with Parvoviral Enteritis Without Clinical Findings**
Klinik Bulgular Görülmeden Parvoviral Enteritis Tanısı Konulan Köpeklerde, CBC, Lipid Profili, Oksidatif Stres Biyobelirteçleri, Total Thiol, Native Thiol ve Disülfid Düzeylerinin Değerlendirilmesi
Efe KURTDEDE, Mehmet Kazım BÖRKÜ, Berna GÜR, Yalçın DOĞAN, Kadir SEVİM, Erman GÜLENDAĞ

103 **Morphometric and Computed Tomographic Investigation of Ligamentum Sacrotuberale in Dogs**
Köpekte Ligamentum Sacrotuberale'nin Morfometerik ve Bilgisayarlı Tomografik İncelenmesi
Cüneyt Tunahan MAVİŞ, Muhammet Lütfi SELÇUK

108 **Acknowledgment of Reviewers**

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.1040814

Endocrinological and Metabolic Profile in Relation to Pregnancy at the First Insemination in Cows Housed Under Cold Conditions

Soğuk Çevre Koşullarında Barındırılan İneklerde İlk Tohumlamada Gebelikle İlgili Endokrinolojik ve Metabolik Profil

Mehmet CENGİZ[1]
Vefa TOHUMCU[1]
Muhammad FURQAN ASGHAR CHACHER[1]
Armağan HAYIRLI[2]
Özgür KAYNAR[3]
Mustafa İLERİTÜRK[4]
Emre YILMAZ[2]
Bulent BALLI[2]
Armağan ÇOLAK[1]

[1]Department of Obstetrics and Gynecology, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey
[2]Department of Animal Nutrition and Nutritional Disorders, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey
[3]Department of Biochemistry, Kastamonu University, Faculty of Veterinary Medicine, Kastamonu, Turkey
[4]Department of Biochemistry, Atatürk University, Faculty of Veterinary Medicine, Erzurum, Turkey

Geliş Tarihi/Received: 23.12.2021
Kabul Tarihi/Accepted: 04.04.2022
Publication Date/Yayın Tarihi: 29.12.2022

Sorumlu Yazar/Corresponding author: Mehmet CENGİZ
E-mail: mehmet.cengiz@atauni.edu.tr

Cite this article as: Cengiz M, Tohumcu V, Asghar Chacher MF, et al. Endocrinological and metabolic profile in relation to pregnancy at the first insemination in cows housed under cold conditions. *Vet Sci Pract* 2022; 17(3), 71-75.

Atıf: Cengiz M, Tohumcu V, Asghar Chacher MF, et al. Soğuk çevre koşullarında barındırılan ineklerde ilk tohumlamada gebelikle ilgili endokrinolojik ve metabolik profil. *Vet Sci Pract* 2022; 17(3), 71-75.

ABSTRACT

This study was performed to investigate the relationship between endocrinological and metabolic profiles and the pregnancy rate at the first insemination in peripartum dairy cows housed under cold conditions. Temperature inside the barn was recorded hourly during the periparturient period. Blood samples were collected before (last 14 day), on the day (0 day), and after parturition (3, 4, 6, 8, 15, 22, and 29 days) from 26 peripartum Simmental cows and analyzed for anti-Müllerian hormone, β-hydroxybutyric acid, insulin-like growth factor 1, insulin, cortisol, malondialdehyde, progesterone, thyroid-stimulating hormone, tri-iodothyronine, and thyroxine concentrations. The cows were divided into 2 groups: pregnant and non-pregnant based on results at the first insemination. The average ambient temperature ranged from −7°C to +11°C in the tent barn. Serum anti-Müllerian hormone (2.00 ± 0.04 vs. 1.89 ± 0.04 mU/L; $P < .006$), insulin (2.10 ± 0.03 vs. 2.51 ± 0.05 ng/mL; $P < .0001$), malondialdehyde (49.0 ± 1.30 vs. 44.0 ± 1.2 ng/mL; $P < .001$), and progesterone (44.1 ± 2.2 vs. 41.7 ± 2.1 pg/mL; $P < .002$) concentrations were different between pregnant and non-pregnant cows. In conclusion, anti-Müllerian hormone and insulin have a determinative role on pregnancy rate in peripartum cows housed under cold condition.

Keywords: Cold stress, cow, fertility, metabolism, pregnancy

ÖZ

Bu çalışma, soğuk koşullarda barındırılan peripartum ineklerde ilk tohumlamada endokrinolojik ve metabolik profil ile gebelik oranları arasındaki ilişkiyi araştırmak amacıyla yapılmıştır. Ortam sıcaklığı, periparturient dönem boyunca saatlik olarak kaydedilmiştir. Anti-Müllerian hormon (AMH), β-hidroksibutirik asit (BHBA), insulin benzeri büyüme faktörü 1 (ILGF-1), insülin, kortizol, malondialdehit (MDA), progesteron (P4), tiroid uyarıcı hormon (TSH), tri-iyodotironin (T3) ve tiroksin (T4) konsantrasyonlarının analizi için 26 adet Simmental inekten doğum öncesi (-14 gün), doğum günü (0 gün) ve doğum sonrası (3, 4, 6, 8, 15, 22 ve 29 gün) farklı aralıklarla kan örnekleri alınmıştır. İnekler, ilk tohumlama sonuçlarına göre geriye dönük olarak gebe (PG) ve gebe olmayan (NPG) olmak üzere iki gruba ayrılmıştır. Çadır ahırda ortalama ortam sıcaklığı −7°C ile +4°C arasında değişmiştir. İneklerin (PG ve NPG) serum AMH (2,00 ± 0,04 vs. 1,89 ± 0,04 mU/L; $P < ,006$), insulin (2,10 ± 0,03 vs. 2,51 ± 0,05 ng/mL; $P < ,0001$), MDA (49,0 ± 1,30 vs. 44,0 ± 1,2 ng/mL; $P < ,001$) ve P4 (44,1 ± 2,2 vs. 41,7 ± 2,1 pg/mL; $P < ,002$) konsantrasyonları farklıydı. Sonuç olarak, AMH ve insulin soğuk şartlarda barındırılan ineklerde gebelik oranları üzerine belirleyici bir rol oynar.

Anahtar kelimeler: Soğuk stresi, inek, fertilite, metabolizma, gebelik

INTRODUCTION

Infrastructure and suprastructure of housing systems are designated to be ecostructural addressing animal welfare and compatible with environmental conditions. Tent-covered barns become common

which provide a microclimate inside while removing harmful gases such as ammonia and methane and also odors.[1] However, the tent-type barns' suitability for geographical regions with severe winter conditions is controversial due to the feature that the tent can only protect against rain and wind. Thus, the effect of cold conditions in tent barns on fertility is worth investigating.

Generally, herds' fertility rate varies depending on genetic, managerial, and environmental factors and their interactions.[2] Stress is one of the most important factors that negatively affect the health and productivity of cows and other farm animals.[3] Stress is the reaction of an organism to external forces that disrupt physiological order by affecting homeostasis[4] and homeorhesis.[5] The extreme heat or cold environments cause stress in cows.[6,7] Both intrinsic (e.g., rumen movements, milk production) and extrinsic factors (e.g., high temperature, high humidity, solar radiation, low wind speed) are involved in the initiation and intensification of heat or cold stress in cows.[7,8]

This experiment was conducted to evaluate the hormonal, metabolic, and reproductive changes during the peripartum period and their effects on pregnancy rate at the first insemination in cows kept in a tent-type barn during the cold season.

MATERIALS AND METHODS

Experimental Design, Housing, and Grouping of Cows

The research was carried out on 26 pregnant (PG) Simmental cows at the end of the third lactation in a private farm (TR250001027418; Nail Cinisli Agriculture Livestock Food Industry and Trade Inc., location: 39°54′N, 40°51′E) in Turkey. Etik kurul tarih: 03.04.2018 Protokol no: 95. Cows were housed in a 2 × 2 free-stall nylon-covered tent barn. In this barn model, the cows were protected only from wind and rain but exposed to outdoor temperature and humidity changes. Cows had *ad libitum* feed and water access to meet the NRC recommendation.[9] Staff observed the cows and noted the changes such as estrus signs, abnormal vaginal discharge, foul odor after calving, and anorexia in the barn. Cows experiencing dystocia, retention of fetal membranes, mastitis, metritis, endometritis, and ovarian pathologies such as cysts were excluded from the study. A computer-assisted automated milking system recorded all milking data. The average milk yield of the cows was measured at 5756 kg for 305-days lactation in the previous lactation.

Pregnant cows were moved into the close-up paddocks and maternity pens, which were in a concrete barn, 14 days before expected parturition. In this area, the temperature was kept in the thermoneutral zone by utilizing the barn's furnace heating system. Three days after parturition, healthy cows were transferred to the main nylon tent barn and kept there for the rest of the lactation cycle.

Ambient Temperature Recording

Two digital thermometer and hygrometer data loggers were placed into the tent and concrete barns to measure and record temperature and humidity on an hourly basis during the entire study period.

Blood Sampling and Serum Analysis

Blood samples were collected from the coccygeal vein (*Vena caudalis*) on the 14th day (before expected parturition), on the day of parturition, and then sampling was extended to 3 , 4, 6, 8, 15, 22, and 29 days after parturition. Blood samples were centrifuged at 4000 rpm for 10 minutes, and serum was stored at −20°C until analyses. Measurements were performed according to the manufacturer's guidelines for β-hydroxybutyric acid (BHBA), insulin-like growth factor 1 (IGF-1), insulin, cortisol, malondialdehyde (MDA), anti-Müllerian hormone (AMH), progesterone (P4), thyroid-stimulating hormone (TSH), tri-iodothyronine (T3), and thyroxine (T4) using the enzyme-linked immunosorbent assay technique.

Reproductive Examination and Artificial Insemination

On the 15th , 22nd, and 29th day postpartum, uterine involution and restart of ovarian activity were monitored using transrectal ultrasonography. Uterine involution was monitored by measuring cervical and uterine horn diameters. The presence of large follicles and corpus luteum (CL) was accepted as the restart of postpartum ovarian activity. All of the cows, which had a healthy postpartum period, were inseminated at first estrus after the voluntary waiting period (>42th day). The same technical staff performed artificial insemination using the same semen throughout the study. The pregnancy checks were made on the 35th day after insemination, and the embryonic and fetal survival was monitored until 120 days of gestation by ultrasonographic examination and rectal palpation.

Statistical Analysis

The temperature was presented as minimum-maximum daily using the UNIVARIATE procedure.[10] The cows were divided into 2 groups as PG and non-pregnant (NPG) according to the first insemination results. Metabolic, hormonal, and reproductive parameters were subjected to a one-way analysis of variance according to the General Linear Model procedure. The linear model was used to analyze the pregnancy status, time, and pregnancy-time effect. Changes over time were determined by the repeated measurement approach. Statistics significance was accepted at the level of $P < .05$.

RESULTS

Ambient Temperature Changes in the Tent and Concrete Barn

At the farm level, the average ambient temperature ranged between −7 °C and +11 °C during the whole study period. It ranged from −7°C to +4°C and from +6°C to +11°C in the tent (postpartum) and concrete (prepartum) barns, respectively (Figure 1).

Evaluation of Fertility Parameters

The cows' fertility status was evaluated with 4 main criteria; pregnancy status at the end of the study, conception at first insemination, number of inseminations per eventual gestation, and days open till eventual gestation (Table 1). A total of 24 out of 26 cows were PG at the end of the study. Twelve of these cows were conceived at first insemination, and none of them showed embryonic and fetal death later on. Days open in the PG cows was 57.9 ± 10.1, whereas it was 193.5 ± 30.4 in the NPG cows (Table 1). For all PG cows, the average number of inseminations per pregnancy was 1.92 ± 1.35, and days open was 94.3 ± 54.0.

Metabolic and Hormonal Profiles

Serum concentrations of AMH (2.00 ± 0.04 vs. 1.89 ± 0.04 ng/mL; $P < .05$), insulin (2.10 ± 0.033 vs. 2.51 ± 0.058 mU/L, $P < .0001$), MDA (44.0 ± 1.2 vs. 49.0 ± 1.3 ng/mL, $P < .001$), and P4 (44.1 ± 2.2 vs. 41.7 ± 2.1 pg/mL, $P < .01$) were significantly different between the PG and NPG cows (Table 2). Serum concentrations of BHBA, IGF-1, cortisol, P4, TSH, T3, and T4 were similar for the PG and NPG cows. Expectedly, these parameters changed during the experimental period ($P < .0001$). Change patterns during the experimental period between the PG and NPG cows tended to

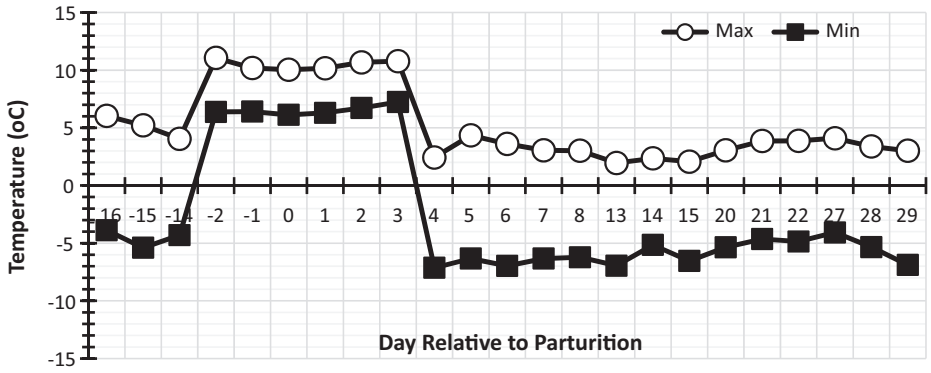


Figure 1. Temperature inside the barn during the experiment process (°C). Max, maximum; Min, minimum.

Table 1. Fertility Status of Cows Upon Insemination During the Cold Season

Pregnancy Status	n	Insemination Number	Days Open
Non-pregnant	2	5.00 ± 1.41	193.5 ± 30.4
Pregnant	24	1.92 ± 1.35	94.3 ± 54.0
	12	1	57.9 ± 10.1
	8	2	98.4 ± 9.2
	1	3	144.0 ± NA
	1	4	171.0 ± NA
	1	5	204.0 ± NA
	1	6	263.0 ± NA

NA, not applicable.

Table 2. Metabolic and Hormonal Profiles of Cows Upon Insemination During the Cold Season

	Group		Statistical Effect		
Parameter	Pregnant	Non-pregnant	Group	DRC	Group × DRC
AMH (ng/mL)	2.00 ± 0.04	1.89 ± 0.04	0.005	0.0001	0.0552
IGF-1 (ng/mL)	2.15 ± 0.032	2.11 ± 0.04	0.5388	0.0001	0.9989
Insulin (mU/L)	2.10 ± 0.033	2.51 ± 0.06	0.0001	0.0001	0.9910
Cortisol (ng/mL)	37.2 ± 1.1	39.6 ± 1.0	0.0898	0.0001	0.3860
P4 (pg/mL)	44.1 ± 2.2	41.7 ± 2.1	0.0024	0.0001	0.9556
TSH (pg/mL)	493 ± 17	532 ± 16	0.1030	0.0001	0.9858
T3 (pg/mL)	372 ± 12	368 ± 9	0.4231	0.0001	0.8203
T4 (ng/mL)	8.85 ± 0.12	8.68 ± 0.14	0.4637	0.0001	0.3540
BHBA (ng/mL)	5.12 ± 0.12	5.19 ± 0.12	0.6353	0.0001	0.9702
MDA (ng/mL)	44.0 ± 1.2	49.0 ± 1.3	0.0011	0.0001	0.5243

DRC, days relative to calving; AMH, anti-Müllerian hormone; IGF-1, insulin-like growth factor 1; P4, progesterone; TSH, thyroid-stimulating hormone; T3, tri-iodothyronine; T4, thyroxine; BHBA, β-hydroxybutyric acid; MDA, malondialdehyde.
DRC, Doğuma kıyasla gün sayısı; AMH, Anti-Müllerian hormon; IGF-1, İnsülin benzeri büyüme faktörü 1; P4, Progesteron; TSH, Tiroid situmile edici hormon; T3, Triiodotironin; T4, Tiroksin; BHBA, Beta-hidroksibütirik asit; MDA, Malondialdehit.

be different for AMH levels (Figure 1; group by time interaction, $P = .0552$). AMH concentrations for the PG cows were higher in the late prepartum period and the first month of early postpartum than that for the NPG cows (Figure 2).

Ultrasonographic Measurements

Diameters of the cervix (41.5 ± 1.29 vs. 42.2 ± 1.50 mm, $P > .05$) and the right (28.6 ± 1.12 vs. 30.6 ± 1.27 mm) and left (25.3 ± 0.97 vs. 27.0 ± 1.15 mm) uterine horns were not different between the PG and NPG cows ($P > .05$; Table 3). No difference was observed clinically in terms of ovarian activities of the cows. All cows showed estrus at least once in their first 42 days of parturition, and at least 1 CL or at least 1 Graafian follicle was present at early 22 days of calving.

Behavioral Changes

Gathering, trembling, and decreased mobility were notable in the first 3-10 days after releasing from maternity pen in the concrete barn to lactating group in tent farm. Thereafter, the intensity of these behaviors gradually diminished. The significant signs of estrus, such as standing for mounting or attempting to mount,

Table 3. Uterine Measurements of Cows Upon Insemination During the Cold Season

	Group		Statistical Effect		
Parameter	Pregnant	Non-pregnant	Group	DRC	Group×DRC
Cervix diameter (mm)	41.5 ± 1.3	42.2 ± 1.5	0.7018	0.0001	0.3645
Right horn diameter (mm)	28.6 ± 1.1	30.6 ± 1.3	0.2010	0.0018	0.8443
Left horn diameter (mm)	25.3 ± 1.0	27.0 ±1.2	0.2339	0.0964	0.9105

DRC, days relative to calving.

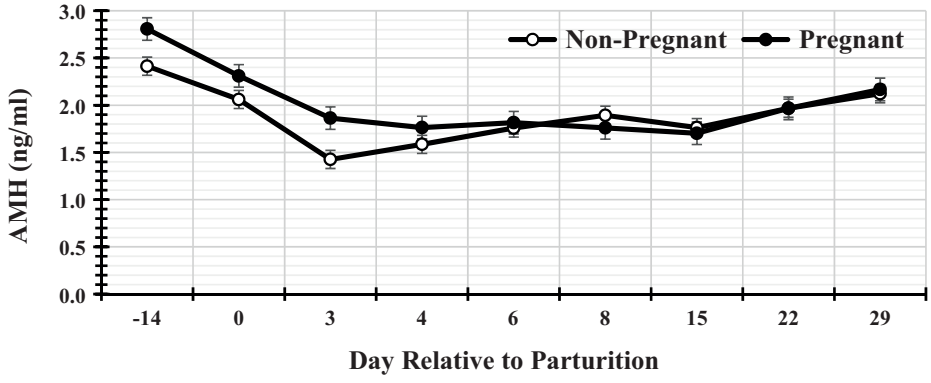


Figure 2. Alterations in AMH concentrations of cows upon insemination during the cold season. AMH, anti-Müllerian hormone.

were not observed in the tent barn. The most considerable sign was sweat and steam on the skin surface of the cows in estrus.

DISCUSSION

Behavioral changes reported in previous studies[11,12] due to acute cold stress exposure were also observed in this study when the cows were transferred from the concrete barn (from the last 2 weeks of gestation to 3-day early postpartum follow-up) to the tent barn. These included gathering, trembling, and reluctant mobility for 3-10 days, which diminished thereafter, probably adapting to a new environment.

The AMH is synthesized by developing antral follicles (3-5 mm follicles) from the ovaries, and it prevents excessive consumption of primordial follicles from the follicular pool. Therefore, it is considered a reliable biomarker of ovarian reserves for cyclicity.[13] The PG cows tended to have a higher serum AMH concentration than the NPG cows at the first insemination (Table 2). This difference was especially significant during the prepartum period and in the first week postpartum (Figure 2). The positive correlation between fertility and AMH concentration was mentioned by Ribeiro et al.[14] However, the difference between the PG and NPG cows decreased in the first week postpartum. This equalization could be due to the failure to achieve extreme cold conditions.

Endocrine IGF-1, which has a positive effect on follicular and embryonic development, is used to monitor the interactions between nutrition and reproduction.[15,16] Previous researchers stated that the cows, which showed physiological ovarian activity and ovulation, has a greater IGF-1 level in the postpartum first week than the cows with inactive ovaries, cystic follicles, and persistent corpus luteum.[17] In the present study, IGF-1 showed the typical pattern as compatible with previous studies.[18,19] It started to increase during the late postpartum, reached the basal level in the first week postpartum, and then gradually increased (data not shown). This could be also due to exclusion of the cows with postpartum abnormal ovarian activity and uterine problems from the study.[20]

Serum insulin was for the PG cows even before calving, which remained high during postpartum. Insulin affects glucose metabolism and steroidogenesis, supports estradiol and P4 production from granulosa cells, and stimulates androgen synthesis from theca cells.[21] The cows with high blood insulin concentration have greater chances of ovulation in the first early 50 days of postpartum and have a lesser interval between calving to the first insemination.[22,23] All these positive effects were interrelated with the positive effects of insulin on follicular development and the production of steroid hormones.[24] However, in the present study, all cows showed estrus at least once in their first 42 days of parturition, and at least 1 CL or at least 1 Graafian follicle was present in the first 22 days of calving.

Stressors can suppress P4 production[25] and may affect reproductive performance, indirectly. P4 is a primary hormone necessary for pregnancy continuation.[26] In this study, serum P4 concentration was consistently higher for the PG cows than that for the NPG cows during the peripartum period. Previous studies have shown a positive correlation between the P4 concentration and ovulation success and pregnancy.[27]

Cortisol is a glucocorticoid derivate, which is an essential regulator of energy metabolism, especially under acute and prolonged stress such as parturition and the postpartum period.[28] Lucy[29] stated that stress negatively impacted fertility and acute and chronic cold conditions were a stressor, leading to an increase in glucocorticoid concentration and the development of secondary changes in productivity. Increased plasma cortisol level was associated with stressful management and environmental factors such as nutrition, housing, and cold stress (acute and chronic) in previous reports.[30]

Thyroid hormones play an essential role in steroidogenesis in granulosa cells,[21] resumption of ovarian activity in post-parturient cows, and the cows with higher plasma T3 and T4 levels have shorter calving to conception interval according to Reist et al.[31] However, a difference was not detected between the groups. The cold exposure did not cause a significant decrease in T3 and T4 levels in the PG and NPG cows.

The BHBA, an indicator of subclinical ketosis, tends to increase in energy-deficient cows.[32] Higher concentrations of BHBA than 1.2 mmol/L in the first postpartum signifies subclinical ketosis.[32] The cold condition might induce lipolysis to elevate the BHBA concentration, especially in the NPG cows.

Upon exposure to the cold environment, development of oxidative stress was attained by the MDA measurement, an endpoint degradation product of lipid peroxidation[33,34] and impacts cell membranes as Castillo et al[33] reported that MDA levels significantly increased in the cows during the transition period, 1 week before and after parturition, Turk et al[34] suggested higher MDA level in prepartum than early and late puerperium. Although higher MDA was expected in the early lactation period due to increased susceptibility to production diseases,[35] prepartum oxidative stress was almost double higher in this study. This result was associated with increased oxidative status, in both advanced pregnancies.

A healthy postpartum process in the uterus and ovarium is essential for reproductive performance. The delayed uterine involution causes delayed bacterial elimination, postpartum uterine infections, profound damage to the endometrium, and infertility.[36,37] In the present study, regular postpartum gynecological examinations were administered, and the cows with postpartum metritis were excluded, purposefully. Thus, the possible adverse effects of delayed involution and metritis on the pregnancy rate could be eliminated, and the potential adverse effects of environmental conditions and endocrinological changes could be evaluated. As a result, a similar involution pattern was observed in both groups. This could be attributed to cows' well-being or failure to achieve the cold stress.

In conclusion, in cold exposure down to −7°C, there were continuous endocrinological and metabolic changes related to stress. High serum AMH, insulin, and P4 concentrations were associated higher rate of conception at the first insemination in the presented conditions. In addition to the reduction in infrastructure cost, the tent-covered barns are suitable for mild/moderate cold environments without adversely affecting welfare and productivity.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Atatürk University (Date: 03.04.2018, Decision No: 2018/95).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.C.; V.T., Design – M.C., A.H.; V.T., Supervision – M.C., V.T., M.F.A.C.; Funding – M.C.,.; Materials – M.C., A.H.; Data Collection and/or Processing – V.T., M.F.A.C., O.K., M.I, E.Y, B.B., A.C.; Analysis and/or Interpretation – O.K., M.I., M.C., A.H.; Literature Review – M.F.A.C. , M.C.; Writing – M.C., V.T., M.F.A.C.; Critical Review – M.C., A.H.

Declaration of Interests: The authors declare that they have no conflict of interest.

Funding: This study was supported by Atatürk University Scientific Research Projects Coordination Unit (Grant no: TSA-2018-6829).

Etik Kurul Onayı: Bu çalışma için Atatürk Üniversitesi Etik Kurulu'ndan onay alınmıştır (Tarih: 03.04.2018, Karar No: 2018/95).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – M.C., V.T.; Tasarım – M.C., A.H., V.T.; Denetleme – M.C., V.T., M.F.A.C.; Kaynaklar – M.C.; Veri Toplanması ve/veya İşlemesi – V.T., M.F.A.C., Ö.K., M.İ., E.Y., B.B., A.Ç.; Analiz ve/veya Yorum – Ö.K., M.İ., M.C., A.H.; Literatür Taraması – M.F.A.C.; M.C.; Yazıyı Yazan – M.C.; V.T., M.F.A.C.; Eleştirel İnceleme – M.C.; A.H.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Funding: Bu çalışma Atatürk Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimince Desteklenmiştir (Grant no: TSA-2018-6829).

REFERENCES

1. Galama PJ, Ouweltjes W, Endres MI, et al. Symposium review: Future of housing for dairy cattle. *J Dairy Sci*. 2020;103(6):5759-5772. [CrossRef]
2. Walsh SW, Williams EJ, Evans ACO. A review of the causes of poor fertility in high milk producing dairy cows. *Anim Reprod Sci*. 2011;123(3-4):127-138. [CrossRef]
3. Polsky L, Von Keyserlingk MAG. Invited review: effects of heat stress on dairy cattle welfare. *J Dairy Sci*. 2017;100(11):8645-8657. [CrossRef]
4. Stott GH. What is animal stress and how is it measured? *J Anim Sci*. 1981;52(1):150-153. [CrossRef]
5. Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J Dairy Sci*. 1980;63(9):1514-1529. [CrossRef]
6. Abdelnour SA, Abd El-Hack ME, Khafaga AF, Arif M, Taha AE, Noreldin AE. Stress biomarkers and proteomics alteration to thermal stress in ruminants: a review. *J Therm Biol*. 2019;79:120-134. [CrossRef]
7. Thatcher WW. Effects of season, climate, and temperature on reproduction and lactation1. *J Dairy Sci*. 1974;57(3):360-368. [CrossRef]
8. Bianca W. Physiology. Cattle in a hot environment. *J Dairy Sci*. 1965;32:291-345.
9. NRC (National Research Council). *Nutrient Requirements of Dairy Cattle*. 7th ed. Washington DC: The National Academy Press; 2001:1-27.
10. SAS. *SAS User's Guide: Statistics*. 9th ed. Cary: Sas Institute; 2002.
11. Webster AJ. The energetic efficiency of metabolism. *Proc Nutr Soc*. 1981;40(1):121-128. [CrossRef]
12. Webster J, Stewart M, Rogers A, Verkerk GA. Assessment of welfare from physiological and behavioral responses of New Zealand dairy cows exposed to cold and wet conditions. *Anim Welf*. 2008;17:19-26.
13. Mossa F, Ireland JJ. Physiology and endocrinology symposium: anti-Müllerian hormone: a biomarker for the ovarian reserve, ovarian function, and fertility in dairy cows. *J Anim Sci*. 2019;97(4):1446-1455. [CrossRef]
14. Ribeiro ES, Bisinotto RS, Lima FS, et al. Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. *J Dairy Sci*. 2014;97(11):6888-6900. [CrossRef]
15. Dutta LJ, Nath KC, Deka BC, et al. Identification and clinico-gynaecological characterization of reproductive disorders in crossbred cows under field conditions. *Indian J Anim Res*. 2020;54(of):593-600. [CrossRef]
16. Velazquez MA, Spicer LJ, Wathes DC. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. *Domest Anim Endocrinol*. 2008;35(4):325-342. [CrossRef]
17. Zulu VC, Nakao T, Sawamukai Y. Insulin-like growth factor-I as a possible hormonal mediator of nutritional regulation of reproduction in cattle. *J Vet Med Sci*. 2002;64(8):657-665. [CrossRef]
18. Radcliff RP, McCormack BL, Crooker BA, Lucy MC. Plasma hormones and expression of growth hormone receptor and insulin-like growth factor-I mRNA in hepatic tissue of periparturient dairy cows. *J Dairy Sci*. 2003;86(12):3920-3926. [CrossRef]
19. Ruprechter G, Noro M, Meotti O, et al. Endocrine and reproductive parameters in sick and healthy primiparous and multiparous dairy cows. *Theriogenology*. 2020;141:173-179. [CrossRef]
20. Beam SW, Butler WR. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biol Reprod*. 1997;56(1):133-142. [CrossRef]
21. Karpova EK, Adonyeva NV, Faddeeva NV, Romanova IV, Gruntenko NE, Rauschenbach IY. Insulin affects reproduction and juvenile hormone metabolism under normal and stressful conditions in Drosophila females. *Dokl Biochem Biophys*. 2013;452(1):264-266. [CrossRef]
22. Spicer LJ, Alonso J, Chamberlain CS. Effects of thyroid hormones on bovine granulosa and thecal cell function in vitro: dependence on insulin and gonadotropins. *J Dairy Sci*. 2001;84(5):1069-1076. [CrossRef]
23. Garnsworthy PC, Fouladi-Nashta AA, Mann GE, Sinclair KD, Webb R. Effect of dietary-induced changes in plasma insulin concentrations during the early postpartum period on pregnancy rate in dairy cows. *Reproduction*. 2009;137(4):759-768. [CrossRef]
24. Gong JG, Lee WJ, Garnsworthy PC, Webb R. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Reproduction*. 2002;123(3):419-427. [CrossRef]
25. Jozwik A, Krzyzewski J, Strzalkowska N, et al. Relations between the oxidative status, mastitis, milk quality and disorders of reproductive functions in dairy cows—a review. *Anim Sci Pap Rep*. 2012;30:297-307.
26. Lonergan P, Sánchez JM. Symposium review: Progesterone effects on early embryo development in cattle. *J Dairy Sci*. 2020;103(9):8698-8707. [CrossRef]
27. Wiltbank MC, Souza AH, Giordano JO, et al. Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. *Anim Reprod*. 2018;9:231-241.
28. Vannucchi CI, Rodrigues JA, Silva LCG, et al. Association between birth conditions and glucose and cortisol profiles of periparturient dairy cows and neonatal calves. *Vet Rec*. 2015;176(14):358. [CrossRef]
29. Lucy MC. Stress, strain, and pregnancy outcome in postpartum cows. *Anim Reprod*. 2019;16(3):455-464. [CrossRef]
30. Gwazdauskas FC. Effects of climate on reproduction in cattle. *J Dairy Sci*. 1985;68(6):1568-1578. [CrossRef]
31. Reist M, Erdin DK, Von Euw D, et al. Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. *Theriogenology*. 2003;59(8):1707-1723. [CrossRef]
32. Puppel K, Kuczyńska B. Metabolic profiles of cow's blood; a review. *J Sci Food Agric*. 2016;96(13):4321-4328. [CrossRef]
33. Castillo C, Hernandez J, Bravo A, Lopez-Alonso M, Pereira V, Benedito JL. Oxidative status during late pregnancy and early lactation in dairy cows. *Vet J*. 2005;169(2):286-292. [CrossRef]
34. Turk R, Juretić D, Gereš D, Svetina A, Turk N, Flegar-Meštrić Z. Influence of oxidative stress and metabolic adaptation on PON1 activity and MDA level in transition dairy cows. *Anim Reprod Sci*. 2008;108(1-2):98-106. [CrossRef]
35. Sharma N, Singh NK, Singh OP, Pandey V, Verma PK. Oxidative stress and antioxidant status during transition period in dairy cows. *Asian Australas J Anim Sci*. 2011;24(4):479-484. [CrossRef]
36. Heppelmann M, Krach K, Krueger L, et al. The effect of metritis and subclinical hypocalcemia on uterine involution in dairy cows evaluated by sonomicrometry. *J Reprod Dev*. 2015;61(6):565-569. [CrossRef]
37. Mateus L, da Costa LL, Bernardo F, Silva JR. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. *Reprod Domest Anim*. 2002;37(1):31-35. [CrossRef]

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.220105

Erzurum Bölgesinde Üretilen Ballarda Bazı Antibiyotik Kalıntılarının Belirlenmesi

Determination of Some Antibiotic Residues in Honey Produced in Erzurum Region

Meryem AYDEMİR ATASEVER[1]
Adil Talha YÜKSEL[2]

[1]Atatürk Üniversitesi, Veteriner Fakültesi, Gıda Hijyeni ve Teknolojisi Anabilim Dalı, Erzurum, Türkiye
[2]Afyonkarahisar İl Gıda Kontrol Laboratuvar Müdürlüğü, Afyonkarahisar, Türkiye

ÖZ

Türkiye, Çin'den sonra dünyanın en büyük bal üreticisidir. Bal, Türkiye'nin en önemli tarımsal ihracat ürünlerinden birisidir. Kontrolsüz antibiyotik satışı ve bilinçsiz kullanımı nedeniyle, Türk balındaki antibiyotik kalıntıları ihracatta önemli sorunlara neden olmaktadır. Balda antibiyotik kalıntılarının varlığı insan sağlığına zararlı olabilir. Baldaki antibiyotik kalıntısı hala dünya çapında önemli bir sorundur. Baldaki antibiyotik kalıntılarının çoğunlukla çevre kaynaklı değil, yanlış arıcılık uygulamalarından kaynaklandığı ortaya çıkmıştır. Bu çalışmanın amacı, Erzurum ilinden toplanan ballardaki neomisin ve tetrasiklin kalıntılarının belirlenmesidir. Bu amaçla, 79 bal numunesi, ELISA 9 metodu ile analiz edilmiş, 79 bal örneğinin 37'sinde (%46,8) 2,1-47,08 ppb arasında ortalama 9,33 ppb düzeyinde tetrasiklin kalıntısı belirlenmiş, kalan 22 örnekte ise tetrasiklin kalıntısının minimum belirleme limitinin altında kaldığı saptanmıştır (<2 ppb). Yetmiş dokuz bal örneğinde neomisin kalıntısı belirlenemedi (minimum tespit limiti 15,63 ng/mL). Türk Gıda Kodeksi yönetmeliğinde bal için belirlenmiş maksimum kalıntı sınırı yoktur; bu ise balın antibiyotik içermemesi gerektiği anlamına gelmektedir. Sonuç olarak; bal üreticilerinin bal üretiminde antibiyotik kullanımının yasal olmadığı konusunda bilgilendirilmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Antibiyotik, ELISA, Erzurum, bal, neomisin, kalıntı, tetrasiklin

ABSTRACT

Turkey is the world's largest honey producer after China. Honey is one of Turkey's most important agricultural export products. Due to uncontrolled antibiotic sale and unconscious use, antibiotic residues in Turkish honey cause important problems in exports. The presence of antibiotic residues in honey can be harmful to human health. Antibiotic residue in honey is still a major problem worldwide. Antibiotic residues in honey were mostly caused by incorrect beekeeping practices, not from the environment. The aim of this study was to determine the neomycin and tetracycline residues in honey collected from the Erzurum province. For this purpose, 79 honey samples were analyzed by the enzyme-linked immunoassay method. Tetracycline residues were found to be between 2.1 and 47.08 ppb in 37 of 79 honey samples (46.8%) and had an average of 9.33 ppb, while 22 of the samples were below the minimum detection limit (<2 ppb). Neomycin residues could not be detected in 79 honey samples (minimum detection limit 15.63 ng/mL). There are no maximum residue limits established for antibiotics in honey according to the Turkish Food Codex regulations, which means honey should not contain antibiotics. As a result, it is believed that honey producers should be informed that it is not legal to use antibiotics in honey production.

Keywords: Antibiotic, ELISA, Erzurum, honey, neomycin, residue, tetracycline

Geliş Tarihi/Received: 07.01.2022
Kabul Tarihi/Accepted: 18.03.2022
Yayın Tarihi/Publication Date: 29.12.2022

Sorumlu Yazar/Corresponding author:
Meryem AYDEMİR ATASEVER
E-mail: meryematasever@atauni.edu.tr

Cite this article as: Aydemir Atasever M, Yüksel AT. Determination of some antibiotic residues in honey produced in Erzurum region. *Vet Sci Pract* 2022; 17(3), 76-80.

Atıf: Aydemir Atasever M, Yüksel AT. Erzurum bölgesinde üretilen ballarda bazı antibiyotik kalıntılarının belirlenmesi. *Vet Sci Pract*. 2022; 17(3), 76-80.

GİRİŞ

Bal, doğal ve sağlıklı bir ürün olarak kabul edilmektedir. Yıllık dünya bal üretiminin yaklaşık %40'ı Asya ülkeleri tarafından gerçekleştirilmektedir. Türkiye ise Çin'den sonra dünyada önemli bal üreticilerindendir.[1,2]

Ancak günümüzde bal, farklı kirlilik kaynakları tarafından kirletilen bir ortamda üretilmektedir. Kirlenme; çevresel ve arı kaynaklı olabilmektedir. Kontaminasyon kaynakları arasında pestisitler, ağır metaller,

bakteriler ve radyoaktivite ele alınmaktadır. Bu kirleticiler hava, su, toprak ve bitkilerde bulunarak arı kovanlarına taşınabilmektedir. Arıcılık uygulamasından kaynaklanan kirletici maddeler arasında akar (başlıca varroa) kontrolü için kullanılan akarisitler, bal hasadında kullanılan arı kovucular, balmumu güvesi için kullanılan pestisitler ve antibiyotikler bulunmaktadır.[3]

Arıcılık sektöründe bakteriyel hastalıkların tedavisi amacıyla antibiyotikler kullanılmaktadır. Özellikle tetrasiklin, yaygın olarak *Paenibacilus* larvaları ve *Streptococcus pluton* bakterilerinin neden olduğu Avrupa yavru çürüklüğü (EFB) ve Amerikan yavru çürüklüğü hastalıklarının (AFB) tedavisnde tercih edilmektedir. Bununla birlikte, yaygın kullanımı nedeniyle bu bakterilerde tetrasikline karşı direnç oluştuğuna dair literatür bilgileri mevcuttur (7, 8). Ayrıca, arıcılıkta eritromisin, linkomisin, neomisin, streptomisin, enrofloksasin vb. gibi diğer antibiyotiklerin kullanıldığı da bildirilmiştir.[4]

Avrupa Birliği (AB) ülkelerinde ve Türkiye'de arıcılıkta antibiyotik kullanımı yasaklanmıştır.[5] Antibiyotik kalıntıları nispeten uzun bir yarı ömre sahip olmaları, aşırı duyarlı kişilerde alerjik reaksiyonlara sebep olmaları, hematopoetik sistem bozukluğuna ve dirençli bakteri suşlarının indüksiyonuna yol açmaları nedeniyle tüketicileri doğrudan etkileyebilirler.[6,7]

Bu çalışmanın amacı, bal üretim kapasitesi ve kalitesinin nispeten yüksek olduğu Erzurum yöresinde üretilen ballarda neomisin ve tetrasiklin antibiyotik varlığını ve seviyelerini belirlemektir. Bu araştırma TYL-2018-6200 kodlu proje ile Atatürk Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından desteklenmiştir.

MATERYAL VE METOT

Örnek Alımı

Bu çalışmada analiz edilen bal örnekleri Erzurum'a bağlı; Aşkale, Aziziye, Çat, Hınıs, Horasan, İspir, Karayazı, Karaçoban, Köprüköy, Narman, Oltu, Olur, Palandöken, Pasinler, Pazaryolu, Şenkaya, Tekman, Tortum, Uzundere, Yakutiye ilçelerinden temin edildi. Toplam 79 adet bal örneği arıcılık yapan bal üreticilerden direkt olarak peteklerden süzülerek alındı. Numuneler oda sıcaklığında muhafaza edildi. Toplanan örnekler, ışığa kapalı ortamda oda ısısında ve ambalajların ağzı ortam nemini almayacak şekilde kapatılarak muhafaza edildi.

Örneklerin tetrasiklin ve neomisin antibiyotik kalıntıları Enzyme-Linked ImmunoSorbent Assay (ELISA) yöntemi ile saptandı.

ELISA Analizi

Tetrasiklin Analizi

Tetrasiklin analizi Elabscience TCs (Tetrasiklin) ELISA kitine ait manuelde belirtilen yönergelere göre yapıldı (Elabscience, USA Tetrasiklin). Bu amaçla deney tüpüne alınan 1 g bal örneği 2 ML% 1 lik trichloroacetic asit ile çözündürüldükten sonra 4000 rpm de oda sıcaklığında 10 dakika santifüj edildi. Daha sonra başka bir deney tüpüne süpernatanttan 100 µL alınarak üzerine 1900 µL reconstition buffer (kit içerisinde bulunan) ilave edilerek 30 saniye çalkalandı. Bu karışımdan 50 µL alınarak analizde kullanıldı.

Mikropleytin ilk 12 kuyucuğuna standartlar paralelli olarak, diğer kuyucuklara da örneklerden 50 µL ilave edildi. Ardından 50 µL antibody solüsyonu ilave edildikten sonra 5 saniye karıştırılarak 37°C lik etüvde 30 dakika (karanlık ortam) inkübe edildi. İnkübasyonu ardından pleytler ters çevirilerek kuyucuklarda bulunan sıvı uzaklaştırıldı. Ardından yıkama solüsyonu ile 5 kez yıkama işlemi yapıldı. Ardından 100 µL HRP konjugate ilave edilerek 37°C lik etüvde 30 dakika (karanlık ortam) inkübe edildi. Daha sonra yıkama işlemi yapıldı. Daha sonra 50 µL substrat reagent A her kuyucuğa ilave edildikten sonra 50 µL substrat reagent B eklenerek hassas 5 saniye çalkalandı. 37°C lik etüvde 15 dakika (karanlık ortam) inkübe edildi. Bu işlemin ardından 50 µL stop solüsyonu ilave edilip çalkalandı. Ardından optik yoğunluğun belirlenmesi için mikroplayt okuyucu da 450 nm de okutuldu. Örneklerin dilüsyon faktörü 40, minimum belirleme dozu (Lod) 2 ppb dir.

Neomisin Analizi

Neomisin analizi Europroxima neomisin 51111 NEO (12)09.15 ELISA kitine ait manuelde belirtilen yönergelere göre yapıldı (Euro Proxima, Arnhem, The Netherlands). Deney tüpüne 1 g bal birlikte 4 mL SDB (SDB; 1 L distile su içerisinde çözündürülmüş 1,15 g Na_2HPO_4; 0,2 g KH_2PO_4; 0,2 g KCl; 30 g NaCl; 0,5 mL Tween 80 (pH 7,4)) eklendi ve vorteks ile iyice karıştırıldı. Bu karışımın berrak kısmından 1 mL alınıp temiz bir tüpe aktarıldıktan sonra üzerine 4 mL SDB ilave edildi ve iyice vortekslendi. Elde edilen bu içerikten 50 µL alınarak ELISA test prosedüründe kullanıldı. Standartlar paralelli olarak pleyte aktarıldı. Daha sonra hazırlanan her bir örnekten alınan 50 µL'lik dilüsyonlar kuyucuklara aktarıldı. Daha sonra 25 µL konjugat (neomisin -HRP) bütün kuyucuklara (kör bulunan kuyucuklar hariç) ilave edildi. Ardından 25 µL antibody solüsyonundan bütün kuyucuklara (kör bulunan kuyucuklar hariç) aktarıldı. Pleyt karıştırıcıda 1 dakika süreyle karıştırıldı. Pleyt 4°C lik karanlık ortamda 1 saat inkübe edildi. Pleyt ters çevirilerek içerisinde bulunan solüsyonlar uzaklaştırıldı. Ardından 3 kez yıkama işlemi uygulandı. Kuyucuklara 100 µL substrat solüsyonundan aktarıldı. 20-25°C de 30 dakika inkübe edildi. Daha sonra her kuyucuğa 100 µL stop solüsyon aktarılarak 450 nm de ELISA okuyucuda absorbans değerleri okundu. Neomisin hesaplamaları için kalibrasyon kurvesi çizilerek konsantrasyonlar hesaplandı.

İstatistiksel Analiz

Örneklerinin analizlerinden elde edilen bulguların ortalama değerleri Exel 2016 programı kullanarak yapıldı.

BULGULAR

Tetrasiklin Analiz Bulguları

İncelenen örneklere ait tetrasiklin kalıntı düzeyleri Tablo 1.'de, tetrasiklin kalıntısı pozitif olan örneklerin ortalaması Şekil 1.de belirtilmiştir.

Neomisin Analiz Bulguları

Yapılan incelemeler sonucunda neomisin açısından pozitif (Limit of Detection (LOD): 15,63 ng/mL) örnek belirlenmedi.

TARTIŞMA

Özellikle arı hastalıklarının önlenmesinde bilinçsiz antibiyotik kullanımı sonucu oluşan kalıntılar gıda güvenliği sorunlarına neden olmakta ve halk sağlığını tehdit etmektedir. Araştırmalar baldaki antibiyotik kalıntılarının çoğunlukla çevreden değil, yanlış arıcılık uygulamalarından kaynaklandığını göstermektedir.[8] Bal örneklerinde antibiyotik kalıntılarına dair birkaç çalışma bulunmaktadır (11-13).

Bu çalışmada kullanılan ELISA kitinin minimum belirleme limiti 2 ppb olup, incelenen 79 örneğin 37 (%46,8) tanesinde 2,1-47,08 ppb arasında değişen konsantrasyonlarda ve ortalama 9,33 ppb düzeyinde söz konusu antibiyotik kalıntısını içerdiği saptandı. Aynı örnekler; minimum belirleme limiti 15,63 ng/mL olan

Tablo 1. Bal örneklerinin tetrasiklin bulguları

İlçeler	Örnek sayısı	Pozitif örnek sayısı	Pozitif örneklerin kalıntı düzeyleri (ppb)	Pozitif örneklerin yüzdesi
Aşkale	3	1	7,32	1/3 (%33)
Aziziye	5	3	4,96	3/5 (%60)
			11,6	
			10,92	
Çat	3	1	26,72	1/3 (%33)
Hınıs	7	3	7,92	3/7 (%42)
			12,36	
			16	
Horasan	3	2	11,72	2/3 (%67)
			47,08	
İspir	7	3	2,1	3/7 (%42)
			5,4	
			5,08	
Karaçoban	5	3	2,72	3/5 (%60)
			6,2	
			9,48	
Karayazı	5	2	6,44	2/5 (%40)
			4,32	
Köprüköy	3	2	5,36	2/3 (%67)
			26	
Narman	3	2	2,64	2/3 (%67)
			5,04	
Oltu	3	1	3,96	1/3 (%33)
Olur	3	2	6,92	2/3 (%67)
			18,16	
Palandöken	3	1	4,44	1/3 (%33)
Pasinler	3	1	5,36	1/3 (%33)
Pazaryolu	5	3	6,6	3/5 (%60)
			6,68	
			15,68	
Şenkaya	3	1	4,72	1/3 (%33)
Tekman	5	2	6,2	2/5 (%40)
			2,84	
Tortum	4	1	10,8	1/4 (%25)
Uzundere	2	1	4,2	1/2 (%50)
Yakutiye	4	2	5,88	2/4 (%50)
			5,28	
Toplam	79	37	9,33	37/79 (%46,8)

ELISA kiti ile neomisin yönünden incelendi ve limiti aşan örneğe rastlanmadı.

Bu çalışmada ballarda tetrasiklin ve bir aminoglikozid grubuna ait bir antibiyotik olan neomisin düzeyleri de araştırıldı. Hayvan hastalıklarının tedavisinde ilaç ve yem katkı maddesi olarak yaygın kullanıldığından; et, süt, bal gibi gıda maddelerinde kalıntı riski oluşturabilmektedir.

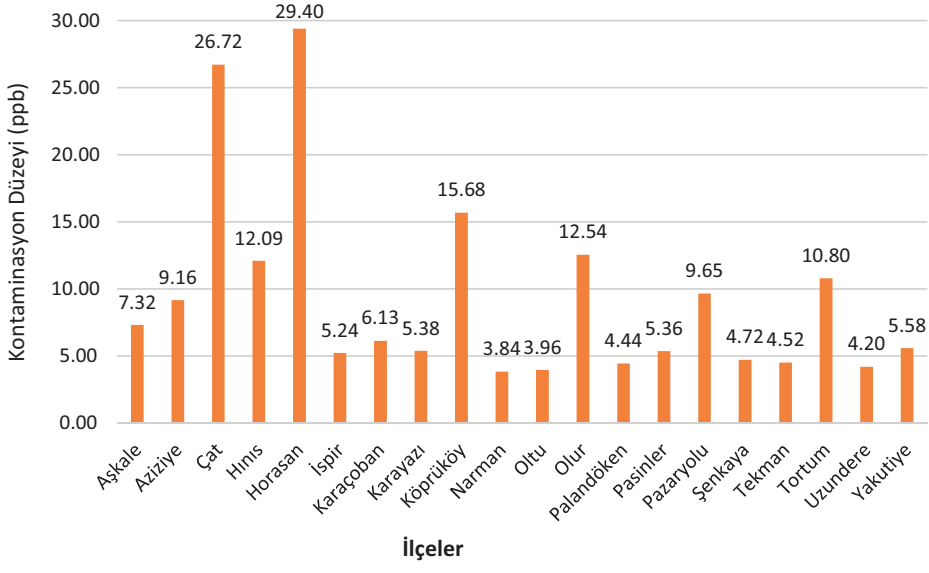
Elde edilen bulgular bal örneklerinde yüksek oranda tetrasiklin belirleyen diğer literatür[9-11] bilgileriyle uyum göstermektedir. Bu durumun halk sağlığı açısından sorun oluşturabileceği görülmektedir. Zira Türkiye'de balda antibiyotik kalıntı düzeyini belirlemeye yönelik yapılan çalışmalarda benzer olgulara rastlanmıştır.

Türkiye'de 2006 yılının ilk altı ayı içerisinde 22 farklı yöreden direkt arıcılardan toplanan örneklerin %10-15'inin tetrasiklin, streptomisin ve sülfonamid antibiyotik kalıntısı içerdiği bildirilmiştir.[9]

Bir başka çalışmada Türkiye'nin batı bölgesinde üreticilerden toplanan 103 balın %23'ünde sülfonamid kalıntısı tespit edilmiş olup, pozitif örneklerin %68'inin sülfametazin, %12'sinin sülfamerazin, ve %20'sinin de sülfametoksazol ile kontamine olduğu bildirilmiştir.[10] Yine ayni bölgede yapılan diğer bir çalışmada ise 59 çam balı örneğinin 35'inin 6-42 ppb arasında tetrasiklin grubu, 31 bal örneğinin ise, 3-32 ppb arasında sülfonamid grubu antibiyotik kalıntısı içerdiği rapor edilmiştir.[11]

Dünyada balda antibiyotik kalıntılarının araştırıldığı bazı çalışmalarda[12-14] düşük düzeylerde antibiyotik kalıntı kontaminasyonun varlığı ortaya konmuştur.

Bal üreticilerinden temin edilen farklı bal türlerinde Liquid Chromatography (LC) yöntemi ile yapılan bir çalışmada bal örneklerinde (belirleme limiti: 15-30 ng/g) tetrasiklin grubu antibiyotik kalıntısı bulunamamıştır.[12] Benzer şekilde High Performance Liquid Chromatography (HPLC) ile yapılan başka bir çalışmada;[14] 36 bal örneğinde oldukça düşük düzeylerde (0,03 ppb) oksitetrasiklin bulunduğu bildirilmiştir. Farklı tür bal örneklerinde (akasya, jojoba,



Şekil 1. Pozitif örneklerin ortalama Tetracycline kalıntı düzeyleri.

pipa ve polifloral) ELISA yöntemi ile tetrasiklin taraması yapılan bir çalışmada ise bal örneklerinin 0,1239 ± 0,1808 ng/mL ile 2,119 ± 0,1576 ng/mL arasında söz konusu antibiyotik ile kontamine olduğunu bildirilmiştir.[15]

Dünyada yapılan birçok çalışmada[4,6,8,16,17] balda yüksek tetrasiklin kontaminasyonu bildirilmiştir.

Belçika'da 2000-2002 yılları arasında ulusal bir tarama programı kapsamında yerel üreticilerden ve çeşitli ülkelerden ithal edilen ballarda farklı antibiyotik (streptomisin, tetrasiklin, sulfanomid, β-laktam, kloramfenikol) düzeyleri incelenmiştir. Yerli (n=248) ve ithal (n=108) bal örneğinin sırasıyla 4 (%1,6) ve 51 (%47,2) streptomisin ile kontamine olduğu belirtilmiştir. Aynı şekilde 72 yerli 98 ithal bal örneğinin ise, sırasıyla 2 (%2,8) ve 29 (%29,6) tetrasiklin ile kontamine olduğu rapor edilmiştir.[8]

Yunanistan'da sıvı kromatografi (LC) ile incelenen 251 bal örneğinin %29'unun tetrasiklin ile kontamine olduğu, genel olarak örneklerin 0,018-0,055 mg/kg düzeyinde tetrasiklin içerdiği ancak bazı örneklerdeki söz konusu kontaminant düzeyinin 0,100 mg / kg'ı aştığı bildirilmiştir.[16]

Hammel ve ark[17] tarafından farklı coğrafi orijin (ABD, Asya, Avrupa) ve farklı bal türleri (çiçek, orman, akasya, ayçiçeği, yonca, çam ağacı) potansiyel kimyasal kirleticiler açısından sıvı kromatografi-elektrosprey iyonizasyon tandem kütle spektrometresi (LC-ESI-MS/MS) ile incelenmiştir. Araştırmada toplam 42 veteriner ilaç kalıntısı (5 tetrasiklin, 7 makrolid, 3 aminoglikozit, 8 β-laktam, 2 amfenikol ve 17 sulfonamid) incelenmiştir. Bal örneklerinin çoğunun birden fazla antibiyotik ile kontamine olduğu belirtilmiştir.

Johnson ve ark[4] Hindistan'da 12 farklı bal örneğini; oksitetrasiklin, kloramfenikol, ampisilin, siprofloksasin, enrofloksasin ve eritromisin kalıntısı yönünden test etmiştir. İncelenen tüm örneklerde aranan antibiyotiklere ait farklı oranlarda kalıntıya rastlanmıştır. İncelenen örneklerde enrofloksasin pozitif örnek yüzdesi %83 olarak belirtilirken, en düşük kontaminasyon yüzdesi, %8 ile siprofloksasin kontaminasyonu şeklinde rapor edilmiştir.

İran 'da yapılan bir çalışmada farklı mevsimlerde temin edilen 135 bal örneğinde enroflaksasin, penisilin, kloramfenikol, gentamisin, tilozin, tetrasiklin ve sülfonamid düzeyleri araştırılmıştır. Buna göre antibiyotik düzeyinin 0-72,1 ng/g aralığında değiştiği, ballarda en sık rastlanan antibiyotiğin enroflaksasin olduğu ve örneklerin %14'ünün 0,2-6,2 ng/g düzeyinde tetrasiklin içerdiği saptanmıştır.[6]

Literatürde aminoglikozitlerin diğer veteriner ilaçlarından farklı fizikokimyasal özelliklere sahip olduğu ve tanımlanmalarının zor olduğu bildirilmektedir.[18] Bu çalışmada neomisin belirlenememesi bu duruma bağlanabileceği gibi üreticilerin bu antibiyotiği tercih etmemeleri ile de ilişkilendirilebilir. Esasında bu çalışmada neomisin düzeyinin belirlenmesinde kullanılan ELISA kitinin minimum belirleme dozunun (15,63 ng/mL) çok düşük olduğu dikkate alındığında bu antibiyotiğin kullanılmama olasılığınıda artırmaktadır.

Literatürde balda neomisin düzeyinin belirlendiği bir çalışmada[19] elde edilen sonuçlar bu çalışma bulguları ile benzerlik göstermektedir.

Çin'deki süpermarketlerden temin edilen ballarda LC-ESI-MS/MS yöntemi ile neomisini de içeren 13 farklı aminoglikozitin analizi yapılmış bal örneklerinde bu bileşiklerin belirlenemediği bildirilmiştir.[19]

Aminoglikozid grubu bir antibiyotik olan streptomisinin de belirlendiği bir çalışmada[20] Hindistan'da arı kovanlarından toplanan nektar ve bal örnekleri antibiyotik kalıntıları yönünden analiz edilmiştir. Nektar ve bal örneklerinde sırasıyla 4-17 ve 11-29 µg/kg streptomisin, 2-29 ve 3-44 µg/kg ampisilin ve 17-34 ve 26-48 µg/kg kanamisin tespit edilirken bölgede arıcılık yapan üreticilerin yoğun antibiyotik kullandığı ifade edilmiştir.

Sonuç olarak bu çalışmada, incelenen bal örneklerinin önemli bir kısmının tetrasiklin kalıntısı içerdiği belirlenmiştir. Bu durum bal tüketicilerinde olası sağlık problemlerine yol açabilir. Halk sağlığının korunması için ulusal kalıntı izleme planı çerçevesinde veteriner ilaçlarının satış ve kullanımının denetlenmesinin büyük önem taşıdığı sonucuna varılmıştır. Ayrıca bal üreticilerinin bal üretiminde antibiyotik kullanımının yasal olmadığı hususunda bilgilendirilmeleri gerektiği düşünülmektedir.

Etik Komite Onayı: Bu çalışma Atatürk Üniversitesi Veteriner Fakültesi Birim Etik Kurulu'ndan onay alınmıştır. (Tarih: 23.05.2017 Karar No: 2017/16).

Hakem Değerlendirmesi: Dış Bağımsız.

Yazar Katkıları: Fikir – M.A.A.; A.T.Y; Tasarım – M.A.A.; Denetleme – M.A.A.; A.T.Y.; Kaynaklar – M.A.A.; A.T.Y.; Malzemeler – M.A.A.; A.T.Y.; Veri Toplanmasıve/veya İşlemesi – M.A.A.; A.T.Y.; Analiz ve/veya Yorum – M.A.A.; A.T.Y.; Literatür Taraması – M.A.A.; A.T.Y.; Yazıyı Yazan – M.A.A.; A.T.Y.; Eleştirel İnceleme – M.A.A.

Çıkar Çatışması: Yazarlar, çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma Atatürk Üniversitesi Bilimsel Araştırmalar Proje Koordinatörlüğü tarafından TYL-2018-6200 proje koduyla desteklenmiştir.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Ataturk University (Date: 23.05.2017, Decision No: 2017/16).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A.A., A.T.Y.; Design – M.A.A., A.T.Y.; Supervision – M.A.A., A.T.Y.; Funding – M.A.A., A.T.Y.; Materials – M.A.A., A.T.Y; Data Collection and/or Processing – M.A.A., A.T.Y., Analysis and/or Interpretation – M.A.A., A.T.Y.; Literature Review – M.A.A., A.T.Y.; Writing – M.A.A., A.T.Y.; Critical Review – M.A.A.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: This study was supported by Atatürk University Scientific Research Projects Coordinatorship with the project code TYL-2018-6200.

KAYNAKLAR

1. FAO, FAOSTAT. "Food and agriculture organization of the United Nations." Rome, URL: http://faostat. fao. org (2018).
2. Subramanian R, Umesh Hebbar H, Rastogi NK. Processing of honey: a review. *Int J Food Prop*. 2007;10(1):127-143. [CrossRef]
3. Bogdanov S. Contaminants of bee products. *Apidologie*. 2006;37(1):1-18. [CrossRef]
4. Johnson S, Jadon N . *Antibiotic Residues in Honey. Report September (2010)*. New Delhi, India: Centre for Science and Environment; 2010.

5. Mutinelli F, Rademacher E. The use of drugs to control varroosis in honey bee colonies and European legislation: the current situation. *Bee World*. 2003;84(2):55-59. [CrossRef]
6. Mahmoudi R, Norian R, Pajohi-Alamoti M.Antibiotic residues in Iranian honey by Elisa. *Int J Food Prop*. 2014;17(10):2367-2373. [CrossRef]
7. Tillotson GS, Doern GV, Blondeau JM.Optimal Antimicrobial Therapy: the balance of potency and exposure. *Expert Opin Investig Drugs*. 2006;15(4):335-337. [CrossRef]
8. Reybroeck W .Residues of antibiotics and sulphonamides in honey on the Belgian market. *Apiacta*. 2003;38:23-30.
9. Sunay AE. Balda Antibiyotik Kalıntısı Sorunu. *Uludağ Arıcılık Derg*. 2006;6(4):143-148.
10. Uludağ R. Ege Bölgesinde Tüketime Sunulan Ballarda Sülfonamid Kalıntılarının Araştırılması. Türkiye: Adnan Menderes Üniversitesi, Sağlik Bilimleri Enstitüsü, Farmakoloji ve Toksikoloji Anabilim Dalı; 2008.
11. Korkmaz SD, Kuplulu O, Cil GI, Akyuz E.Detection of sulfonamide and tetracycline antibiotic residues in Turkish pine honey. *Int J Food Prop*. 2017;20(suppl1):S50-S55. [CrossRef]
12. Viñas P, Balsalobre N, López-Erroz C, Hernández-Córdoba M. Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residues in honey. *J Chromatogr A*. 2004;1022(1-2):125-129. [CrossRef]
13. Wang J, Leung D. Analyses of macrolide antibiotic residues in eggs, raw milk, and honey using both ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry and high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2007 ;21(19):3213-3222. [CrossRef]
14. Radia D, Azzedine C, Nicoletta D, et al. Physicochemical parameters and antibiotics residuals in Algerian honey. *Afr J Biotechnol*. 2015;14(14):1242-1251. [CrossRef]
15. Wang J, Leung D . Analyses of macrolide antibiotic residues in eggs, raw milk, and honey using both ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry and high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2007;21(19):3213-3222. [CrossRef]
16. Saridaki-Papakonstadinou M, Andredakis S, Burriel A, Tsachev I. Determination of tetracycline residues in Greek honey. *TJS*. 2006;4(1):33-36.
17. Hammel YA, Mohamed R, Gremaud E, LeBreton MH, Guy PA. Multi-screening approach to monitor and quantify 42 antibiotic residues in honey by liquid chromatography–tandem mass spectrometry. *J Chromatogr A*. 2008;1177(1):58-76. [CrossRef]
18. Kumar P, Rúbies A, Companyó R, Centrich F. Determination of aminoglycoside residues in kidney and honey samples by hydrophilic interaction chromatography-tandem mass spectrometry. *J Sep Sci*. 2012;35(20) :2710-2717. [CrossRef]
19. Zhu WX, Yang JZ, Wei W, Liu YF, Zhang SS. Simultaneous determination of 13 aminoglycoside residues in foods of animal origin by liquid chromatography–electrospray ionization tandem mass spectrometry with two consecutive solid-phase extraction steps. *J Chromatogr A*. 2008;1207(1-2):29-37. [CrossRef]
20. Solomon RJ, Santhi VS, Jayaraj V. Prevalence of antibiotics in nectar and honey in South TamilNadu, India. *Integr Biosci*. 2006;10(3):163-167. [CrossRef]

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.221830

Investigation of the Effectiveness of Chromogenic Media in the Isolation of *Pasteurella multocida* and *Mannheimia haemolytica* from Calf Nasal Swab Samples

Buzağı Nasal Svap Örneklerinden *Pasteurella multocida* ve *Mannheimia haemolytica* İzolasyonunda Kromojenik Besiyerinin Etkinliğinin Araştırılması

Osman Yaşar TEL[1]
Songül ÖTKÜN[2]
Ayfer Güllü YÜCETEPE[1]
Oktay KESKİN[1]

[1]Departmant of Veterinary Microbiology, Harran University, Faculty of Veterinary Medicine, Şanlıurfa, Turkey
[2]Departmant of Veterinary Microbiology, Siirt University, Faculty of Veterinary Medicine, Siirt, Turkey

Received/Geliş Tarihi: 26.07.2022
Accepted/Kabul Tarihi: 18.10.2022
Publication Date/Yayın Tarihi: 29.12.2022

Corresponding author/Sorumlu Yazar: Songül ÖTKÜN
E-mail: songulotkun@yahoo.com

Cite this article as: Tel OY, Ötkün S, Yücetepe AG, Keskin O. Investigation of the effectiveness of chromogenic media in the isolation of *Pasteurella multocida* and *Mannheimia haemolytica* from calf nasal swab samples. *Vet Sci Pract.* 2022; 17(3), 81-86.

Atıf: Tel OY, Ötkün S, Yücetepe AG, Keskin O. Buzağı nasal svap örneklerinden *Pasteurella multocida* ve *Mannheimia haemolytica* izolasyonunda kromojenik besiyerinin etkinliğinin araştırılması. *Vet Sci Pract.* 2022; 17(3), 81-86.

ABSTRACT

Accurate and rapid diagnosis of disease agents is the most important step in terms of control practices. Chromogenic media are culture media that allow the formation of colonies in colors specific to target microorganisms. Because they are target-specific, they do not require validation of results but provide ease of use and time savings. In this study, it was aimed to evaluate the effectiveness of a chromogenic medium (Pasteurella BDR kit) in the detection of *Pasteurella multocida* and *Mannheimia haemolytica* agents in the *Pasteurelleceae* family, which cause respiratory disease in cattle. In this study, nasal swab samples taken from calves showing symptoms of respiratory disease were cultured in chromogenic and standard media. Suspicious growing colonies were confirmed by polymerase chain reaction for *P. multocida* and *M. haemolytica*. While 31 (36.9%) samples formed colonies with the chromogenic medium in the color specific to the target bacteria, 28 (33.3%) samples were positively determined for the target bacteria using the standard cultural method. The results of 26 samples were positive by both cultural diagnosis methods. When the results were compared with the traditional cultural diagnosis, agreement was found to be 92.86%. All colored colonies grown on the chromogenic medium were also tested by polymerase chain reaction (PCR). It was determined that the chromogenic medium detected *P. multocida* at a rate of 92.86% (n = 26) and *M. haemolytica* at a rate of 100% (n = 2) by forming colonies with a family-specific color. As a result, it was concluded that the use of chromogenic media is beneficial in the practical, rapid, and high-accuracy diagnosis of target agents.

Keywords: Chromogenic medium, Pasteurella, bovine respiratory disease, culture

ÖZ

Hastalık etkenlerinin doğru ve hızlı teşhisi, kontrol uygulamaları açısından en önemli basamaktır. Kromojenik besiyerleri hedef mikroorganizmalara özgü renkte kolonilerin meydana gelmesini sağlayan kültür ortamlarıdır. Bunlar hedefe özgü olduklarından sonuçların doğrulama ihtiyacı duymamakla birlikte, kullanım kolaylığı ve zaman tasarrufu sağlarlar. Bu çalışmada, sığırlarda solumun hastalığına neden olan *Pasteurelleceae* ailesinde bulunan *Pasteurella multocida* ve *Mannheimia haemolytica* etkenlerinin tespitinde, kromojenik besiyerinin (Pasteurella BRD Kit) etkinliğini değerlendirmek amaçlandı. Çalışmada solunum hastalığı belirtisi gösteren buzağılardan alınan nasal svap örnekleri kromojenik ve standart besiyerlerinde kültüre edildi. Üreyen şüpheli koloniler *Pasteurella multocida* ve *Mannheimia haemolytica* yönünden Polimeraz Zincir Reaksiyonu (PCR) ile doğrulandı. Kromojenik besiyeri ile 31 (%36,9) numune, hedef bakterilere özgü renkte koloni oluştururken, standard kültürel yöntem ile 28 (%33,3) numune hedef bakteriler yönünden pozitif olarak belirlendi. Her iki kültürel tanı yöntemiyle 26 numunenin sonuçları *pozitif* olarak tespit edildi. Sonuçlar geleneksel kültürel tanı ile karşılaştırıldığında %92,86 oranında uyumlu sonuç

bulundu. Kromojenik besiyerinde üreyen renkli kolonilerin tamamı PCR ile de test edildi. Kromojenik besiyeri *P. multocida*'yı %92,86 (n:26), *M. haemolytica*'yı %100 (n:2) oranında aileye özgü renkte koloni meydana getirme suretiyle tespit ettiği belirlendi.

Sonuç olarak, hedef etkenin pratik, hızlı ve yüksek doğrulukla teşhisinde kromojenik besiyerinin kullanımının yararlı olduğu kanısına varıldı.

Anahtar Kelimeler: Kromojenik besiyeri, *Pasteurella*, sığır solunum hastalığı, kültür

INTRODUCTION

Following the rapid and accurate diagnosis of microorganisms causing disease in animals, the implementation of an appropriate control program is important in many ways, such as preventing epidemics, protecting animal welfare, and reducing antimicrobial resistance and economic losses affecting animal health and welfare worldwide.[1-3] The etiology of the disease, which is an important cause of death in cattle, is multifactoral, and bacterial agents such as *Pasteurella multocida, Mannheimia haemolytica*, and *Mycoplasma bovis* act as opportunistic pathogens and adversely affect the prognosis, especially in calves.[4,6] The diagnosis of bovine respiratory tract disease is made by cultural, molecular, or serological examination of samples taken from the animal.[7,8] These diagnostic methods are widely used and play an important role in the investigation of the disease,[2,3,9-11] but these methods are time-consuming and require a well-equipped laboratory and experienced personnel.

In recent years, highly specific chromogenic media targeting pathogenic or resistant microorganisms have been developed for rapid diagnosis and treatment of diseases.[12-14] These media make it possible to distinguish microorganisms according to colony color, as they contain chromogenic substrates that allow the formation of colonies with a color specific to the target microorganisms. Compared to standard methods, these culture media are easy to use and the results are easy to read, and the need for confirmation testing is less. In addition, chromogenic media provides cost and time savings with high accuracy and sensitivity, as well as fast and practical identification in samples where contamination is possible.[12,15-17] The performance of chromogenic media has been evaluated in many studies, such as mastitis, urinary tract pathogens, and the diagnosis of resistant microorganisms.[18-20]

The aim of this study was to compare the performance of chromogenic media in the isolation of *P. multocida* and *M. haemolytica* with the standard cultural diagnosis method using nasal swab samples from calves with respiratory tract disease.

MATERIAL AND METHODS

The study was carried out with samples brought to our laboratory for diagnosis purposes. Nasal swab samples were taken from 84 calves with the suspicion of respiratory disease outbreak, such as difficulty in breathing and coughing among young animals on a farm. Samples in transport medium and cold chain were delivered to the Microbiology Department laboratory.

Microbiological Culture

Standard microbiological methods were applied to the samples in the laboratory for microbiological analysis. In summary, 84 nasal swab specimens were seeded on 5% sheep blood agar and MacConkey agar (Merck, Germany) and incubated aerobically at 37°C for 24-48 hours. The growing microorganisms were evaluated in terms of hemolysis features, Gram staining, and other biochemical features and their identifications were made.[21]

The samples were simultaneously cultivated of a selective and chromogenic medium (Pasteurella BRD Kit, Arbilim Biyoteknoloji, Türkiye) developed to facilitate the detection of *Pasteurellaceae* and incubated at 37°C for 24-48 hours under aerobic conditions. On chromogenic media, members of the *Pasteurellaceae* family form colonies ranging in color from pink to lilac, while inhibiting coliforms form blue-steel-blue colonies. Other Gram (–) bacteria are inhibited or form colorless colonies. The growth of yeasts and Gram (+) microorganisms is suppressed.

Molecular Diagnosis

DNA Extraction

The boiling method was used for DNA extraction. Suspected bacteria were incubated aerobically overnight at 37°C in a brain heart infusion medium (Merck), and 2 mL of bacterial suspension was incubated at –20°C for 10 minutes. Then, the thawed suspension was centrifuged and the pellet was homogenized with 200 µL of ddH_2O and boiled in a water bath for 10 minutes, and then the centrifugation was repeated and the supernatant was stored at 4°C to be used for polymerase chain reactions (PCRs).[22]

Polymerase Chain Reaction Step

The reaction was performed according to the method reported by Deressa et al.[23] comprising a PCR mix, 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$), 0.2 mM dNTP, 0.5 mM *P. multocida* primers and 2 mM *M. haemolytica* primer, 2 units of Taq polymerase and then the final product was prepared in a volume of 25 µL using 1 µL of template DNA as a sample (Table 1).

Table 1. Primers Used in PCR and Length of Amplification Product (Base pair)

Target Bacteria	*Pasteurella multocida*	*Mannheimia haemolytica*
Oligonucleotides (5'-3')	GCTGTAAACGAACTCGCCC ATCCGCTATTTACCCAGTGG	GTTTGTAAGATATCCCATTT CGTTTTCCACTTGCGTGA
Amplicon length	460 bp	1022 bp

The PCR amplification consisted of 30 cycles of 1 minute at 95°C, 1 minute at 48°C, and 30 seconds at 72°C, followed by 3 minutes of pre-denaturation at 95°C.

In order to evaluate the PCR products, the presence of specific bands of 460 bp for *P. multocida* and 1022 bp for *M. haemolytica* was investigated by running it on 2% agarose gel and staining it with ethidium bromide.

RESULTS

In this study, the effectiveness of chromogenic medium in the isolation of *P. multocida* and *M. haemolytica* from nasal swab samples taken from young animals with respiratory problems was determined by comparing them with standard diagnostic methods.

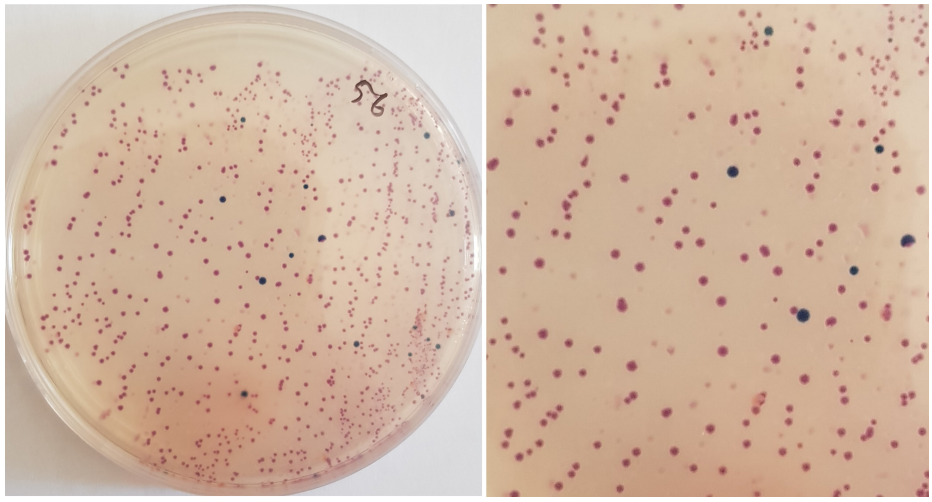


Figure 1. Colony morphology of bacteria in Pasteurella BRD Kit. Pink-mauve colonies: Pasteurellaceae, blue-steel-blue colonies: Coliforms.

All of the 84 nasal swab samples from calves were seeded on standard media and chromogenic agar simultaneously. A history of antibiotic treatment was obtained in 16 (19.05%) of the sampled calves. Although bacterial growth was observed in the blood agar medium in all of the samples, growth was observed in 14 (16.66%) samples on the MacConkey agar medium. While 2 of the growing bacteria were identified as *M. haemolytica*, 28 (33.3%) of the suspicious colonies grown on blood agar were found to be positive for *P. multocida* according to standard identification methods (Table 2). *M. haemolytica*-isolated samples (n = 2) were also positive for *P. multocida,* which was also isolated and identified from 2 of the samples (n = 16) taken from animals that were started on antibiotic treatment.

A total of 31 (36.9%) specimens formed colonies (pink-lilac in color) specific to the *Pasteurellaceae* family in cultivation on chromogenic medium (Figure 1). In the evaluation, 4 (4.76%) samples were found to be suspicious and there were 49 (58.33%) negative samples (Table 2). Moreover, 5 samples found positive in the chromogenic medium were found to be negative by standard methods, 1 sample that was found to be positive by standard methods was found to be negative in the chromogenic medium, and 1 sample was suspicious. Of the samples, 30.95% (26) were found positive with the same results in standard and chromogenic media, and the agreement between the standard identification and chromogenic media was 92.86% (Table 2).

Bacteria found to be positive and suspicious by both cultural methods (62 in total) were molecularly analyzed for *M. haemolytica* and *P. multocida* by PCR. The PCR results were fully compatible with the standard method. It was found that 28 (33.3%) samples formed bands compatible with *P. multocida* (Figure 2). Of these, 26 were positive, 1 was negative, and 1 was suspicious on chromogenic medium. Moreover, 2 samples were culturally and molecularly identified as *M. haemolytica*, (Figure 3) and they were also positive for *P. multocida*. It was found that 5 of the PCR results were negative for *P. multocida* and *M. haemolytica*, which produced a color specific to the *Pasteurellaceae* family in chromogenic culture.

DISCUSSION

The animal and human upper respiratory tracts are home to a wide variety of potentially pathogenic commensal microorganisms that are in competition with each other. Among these microorganisms, which are defined as pathobionts, members of the *Pasteurellaceae* family are commonly found.[24,25] There are many pathogenic species in this family, and *P. multocida* and *M. haemolytica* cause fatal pneumonia, especially in calves younger than 6 months old and under stress.[24,26,27] In this study, the diagnosis performance of chromogenic medium developed to detect bacteria in the *Pasteurellaceae* family was tested and compared with the standard culture method. When the results were compared, it was seen that there was a high rate of agreement (92.86%).

Payne and Roscoe[19] compared the performance of 2 different commercial chromogenic agars (chromID CPS and UriSelect), which detect many important pathogens, such as *Escherichia coli, Proteus mirabilis, and Enterococcus spp.*, in clinical urine samples (human) with standard culture at rates of 93% and 93.1%, and obtained consistent results. In the current study, in which the performance of the chromogenic medium developed for the detection of agents in the *Pasteurellaceae* family was compared with the traditional culture, the compatibility in the diagnosis of nasal swab samples was 92.86%. Cole et al[28] found 96.6% compatible results in their study where they compared cat and dog urine samples with traditional culture using choromogenic triplates (UTid+), which was developed for dairy culture purposes and allows for the identification of many microorganisms, such as *E. coli, Staphylococcus* spp., and *Enterococcus* spp. When the

Table 2. Results of the Pasteurella BRD Kit and Standard Cultural Diagnosis

		Standard Culture Result		
		Pasteurella	*Pasteurella* Negative	Total
Pasteurella Kit growth result	Positive count/percentage	26/30.95%	5/5.95%	31/36.9%
	Negative count/percentage	1/1.19%	48/57.14%	49/58.33%
	Suspect count/percentage	1/1.19%	3/3.57%	4/4.76%
	Total/percentage	28/33.33%	56/66.66%	84/100%

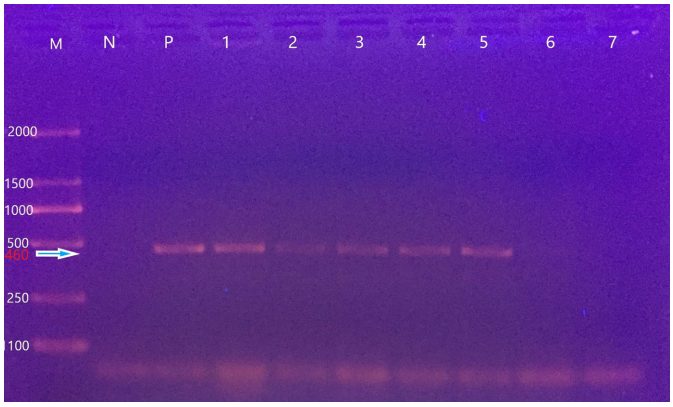


Figure 2. *Pasteurella multocida* polymerase chain reaction results: M, ladder; N, negative control; P, positive control; 1-5, positive samples; 6 and 7: negative samples.

results were compared, despite the clinically compatible results, it was thought that the small differences could have been caused by many variables, such as the chromogenic environment and target microorganisms, sampling method, researcher, and laboratory. The performance of chromogenic media in rapid microbiological identification of mastitis has been evaluated in many studies and its importance in diagnosis has been demonstrated.[17,18]

The number of studies investigating the effectiveness of chromogenic culture media in the detection of agents in respiratory system diseases is limited.[29] As a result of culturing 84 nasal swab specimens in this study in chromogenic media, 31 (36.9%) specimens formed a pink-lilac-colored colony specific to the *Pasteurellaceae* family (Figure 1). The results were consistent with traditional cultural diagnosis and PCR. Many studies[18,28,30] have concluded that, similar to the results of this study, chromogenic environments will play an important role in clinical diagnosis in terms of rapid screening of pathogens.

P. multocida and *M. haemolytica* are frequently isolated bacterial agents in pneumonia.[31] Since the role of PCR in the accurate and rapid detection of these agents is undeniable,[32] suspicious bacteria were examined by PCR for the presence of *P. multocida* and *M. haemolytica*. It was found that 26 samples were positive for *P. multocida* (Figure 2) and 2 samples were positive for *P. multocida* and *M. haemolytica*. In these studies, the isolation rates of the 2 pathogens were found in different numbers in healthy animals and animals showing respiratory disease symptoms, and

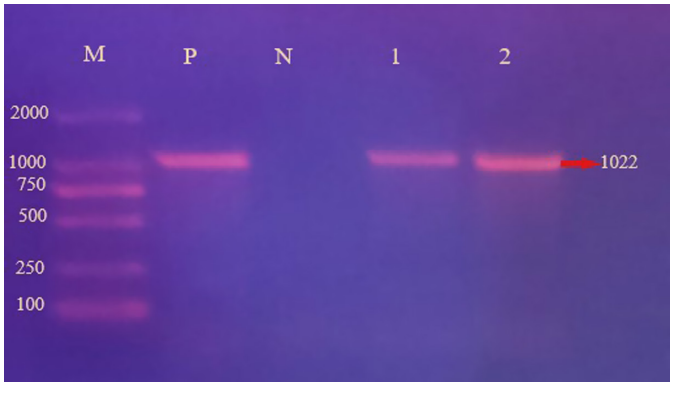


Figure 3. *Mannheimia haemolytica* polymerase chain reaction result. M, ladder; P, positive control; N, negative control; 1 and 2, positive samples.

the complex etiological situation played a big role in this. However, these 2 bacterial agents were found to be significantly lower in the healthy animals when compared to the sick animals.[31,33-35] Tel and Keskin[36] isolated and identified *M. haemolytica* at a rate of 12.5% and *P. multocida* at a rate of 31.6% in their study with 240 pneumonic lung samples. In the current study, *P. multocida* was found at a rate of 33.3% and *M. haemolytica* was found at a rate of 2.38% by PCR from 84 calves showing respiratory system disease. Although the isolation rate of *P. multocida* was close to the findings of Tel and Keskin,[36] the difference in the rate of *M. haemolytica* was thought to be related to variables such as age, geographical region, and sampling time (Figure 3).

One of the important aims of this study was to test the performance of the chromogenic medium in detecting *P. multocida* and *M. haemolytica*. Of the 31 samples that formed pink-mauve colonies specific to the *Pasteurelleceae* family on chromogenic media, 26 were identified as *P. multocida* and 2 were identified as *M. haemolytica* by PCR. One of the 2 samples identified as *P. multocida* by PCR was identified as negative in the chromogenic medium, and in the other, it was identified as suspicious. Based on these data, it was observed that the chromogenic medium formed colonies with a family-specific color at a high rate of 92.86% (n = 26) for *P. multocida* and 100% (n = 2) for *M. haemolytica*.

There are many bacterial genera and species in the *Pasteurellaceae* family.[37] Of the samples, 5 that formed colonies with a color specific to the *Pasteurellaceae* family on chromogenic media were found to be negative for *P. multocida* and *M. haemolytica* by PCR, and no evaluation was made in terms of other possible factors.

As a result, chromogenic medium was used to detect *P. multocida* and *M. haemolytica* from the nasal swabs of calves with high accuracy. In the preparation and visual evaluation and reading of the results, the use of chromogenic medium, which does not require special training and gives results in a short time, was found to be advantageous. Farms will benefit from accurate and rapid diagnosis and treatment in places where contamination is highly likely, which do not have equipped laboratories such as clinics and sufficient specialized personnel. In addition, it has been predicted that the use of such media will play an important role in preventing unnecessary drug use and antimicrobial resistance.

Ethics Committee Approval: This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees."

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.Y.T.; Design – O.Y.T.; Supervision – O.Y.T.; Resources – O.Y.T., A.G.Y.; Data Collection and/or Processing – O.Y.T., A.G.Y., S.Ö.; Analysis and/or Interpretation – O.Y.T., S.Ö.; Literature Search – S.Ö., O.Y.T.; Writing – S.Ö.; Critical Review – O.K., O.Y.T.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir– O.Y.T; Tasarım – O.Y.T; Denetleme – O.Y.T.; Kaynaklar – O.Y.T., A.G.Y.; Malzemeler – O.Y.T.; Veri Toplanması ve/veya İşlemesi – O.Y.T., A.G.Y., S.Ö.; Analiz ve/veya Yorum – O.Y.T., S.Ö.; Literatür Taraması – S.Ö., O.Y.T.; Yazıyı Yazan – S.Ö.; Eleştirel İnceleme – O.Y.T., O.K.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmiştir.

REFERENCES

1. Peel DS. The effect of market forces on bovine respiratory disease. *Vet Clin North Am Food Anim Pract*. 2020;36(2):497-508. [CrossRef]
2. Kudirkiene E, Aagaard AK, Schmidt LMB, Pansri P, Krogh KM, Olsen JE. Occurrence of major and minor pathogens in calves diagnosed with bovine respiratory disease. *Vet Microbiol*. 2021;259:109135. [CrossRef]
3. Gorden PJ, Plummer P. Control, management, and prevention of bovine respiratory disease in dairy calves and cows. *Vet Clin North Am Food Anim Pract*. 2010;26(2):243-259. [CrossRef]
4. Pratelli A, Cirone F, Capozza P, et al. Bovine respiratory disease in beef calves supported long transport stress: an epidemiological study and strategies for control and prevention. *Res Vet Sci*. 2021;135:450-455. [CrossRef]
5. Chernitskiy AE, Safonov VA. Early detection of bovine respiratory disease in calves by induced cough. *In IOP Conference Series. IOP Conf Ser.: Earth Environ Sci*. 2021;677(4). [CrossRef]
6. Dorso L, Rouault M, Barbotin C, Chartier C, Assié S. Infectious bovine respiratory diseases in adult cattle: an extensive necropsic and etiological study. *Animals (Basel)*. 2021;11(8):2280. [CrossRef]
7. Zhang M, Hill JE, Alexander TW, Huang Y. The nasal viromes of cattle on arrival at Western Canadian feedlots and their relationship to development of bovine respiratory disease. *Transbound Emerg Dis*. 2021;68(4):2209-2218. [CrossRef]
8. Bell CJ, Blackburn P, Elliott M, et al. Investigation of polymerase chain reaction assays to improve detection of bacterial involvement in bovine respiratory disease. *J Vet Diagn Invest*. 2014;26(5):631-634. [CrossRef]
9. Gülaydın O, Gürtürk K. Identification of Pasteurella multocida strains isolated from respiratory tract of healthy and diseased cattle and determination of capsular types by PCR. *Van Veterin J*. 2018;29(3):143-146.
10. McGuirk SM. Disease management of dairy calves and heifers. *Vet Clin North Am Food Anim Pract*. 2008;24(1):139-153. [CrossRef]
11. Çelik M, Erdenliğ-Gürbilek S. Mannheimia haemolytica suşlarının farklı besiyerlerinde üreme ve lökotoksin oluşturma özelliklerinin incelenmesi. *J Adv Vetbiosci Tech*. 2020;5(2):33-42.
12. Perry JD, Freydière AM. The application of chromogenic media in clinical microbiology. *J Appl Microbiol*. 2007;103(6):2046-2055. [CrossRef]
13. Reissbrodt R. New chromogenic plating media for detection and enumeration of pathogenic *Listeria* spp.—an overview. *Int J Food Microbiol*. 2004;95(1):1-9. [CrossRef]
14. Alizadeh N, Rezaee MA, Kafil HS, et al. Detection of carbapenem-resistant *Enterobacteriaceae* by chromogenic screening media. *J Microbiol Methods*. 2018;153:40-44. [CrossRef]
15. Rank EL. Chromogenic agar media in the clinical, food, and environmental testing arenas, part I. *Clin Microbiol Newsl*. 2012;34(6):43-47. [CrossRef]
16. Perry JD. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. *Clin Microbiol Rev*. 2017;30(2):449-479. [CrossRef]
17. Garcia BLN, Fidelis CE, Freu G, Granja BM, Dos Santos MV. Evaluation of chromogenic culture media for rapid identification of Gram-positive bacteria causing mastitis. *Front Vet Sci*. 2021;8:662201. [CrossRef]
18. Granja BM, Fidelis CE, Garcia BLN, Dos Santos MV. Evaluation of chromogenic culture media for rapid identification of microorganisms isolated from cows with clinical and subclinical mastitis. *J Dairy Sci*. 2021;104(8):9115-9129. [CrossRef]
19. Payne M, Roscoe D. Evaluation of two chromogenic media for the isolation and identification of urinary tract pathogens. *Eur J Clin Microbiol Infect Dis*. 2015;34(2):303-308. [CrossRef]
20. Pletinckx LJ, De Bleecker Y, Dewulf J, Rasschaert G, Goddeeris BM, De Man I. Evaluation of salt concentrations, chromogenic media and anatomical sampling sites for detection of methicillin-resistant *Staphylococcus aureus* in pigs. *Vet Microbiol*. 2012;154(3-4):363-368. [CrossRef]
21. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, Fitzpatrick EI. *Veterinary Microbiology and Microbial Disease*. Chichester, UK: John Wiley & Sons; 2011.
22. Ewers C, Lübke-Becker A, Bethe A, Kießling S, Filter M, Wieler LH. Virulence genotype of Pasteurella multocida strains isolated from different hosts with various disease status. *Vet Microbiol*. 2006;114(3-4):304-317. [CrossRef]
23. Deressa A, Asfaw Y, Lubke B, Kyule MW, Tefera G, Zessin KH. Molecular detection of *Pasteurella multocida* and *Mannheimia haemolytica* in sheep respiratory infections in Ethiopia. *J Appl Res Vet Med*. 2010;8(2):101.
24. Thomas AC, Bailey M, Lee MRF, et al. Insights into *Pasteurellaceae* carriage dynamics in the nasal passages of healthy beef calves. *Sci Rep*. 2019;9(1):11943. [CrossRef]
25. Bosch AA, Biesbroek G, Trzcinski K, Sanders EA, Bogaert D. Viral and bacterial interactions in the upper respiratory tract. *PLoS Pathog*. 2013;9(1):e1003057. [CrossRef]
26. Pansri P, Katholm J, Krogh KM, et al. Evaluation of novel multiplex qPCR assays for diagnosis of pathogens associated with the bovine respiratory disease complex. *Vet J*. 2020;256:105425. [CrossRef]
27. Cirone F, Padalino B, Tullio D, et al. Prevalence of pathogens related to bovine respiratory disease before and after transportation in beef steers: preliminary results. *Animals (Basel)*. 2019;9(12):1093. [CrossRef]
28. Cole SD, Swiderski M, Dietrich J, McGonigle KM. Comparison of a chromogenic urine culture plate system (UTid+) and conventional urine culture for canine and feline specimens. *Vet Sci*. 2022;9(3):138. [CrossRef]
29. Al-Anbagi NA. Isolaton and ıdentıfıcatıon some bacterıal causes of lung abscessessheep by chromogenıc medıa. *Basrah J Vet Res*. 2016;15(2):360-370.
30. Beehan DP, McKinnon AO. How to Diagnose Common Equine Reproductive Tract Bacterial Pathogens Using Chromogenic Agar. *Proc Am Assoc Eq Pract*. 2009;55:320-325.
31. DeRosa DC, Mechor GD, Staats JJ, Chengappa MM, Shryock TR. Comparison of *Pasteurella* spp. simultaneously isolated from nasal and transtracheal swabs from cattle with clinical signs of bovine respiratory disease. *J Clin Microbiol*. 2000;38(1):327-332. [CrossRef]
32. Abed AH, El-Seedy FR, Hassan HM, et al. Serotyping, genotyping and virulence genes characterization of *Pasteurella multocida* and *Mannheimia haemolytica* isolates recovered from pneumonic cattle calves in North Upper Egypt. *Vet Sci*. 2020;7(4):174. [CrossRef]
33. Cengiz S, Adıgüzel MC, Dinç G. Detection of *Pasteurella multocida*, *Mannhemia haemolytica*, *Histophilus somni* and *Mycoplasma bovis* in cattle lung. *Rev Mex Cienc Pecuarias*. 2021;12(3):710-720. [CrossRef]
34. Timsit E, Hallewell J, Booker C, Tison N, Amat S, Alexander TW. Prevalence and antimicrobial susceptibility of *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni* isolated from the lower respiratory tract of healthy feedlot cattle and those diagnosed with bovine respiratory disease. *Vet Microbiol*. 2017;208:118-125. [CrossRef]

35. Jamali H, Rezagholipour M, Fallah S, et al. Prevalence, characterization and antibiotic resistance of *Pasteurella multocida* isolated from bovine respiratory infection. *Vet J*. 2014;202(2):381-383. [CrossRef]
36. Tel OY, Keskin O. Koyun akciğerlerinden *Pasteurella multocida* ve *Mannheimia haemolytica* izolasyonu ve antibiyotiklere duyarlılığı. *Yüzüncü Yıl Univ Vet Fak Derg*. 2010;21(1):31-34.
37. Christensen H, Kuhnert P, Norskov-Lauritsen N, Planet PJ, Bisgaard M. The family *Pasteurellaceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, eds. *The Prokaryotes*. 4th ed. Berlin, Germany: Springer; 2014:535-564.

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.2204273

Tavşanlarda Kronik Unilateral Kısmi Üreteral Obstrüksiyonun Diüretik Doppler Ultrasonografi ile Değerlendirilmesi

Evaluation of Diuretic Renal Doppler Ultrasonography Findings in Chronic Unilateral Partial Ureteral Obstruction in Rabbits

Mahir KAYA[1]
Ali BUMIN[2]

[1]Akdeniz Üniversitesi, Sağlık Hizmetleri Meslek Yüksekokulu, Tıbbi Görüntüleme Teknikleri, Antalya, Türkiye
[2]Ankara Üniversitesi, Veteriner Fakültesi, Cerrahi Anabilim Dalı, Ankara, Türkiye

ÖZ

Bu deneysel çalışma, kronik unilateral kısmi üreteral obstrüksiyonun (UO) tanısında diüretik kullanımının renal Doppler parametreleri üzerine etkisini değerlendirmek amacıyla yapılmıştır. Yeni Zellanda Tavşanında (n=10) sol üreter daraltılarak, kısmi UO oluşturuldu. Böbreklerin Doppler ultrasonografik değerlendirilmesi ile preoperatif ve postoperatif 1. ve 4. haftalarda rezistif indeks (RI), RI farkı (RI) ve RI oranı (RIr) elde edildi. Preoperatif sol böbreğin RI değeri ile kıyaslandığında obstruktif (sol) böbreğin RI değerinin, postoperatif 1. haftadaki artışı istatistiksel yönden önemliydi (P = ,04). Diüretik uygulaması sonrasında non-obstruktif (sağ) böbreğin ortalama RI değeri, her iki postoperatif ölçüm zamanında azaldı ve buna bağlı olarak obstruktif ve non-obstruktif böbrek arasında istatistiksel fark belirlendi (P < ,002). Obstruktif böbreğin ortalama RI değerleri, postoperatif hem doğrudan hem de diüretik ölçümlerde 0,70 eşik değerini aşmadı. Postoperatif ölçümlerde diüretik uygulaması, ortalama RIΔ'nı eşik değerin (≥ 0,11) üzerine çıkmasını sağladı ve eşik değerin (≥ 1,1) üzerinde olan ortalama RIr'nı daha da arttırdı. Bu çalışmanın sonuçları, eşik değeri aşmayan tek taraflı yüksek renal RI değerlerinde diüretik uygulamasının, RIΔ'nı ve RIr'yı yükselterek, kronik unilateral kısmi UO'nun tanısal doğruluğunu artırabileceğini göstermektedir.

Anahtar Kelimeler: Doppler ultrasonografi, diüretik, obstruktif üropati, tavşan

ABSTRACT

The experimental study was conducted to evaluate the effect of furosemide on renal Doppler parameters in the diagnosis of chronic unilateral partial ureteric obstruction. Partial ureteral obstruction (UO) was induced by narrowing the left ureter in New Zelland Rabbits (n=10). Resistive index (RI), RI difference (RIΔ) and RI ratio (RIr) values were obtained by Doppler utrasonographic (DU) evaluation of the kidneys preoperatively and the 1st and 4th weeks postoperatively. According to the mean RI value of preoperaitve left kidney, the increase in the mean RI value of the obstructive (left) kidney at the postoperative 1st week was statistically significant (P < .04). After furosemide administration, the mean RI value of the non-obstructive kidney decreased at both postoperative measurement times, and accordingly, a statistical difference was determined between the obstructive and non-absturctive kidneys (P < .002). The mean RI values of the postoperative measurement did not exceed the 0.70 threshold for both direct and diuretic measurements. At the postoperative measurement times, diuretic administration caused the mean RIΔ above threshold value (≥ 0.11) and further increased the RIr, which was above the threshold value (≥ 1.1). The results of this study demonstrate that diuretic administration can increase the diagnostic accuracy of chronic unilateral partial UO by increasing RIΔ and RIr in unilateral high renal RI values that do not exceed the threshold value.

Keywords: Doppler ultrasonography, diuretic, obstructive uropathy, rabbit

GİRİŞ

Üriner bölgenin ana patolojilerinden birisi olan üreteral obstrüksiyon (UO), idrar akışının farklı etiyolojik nedenler ile kısıtlanması veya engellenmesi sonucunda üremik krize, böbrek ve üreter yapısında değişime ve böbrek işlevinde kayba neden olabilir. Bu hastalık, yapısal empedans olarak tanımlanan obstruktif üropati ile sonuçlanabilir. Bu empedansın bir sonucu olarak, böbrekte hasara neden olacak pelvikalisiyel dilatasyon meydana gelir ve bu dilatasyon türü (obstruktif dilatasyon) hidronefroz; obstruktif üropatiden dolayı böbrekte oluşan hasar da obstruktif nefropati olarak adlandırılır.[1] Üreteral obstrüksiyon doğmasal veya edinsel, kısmi veya tam, unilateral veya bilateral, akut veya kronik, statik veya dinamik ya da intraluminal, intramural veya ekstramural olarak sınıflandırılabilir.[2]

Unilateral ve kısmi UO'da kollateral böbreğin işlevi normal ise hastada azotemi oluşmayabilir, hasta sahipleri tarafından renal kolik fark edilemeyebilir ve letarji, anoreksi, kusma, kilo kaybı gibi diğer non-spesifik klinik bulgular da tanı konulması için yetersiz kalabilir. Bundan dolayı evcil hayvanlarda obstruktif üropati sıklıkla kronikleşme eğiliminde olup, tanı konulduğunda özellikle kollateral böbrekte de işlev kaybı varsa, hastanın durumu ciddidir.[3] Bu tür olgularda böbrek morfolojisinin değerlendirilmesi kadar her iki böbreğin işlevinin de bilinmesi önemlidir.

*Bu makale Mahir Kaya'nın doktora tezinden üretilmiştir.

Geliş Tarihi/Received: 22.11.2022
Kabul Tarihi/Accepted: 21.12.2022
Yayın Tarihi/Publication Date: 29.12.2022

Sorumlu Yazar/Corresponding Author: Mahir Kaya
E-posta: kayamahir@gmail.com

Atıf: Kılıç Erkek Kaya M, Bumin A. Tavşanlarda Kronik Unilateral Kısmi Üreteral Obstrüksiyonun Diüretik Doppler Ultrasonografi ile Değerlendirilmesi. *Vet Sci Pract*. 2022; 17(3), 87-90.

Cite this article: Kaya M, Bumin A. Evaluation of diuretic renal doppler ultrasonography findings in chronic unilateral partial ureteral obstruction in rabbits. *Vet Sci Pract*. 2022; 17(3), 87-90.

Üreteral obstrüksiyonun tanısından farklı görüntüleme yöntemleri kullanılmaktadır: intravenöz pyelografi, antegrad pyelografi, bilgisayarlı tomografi, manyetik rezonans görüntüleme, gri-skala ultrasonografi (US), Doppler US.[4,5] Gri-skala ve Doppler US hariç, bu tanı yöntemlerinin ana dezavantajı iyonize radyasyon ve radiokontrast madde kullanılmasıdır.[2] Gri-skala ultrasonografi ile böbrek morfolojisi değerlendirilerek UO tanısı konulabilir. Ancak böbrek korteks kalınlığında değişim yoksa , toplayıcı sistemde dilatasyon görülmüyorsa ve/veya akustik gölge vermen obstruktif lezyon saptanmıyorsa, bu görüntüleme yöntemi yetersiz kalır. Diğer taraftan aşırı mesane dolgunluğu gibi bazı fizyolojik durumlarda veya pyelonefritis gibi medikal renal hastalıklarda, obstrüksiyondan bağımsız olarak renal toplayıcı sistemde ve üreterde non-obstruktif dilatasyon belirlenebilir. Bundan dolayı, gri-skala US, özellikle deneyimsiz uygulayıcıları hatalı tanıya sürükleyebilir.[6,7] Sadece böbrek morfolojinin değil, aynı zamanda obstruktif üropatiden dolayı oluşan böbrek perfüzyonundaki değişim de Doppler US ile değerlendirilerek böbrek işlevi hakkında bilgi elde edilebilir .[8] Bu amaç doğrultusunda en yaygın kullanılan Doppler US parametrelerinden birisi de rezistif indekstir (RI).[9]

Bu çalışmada kronik unilateral kısmi UO'nun tanısında diüretik kullanımının RI, RI farkı (RIr) ve RI oranı (RIr) üzerine etkisinin değerlendirilmesi amaçlanmıştır.

MATERYAL ve METOT

Hayvanlar ve Etik Kurul Onayı

Çalışmanın hayvan materyalini, Lalahan Araştırma Enstitüsünden (Lalahan-Ankara) temin edilen Yeni Zellanda ırkı, ortalama 3,25 kg ağırlığındaki sağlıklı, yetişkin 10 erkek tavşan oluşturdu. Tavşanlar 25°C'de, 12 saat karanlık/ışık aydınlatma ortamında ayrı kafeslerde standart yeme ve suya serbest erişimleri sağlanacak şekilde barındırıldı. Bu deneysel çalışma, Ankara Üniversitesi Veteriner Fakültesi Etik Kurulunun uygun görmesi ile yapıldı (Tarih: 06 Temmuz 2004, Protokol Numarası: 2004-35).

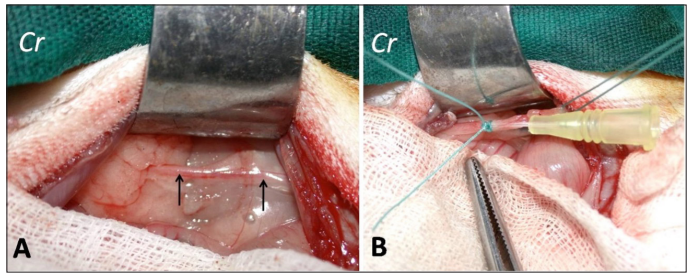
Kısmi UO'un oluşturulması ve intravenöz pyelografi (İVP) ile doğrulanması

İntramusküler xylazine HCl (20 mg/kg, Alfazine®, EgeVet, Türkiye) ve ketamine HCl (50 mg/kg, Alfamine®, Pfizer, Türkiye) anestezisi altındaki tüm hayvanlara laparotomi yapıldı. Sol üreterin orta kısmı açığa çıkarıldı, lümeni içerisine 24 gauge'lik (0,7 mm) anjiyoket yerleştirildi ve dışarıdan 4-0 emilmeyen iplikle (Polyester, Ti-Cron™, Türkiye) bağlanarak, üreter 0,7 mm'lik çapta daraltıldı ve böylece kısmi UO oluşturuldu (Şekil 1). Anjiyoketin silikon kısmı uzaklaştırıldıktan sonra linea alba, derialtı doku ve deri primer kapatıldı.

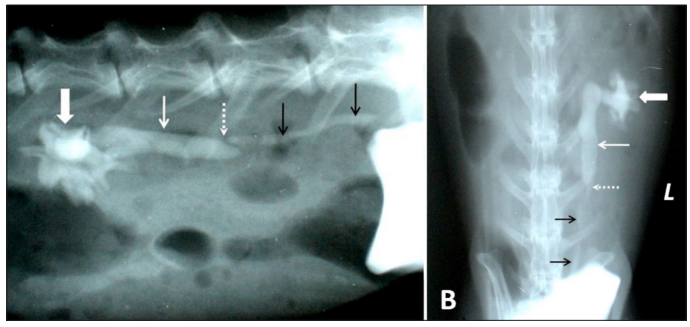
Postoperatif 1. haftada kısmi UO'un varlığını doğrulamak için İVP yapıldı. İyonik kontrast madde (sodyum-meglumin amidotrizoat; Urografin % 76; Schering, Almanya), 880 l/kg dozunda intravenöz yolla uygulandı. Uygulama sonrasındaki 2 saat içerisinde 40 dakikalık periyotlar ile sol lateral (L) ve ventrodorsal (VD) pozisyonda radyogramlar alındı. Unilateral kısmi UO, böbreğin toplayıcı sisteminde ve proksimal üreterde dilatasyonun, obstrüksiyon yerinin ve distal üreterin izlenmesi ile doğrulandı (Şekil 2). Bu 3 kritere uyan 10 hayvana renal Doppler US yapıldı.

Doppler US parametrelerin ölçülmesi

Renal Doppler US'de (ESAOTE, AU5 model, Esaote Biomedica, Genova, İtalya) multifrekans (7,5-10 MHz) özelliğine sahip lineer prop kullanıldı. Preoperatif ve postoperatif 1. ve 4. haftalarda yapılan Doppler US incelemelerinde duvar filtresi ve örnekleme aralığı asgari düzeyde tutuldu ve ölçümler 60° ile yapıldı. Postoperatif



Şekil 1. a, b. A- Retroperitoneal boşlukta sol üreterin kranialden (Cr) kaudale doğru seyri (oklar) . B- Diseke edilen üreter, askıya alındıktan sonra lümenine yerleştirilen anjiyoketin 0,7 mm'lik çapı ölçüsünde dışarından uygulanan ligatürle daraltılması.



Şekil 2. a, b. İntravenöz ürografi ile unilateral kısmi üreteral obstrüksiyonun doğrulanması. Postoperatif 1. haftada intravenöz ürografinin 120. dakikasında elde edilen sol lateral (A) ve ventrodorsal (B) radyogramlarda böbreğin toplayıcı sisteminde (beyaz kalın ok) ve proksimal üreterde (beyaz ok) dilatasyon izlenmektedir. Üreterdeki obstrüksiyon yeri, kesikli beyaz okla ve normal çaptaki distal üreter ise siyah oklarla gösterilmektedir. L, sol taraf.

ölçüm zamanlarında 1 mg/kg dozunda intravenöz furosemid (Lasix®, Sanofi, Şişli-İstanbul) uygulamasından önce ve sonra duplex Doppler US ile elde edilen spektral örnekler, her iki böbreğin üç farklı yerindeki aa. arcuatae veya aa. interlobares'lerden alındı. Bu arteriyel spektrumdan en yüksek sistolik (PSV) ve en düşük diastolik (EDV) hızlar belirlenerek, RI değeri [(PSV – EDV) / PSV] elde edildi. RI farkı (Δ) (obstruktif böbreğin RI değeri – non-obstruktif böbreğin RI değeri) ve RI oranı (r) (obstruktif böbreğin RI değeri / non-obstruktif böbreğin RI değeri) hesaplandı. Her böbreğin üç farklı yerinden elde edilen Doppler parametrelerinin ortalaması, istatistiksel değerlendirmede kullanıldı.

Kronik unilateral kısmi UO'un Doppler US tanısında eşik değer olarak RI için ≥ 0,70, RIΔ için ≥ 0,11 ve RIr için ≥ 1,1 kabul edildi.[9]

İstatistiksel Analiz

t-testi ve tekrarlayan ölçümlerle 2-yollu ANOVA Statistical Package for the Social Sciences software 10.0 (IBM Inc, Chicago, IL, USA) kullanılarak ortalama RI değerlerinin tüm ölçüm zamanları arasındaki ve her ölçüm zamanı için obstruktif (sol) ve non-obstruktif (sağ) böbrekler arasındaki fark değerlendirildi. $P < ,05$ istatistiksel önemde kabul edildi.

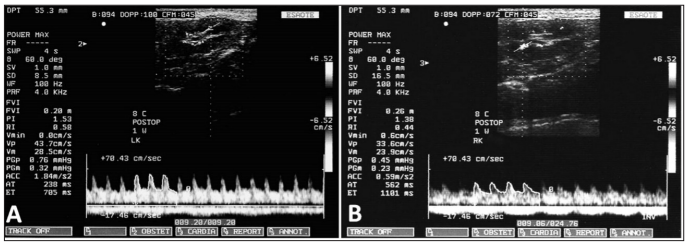
BULGULAR

Ölçüm zamanlarına göre ortalama RI, RIΔ ve RIr değerleri Tablo 1'de sunulmuştur. Sol ve sağ böbreklerin preoperatif ortalama RI değerleri arasında istatistiksel fark saptanmadı. Preoperatif ortalama RI değerlerine göre postoperatif dönemlerde doğrudan ölçümle elde edilen ortalama RI değeri obstruktif (sol) böbrekte artmasına rağmen, sadece postoperatif 1. haftadaki fark önemliydi (*P*

Tablo 1. Ölçüm zamanlarına ve diüretik uygulamasına göre Doppler ultrasonografi parametreleri

		Ölçüm zamanları				
			Postoperatif (hafta)			
			1		4	
Parametreler	Böbrek	Preoperatif	Doğrudan	Diüretik	Doğrudan	Diüretik
RI	L	0,51±0,02	0,62±0,03*	0,59±0,01†	0,59±0,01	0,59±0,01†
	R	0,50±0,02	0,54±0,02	0,44±0,02	0,52±0,01	0,45±0,01
RIΔ		-	0,09±0,02	0,16±0,03	0,07±0,02	0,14±0,02
RIr		-	1,19±0,02	1,34±0,03	1,13±0,02	1,32±0,03

Veriler, ortalama±standart deviasyon olarak sunulmuştur. RI: rezistif indeks, RIΔ: RI farkı, RIr: RI oranı, L: obstruktif (sol) böbrek, R: non-obstruktif (sağ) böbrek.
*Aynı satırda preoperatif ve postoperatif ortalama RI değerleri arasındaki istatistiksel fark, P < ,04. †Aynı sütunda obstruktif ve non-obstruktif ortalama RI değerleri arasındaki istatistiksel fark, P < ,002.



Şekil 3. a, b. A- Retroperitoneal boşlukta sol üreterin kranialden (Cr) kaudale doğru seyri (oklar) . B- Diseke edilen üreter, askıya alındıktan sonra lümenine yerleştirilen anjiyoketin 0,7 mm'lik çapı ölçüsünde dışarından uygulanan ligatürle daraltılması.

= ,04). Non-obstruktif (sağ) böbreğin preoperatif ile postoperatif dönemlerinde doğrudan ölçümle elde edilen ortalama RI değerleri arasında önemli fark tespit edilmedi. Diüretik uygulaması sonrasında obstruktif böbreğin ortalama RI değerleri değişmezken, non-obstruktif böbreğin ortalama RI değerleri, her iki postoperatif ölçüm zamanında da azaldı ve böylece obstruktif ve non-obstruktif böbreklerin ortalama RI değerleri arasında istatistiksel fark belirlendi (*P* < ,002). Postoperatif dönemlerde diüretik uygulamasına bağlı aynı taraftaki böbreklerin ortalama RI değerleri arasında fark belirlenmedi. Postoperatif dönemlerde obstruktif böbreğin ortalama RI değerleri, hem doğrudan hem de diüretik ölçümlerde 0,70 eşik değerini aşmadı (Şekil 3). Ortalama RIΔ, sadece diüretik uygulaması sonrasındaki postoperatif ölçümlerde 0,11 eşik değerinin üzerine çıkarken, doğrudan ölçümlerde 1.1 eşik değerinin üzerinde ortalama RIr değeri daha da arttı. Postoperatif dönemde doğrudan ve diüretik ölçümler ile elde edilen Doppler parametrelerinde zamana göre değişimin önemli yoktu.

TARTIŞMA

Böbreğin toplayıcı sisteminde dilatasyon ile ilişkili perfüzyon değişimleri Doppler US kullanılarak değerlendirilebilir. Bu amaçla hem insanlarda hem de hayvanlarda renal hastalıkları değerlendirmek için RI değeri kullanılmaktadır.[10-12] Yüksek renal RI değerleri (> 0.65) şiddetli renal fibrozis ve böbrek işlevinde azalma ile ilişkilidir.[13] İlaveten renal cerrahi öncesinde elde edilenRI değerleri ile böbrek işlevinin geri kazanılabilirliği arasında pozitif korelasyon olduğu da gösterilmiştir.[14] Bu indeks, böbrek hasarı için özel bir belirteçtir hem de böbrek işlevinin prognostik bir indikatörüdür.[15] Obstruktif üropatinin Doppler US ile değerlendirilmesinde ≥ 0,7, renal RI için eşik değerdir.[16] Yine de obstruktif üropatinin değerlendirilmesinde kullanılan renal RI'nin bazı sınırları vardır: *i*- obstrüksiyondan 6 saat sonra yüksek RI değerlerinin elde edilmesi, *ii*- obstrüksiyonun şiddet derecesi yüksekse veya obstrüksiyon süresi 12 saati aşmışsa 0,7'nin altında renal RI değerlerinin saptanması, *iii*- kısmi UO'da referans aralıkta renal RI değerleri saptanması.[2,4,17,18] Sunulan çalışmada preoperatif değerlere göre her iki postoperatif doğrudan ölçüm zamanında yüksek RI değerleri elde edilmesine ve 1 haftada istatistiksel fark (*P* < .04) belirlenmesine rağmen ortalama RI, 0,7 eşik değerini aşmadı. Bununla birlikte postoperatif doğrudan ölçüm zamanlarındaki ortalama RIΔ değerleri, unilateral UO için eşik sınır olarak kabul edilen 0,1 değerinin altındaydı. Obstruktif dilatasyondan non-obstruktif dilatasyonu ayırt etmekte RIr için eşik değer olarak ≥ 1.1[19] daha yaygın kullanılmasına rağmen, Riahinezhad ve ark.[20] > 1.08 değerinin % 83 spesifiteye sahip olduğunu bildirmiştir. Sunulan çalışmada postoperatif doğrudan ölçüm zamanlarındaki RIr değerleri 1.13'e eşit veya üzerindeydi.

Diüretik uygulamaları, hem non-obstruktif hem de obstruktif böbreğin RI değerini olumlu yönde değiştirdiği ve aynı zamanda kısmi UO'da anlamlı olmayan Doppler US parametrelerini tanının konmasında kullanılabilecek düzeye getirebildiği bildirilmiştir.[9,21] Akut[17,18] veya kronik[4] unilateral kısmi UO çalışmalarında diüretik uygulamasının, tanıya olumlu yönde katkıda bulunacak şekilde RIΔ değerini değiştirdiği gösterilmiştir. Sunulan çalışmada postoperatif doğrudan ölçümlerde eşik değerin altındaki (< 0,1) RIΔ değeri, diüretik uygulamasına bağlı olarak postoperatif hem 1. hem de 4. haftalarda eşik değerin üzerine çıktı. Aynı zamanda diüretik uygulaması, eşik değerin üzerinde olan RIr değerinin daha da yükselmesine neden oldu. Diüretik uygulamasının bu iki değer üzerindeki olumlu etkisi, Yokoyama ve ark.'dan[4] farklı olarak, obstrüktif böbrek RI değerlerinde değişime neden olmadan, hem erken kronik (1. hafta) hem de geç kronik (4. hafta) dönemlerde non-obstruktif böbrekteki ortalama RI değerlerini düşürmesinden kaynaklanmaktaydı. Ancak zamana göre diüretik uygulamasının Doppler parametreleri üzerine etkisi olmadı.

Sonuç olarak, unilateral kısmi UO'nun Doppler US değerlendirilmesinde bir tanı kriteri olarak renal RI için 0,7 eşik sınırın tek başına yetersiz kaldığı görüldü. Yine de, tek taraflı yüksek renal RI değerleri belirlenen UO şüpheli olgularda diüretik uygulaması, RIΔ ve RIr değerlerinin eşik değerlerin üzerine çıkmasını sağlayarak, kronik unilateral kısmi UO'nun tanısal doğruluğunu artırabilir.

Etik Komite Onayı: Bu çalışma için gerekli etik izin, Ankara Üniversitesi Veteriner Fakültesi Etik Kurulu'ndan alınmıştır. (Tarih: 06.07.2004, Karar No: PAUHADYEK 2004-35).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - M.K., A.B.; Tasarım - M.K., A.B.; Denetleme - M.K., A.B.; Kaynaklar - M.K., A.B.; Malzemeler - M.K., A.B.; Veri Toplanmasıve/veya İşlemesi - M.K., A.B.; Analiz ve/veya Yorum -; M.K., A.B.; Literatür Taraması - M.K., A.B.; Yazıyı Yazan - M.K., A.B.; Eleştirel İnceleme - M.K., A.B.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Ethics Committee Approval: This study was approved by the ethical committee of Ankara University Faculty of Veterinary. (Date; 10.02.2022, Decision No: PAUHADYEK-2021/51).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.K., A.B.; Design - M.K., A.B.; Supervision - M.K., A.B.; Resources - M.K., A.B.; Materials - M.K., A.B.; Data Collection and/or Processing - M.K., A.B.; Analysis and/or Interpretation - M.K., A.B.; Literature Search - M.K., A.B.; Writing Manuscript - M.K., A.B.; Critical Review - M.K., A.B.; Other – M.K., A.B.;

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The authors declared that this study has received no financial support.

KAYNAKLAR

1. Apoku, IN, Ayoola OO, Salako AA, Idowu BM. Ultrasound evaluation of obstructive uropathy and its hemodynamic responses in southwest Nigeria. *International Braz J Urol* 2015;41:556-561. [Crossref]
2. Hayan F, Bacha R, Farooq MY, et al. Doppler comparison between ureteric obstruction and ureteric jet velocity. *EAS J Radiol Imaging Technol*. 2019;1:106-112. [Crossref]
3. Clarke DL. Feline ureteral obstructions part 1: medical management. *J Small Anim Pract*. 2018;59:324-33. [Crossref]
4. Yokoyama H, Tsuji Y. Diuretic Doppler ultrasonography in chronic unilateral partial ureteric obstruction in dog. *BJU Int*. 2002;90:100-104. [Crossref]
5. Müslümanoğlu AY, Tepeler A. Renal kolik tanısı ve tedavisi. *Marmara Med J*. 2008;21:187-192.
6. Lemieux C, Vachon C, Beauchamp G, Dunn ME. Minimal renal pelvis dilatation in cats diagnosed with bening ureteral obstruction by antegrade pyelograohy: a retrospective study of 82 cases (2012-2018). *J Fenile Med Surg*. 2021;23:892-899. [Crossref]
7. Clarke DL. Medical and surgical management of ureteral obstructions. *Adv Small Anim Care*. 2021;85-100. [Crossref]
8. Ikee R, Kobayashi S, Hemmi N, Imakiire T, Kikuchi Y, Moriya H, Suzuki S, Miura S. Correlation between the resistive index by Doppler ultrasound and kidney function and histology. *Am J Kidney Dis*. 2005;46:603-609. [Crossref]
9. Kaya M. The evaluation of renal hemodynamics with Doppler ultrasonography. In: Artis, AS (Ed), Hemodynamics-new diagnostic and therapeutic approaches. 1*st* ed. InTech, Rijeka, Croatia. 2012;1-30. [Crossref]
10. Cruz EG, Broca Garcia BE, Sandoval DM, et al. Renal resistive index as a predictor of acute kidney injury and Mortality in COVID-19 critically III. *Blood Purif*. 2022;51:309-316. [Crossref]
11. WU H, Liu K, Darko IN, et al. Predictive value of renal resistive index for the onset of acute kidney injury and its non-recovery: a systematic review and meta-analysis. *Clinical Nephrol*. 2020;93:172-186. [Crossref]
12. Evangelista GCL, Viana AG, Neves MM, Reis ECC. Resistivity and pulsatility indexes in feline kidney disease: a systematic review. *Vet Radiol Ultrasound*. 2022;63:306-318. [Crossref]
13. Halbgewachs C, Domes T. Postobstructive diuresis: Pay close attention to urinary retention. *Can Fam Physician*. 2015;61:137-42.
14. Li XD, Wu YP, Wei Y, Chen SH, Zheng QS, Cai H, Xue XY, Xu N. Predictors of recoverability of renal function after pyeloplasty in adults with ureteropelvic junction obstruction. *Urol Int*. 2018;100:209-215. [Crossref]
15. Di Nicolo P, Granata A. Renal intraparenchymal resistive index: the ultrasonographic answer to many clinical questions. *J Nephrol*. 2019;32:527-538. [Crossref]
16. Platt JF, Rubin JM, Ellis JH. Distinction between obstructive and nonobstructive pyelocaliectasis with duplex Doppler sonognaphy. *Am J Roentgenol*. 1989;153:997-1000. [Crossref]
17. Kaya M, Bumin A, Sen Y, Alkan Z. Comparison of Excretory Urography, Ultrasonography-Guided Percutaneous Antegrade Pyelography, and Renal Doppler Ultrasonography in Rabbits with Unilateral Partial Ureteral Obstruction: An Experimental Study. *Kafkas Üni Vet Fak Derg*. 2010;16:735-741. [Crossref]
18. Kaya M, Çetinkaya MA. Evaluation of acute partial unilateal obstruction based on the renal venous impedance index in rabbit: an experimental study. *Ankara Univ Vet Fak Derg*. 2022;69:259-264. [Crossref]
19. Lim GY, Jang HS, Lee EJ, Lim YS, Jung SE, Park SH. Utility of the resistance index ratio in differentiating obstructive from nonobstructive hydronephrosis in children. *J Clin Ultrasound*. 1999;27:187-193. [Crossref]
20. Riahinezhad M, Sarrami AH, Gheisari A, Shafaat O, Merikhi A, Karami M, Farghadani M, Moslehi M. How may Doppler indices help in the differentiation of obstructive from nonobstructive hydronephrosis? *J Res Med Sci*. 2018;23:76. [Crossref]
21. Shokeir AA, Nijman RJM, El-Azab M, Provoost AP. Partial ureteral obstruction. Effect of intravenous normal saline and furosemide upon the renal resistive index. *J Urol*. 1997;157:1074-1077. [Crossref]

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.223353

Systemic Effect of Metformin on Bone Healing at Bone Defect in Rabbits Using Radiographical and Serum Alkaline Phosphatase Assessment

Kemik Defektli Tavşanlarda Metforminin Kemik İyileşmesi Üzerindeki Sistemik Etkisinin Radyografik ve Serum Alkalin Fosfataz Kullanılarak Değerlendirilmesi

Raad MAHMOOD HUSSEIN[1]
Ghada ABDULRAHMAN TAQA[2]

[1]Ministry of Health, Kirkuk Health Directorate, Kirkuk, Iraq
[2]Department of Dental Basic Sciences, University of Mosul, College of Dentistry, Mosul, Iraq

ABSTRACT

Clinical therapy for disorders of bone healing present serious challenges. The delivery of the bone filling materials necessitates surgical implantation at the fracture site, which could lead to local complications. As a result, taking osteogenic medications will offer a great way to heal bone lesions. Increased osteoblasts and decreased osteoclasts are 2 ways that metformin has an osteogenic impact. The aim of the study is to determine the systemic impact of metformin on bone healing at the site of a bony defect using radiographic and serum alkaline phosphatase testing. A total of 20 mature male rabbits, divided into 2 groups of 10 each for the treatment group and the control group, were utilized. All rabbits underwent identical surgical procedures under general anesthesia. Two holes that were 3 mm in diameter and 3 mm deep were created and left empty after the femur has been surgically exposed. The study was conducted over 28 days. The rabbits in the treatment group received 50 mg/kg of metformin orally daily for 28 days. The animals were sacrificed at 2 different time points, according to their groups, on the 14th and 28th day after surgery. Bone samples from the defect site of the femur were isolated, sectioned, assessed radiographically, and blood was drawn for serum alkaline phosphatase measurement. There was an increase in bone mineral density and osseointegration. In addition, serum alkaline phosphatase increased in the animals of the group treated with metformin than in the control group at both study time periods. Metformin increases bone healing and regeneration at the bone defect sites and enhances the process of osteogenesis and osseointegration more than the control untreated rabbits.

Keywords: Bone mineral density; bone defect; bone healing; metformin; serum alkaline phosphatase

ÖZ

Kemik iyileşme bozukluklarının klinik tedavisi ciddi zorluklar barındırır. Ancak, kemik dolgu malzemelerinin uygulanması yaralanma yerinde cerrahi implantasyon gerektirir, bu da yerel komplikasyonlara yol açabilir. Bu nedenle, osteojenik ilaçları almak kemik lezyonlarının iyileşmesine yardımcı olacaktır. Artan osteoblast ve azalan osteoklastlar metforminin osteojenik etkisini temsil eden iki yoldur. Çalışmanın amacı, metformin uygulamasının kemik defekti bölgesinde kemik iyileşmesi üzerindeki sistemik etkisini radyografik ve serum alkalin fosfataz testleri kullanarak belirlemektir. Çalışmada, toplam 20 yetişkin erkek tavşan onarlı iki gruba bölünerek kullanılmıştır. Tavşanların tamamı genel anestezi altında aynı cerrahi prosedüre maruz kaldılar. Femur cerrahi olarak ekspoze edildikten sonra üzerine 3 mm çapında ve 3 mm derinliğinde iki delik açıldı ve delikler boş bırakıldı. Çalışma 28 gün sürdü. Çalışma grubundaki tavşanlar, operasyonun ardından 28 gün boyunca günde 50 mg/kg oral metformin aldı. Hayvanlar araştırmadaki numaralarına ve gruplarına göre iki farklı zaman aralığında, operasyondan sonraki 14'üncü ve 28'inci günlerde sakrifiye edildiler. Femur defekt bölgesinden alınan kemik örnekleri, ayrıştırıldı, kesildi, radyografik olarak değerlendirildi ve serum alkalin fosfataz ölçümü için kan alındı. Metformin alan hayvanların kontrol grubuna kıyasla çalışma zamanlarının her ikisinde de kemik mineral yoğunluğu ve osseointegrasyon artışı olduğunu ve ayrıca serum alkalin fosfataz artışı olduğunu göstermiştir. Sonuç olarak, metformin, kontrol grubunda tedavi uygulanmayan tavşanlara kıyasla kemik defekti bölgesinde kemik iyileşmesini ve yenilenmesini artırmakta ve osteogenezis ve osseointegrasyon sürecini daha fazla güçlendirmektedir.

Anahtar Kelimeler: Metformin, BMD, serum alkalin fosfataz, kemik iyileşmesi, kemik defekti

Received/Geliş Tarihi: 29.09.2022
Accepted/Kabul Tarihi: 20.12.2022
Publication Date/Yayın Tarihi: 29.12.2022

Corresponding Author/Sorumlu Yazar: Raad Mahmood Hussein
E-mail: azawdraad015@gmail.com

Cite this article: Mahmood Hussein R, Abdulrahman Taqa G. Systemic Effect of metformin on bone healing at bone defect in rabbits using radiographical and serum alkaline phosphatase assessment. *Vet Sci Pract*. 2022; 17(3), 91-97.

Atıf: Mahmood Hussein R, Abdulrahman Taqa G. Kemik defektli tavşanlarda metforminin kemik iyileşmesi üzerindeki sistemik etkisinin radyografik ve serum alkalin fosfataz kullanılarak değerlendirilmesi. *Vet Sci Pract*. 2022; 17(3), 91-97.

INTRODUCTION

Bones provide a structural function and cover vital organs. In addition, bones serve as storage for minerals and growth factors as well as a site for the creation of blood cells. Their physiological function is closely linked to the presence of stem cells, which are crucial regulators of cell activities.[1]

One of the most frequent traumatic injuries is a bone fracture. Although the majority of minor fractures recover within a few weeks, 5% to 10% of long bone fractures take 6–8 months to heal properly. Current therapies, including autologous bone transplants, intramedullary nails or fixation plates, and bone morphogenetic protein (BMP)-based therapy do not always enable full bone restoration.[2]

Bone regeneration can occur either by endochondral ossification or by intramembranous ossification processes. Mesenchymal stem cells (MSCs) immediately differentiate into osteoblasts upon intramembranous ossification, which leads to the deposition of mineralized extracellular matrix. Endochondral ossification goes through stages such as inflammation, development of soft to hard callus, and remodeling of the fracture site.[3]

Bone is a highly dynamic tissue. Maintaining homeostatic bone metabolism and skeletal strength requires a series of coordinated steps known as "bone remodeling."[4] Clinical judgments, radiographic analyses, and serological tests are used to evaluate bone healing.[5] Malignant bone tumors and serious trauma can remove a considerable amount of bone, resulting in severe bone defect. In addition, bone abnormalities that necessitate bone augmentation treatments are typically present with fractures sustained from high energy trauma and osteoporotic fractures.[6]

Several drugs with very different indications and exhibiting a pleiotropic spectrum of actions are used to target the local and systemic regulation of bone metabolism. These include antihyperlipidemic drugs such as (HMG-CoA reductase inhibitors), antihypertensive drugs (such as ACE inhibitors), drugs for osteoporosis (bisphosphonates), cancer drugs (inhibitors proteasome), and other drugs.[7] Metformin works by preventing the production of hepatic gluconeogenesis, increasing the density of low and high affinity insulin receptors, and lowering resistance to the peripheral effects of insulin.[8] Metformin treatment for diabetic patients has been shown to reduce TNF expression. The pharmacological action of metformin goes beyond simple glycemic control, decreasing the markers of inflammation, and contributing to the reduction of oxidative stress with a confirmed anti-inflammatory effect.[9] In the setting of chronic periodontal inflammation, MSCs have a significantly reduced potential for osteogenic differentiation. A highly effective strategy to stimulate or restore the osteogenic potential of MSCs in an inflammatory environment remains an unrealized goal.[10]

Metformin belongs to a group of drugs known as biguanides, which work well both alone and in conjunction with other hypoglycemic medications. Metformin is typically well accepted, has few adverse effects, and is relatively inexpensive.[11] Therefore, this study focuses on the effect of metformin on bone regeneration.

During the maturation and mineralization phases of the newly formed osteoid, alkaline phosphatase (ALP), which can be detected in serum, is secreted into extracellular fluid by osteoblasts. However, only around 50% of the ALP activity in healthy people's blood is derived from bone, with the remaining 50% largely coming from the liver. The bone-derived isoform (BALP) can be more specifically detected by some techniques.[12]

MATERIAL AND METHODS

Experimental Model

Twenty adult male New Zealand rabbits weighing 1.75 kg to 2 kg and aged between 6 and 8 months were utilized. The animals were sacrificed at the end of the experiments (at 14 and 28 days) using an overdose of general anesthesia (ketamine 200 mg/kg + xylazine 40 mg/kg)[13]. The study was carried out in agreement with the Institutional Animal Research Ethics Board's guidelines as of June 19, 2022 (UoM.Dent/A.L.58/22).

Medication

The drug used in this study is metformin tablets 500 mg (the smallest dose available to control the administered dose)

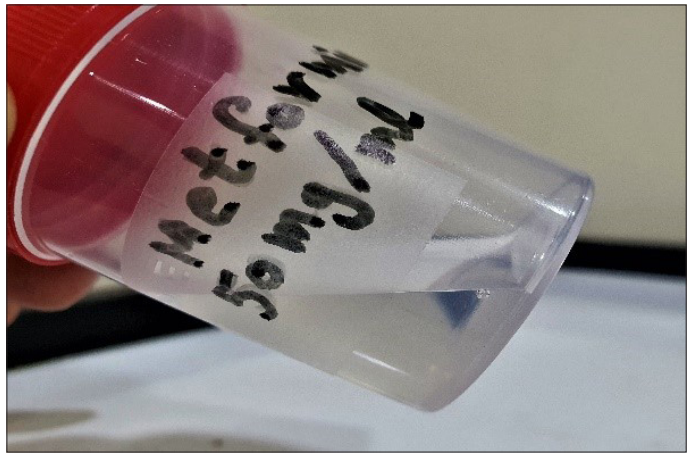


Figure 1. Glucophage®(metformin 500 mg) tablets / prepared liquid metformin.

produced by the well-known German brand company, MERCK under the trade name, Glucophage.

Metformin in Iraq and most of countries is available as tablets only. Therefore, we were prepared it in the form of liquid by fine grinding of the tablets to obtain fine powder. Each 500 mg tablet was ground alone; the resultant fine powder of this tablet was filled into a hard gelatin capsule to control the amount of drug (500 mg per capsule). For oral administration of the drug, the content of each capsule was dissolved in 10 mL of distilled water with vigorous shaking for at least 2 mins to obtain homogenous solution containing 500 mg/10 mL of the drug (Figure 1). Though metformin is essentially insoluble in organic solvents like acetone, ether, and chloroform, it is readily soluble in water.[14] Metformin liquid was administered to the rabbits orally in a dose of 50 mg/kg once daily using a feeding tube and pushed through a graduated syringe to give the exact and accurate dose.[15] The rabbits in the treatment group received 50 mg/kg of metformin orally daily for 28 days.

Study Design

Group 1: Control group that received no medication (n = 10). This group was subdivided into 2 groups of 5 rabbits for each period, according to the sacrifice date (day 14 and 28 post-surgery).

Group 2: Treatment group that was systemically treated with metformin (n = 10). After the surgical procedure, metformin was administered orally in a once daily dose of (50 mg/kg) body weight [15] using feeding tube. This group was subdivided into 2 groups of 5 rabbits for each period, according to the sacrifice date (day 14 and 28 post-surgery).

Preparation of Animals for Surgery

All the 20 rabbits received anesthesia by intramuscular injection. Each rabbit received an intramuscular injection of a mixture of ketamine (KETALROM-50), 40 mg/kg and xylazine (Holland), 4 mg/kg.[16]

Animal Surgical Procedure

After 20 mins, the animal gained anesthesia,[17] and the surgery was performed on the left femur bone.

Two holes, 3 mm in diameter and 3 mm deep, were created in the exposed femur diaphysis, using 2000 rpm low speed straight surgical hand piece with a 3 mm round carbide bur and continuous normal saline irrigation (Figure 2).[18]

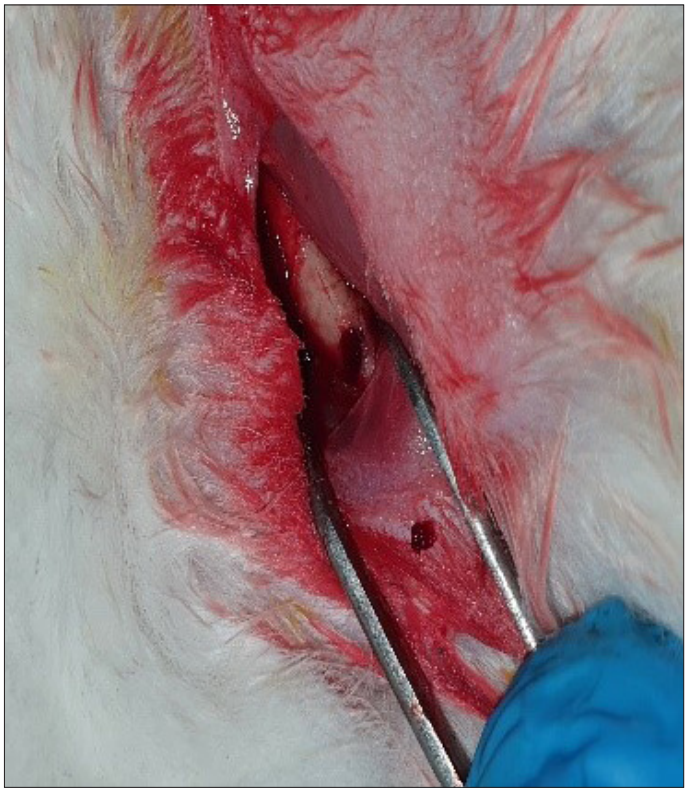


Figure 2. Two holes were made in the femur bone of each of the 20 rabbits.

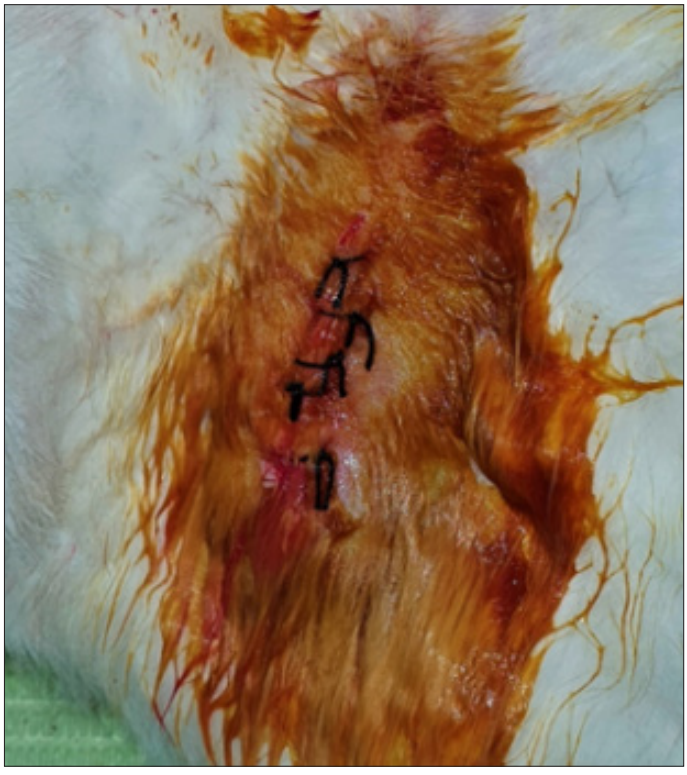


Figure 3. Suturing and disinfection of the wound

The bone defects (holes) were left empty without any material. The wound was closed using a 3/0 black silk suture and rubbed well with povidone iodine 10% as shown in (Figure 3).

Postoperative Care of Animals

After the surgical procedure, the animals were given oxytetracycline 20% injection (Limoxin-200 LA® [Holland]) as a prophylactic antibiotic for wound healing, administered as a single daily dose of 0.5 mL/ kg intramuscularly for 3 consecutive days from the operation day.

Criteria of the Study

Biochemical Criteria

Blood Sample Preparation

After the surgery, on days 14 and 28, the animals were sacrificed and blood was drawn directly from the jugular vein. The serum was then separated and stored until it was used for analysis of serum ALP.

Determination of Serum Alkaline Phosphatase for Rabbits Using the Spectrophotometer

Materials Supplied:

The whole kit was stored in a refrigerator at temperatures between 2°C and 8°C until the time of analysis, during which the kit should reach to room temperature (20°C–25°C).

Reagents:

1- Vial R1 (substrate-buffer):

Disodium phenyl phosphate 5 mmol/L

Carbonate-bicarbonate buffer pH 10 50 mmol/L

Stabilizer

2- Vial R2 (standard):

Phenol corresponding to 20 U king and kind

3- Vial R3 (blocking reagent) (Toxic)

4-amino antipyrine 60 mmol/L

Sodium arsenate 240 mmol/L

4- Vial 4 (dye reagent)

Procedure

Reagents and specimens were at room temperature. Tubes were prepared as shown in Table 3.

Reagent 1 was prepared and incubated for 5 mins at 37°C. Reagent 2 was prepared and was let to stand exactly 15 mins at 37°C.

The reagents were mixed and then incubated for 10 mins at room temperature and were stored away from light. Read absorbances of the blank specimen, standard, and assay at 510 nm against reagent blank. Coloration was stable for 45 mins away from light.

Radiological Criteria

At the time of animal sacrificing, the site of bone defect (hole) and around it in the femur bone were taken directly for radiographical examination. To assess the degree of bone development, the specimens were examined using a digital radiography machine.

In this criterion, the linear measurement was not used, instead the densitometric analysis were used to measure the bone mineral density at the desired area in the hole. (Figure 4).

RESULTS

Serum Alkaline Phosphatase

On day 14, serum ALP was measured for both groups. That of the control group was used as a reference to compare with the

Figure 4. Radiographical machine, sensor, and control.

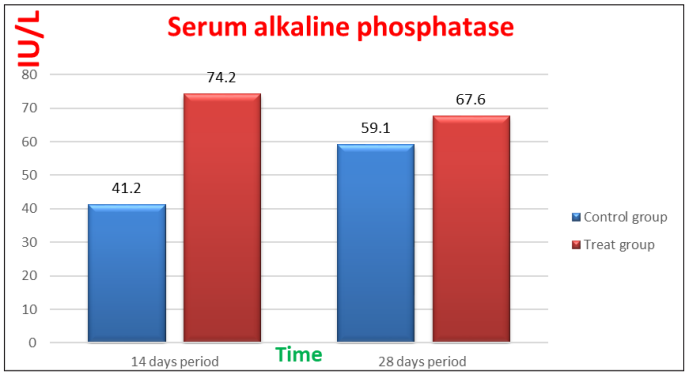


Figure 5. The histogram of the statistical analysis of serum alkaline phosphatase measured in (IU/L).

Table 1. Alkaline phosphatase levels at both groups at different periods.

Group Time	14 days period	28 days period
Control group	41.2 ± 0.73	59.1 ± 0.9
Treat group	74.2 ± 1.53	67.6 ± 1.36
P value	.0001	.0001

treatment group. An independent sample *t* test (using IBM SPSS 26) showed a highly significant difference in serum ALP at day 14 between the control group (41.2 ± 0.73) IU/L and the treatment group (74.2 ± 1.53) IU/L ($P \leq .001$), as shown in Table 1 and Figure 5.

Independent sample *t* test showed highly significant difference in serum ALP on day 28 between the control group (59.1 ± 0.9) IU/L and the treatment group (67.6 ± 1.36) IU/L ($P \leq .001$), as shown in Table 1 and Figure 5.

Radiological Results

In this criterion, densitometric analysis were used to measure the bone mineral density at the desired area in the hole. Also, gross radiographical assessment was used to observe the effect between various groups. Radiological criteria are a good indication of new bone formation at the site of the hole and for comparison between the holes of different sites.

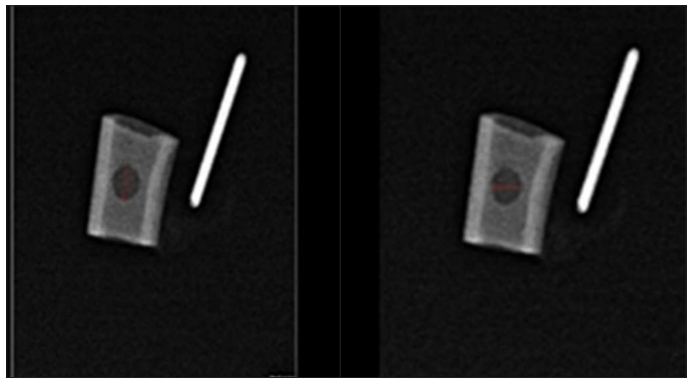


Figure 6. Densitometric study of the femur bone hole in the control group after 14 days.

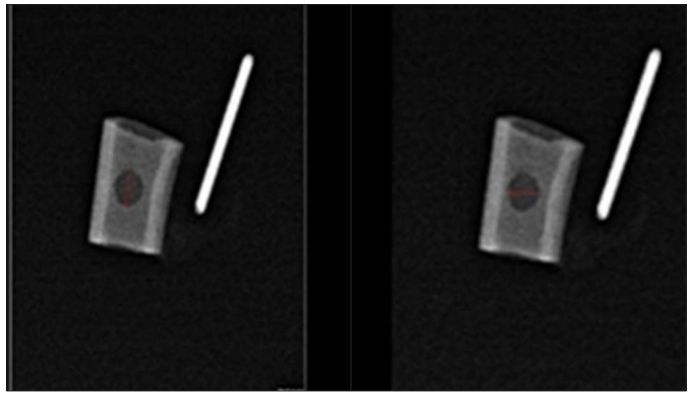


Figure 7. Densitometric evaluation of the treated group's 14-day femur bone hole.

Table 2. Bone mineral density mean ± standard error at different time periods.

Group Time	14 days	28 days
Control group	134.5 ± 1.5	160.5 ± 1.7
Treated group	135.75 ± 1.3	171.25 ± 0.85
P	0.085	0.001

On day 14, independent sample *t* test showed that there was no significant difference ($P \geq .05$) in bone mineral density (BMD) between the control group (134.5 ± 1.5) the metformin-treated group (135.75 ± 1.3). The treatment group slightly exceeded the control group in BMD, and there was gross observable difference in radiopacity between these 2 groups, as shown in Table 2, and Figures 6 and 7.

On day 28, obvious difference was observed in gross radiopacity between the control and treatment groups. In addition, there was a significant difference in BMD at day 28 between the control group (160.5 ± 1.7) and the treatment group (171.25 ± 0.85) ($P \leq .05$), as shown in Table 2 and Figures 8 and 9.

DISCUSSION

In this study, serum ALP levels indicate that the metformin-treated group experienced an increase in the level of enzyme more than the control group during both time intervals of 14 and 28 days. Serum ALP can be used as an indicator to measure the activity of bone formation. Studies suggest that assessing markers of bone formation such as serum ALP throughout the healing of fractures could increase the accuracy of the evaluation of the

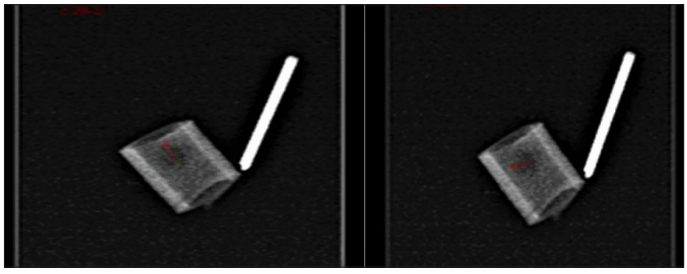


Figure 8. Densitometric analysis of the femur bone hole of the control group at 28 days period.

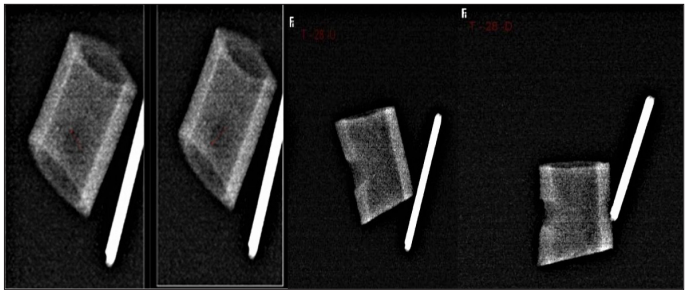


Figure 9. Densitometric analysis of the femur bone hole of the treated group at 28 days period.

Table 3. Manual procedure of serum alkaline phosphatase measurement.

	Reagent blank	Specimen blank	Standard	Assay
Reagent R1	2 mL	2 mL	2 mL	2 mL
Incubated for 5 mins at 37°C				
Specimen				50 µL
Reagent 2 (standard)			50 µL	
Let to stand exactly 15 mins at 37°C				
Reagent R3	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Mixed well				
Reagent R4	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Specimen		50 µL		
Demineralized water	50 µL			

bone healing stage and enable early identification of patients at risk for developing delayed union or nonunion.[19] This supports the choice of serum ALP as a marker in our study.

Because of its ease of use in individuals with normal liver function, total serum ALP is a frequently applied marker of bone metabolism. ALP levels also suggest normal osteoblast activity and new bone development.[20]

This study uses serum ALP as an indicator of bone healing by comparing its level to that of the control group (baseline), despite the fact that there are few studies establishing the role of this enzyme in the evaluation of the healing process following fractures. The bone-isoenzyme of ALP (BsALP), which is believed to be a more accurate marker of bone formation, was not available at our center and had a high cost. The healing of fractures is highly associated with serum ALP levels. Tissue-nonspecific ALPs make up the majority of the enzyme fraction circulating in serum, which is of therapeutic importance.[21]

The circulating enzyme in healthy people is mostly derived from liver and bone. The hydrolysis of organic phosphate esters that are present in the extracellular space is catalyzed by a group of isoenzymes called ALPs, which are found on the outer layer of the cell membrane. The cofactors magnesium and zinc are essential for this enzyme. Though ALPs are present in a variety of body regions and exhibit a variety of physiochemical properties, they are actual isoenzymes because they catalyze the same biochemical reaction. More than 80% of the ALP in serum is released from the liver and bone, with small amounts from the intestines.[22]

Serum total ALP activity is frequently used as a biochemical marker of osteoblast function, however, because of contribution of its activity, it lacks great specificity. It is particularly derived from the liver. About half of the serum ALP activity in healthy people comes from bone and the other half from the liver.[19]

The results of our study in which there was an increase in serum ALP at both time intervals of the study, seems to correspond with a study on humans after orthognathic surgery in which ALP and bone-specific ALP were used as markers of bone formation and osteoblastic activity. Enzyme levels significantly decreased 1 day and from 1 day to 1 week, respectively, after surgery. They then increased to their maximum values by 1 month before gradually returning to preoperative levels by six months.[23]

In agreement with this study, the biochemical parameters were assessed during fracture healing in 7 dogs with femoral fractures stabilized using string of pearls locking plates, in another study. Serum ALP was tested on the day prior to surgery, as well as on days 15 and 45 after the surgical operation. The results of a one-way ANOVA statistical analysis showed that there was a highly significant difference between the 3 intervals. The 15th postoperative day showed the highest value, whereas the 45th postoperative day showed the lowest value.[24]

Metformin significantly stimulated the gene and protein expression of essential osteoblastic transcription factors such as Runt-related transcription factor2 (RUNX2), enhanced ALP activity, and mineral deposition.[25] The increase in ALP enzymatic activity is a result of metformin's ability to stimulate cell proliferation and promote osteogenic differentiation. The stimulation of cells' proliferative activity and their differentiation into osteogenic cells appear to be solely dependent on metformin concentration, and this is according to one of the key findings of *in vitro* research that examine metformin-related osteogenic activity[26] and the dose used in our study exhibit osteogenecity.

Metformin increases BMP-2 expression through increasing ALP and osteocalcin (OCN) secretion, and this will increase BMD. Through the transactivation of genes via adenosine monophosphate-activated protein kinase (AMPK) regulation, metformin promotes osteoblast differentiation. The major osteogenic genes such ALP have been demonstrated to be expressed more strongly when AMPK is activated.[27]

All the previously mentioned studies agree with the results of the present study, which confirms the role of systemic metformin administration at a dose of 50 mg/kg on increasing serum ALP levels at 14 and 28 days after the surgical induction of bone defect.

It is observed in this study that the animals treated with metformin showed greater bone growth and mineralization than the control group. Despite the small and insignificant difference between the 2 groups on day 14, there was a significant difference between metformin and the untreated group on day 28.

The production of calcium and phosphorus-rich deposits in mineralized extracellular matrix is another sign of metformin's osteogenic activity as determined by an *in vitro* model.[28]

It appears that metformin's anti-adipogenic properties may be linked to its protective effects on bone tissue.[29]

Independent of age, BMI, or GFR, metformin administration is linked to a decreased incidence of osteopenia and osteoporosis, particularly in the female population.[30]

Other animal studies that examined the impact of metformin on bone and supported our findings revealed that metformin can raise bone mineral density and lessen bone loss in rats. In addition, both diabetic and nondiabetic rats treated with metformin had better bone repair.[31]

A recent study examined the effectiveness of metformin and glucosidase inhibitors on BMD in patients with type 2 diabetes mellitus (T2DM) and explored its mechanism of action in treating osteoporosis induced by T2DM, thereby providing references for the prevention and treatment of osteoporosis. It is obvious from this study's data and results that metformin increases the amount of bone formed and plays a critical role in preventing osteoporosis.[32] By reducing systemic inflammation and encouraging the production of osteoclasts, metformin can prevent osteoporosis.[33] Also, by enhancing BMP-2 expression and raising ALP and OCN secretion, it increased BMD.[34] Metformin promotes stimulation of osteopontin (OPN) and lowers RANKL expression in osteoblasts in rats with ovariectomies; according to Mai *et al*., osteogenesis was controlled by AMPK through OPN, whereas adipogenesis was suppressed.[35]

There is expectation that metformin can help prevent fractures induced by osteoporosis. Through a variety of receptors, including AMPK, which is crucial for bone regeneration, metformin regulates bone quality. In addition, metformin blocks the production of advanced glycation end products (AGEs), which may enhance bone turnover because AGEs inhibit osteoblasts (bone-forming cells) and activate osteoclasts.[36]

These data demonstrate that more complicated metabolic processes, particularly in T2DM, are the cause of fragility. The generation of AGEs and reactive oxygen species is induced by hyperglycemia. These substances may lead to improper bone homeostasis through oxidative stress.[37]

Our radiographic findings were consistent with a study showing that systemic metformin use improves osseointegration by raising bone filling percentages. During the 4-week osseointegration period, statistically significant variations in bone filling were found between the dental implants in the control and metformin-treated groups.[38]

Metformin has been proven in numerous studies to have anti-inflammatory and antioxidative stress effects on a number of disorders, including rheumatoid arthritis, neuropathic pain, renal problems, and Ankylosing spondylitis.[39] Therefore, metformin as it seems counteracts the oxidative stress and inflammation at bone lesion sites and promote osteogenesis.

Metformin use consistently increases the level of bone formation markers such as serum ALP, bone mineral density, and greater radiopacity observed by radiographic evaluation in metformin-treated animals compared to that of the control group at 2 different time intervals.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Institutional Animal Research Ethics Board's (Date: 19.06.2022 Decision No: UoM.Dent/A.L.58/22).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.R.M., T.G.A.; Design - H.R.M., T.G.A.; Supervision - T.G.A.; Resources - H.R.M.; Materials - H.R.M.; Data Collection and/or Processing - H.R.M.; Analysis and/or Interpretation - H.R.M.; Literature Search - H.R.M.; Writing Manuscript - H.R.M.; Critical Review - T.G.A.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Hayvan Hakları Etik Kurulu'ndan alınmıştır (Tarih: 19.06.2022, Karar No: UoM.Dent/A.L.58/22).

Hakem değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - H.R.M., T.G.A.; Tasarım - H.R.M., T.G.A.; Denetleme - T.G.A.; Kaynaklar - H.R.M.; Malzemeler - H.R.M.; Veri Toplanmasıve/veya İşlemesi - H.R.M.; Analiz ve/veya Yorum - H.R.M.; Literatür Taraması - H.R.M.; Yazıyı Yazan - H.R.M.; Eleştirel İnceleme - T.G.A.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Toosi S, Behravan J. Osteogenesis and bone remodeling: A focus on growth factors and bioactive peptides. *Biofactors*, 2020;46(3), 326-340. [Crossref]
2. Schlundt C, Bucher CH, Tsitsilonis S, Schell H, Duda GN, Schmidt-Bleek K. Clinical and research approaches to treat non-union fracture. *Curr Osteoporos Rep*, 2020;16(2),155-168. [Crossref]
3. Pajarinen J, Lin T, Gibon E, Kohno Y, Maruyama M, Nathan K. and Goodman, S. B. Mesenchymal stem cell-macrophage crosstalk and bone healing. *Biomaterials*, 2019;196,80-89. [Crossref]
4. Raggatt LJ, and Partridge NC. Cellular and molecular mechanisms of bone remodeling. *J Biol Chem*, 2010;285(33), 25103-25108. [Crossref]
5. Voller T, Ahmed M, Ho S, Kozhikunnath A, Bendall S. Measuring bone healing in fractures and fusions. *Orthop Trauma,* 2022;36(4), 218-222. [Crossref]
6. Nauth A, Schemitsch E, Norris B, Nollin Z, Watson JT. Critical-size bone defects: is there a consensus for diagnosis and treatment?. *J Orthop Trauma,* 2018;32,7-11. [Crossref]
7. Rothe R, Schulze S, Neuber C, Hauser S, Rammelt S, Pietzsch J. Adjuvant drug-assisted bone healing: Part III–Further strategies for local and systemic modulation. *Clin Hemorheol Microcirc*, 2019;73(3),439-488. [Crossref]
8. Bucher S, Bauduceau B, Benattar-Zibi L, Bertin P, Berrut G, Corruble E, Becquemont L. Primary care management of non-institutionalized elderly diabetic patients: *The S. AGES cohort–Baseline data. Prim Care Diabetes.* 2015;9(4),267-274. [Crossref]
9. Andrews M, Soto N, Arredondo M. Effect of metformin on the expression of tumor necrosis factor-α, Toll like receptors 2/4 and C reactive protein in obese type-2 diabetic patients. *Rev Med Chil*, 2012;140(11), 1377-1382. [Crossref]

10. Ren C, Hao X, Wang L, Hu Y, Meng L, Zheng S, Sun H. Metformin Carbon Dots for Promoting Periodontal Bone Regeneration via Activation of ERK/AMPK Pathway. *Adv Healthc Mater,* 2021;10(12), 2100196. [Crossref]
11. Sanchez-Rangel E, Inzucchi SE. Metformin: clinical use in type 2 diabetes. *Diabetologia*, 60(9), 1586-1593 (2017). [Crossref]
12. Vasikaran S, Eastell R, Bruyere O, Foldes AJ, Garnero P, Griesmacher A, Kanis JA. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. *Osteoporos Int*, 2011;22(2),391-420. [Crossref]
13. De Lima Santos A, da Silva CG, de Sá Barreto LS, Leite KRM, Tamaoki MJS, Ferreira LM, Faloppa F. A new decellularized tendon scaffold for rotator cuff tears–evaluation in rabbits. *BMC Musculoskelet Disord*, 2020;21(1), 1-12. [Crossref]
14. Kumar AB, Mohan S, Sakthi RS, Ramkanth S. A *Review On Clinical Pharmacokinetic And Pharmacodynamic Profile Of Metformin* (2020).
15. Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Restoration of plasma markers of liver and kidney functions/integrity in alloxan-induced diabetic rabbits by aqueous extract of Pleurotus tuberregium sclerotia. *Biomed Pharmacother.* 2017;95, 1809-1814. [Crossref]
16. Ahirwar LK, Kalra P, Sharma S, Mohamed A, Mittal R, Das S, Bagga B. Linezolid shows high safety and efficacy in the treatment of Pythium insidiosum keratitis in a rabbit model. *Exp Eye Res*, 2021;202,108345. [Crossref]
17. Jiron JM, Mendieta Calle JL, Castillo EJ, Abraham AM, Messer JG, Malphurs WL, Aguirre JI. Comparison of isoflurane, ketamine–dexmedetomidine, and ketamine–xylazine for general anesthesia during oral procedures in rice rats (Oryzomys palustris). *J Am Assoc Lab Anim Sci,* 2019;58(1),40-49. [Crossref]
18. Nguyen-Thanh T, Nguyen-Tran BS, Cruciani S, Dang-Cong T, Maioli M. A rabbit femoral trochlear defect model for chondral and osteochondral regeneration. *Acta Veterinaria Brno*, 2022;91(3), 293-301. [Crossref]
19. Sipani AK, Dhar A, Sungte N. Serum alkaline phosphatase: A prospective biomarker for assessment of progress of fracture healing in diaphyseal fractures of long bones in adult patients. *Int J Orthop*, 2020;6(2),248-251. [Crossref]
20. Seibel MJ. Biochemical markers of bone turnover part I: biochemistry and variability. The Clinical biochemist. *Reviews/Australian Association of Clinical Biochemists*., 2005;26(4),97.
21. Castells L, Cassanello P, Muñiz F, de Castro MJ, Couce ML. Neonatal lethal hypophosphatasia: A case report and review of literature. *Medicine*, 2018;97(48). [Crossref]
22. Pinart M, Kunath F, Lieb V, Tsaur I, Wullich B, Schmidt S. Prognostic models for predicting overall survival in metastatic castration-resistant prostate cancer: a systematic review. *World J Urol*, 2020;38(3), 613-635. [Crossref]
23. Abe Y, Chiba M, Yaklai S, Pechayco RS, Suzuki H, Takahashi T. Increase in bone metabolic markers and circulating osteoblast-lineage cells after orthognathic surgery. *Sci Rep*, 2019;9(1),1-10. [Crossref]
24. Reddy GAK, Kumar VG, Raghavender, KBP, Kumar DP. Evaluation of haemato-biochemical parameters for assessment of fracture healing in dogs. *Pharma Innovation J*, 2020;9(9),123-125. [Crossref]
25. Wang P, Ma T, Guo D, Hu K, Shu Y, Xu HH, Schneider, A. Metformin induces osteoblastic differentiation of human induced pluripotent stem cell‐derived mesenchymal stem cells. *J Tissue Eng Regen Med*, 2018;12(2),437-446. [Crossref]
26. Molinuevo MS, Schurman L, McCarthy AD, Cortizo AM, Tolosa MJ, Gangoiti MV, Sedlinsky C. Effect of metformin on bone marrow progenitor cell differentiation: in vivo and in vitro studies. *J Bone Miner Res*, 2010;25(2),211-221. [Crossref]
27. Jang WG, Kim EJ, Bae IH, Lee KN, Kim YD, Kim DK, Koh JT. Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. *Bone*, 2011;48(4), 885-893. [Crossref]
28. Śmieszek A, Tomaszewski KA, Kornicka K, Marycz K. Metformin Promotes Osteogenic Differentiation of Adipose-Derived Stromal Cells and Exerts Pro-Osteogenic Effect Stimulating Bone Regeneration. *J. Clin. Med*, 2018;7,482. [Crossref]
29. Luo T, Nocon A, Fry J, Sherban A, Rui X, Jiang B, Zang M. AMPK activation by metformin suppresses abnormal extracellular matrix remodeling in adipose tissue and ameliorates insulin resistance in obesity. *Diabetes*, 2016;65(8), 2295-2310. [Crossref]
30. Sun J, Liu Q, He H, Jiang L, Lee KO, Li D, Ma J. Metformin treatment is associated with an increase in bone mineral density in type 2 diabetes mellitus patients in China: a retrospective single-center study. *Diabetes Metab*, 2022;101350. [Crossref]
31. Mai QG, Zhang ZM, Xu S, Lu M, Zhou RP, Zhao L, Bai XC. Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. *J Cell Biochem*, 2011;112(10),2902-2909. [Crossref]
32. Kendler D, Chines A, Clark P, Ebeling PR, McClung M, Rhee Y, Stad RK. Bone mineral density after transitioning from denosumab to alendronate. *J Clin Endocrinol Metab*, 2020;105(3), e255-e264. [Crossref]
33. Zhou Q, Guan Z, Liu S, Xuan Y, Han G, Chen H, Guan Z. The effects of metformin and alendronate in attenuating bone loss and improving glucose metabolism in diabetes mellitus mice. *Aging (Albany NY),* 2022;14(1), 272. [Crossref]
34. Zheng L, Shen X, Ye J, Xie Y, Yan S. Metformin alleviates hyperglycemia-induced apoptosis and differentiation suppression in osteoblasts through inhibiting the TLR4 signaling pathway. *Life sciences*, 2019;216, 29-38. [Crossref]
35. Wang YG, Qu XH, Yang Y, Han XG, Wang L, Qiao H, Dai KR. AMPK promotes osteogenesis and inhibits adipogenesis through AMPK-Gfi1-OPN axis. *Cell Signal*, 2016;28(9),1270-1282. [Crossref]
36. Gonnelli S, Caffarelli C, Giordano N, Nuti R. The prevention of fragility fractures in diabetic patients. *Aging Clin and Exp Res*, 2015;27(2), 115-124. [Crossref]
37. Yang P, Feng J, Peng Q, Liu X, Fan Z. Advanced glycation end products: potential mechanism and therapeutic target in cardiovascular complications under diabetes. *Oxid Med Cell Longev,* (2019). [Crossref]
38. Yıldırım TT, Dündar S, Bozoğlan A, Karaman T, Kahraman OE, Özcan EC. The effects of metformin on the bone filling ration around of TiAl6Va4 implants in non diabetic rats. *J Oral Biol Craniofac Res*, 2020;10(4),474-477. [Crossref]
39. Afshari K, Dehdashtian A, Haddadi NS, Haj-Mirzaian A, Iranmehr A, Ebrahimi MA, et al. Anti-inflammatory effects of metformin improve the neuropathic pain and locomotor activity in spinal cord injured rats*:* introduction of an alternative therapy. *Spinal cord*, 2018;56(11), 1032-1041. [Crossref]

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.222745

Evaluation of CBC, Lipid Profile, Oxidative Stress Biomarkers, Total Thiol, Native Thiol, and Disulfide Levels in Dogs Diagnosed with Parvoviral Enteritis Without Clinical Findings

Klinik Bulgular Görülmeden Parvoviral Enteritis Tanısı Konulan Köpeklerde, CBC, Lipid Profili, Oksidatif Stres Biyobelirteçleri, Total Thiol, Native Thiol ve Disülfid Düzeylerinin Değerlendirilmesi

Efe KURTDEDE[1]
Mehmet Kazım BÖRKÜ[2]
Berna GÜR[2]
Yalçın DOĞAN[2]
Kadir SEVİM[2]
Erman GÜLENDAĞ[3]

[1]Department of Biochemistry, Ankara University, Faculty of Veterinary Medicine, Ankara, Türkiye
[2]Department of Internal Medicine, Ankara University, Faculty of Veterinary Medicine, Ankara, Türkiye
[3]Department of Biostatistics, Siirt University, Faculty of Veterinary Medicine, Siirt, Türkiye

Received/Geliş Tarihi: 28.07.2022
Accepted/Kabul Tarihi: 14.12.2022
Publication Date/Yayın Tarihi: 29.12.2022

Corresponding author/Sorumlu Yazar:
Efe KURTDEDE
E-mail: efekurtdede@gmail.com

Cite this article as: Kurtdede E, Börkü MK, Gür B, Doğan Y, Sevim K, Gülendağ E. Evaluation of CBC, lipid profile, oxidative stress biomarkers, total thiol, native thiol, and disulfide levels in dogs diagnosed with parvoviral enteritis without clinical findings. *Vet Sci Pract.* 2022; 17(3), 98-102.

Atıf: Kurtdede E, Börkü MK, Gür B, Doğan Y, Sevim K, Gülendağ E. Klinik bulgular görülmeden parvoviral enteritis tanisi konulan köpeklerde, CBC, lipid profili, oksidatif stres biyobelirteçleri, total thiol, native thiol ve disülfid düzeylerinin değerlendirilmesi. *Vet Sci Pract.* 2022; 17(3), 98-102.

ABSTRACT

In this study, whole blood cell count, metabolic and inflammatory biomarkers, and oxidative stress parameters were evaluated in dogs diagnosed with canine parvoviral enteritis by stool antigen testing performed 24-48 hours before any clinical findings arose. This study aimed to conduct effective evaluations of the status of the disease, the selection of treatment methods, and the prognosis of the disease. Thirty dogs with canine parvoviral enteritis positivity according to stool antigen test results 24-48 hours before the appearance of any clinical signs associated with parvoviral enteritis (lethargy, vomiting, diarrhea, dehydration) were evaluated together with 10 healthy dogs. In analyses of the dogs with canine parvoviral enteritis, significant decreases were found for white blood cell ($P < .001$), neutrophil ($P < .001$), and monocyte ($P = .011$) counts and significant increases were found for alanine aminotransferase and aspartate aminotransferase ($P < .001$ for both). Significant increases ($P < .001$) were also determined for high-density lipoprotein, cholesterol, and triglyceride levels among the studied lipid metabolites and C-reactive protein, malondialdehyde, and paraoxonase-1 among inflammatory biomarkers. Significant decreases were found for total thiol ($P = .002$) and native thiol ($P < .001$), with which antioxidant levels were evaluated, and a significant increase was found for disulfide ($P < .001$), with which oxidation was evaluated. It was concluded that early changes in blood samples taken 24-48 hours before the onset of clinical symptoms in dogs with CPV, as reflected by values of CBC and metabolic and inflammatory biomarkers, may provide information about the severity of the disease, the choice of medical treatments to be applied, and prognosis.

Keywords: Canine parvoviral enteritis, disulfide, malondialdehyde, paraoxonase-1, thiol

ÖZ

Bu çalışmada, klinik bulgular görülmeden 24-48 saat önce, dışkıda yapılan antijen testi ile parvoviral enteritis (CPV) tanısı konulan köpeklerde, tam kan hücreleri (CBC) sayıları, metabolik ve inflamasyon biyobelirteçleri ve oksidatif stres parametreleri birlikte değerlendirilerek; hastalığın durumu, sağaltım yöntemlerinin seçimi ve hastalığın prognozu hakkında etkin değerlendirmelerin yapılması amaçlandı. Çalışmada, parvoviral enteritis ile ilişkili klinik belirtilerin (durgunluk, kusma, ishal, dehidratasyon) görülmesinden 24-48 saat önceki dışkı antijen testi sonucuna göre CPV pozitif olan 30 köpek ile, 10 sağlıklı köpek değerlendirildi. CPV'li köpeklerde yapılan analizlerde, WBC ($P < ,001$), nötrofil (neut) ($P < ,001$) ve monosit (mono) ($P = ,011$) sayılarında anlamlı düşüşler, ve alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) değerlerinde anlamlı artışlar ($P < ,001$) saptandı. Lipid metabolitlerinden yüksek dansiteli lipoprotein (HDL), kolesterol (Chl) ve trigliserit (Tg) düzeylerinde ve inflamasyon biyobelirteçlerinden C-reaktif protein (CRP), malondialdehit (MDA) ve paraoksanaz-1 (PON-1) değerlerinde anlamlı artışlar ($P < ,001$) belirlendi.

Antioksidan düzeyinin değerlendirildiği total thiol (*P*=,002) ve native thiol (*P* < ,001) değerlerinde anlamlı düşüşler, ve oksidasyonun değerlendirildiği disülfit düzeyinde anlamlı (*P* < ,001) artış saptandı. CPV'li köpeklerde klinik semptomların başlangıcından 24-48 saat önce alınan kan örneklerinde, CBC sayısında ve metabolik ve inflamasyon biyobelirteçlerinin düzeylerinde meydana gelen erken dönem değişikliklerin hastalığın şiddetine, uygulanacak tıbbi tedavilerin seçimine ve prognoza yön verebileceği sonucuna varıldı.

Anahtar Kelimeler: Canine parvoviral enteritis, disulfide, MDA, PON-1, thiol

INTRODUCTION

Canine parvoviral enteritis (CPV) infection is an important disease that is commonly seen in domestic and wild dogs, especially between the ages of 6 weeks and 6 months, in Turkey and around the world. It is characterized by high morbidity and mortality rates despite vaccination. Sivas Kangal, Rottweiler, Doberman, Pincher, Labrador Retriever, and German Shepherd dogs are particularly susceptible to this disease.[1,2]

Some pathophysiological disorders developing in the course of the disease include intravascular coagulopathy, the death of myeloproliferative cells and immunosuppression due to thymic lymphocytosis, septicemia due to intestinal mucosal destruction, malabsorption due to villus atrophy, and dehydration and shock due to diarrhea and vomiting.[3-5] Good response to treatment is directly proportional to effective treatment in the early period and the speed of the patient's recovery from intestinal disorders.[6,7]

Determination of whole blood cell count (CBC) parameters including lymphocyte (Lym), monocyte (Mono), and neutrophil (Neut) counts together with oxidative stress status, some liver parameters, and lipid profile is important in evaluating the pathophysiological status of patients. In dogs with CPV, systemic inflammation as reflected by C-reactive protein (CRP) and procalcitonin (PCT); liver parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), and albumin (Alb); lipid profile as reflected by high-density lipoprotein (HDL), cholesterol (Chol), and triglyceride (Tg); and biomarkers associated with oxidative stress such as malondialdehyde (MDA) and paraoxonase-1 (PON-1) can be evaluated and treatment protocols that will help clinical veterinarians in choosing treatment practices can be recommended.[2,6,8,9]

One of the most important findings in the early period of CPV infection is the increase in serum CRP, which is an acute-phase protein.[10,11] It is also recommended to evaluate the changes in serum MDA resulting from the peroxidation of polyunsaturated fatty acids abundant in erythrocyte membranes, the changes in PON-1 and HDL levels associated with redox status and degradation of oxidized lipids, and oxidative pathobiological changes in dogs with CPV.[12,13] Since plasma thiols contain sulfhydryl groups with antioxidant/prooxidant properties, determining the level of thiol and disulfide hemostasis in patients is also valuable in the evaluation of oxidative stress.[14,15] The plasma thiol level in the body is often affected by oxidative stress and other disorders. When subjected to oxidation reactions, thiol compounds form disulfide bonds, which have important functions in the response to upregulated oxidative stress markers, metabolic functioning, and the maintenance of hemostasis.[16,17] The plasma thiol pool, reflecting thiol/disulfide homeostasis, is evaluated based on the levels of Alb and thiols (proteins and compounds of low molecular weight).[18] Molecules found in the dynamic plasma thiol pool are affected by oxidative stress and other disease processes, triggering changes in thiol/disulfide homeostasis. In addition to their antioxidant effects, these molecules also have important effects on metabolic reactions, signal transduction, the scavenging of toxins, gene expression, and cell signaling.[19]

In this study, dogs diagnosed with CPV were evaluated according to stool antigen test results obtained 24-48 hours before the appearance of clinical findings. Whole CBC results, liver parameters (ALT, AST, TP, and Alb), metabolic (HDL, Chol, and Tg) and inflammatory biomarkers (CRP, PCT, and PON-1), and oxidative stress (MDA, total thiol, native thiol) and oxidation (disulfide) parameters were considered together with the aim of performing effective evaluations of the status of the disease, the selection of treatment methods, and the prognosis of the disease.

METHODS

Ethical approval of this study was obtained from the Ankara University Animal Experiments Local Ethics Committee with decision number 2020-5-43 and decision date 04-03-2020. Forty owned dogs of different breeds and both sexes, ranging in age between 6 weeks and 6 months and brought to the Animal Hospital of Ankara University's Veterinary Faculty for general health examinations and vaccination purposes, were included in the study after obtaining written permission and approval from their owners.

Thirty dogs with positive fecal CPV antigen test results in the first examination and the beginning of clinical signs of CPV (lethargy, vomiting, diarrhea, dehydration) 24-48 hours after that initial examination constituted the group of dogs with CPV infection. Ten dogs determined to be healthy according to clinical and laboratory examination findings constituted the control group. In dogs with suspected CPV, the diagnosis was confirmed by detecting the CPV antigen in stools with a rapid test kit (Canivet CPV-CCV Ag Combo Test, Vet Diagnostix, China).

Blood samples were collected from the cephalic vein for all dogs included in the study, with 1 mL obtained for each tube with anticoagulant and 5 mL for each tube without anticoagulant.

Whole blood cell count values were determined from blood samples with anticoagulant using an automatic hematology analyzer (Mindray BC 5000). Within 2 hours after blood collection, blood samples without anticoagulant were centrifuged at 3000 rpm for 10 minutes and serum was obtained. These samples were placed in small containers and kept at −20°C until biochemical analysis was performed. The levels of ALT, AST, TP, Alb, urea, and creatinine in blood serum samples thawed at room temperature before analysis were determined spectrophotometrically with an autoanalyzer (Mindray BS-120) using the relevant kits.

Blood serum MDA level was calculated according to the method specified by Draper and Hadley.[19] For this, 2.5 mL of 10% trichloroacetic acid was mixed with 0.5 mL of serum in a tube. The reaction

solution was kept in a hot water bath for 15 minutes. The solution was then held at room temperature and centrifuged at 400× g for 10 minutes. The supernatants were removed in volumes of 2 mL and combined with 1.0 mL of 0.67% TBA in separate tubes, and those mixtures were incubated in boiling water baths for 15 minutes. The mixtures were brought to room temperature and absorbance was measured at 532 nm. Malondialdehyde activity was expressed as nmol/g protein.

Paraoxonase-1 activity was determined by the method developed by Eckerson et al.[20] This method is based on the conversion of paraoxon (diethyl p-nitrophenyl phosphate) to diethyl phosphate and p-nitrophenol with the effect of paraoxonase in the serum with measurement of the absorbance of the formed p-nitrophenol using a spectrophotometer at a wavelength of 412 nm. Paraoxonase-1 activity was expressed as U/L.

Thiol-disulfide levels (thiol [–SH] and disulfide [–S–S]) were determined separately spectrophotometrically according to the method specified by Erel and Neselioglu.[15] With this method, $NaBH_4$ is used to reduce sulfide bonds in serum samples and form thiol groups in the free functional state. Formaldehyde was used to remove the unused portion of $NaBH_4$, and with DNTB, thiol groups were determined at 415 nm as total thiol and native thiol. The disulfide value (μmol/L) was calculated with the following formula: (total thiol [μmol/L] – native thiol [μmol/L])/2.

Statistical Analysis

Results were analyzed using the Shapiro-Wilk test for normality and the Levene test for homogeneity of variances. One-way analysis of variance and Kruskal-Wallis tests were conducted accordingly to determine the presence of similarities and differences between experimental groups. The differences between the groups were identified by Tukey HSD and Dwass-Steel-Critchlow-Fligner pairwise tests. All data were analyzed using IBM Statistical Package for Social Sciences Statistics 26.0 (IBM Corp., Armonk, NY, USA). Values of $P < .05$ were considered significant for all analyses.

RESULTS

In this study, CBC results, ALT and AST levels, metabolic biomarkers (HDL, Chol, and Tg), systemic inflammatory biomarkers (CRP, PCT, and PON-1), and oxidative stress (MDA, total thiol, and native thiol) and oxidation (disulfide) parameters were obtained as presented in Tables 1-3.

DISCUSSION

Canine parvoviral enteritis is a highly contagious disease involving acute hemorrhagic enteritis in dogs with high morbidity and mortality rates. Veterinary clinicians use blood cell counts and some laboratory analysis results obtained from blood serum in evaluating the severity of this disease, choosing the treatment options to be applied, and determining the prognosis of the patient. For this reason, it is important to consider analytical results that reveal the levels of systemic inflammation, metabolic processes, and oxidative stress in dogs with CPV.[10] It is necessary to evaluate CBC values and parameters related to systemic inflammation, metabolic processes, and oxidative stress.[20,21]

Previous studies have noted the importance of determining the values of certain hematological parameters in cases of CPV and monitoring them throughout the treatment process. Kocatürk et al[22] reported that determining the level of CRP, an acute-phase protein, in the evaluation of systemic inflammation is important in predicting the severity and prognosis of the disease, and a direct correlation was found between the severity of the disease and increased CRP levels in dogs with CPV. Şimşek et al[23] stated that CRP levels were statistically significantly increased in dogs with this disease, while Dinler et al[24] found PCT and CRP levels to be higher in cases of CPV compared to a control group. Similarly, in the present study, a significant increase ($P < .001$) was found in CRP levels in dogs with CPV compared to the healthy control group, revealing inflammation, while the increase in PCT was not statistically significant.

Many other studies have reported the importance of determining the values of hematological parameters and monitoring them during the treatment of dogs with CPV. Şimşek et al[23] and Goddard et al[10] found WBC, Lym, Mono, and granulocyte counts to be significantly lower in cases of CPV compared to healthy dogs.

Table 1. WBC, Lym, Mono, and Neut Counts of the CPV and Control Groups

	Hematological Parameters (10^9/L)			
Groups	WBC	Lym	Mono	Neut
Control (n = 10)	8.41 ± 0.41	2.12 ± 0.5	0.47 ± 0.06	5.52 ± 0.31
CPV (n = 30)	4.714 ± 0.59**	1.774 ± 0.23	0.28 ± 0.04*	2.47 ± 0.31**
P	<.001		.011	<.001

*The Mono counts of the CPV and control groups were significantly different ($P = .011$).
**The WBC and Neut counts of the CPV and control groups were significantly different ($P < .001$).
CPV, canine parvoviral enteritis; Lym, lymphocytes; Mono, monocytes; Neut, neutrophils; WBC, white blood cell count.

Table 2. ALT, AST, TP, Alb, Chol, Tg, and HDL levels of the CPV and control groups

	Biochemical Parameters						
Groups	ALT (U/L)	AST (U/L)	TP (g/dL)	Alb (g/dL)	Chol (mg/dL)	Tg (mg/dL)	HDL (mg/dL)
Control (n = 10)	40.68 ± 4.04	48.15 ± 0.83	5.23 ± 0.15	2.90 ± 0.17	234.5 ± 3.81	57.75 ± 1.4	49.25 ± 0.96
CPV (n = 30)	61.48 ± 1.04	61.23 ± 1.01	5.17 ± 0.11	2.84 ± 0.06	237.77 ± 4.92	72.45 ± 1.35	56.636 ± 0.64
P	<.001	<.001				<.001	<.001

*The ALT, AST, Tg, and HDL levels of the CPV and control groups were significantly different ($P < .001$).
Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Chol, cholesterol; CPV, canine parvoviral enteritis; HDL, high-density lipoprotein; TP, total protein.

Table 3. CRP, PCT, MDA, PON-1, total thiol, disulfide, and native thiol levels of the CPV and control groups

	Biochemical Parameters						
Groups	CRP (mg/L)	PCT (%)	MDA (nmol/g protein)	PON-1 (U/L)	Total Thiol (μmol/L)	Disulfide (μmol/L)	Native Thiol (μmol/L)
Control (n = 10)	9.84 ± 0.26	0.18 ± 0.05	1.878 ± 0.03	157.05 ± 2.9	236 ± 1.34	12.037 ± 0.21	204.299 ± 1.82
CPV (n = 30)	103.5 ± 1.84	0.28 ± 0.04	2.9 91 ± 0.04	118.883 ± 2.46	217.767 ± 4.92	15.307 ± 0.25	169.907 ± 1.91
P	<.001		<.001	<.001	.002	<.001	<.001

* The total thiol levels of the CPV and control groups were significantly different ($P = .002$).
**The CRP, MDA, PON-1, disulfide, and native thiol levels of the CPV and control groups were significantly different ($P < .001$).
CPV, canine parvoviral enteritis; CRP, C-reactive protein; MDA, malondialdehyde; PCT, procalcitonin; PON-1, paraoxonase-1.

Kubesy et al[25] found decreased WBC, Neut, and Lym counts and increased Mono counts in dogs with CPV compared to a control group. Arora et al[26] determined decreases in total leukocyte and Neut counts in dogs with CPV. Decreases in WBC, Lym, and Neut counts, which are common findings in studies of dogs with CPV, were also determined in this study. The decrease in Mono count obtained here is consistent with the findings of Şimşek et al[23] and Goddard et al.[10] Alves et al[27] stated that increases in total leukocyte, Lym, and Mono counts in dogs with CPV are signs that the prognosis of the patient will be good. In the present study, the significant decreases in WBC count ($P = .011$) and Neut and Mono counts ($P < .001$) and insignificant decrease in Lym count in dogs with CPV were thought to be due to the destruction of the bone marrow and lymphoproliferative organs, together with the development of the inflammatory response.

In dogs with CPV, it is important to determine the levels of serum ALT, AST, TP, and Alb, which are some of the liver parameters used to evaluate metabolic profiles. Serum urea and creatinine levels should also be used to evaluate kidney function. Öcal and Ünsüren[28] reported that dogs with CPV had lower serum TP and Alb levels and higher serum blood urea nitrogen, creatinine, ALT, and AST levels compared to healthy dogs. Abdullaziz et al[29] found significant decreases in TP and Alb values and significant increases in ALT, AST, urea, and creatinine values in dogs with CPV compared to healthy dogs. Thakur and Thakur[30] found increases in serum AST and ALP levels and decreases in Alb levels in dogs with CPV. Arora et al[26] found increases in AST and ALP values and decreases in Alb values in dogs with CPV. Abdullaziz et al[29] and Khare et al[21] concluded that increased ALT and AST levels in dogs with CPV were related to the absorption of toxic substances resulting from hepatic hypoxia caused by hypovolemia or intestinal barrier disorders. In the present study, on the other hand, mild increases in serum ALT and AST were seen in dogs whose physical clinical examinations did not reveal any signs of disease but whose fecal CPV screening test results were positive. This finding suggests that hypovolemia began to form in these dogs together with deterioration of the intestinal barrier.

Metabolic, systemic inflammatory, and oxidation processes of dogs with CPV are evaluated by monitoring the levels of HDL, Tg, and Chol, which are biomarkers of lipid metabolism. Salarpour et al[31] found statistically insignificant increases in HDL and Tg levels and insignificant decreases in Chol levels in 27 dogs with CPV. Yılmaz and Senturk[32] found significant decreases in HDL and Chol levels and significant increases in Tg levels in dogs with CPV compared to healthy dogs. In the present study, significant increases ($P < .001$) in HDL and Tg levels in dogs with CPV were evaluated as indications that the level of Chol transport was high in these animals and the effective prevention of lipid peroxidation was increased. Furthermore, this result highlights the necessity of evaluating the effects of oxidative stress in relation to the severity of CPV, and at the same time, the effects of increased HDL and Chol levels on liver performance, changes in protein structure,[33] and the development of inflammatory and oxidative stress responses[22,23] with the beginning of the deterioration of the intestinal barrier.

Serum MDA, PON-1, total thiol, disulfide, and native thiol levels, which reveal oxidative stress, should also be determined in order to evaluate the oxidative balance in patients and to identify the necessary medical applications.[22,34-36] In comparative studies with healthy dogs, Kocatürk et al[22] reported that PON-1 levels were lower in dogs with CPV, while Harizan et al[34] found that MDA levels were higher. Panda et al[35] found a significant difference in MDA levels in dogs with CPV. Değirmençay et al[36] determined that the levels of total thiol, disulfide, and native thiol of infected dogs were lower than those of healthy dogs. In the dogs with CPV in this study, significant increases ($P < .001$) in MDA, PON-1, and disulfide were detected, while significant decreases in total thiol ($P = .002$) and native thiol ($P < .001$) showed that noteworthy levels of oxidative stress had developed in the dogs with CPV. The decrease in PON-1 activity is thought to occur in response to the occurrence of lipid peroxidation via HDL oxidation. Kocatürk et al[22] emphasized that determining the level of CRP, one of the acute-phase proteins that reveal the degree of systemic inflammation in dogs with CPV, is important in terms of evaluating the severity and prognosis of the disease, and they found a direct correlation between the severity of CPV and the increase in CRP. Şimşek et al[23] stated that CRP levels were statistically significantly higher in dogs with parvoviral enteritis, while Dinler et al[24] found PCT and CRP levels to be higher in dogs with parvoviral enteritis compared to a control group. In the present study, a significant increase ($P < .001$) in CRP, revealing inflammation, was found in dogs with CPV compared to the control group, while the increase in PCT was not statistically significant.

Based on the findings of the present study, it is clear that the evaluation of changes in inflammatory, metabolic, and oxidative stress parameters in the early phase of CPV will provide important data for clinical veterinarians. In the early phase of CPV infection, the increase in disulfide level, which indicates increased oxidation, and the decrease in total thiol and native thiol levels, which indicate decreased antioxidant levels, are particularly noteworthy. In addition, the increase in the level of MDA, which is the end product of lipid peroxidation, and the decrease in the level of PON-1, which protects lipid metabolites from oxidation, indicate that significant oxidative stress develops in dogs in the early stage of CPV without clinical signs of the disease. Other indicators of oxidative stress that developed in dogs with CPV in this study were increased HDL, Chol, and Tg, which are lipid metabolites.

In conclusion, the data obtained in this study by evaluating the changes in the levels of CBC results, metabolic and inflammatory biomarkers, and oxidative stress-related parameters that show the pathophysiological status of dogs 24-48 hours before the appearance of clinical findings of CPV suggest that these variables can provide guidance in determining the seriousness of the disease in the early period and selecting the treatment methods to be applied. This study was carried out with a limited number of dogs. If similar studies are conducted in the future with more dogs with CPV, it will be possible to make more comprehensive generalizations of the obtained data.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara University (Date 04.03.2020, Decision No: 2020-5-43).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Design – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Supervision – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Resources – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Materials – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Data Collection and/or Processing – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Analysis and/or Interpretation – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Literature Search – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Writing Manuscript – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Critical Review – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Other – E.K., M.K.B., B.G., Y.D., K.S., E.G.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Ankara Üniversitesi'nden alınmıştır. (Tarih: 04.03.2020, Karar No: 2020-5-43)

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Tasarım – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Denetleme – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Kaynaklar – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Malzemeler – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Veri Toplanması ve/veya İşlemesi – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Analiz ve/veya Yorum – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Literatür Taraması – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Yazıyı Yazan – E.K., M.K.B., B.G., Y.D., K.S., E.G.; Eleştirel İnceleme – E.K., M.K.B., B.G., Y.D., K.S., E.G.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Prittie J. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *J Vet Emerg Crit Care*. 2004;14(3):167-176. [CrossRef]
2. Decaro N, Buonavoglia C. Canine parvovirus--a review of epidemiological and diagnostic aspects, with emphasis on type 2c. *Vet Microbiol*. 2012;155(1):1-12. [CrossRef]
3. Tuteja D, Banu K, Mondal B. Canine parvovirology - A brief updated review on structural biology, occurrence, pathogenesis, clinical diagnosis, treatment and prevention. *Comp Immunol Microbiol Infect Dis*. 2022;82(82):101765. [CrossRef]
4. Radzykhovskyi M, Sokulskiy I, Dyshkant O, Antoniuk A, Gutyj B, Sachuk R. Experimental study of tropism of cultivated canine parvovirus in the immunogenesis organs of puppies. *Regul Mech Biosyst*. 2022;13(3):241-246.
5. Engelbrecht M, Atkinson B, Goddard A, Pazzi P, McClure V. Mean platelet volume and platelet volume distribution width in canine parvoviral enteritis. *Front Vet Sci*. 2021;8(8):722280. [CrossRef]
6. Aktaş MS, Özkanlar Y, Erzurum KA, Sahipli Köpeklerin ÇKG. Parvoviral Enteritisini Etkileyen risk Faktörleri üzerinde bir araştırma. *Atatürk Univ Vet Bil Derg*. 2011;6(1):1-8.
7. Uchańska O, Ochota M, Eberhardt M, Niżański W. Dead or alive? A review of perinatal factors that determine canine neonatal viability. *Animals (Basel)*. 2022;12(11):1402. [CrossRef]
8. Kataria D, Agnihotri D, Kumar S, Lohiya A. Comparative assessment of oxidative stress in dogs regarding CPV infection. *Innov J*. 2020;9(2):115-117. [CrossRef]
9. Szarkova A, Tóthová C, Weissová T, et al. Evaluation of acute phase proteins in dogs suffering from various diseases. *Int J Vet Sci*. 2023;12(1):82-88.
10. Goddard A, Leisewitz AL, Christopher MM, Duncan NM, Becker PJ. Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis. *J Vet Intern Med*. 2008;22(2):309-316. [CrossRef]
11. Covin MA, Steiner JM. Measurement and clinical applications of C-reactive protein in gastrointestinal diseases of dogs. *Vet Clin Pathol*. 2022;50(suppl 1):29-36. [CrossRef]
12. Tomsič K, Seliškar A, Lukanc B, Nemec Svete A. Plasma total Antıoxıdant Capacıty and Actıvıtıes of blood Glutathıone Peroxıdase and Superoxıde Dısmutase Determıned in healthy dogs by Usıng Commercıally Avaılable Kıts. *Acta Vet Beograd*. 2016;66(4):534-548. [CrossRef]
13. Elsayed NM, Kubesy AA, Salem NY. Altered blood oxidative stress biomarkers in association with canine parvovirus enteritis. *Comp Clin Pathol*. 2019:1-5.
14. Pugliese M, Biondi V, Merola G, Landi A, Passantino A. Oxidative stress evaluation in dogs affected with canine monocytic ehrlichiosis. *Antioxidants (Basel)*. 2022;11(2):328. [CrossRef]
15. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*. 2014;47(18):326-332. [CrossRef]
16. Asoğlu M, Kiliçaslan F, Beginoğlu Ö, et al. Thiol/disulphide homeostasis as a new oxidative stress marker in untreated patients with generalized anxiety disorder. *Anadolu Psikiyatr de*. 2018;19:143-149.
17. Behringer S, Wingert V, Oria V, et al. Targeted metabolic profiling of methionine cycle metabolites and redox thiol pools in mammalian plasma, cells and urine. *Metabolites*. 2019;9(10):235. [CrossRef]
18. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Radic Biol Med*. 2013;65:244-253. [CrossRef]
19. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*. 1990;186:421-431. [CrossRef]
20. Eckerson HW, Romson J, Wyte CM, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. *Am J Hum Genet*. 1983;35(2):214-227.
21. Khare DS, Gupta DK, Shukla PC, Das G, Meena NS, Khare R. Clinical and haemato-biochemical changes in canine parvovirus infection. *J Pharmacogn Phytochem*. 2020;9(4):1601-1604.
22. Kocatürk M, Tvarijonaviciute A, Martinez-Subiela S, et al. Inflammatory and oxidative biomarkers of disease severity in dogs with parvoviral enteritis. *J Small Anim Pract*. 2015;56(2):119-124. [CrossRef]
23. Şimşek E, ile Enfekte P, Reaktif KSC. *Protein Değerlerinin Belirlenmesi Türkiye Cumhuriyeti Kirikkale* [Dissertation]. Kırıkkale Univerity; 2018.
24. Dinler AC, Tuna EG, Ekren GS, Ulutas B, Voyvoda H. Serum intestinal fatty acid-binding protein and calprotectin concentrations to assess clinical severity and prognosis of canine parvovirus enteritis. *Kafkas Univ Vet Fak Derg*. 2022;28(1):105-114.
25. Kubesy AA, Rakha GM, Salem SI, Jaheen AH. Altered blood procalcitonin, C-reactive protein, and leucocytes count in association with canine parvovirus (CPV) enteritis. *Comp Clin Pathol*. 2019;28(4):1095-1099. [CrossRef]
26. Arora R, Tyagi A, Shekthar S, Rajora VS, Arora N. Haemato-biochemical alterations in gastroenteritis affected dogs. *J Entomol Zool Stud*. 2018;6(5):972-974.
27. Alves FdS, Barbosa BC, Coelho NDdG, et al. Clinical and hematological prognostic factors in dogs with parvoviral enteritis and sepsis. *Semin Cienc Agrar*. 2019;40(4):1477-1488. [CrossRef]
28. Öcal N, Ünsüren H. Parvoviral Hemorajik Gastroenteritli köpeklerin Sağıltımında total parenteral Beslemenin etkisi. *Kafkas Univ Vet Fak Derg*. 2009;15(2):237-244.
29. Abdullaziz I, Aly M, Elshahawy I. Clinical, haemato-biochemical alterations with acute phase response in canine parvoviral enteritis. *Damanhour J Vet Sci*. 2022;7(1):23-27. [CrossRef]
30. Thakur M, Thakur R. Haemato-biochemical alterations in canine Parvo Virus infection affected pups. *Himachal J Agric Res*. 2017;43(2):131-134.
31. Salarpour R, Ahmadmahmudi MZ, Rezaei M, Eskandarzade N. Paraoxonase activity assessment in dogs suffering from Parvovirus infection. *J Hellenic Vet Med Soc*. 2021;71(4):2399-2504. [CrossRef]
32. Yilmaz Z, Senturk S. Characterization of lipid profiles in dogs with parvoviral enteritis. *J Small Anim Pract*. 2007;48(11):643-650. [CrossRef]
33. Marsche G, Saemann MD, Heinemann A, Holzer M. Inflammation alters HDL composition and function: implications for HDL-raising therapies. *Pharmacol Ther*. 2013;137(3):341-351. [CrossRef]
34. Harizan IM, Khinchi RK, Manju SSK, Meena VK. Alterations in haemato-biochemical and oxidative stress indices in dogs affected with parvoviral enteritis. *The Pharma. Innov J*. 2021;10(8):428-431.
35. Panda D, Patra RC, Nandi S, Swarup D. Oxidative stress indices in gastroenteritis in dogs with canine parvoviral infection. *Res Vet Sci*. 2009;86(1):36-42. [CrossRef]
36. Değirmençay Ş, Çamkerten G, Çamkerten İ, Aktaş MS. An investigation of thiol/disulfide homeostasis and ischemia-modified albumin levels to assess the oxidative stress in dogs with canine distemper. *Vet Arhiv*. 2021;91(1):39-49. [CrossRef]

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.221725

Morphometric and Computed Tomographic Investigation of Ligamentum Sacrotuberale in Dogs

Köpekte Ligamentum Sacrotuberale'nin Morfometerik ve Bilgisayarlı Tomografik İncelenmesi

Cüneyt Tunahan MAVİŞ[1]
Muhammet Lütfi SELÇUK[2]

[1]Agriculture and Rural Development Support Institution, Konya, Turkey
[2]Department of Physiotherapy and Rehabilitation, Karamanoğlu Mehmetbey University, Faculty of Health Sciences. Karaman, Turkey.

ABSTRACT

The ligamentum sacrotuberale is an anatomical structure that is frequently used in clinical hip dislocations and perineal hernias in dogs. However, various differences have been identified in the description of its anatomical location in the literature. The aim of this study is to contribute to the literature by determining the accuracy of different information specified in the literature and determining its morphology. The study was performed on the cadavers of 7 healthy adult dogs (3 females and 4 males). Three-dimensional reconstruction of the ligamentum sacrotuberale was performed on the images obtained by computed tomography in dogs. Dogs were then dissected and morphometric measurements were made. As a result of the analyses performed, no statistical difference was found when the right and left ligamentum sacrotuberale were compared ($P > .05$). In our study, it was determined that in all of our dissections performed on dogs, ligamentum sacrotuberale originated from the caudolateral surface of the last sacral vertebra and the craniolateral of the first tail vertebra and ended on the dorsal surface of the tuber ischidicum.

Keywords: Computed tomography, dog, 3D modeling, ligamentum sacrotuberale

ÖZ

Ligamentum sacrotuberale köpeklerde klinik kalça çıkıkları ve perineal fıtıklarda sıklıkla kullanılan anatomik bir yapıdır. Ancak literatürde anatomik yerleşiminin tanımlanmasında çeşitli farklılıklar tespit edilmiştir. Bu çalışmanın amacı, literatürde belirtilen farklı bilgilerin doğruluğunu ve morfolojisini belirleyerek literatüre katkı sağlamaktır. Çalışma 7 yetişkin köpeğin (3 dişi ve 4 erkek) kadavrası üzerinde yapıldı. Köpeklerde bilgisayarlı tomografi ile elde edilen görüntüler üzerinde lig. sacrotuberale'nin üç boyutlu rekonstrüksiyonu yapıldı. Köpekler daha sonra diseke edildi ve morfometrik ölçümler yapıldı. Yapılan analizler sonucunda sağ ve sol ligamentum sacrotuberale karşılaştırıldığında istatistiksel olarak fark bulunmadı ($P > .05$). Çalışmamızda köpeklerde yaptığımız tüm diseksiyonlarda lig. sacrotuberale'nin son sakral omurun kaudolateral yüzeyinden ve birinci kuyruk omurunun craniolateral yüzeyinden köken aldığı ve tuber ischidicum'un dorsal yüzeyinde sona erdiği belirlendi.

Anahtar Kelimeler: Bilgisayarlı tomografi, köpek, 3D modelleme, ligamentum sacrotuberale

Received/Geliş Tarihi: 31.05.2022
Accepted/Kabul Tarihi: 16.11.2022
Publication Date/Yayın Tarihi: 29.12.2022

Corresponding author/Sorumlu Yazar: Cüneyt Tunahan MAVİŞ
E-mail: cuneyttunahan@gmail.com

Atıf: Maviş CT, Selçuk ML. Köpekte ligamentum sacrotuberale'nin morfometerik ve bilgisayarlı tomografik incelenmesi. *Vet Sci Pract*. 2022; 17(3), 103-107.

Cite this article as: Maviş CT, Selçuk ML. Morphometric and computed tomographic investigation of ligamentum sacrotuberale in dogs. *Vet Sci Pract*. 2022; 17(3), 103-107.

INTRODUCTION

Ligamentum (lig.) sacrotuberale, in the form of a fibrous cord, was used in surgical operations in dogs.[1-4] Traumatic coxofemoral luxation was a common problem in dogs, and for the treatment of luxation, transposition method of the lig. sacrotuberale was used as an intra-articular procedure. This technique provided latero-medial stability of the joint.[5,6] Besides sacrotuberale hip dislocations, it was also used in muscle hernias. Depending on the type of herniation, simple placement of the abdominal muscles was not possible due to atrophy of the perineal diaphragm muscles in most cases; therefore, the lig. sacrotuberale was often included in surgical operations to close the ventral and lateral part of the hernial deficit.[7,8] Although lig. sacrotuberale was used in surgical operations performed in the perineal

region, different definitions were given in the literature review to describe its anatomical location.

There were literatures stating that the lig. sacrotuberale was located between the caudo-lateral of the sacrum and the tuber ischiadicum,[1,2,9] while there were literatures stating that it was located between the cauodolateral of the sacrum and the processustransversus of the first tail vertebrae and the lateral surface of the tuber ischiadicum.[3,10,11] In another article, it was reported that the lig. sacrotuberale extended from the last sacral vertebra and the first 2 tail vertebrae to the outer surface of the tuber ischiadicum. The ligament partially or completely serves as the origin of the musculus abductor cruris caudalis, musculus biceps femoris, musculus gluteus superficialis, and musculus piriformis.[4] Ligamentum sacrotuberale has a very sensitive and important anatomical position. Due to its anatomical location, many surgeons use this ligament in the treatment of various ailments in dogs.[5,12,13]

In this study, a dissection procedure was applied in dogs and 2-dimensional (2D) images were obtained by computerized tomography (CT) imaging methods, and a 3-dimensional (3D) model on a computer program was created to determine the anatomical location of the lig. sacrotuberale. The aim of this study was to contribute to the literature by determining the accuracy of different information specified in the literature and determining its morphology.

MATERIALS AND METHODS

The study was conducted on cadavers of 7 adult healthy dogs of age between 1 and 10 years old (3 female Pariah dogs—mongrel and 4 male Pariah dogs—mongrel). This study was carried out on cadavers that died in the animal shelter of Konya Metropolitan Municipality Environmental Protection and Control Department for various reasons and were given as cadavers to Selcuk University Veterinary Faculty Anatomy Department. The ethical approval for investigation was obtained from Karamanoglu Mehmetbey University Faculty of Health Science Ethics Committee (October 27, 2021/06-34).

Computed Tomography Scanning

Images were obtained by placing the dogs in the prone position on multi-detector computed tomography (Siemens Dual Source, Somatom Definition Flash, Germany). Images were kVp 120, mAs 150-200 and parallel slice thickness of 0.6 mm, reconstruction interval of 0.5 mm, diameter FOV(field of view) of 30 cm, and interval value between 1 and 1.5. Computerized tomography images of dogs were recorded on CD-ROMs in digital imaging and communication in medicine (DICOM) format.[14-19]

Segmentation and 3-Dimensional Modeling

Three-dimensional reconstructions of os coxae, os sacrum, first coccygeal vertebra, and lig. sacrotuberale were obtained using Mimics 20.01 (The Materialize Group, Leuven, Belgium). First, images in DICOM format were opened with the Mimics 21.0 (The Materialize Group),[20] and 3D models were created by adjusting the threshold feature of the bones forming the pelvis cavity to a gray level of 220-3050 HU. Then, the gray level threshold was set to 410-2170 HU, and lig. sacrotuberale was manually marked using the editing mask feature of the program and a 3D model was created (Figure 1).

Macro-Anatomic and Cross-Sectional Anatomy Study

In the dissection performed on canine cadavers, an incision was made in the caudodorsal skin from the caudal aspect of the greater trochanter to the last sacral vertebra. In order not to damage lig. sacrotuberale, blunt dissection of the muscles was performed by palpating between the processus transversus of the last sacral vertebra and the tuber ischiadicum (Figure 2). After the ligament was exposed, the thickness of the origin, midpoint, insertio, and length of the ligament were measured by a digital caliper. The height of dogs was obtained by measuring the tip of the nose from the tuber ischiadicum.[21,22] Following the dissection procedure, previously obtained CT images and dissection findings were compared and anatomical formations were named on the CT images (Figures 3 and 4). The nomenclature was made according to Nomina anatomica veterinaria.

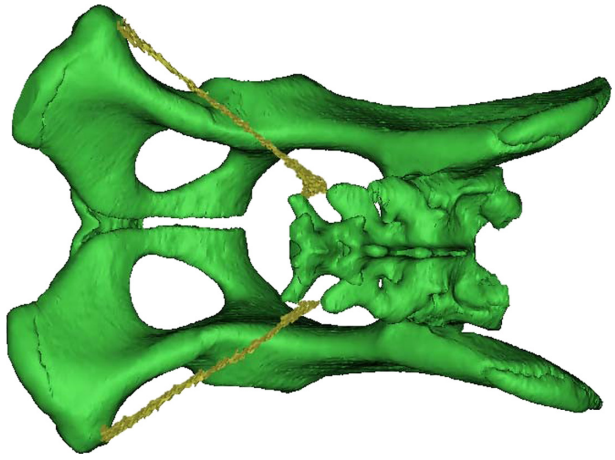


Figure 1. Modeling of the lig. sacrotuberale with the Mimics program.

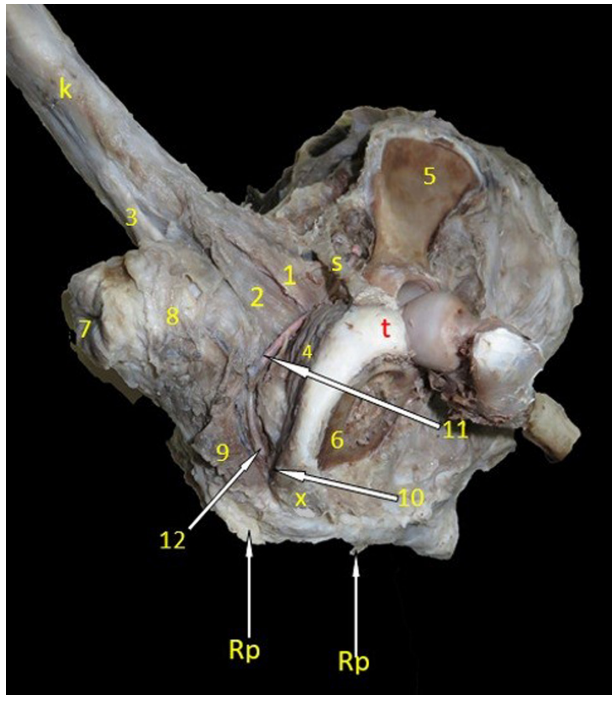


Figure 2. Regio perinealis topography. 1: M. coccygeus, 2: M. levator ani, 3: M. rectococcygeus, 4: M. obturatorius internus, 5: Ala ossis ilii, 6: Ala ossis ischii, 7: Anus (zona cutanea), 8: M. sphincter ani externus, 9: M. bulbospongiosus, 10: M. ischiourethralis, 11: A. pudenda interna, 12: A. dorsalis penis, x: M. ischiocavernosus, s: Lig. sacrotuberale, t: Tuber ischiadicum, k: Caudae, Rp: M. retractor penis.

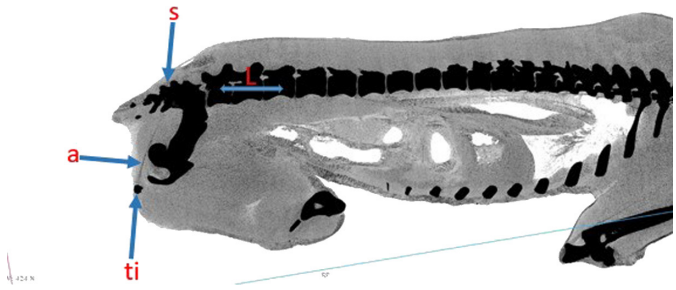


Figure 3. Position of the ligamentum sacrotuberale in horizontal section of the computerized tomography. a: Lig. sacrotuberale, s: 3.Sacral vertebrae, L: Vertebrae lumbales, ti: Tuber ischiadicum.

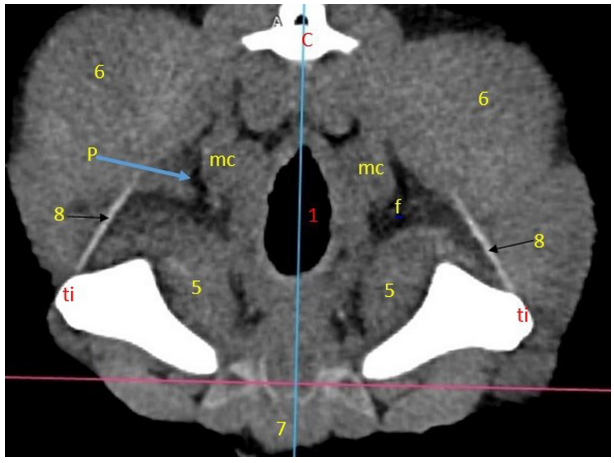


Figure 4. Ischiorectal fossa computerized tomography transversal section. mc: Coccygeal muscle, ti Tuber ischiadicum, 1: Rectum, C: 2. caudal vertebra, 5: M. obturatorius internus, f: Fossa ischiorectalis, 6: M.gluteus superficialis, 7: M. bulbospongiosus, 8: Lig. sacrotuberale, P:Canalis pudendalis.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences version 21.0. (IBM SPSS Corp.; Armonk, NY, USA). The conformity of the variables to the normal distribution was examined using visual and analytical methods. Data obtained from the right and left lig. sacrotuberale were compared with paired sample *t*-test. $P < .05$ was accepted statistically significant. Data are expressed as mean ± standard error (mean ± SE).

RESULTS

Information about age, height, and weight of male and female animals used in the study is given in Table 1, and morphometric measurements of lig. sacrotuberale are given in Table 2. In the study, the average height of the dogs was 93.28 ± 3.70 cm and their average weight was 20.00 ± 1.72 kg.

Table 1. Age, Body Height, and Weight of the Animals Used in the Study

	Breed	Age	Height (cm)	Weight (kg)
First male	Pariah dog–mongrel	8-10	86	23
Second male	Pariah dog–mongrel	6-8	94	25
Third male	Pariah dog–mongrel	1-3	80	17
Fourth male	Pariah dog–mongrel	1-3	89	14
First female	Pariah dog–mongrel	5-7	107	22
Secondfemale	Pariah dog–mongrel	1-3	92	15
Third female	Pariah dog–mongrel	3-5	105	24

In the thickness measurements made in the lig. sacrotuberale, the mean origo thickness was 12.20 ± 1.37 mm on the left, 12.06 ± 1.34 mm on the right, the mean midpoint thickness was 4.30 ± 0.48 mm on the left, 4.21 ± 0.48 mm on the right, the mean insertio thickness was 8.60 ± 1.18 mm on the left side, and 8.57 ± 1.19 mm on the right side. The mean length of the left ligament was 93.39 ± 4.33 mm, and 93.51 ± 4.32 mm on the right. When the lig. sacrotuberale length of the right and left sides were compared, no statistical difference was found ($P > .05$).

In all of our dissections performed on dogs in the study, it was determined that the lig. sacrotuberale originated from the caudolateral surface of the last sacral vertebra and the craniolateral of the first coccygeal vertebra and had a termination point on the dorsal surface of the tuber ischiadicum (Figures 1-3). It was observed that it started in the form of a flat fan from the caudolateral of the last sacral vertebra, which was the starting point of the ligament, and progressed in a rounder and thicker manner after approximately 25-35 mm. The ligament termination on the tuber ischiadicum was wide and flattened like at the origin point, but the ligament was thinner and weaker than the origin in this region. In the measurements made regarding the lig. sacrotuberale, it was observed that the length value of the ligament increased and took on a wider and more flexible structure, while the width of the insertio increased significantly with the advancing age of dogs. In the study, it was determined that the nerve ischiadicus was located 25-40 mm craniomedial from the midpoint of a 45° straight line drawn between the last sacral vertebra and the tuber ischiadicum and was 50-70 mm deep of the region muscles (musculus gluteus superficialis and musculus biceps femoris (Figures 5 and 6)). The vascular nerve bundle consisting of nerve pudendus, arteria pudenda interna, and vena pudenda interna was located 40-50 mm inside the fat

Table 2. Ligamentum Sacrotuberale Measurement Values (Mean ± Standard Error)

	Thickness of the Origo (mm)		Thickness of the Midpoint (mm)		Thickness of the Insertio		Length of the Ligament (mm)	
Gender	Left	Right	Left	Right	Left	Right	Left	Right
First male	15.96	15.89	5.61	5.47	13.04	13.01	109.3	108.8
Second male	9.68	9.99	5.29	5.19	12.87	12.95	106.04	106.90
Third male	14.62	14.32	3.51	3.08	5.55	5.63	85.97	86.38
Fourth male	7.59	7.52	2.20	2.27	5.84	5.80	80.62	80.72
Mean ± SE	11.96 ± 1.98	11.93 ± 1.93	4.15 ± 0.79	4.01 ± 0.78	9.32 ± 2.09	9.35 ± 2.09	95.4 8± 7.15	95.70 ± 7.12
First female	14.75	14.77	5.51	5.54	8.11	8.20	93.32	93.24
Second female	7.98	7.76	4.41	4.39	6.91	6.78	81.44	81.56
Third female	14.85	14.16	3.57	3.52	7.89	7.65	97.07	96.99
Mean ± SE	12.53 ± 2.27	12.23 ± 2.24	4.49 ± 0.56	4.48 ± 0.58	7.64 ± 0.37	7.54 ± 0.41	90.61 ± 4.71	90.5 9 ± 4.64

SE, standard error.

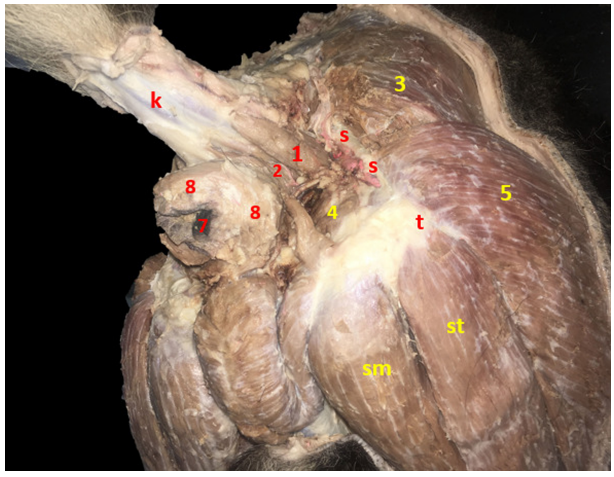


Figure 5. Position of the ligamentum sacrotuberale in male dog. 1: M. coccygeus, 2: M. levator ani, 3: M. gluteus superficialis, 4: M. obturatorius internus, 5: M. biceps femoris, sm: M. semimembranosus, st: M. semitendinosus, 7: Anus (zona cutanea), 8: M. sphincter ani externus, s: Lig. sacrotuberale, t: Tuber ischiadicum, k: Caudae.

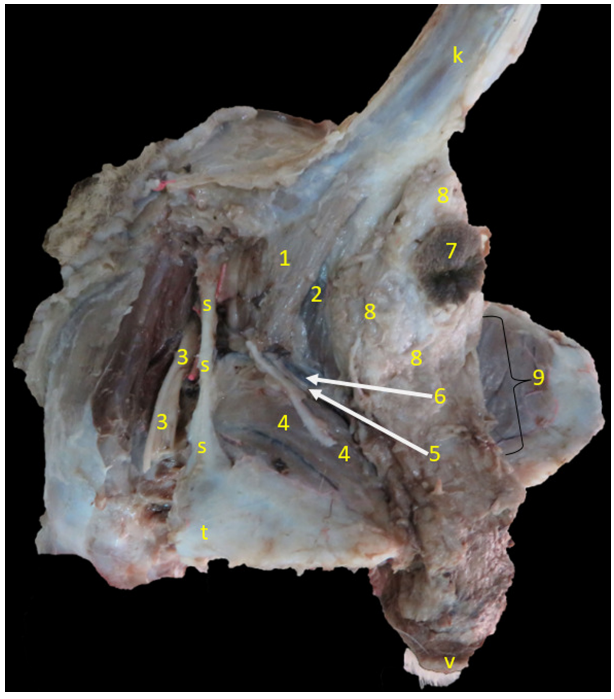


Figure 6. Position of the ligamentum sacrotuberale in female dog. 1: M. coccygeus, 2: M. levator ani, 3: N. ischiadicus, 4: M. obturatorius internus, 5: N. pudendus, 6: A. et V. pudenda interna , 7: Anus (zona cutanea), 8: M. sphincter ani externus, 9: Fossa ischiorectalis, s: Lig. sacrotuberale, t: Tuber ischiadicum, v: Vulva, k: Caudae.

layer filling the fossa ischiorectalis and caudolateral to the musculus coccygeus (Figure 6).

DISCUSSION

Ligamentum sacrotuberale is used in cases of hip dislocation, which is frequently encountered in dogs.[5,6,13] It can be used to strengthen repair, as the inclusion of the lig. sacrotuberale in a herniorrhage does not increase the risk of complications or nerve injury.[7,23] In studies using the transposition method of the lig. sacrotuberale, the ligament was cut from the origin point and directed to the coxo-femoral region, passed through the holes opened on the acetabulum, and served as a support.[6,13] In order to contribute to the transposition method of the ligament applied by the surgeons, as a result of our dissections, placing additional support sutures along the fixed ligament would be a more complementary and long-lasting approach, since the tuber ischiadicum, where this ligament ended, was weaker on the apex surface. In the surgical operation that includes the method of placing sutures around the lig. sacrotuberale for perineal hernia, the position of the anatomical structures of nerve ischiadicus, nerve pudendus, arteria pudenda interna, and vena pudenda interna should be well known.[7,12,24-27]

Evans and de Lahunta[3] stated that the lig. sacrotuberale consisted of a flattened fibrous stripe at both ends and was extending between the caudolateral endpoint of the sacrum and the processus transversus of the first caudal vertebra to the lateral part of the tuber ischiadicum. Takci and Ozcan[4] stated that the origin of lig. sacrotuberale started from the last sacral vertebrae and the first 2 coccygeal vertebrae. While the information obtained in the dissections and CT images in our study was compatible with Evans and de Lahunta,[3] it was not in line with Takci and Ozcan.[4]

It was stated that the lig. sacrotuberale anatomically served as the origin for musculus abductor cruris caudalis, musculus biceps femoris, musculus Piriformis, and musculus gluteus superficialis.[2-4] Similar findings were also reported in our study.

Takci and Ozcan[4] reported that the length of the lig. sacrotuberale was 88 mm. In the present study, this value was determined to be 95.48 ± 7.15 mm on the right side and 95.70 ± 7.12 mm on the left side in male dogs, and in female dogs, 90.61 ± 4.71 mm on the left and 90.59 ± 4.64 mm on the right. It was concluded that the findings of Takci and Ozcan[4] could only be found in small dogs. Miller[3,28] stated that the midpoint width of the ligament in large dogs was at most 3 mm. In the present study, the midpoint width of the ligament was determined as 4.15 ± 0.79 mm on the left and 4.01 ± 0.78 mm on the right in male dogs and 4.49 ± 0.56 mm on the left and 4.48 ± 0.58 mm on the right in female dogs. This difference was thought to be due to the breed of dogs used.

In conclusion, it is critical to specify the anatomical dimensions of this ligament, its structure, and the position of adjacent anatomical structures in newly developed techniques such as perineal hernia repair and hip luxation treatment to be performed in dogs, using the lig. sacrotuberale. With the study, the exact location of the lig. sacrotuberale was determined by dissections and 2D radiological and 3D modeling on the CT images obtained,[24] and data of the ligament were created with morphometric measurements. With the present study, determining the location of the ligament with various methods has enabled the accuracy of different information specified in the literature to be tested, and it is thought that the measurement data created will be important in clinical studies. The data obtained will be able to contribute to the studies to be done in surgery and on the region.

Ethics Committee Approval: The ethical approval for investigation was obtained by Karamanoglu Mehmetbey University Faculty of Health Sciences Ethics Committee (Date: 27.10.2021, Decision No: 06-34).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – C.T.M.; Design – C.T.M.; Supervision – M.L.S.; Resources – C.T.M.; Materials – C.T.M.; Data Collection and/or Processing – M.L.S.; Analysis and/or Interpretation – M.L.S.; Literature Search – M.L.S.; Writing Manuscript – C.T.M.; Critical Review – M.L.S.

Declaration of Interests: The authors declared that there is no conflict of interest.

Funding: The authors declared that this study has received no financial support.

Etik Komite Onayı: Bu çalışma için etik komite onayı Karamanoglu MehmetbeyÜniversitesi'nden (Tarih: 27.10.2021, Karar No: 06-34) alınmıştır.

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir – C.T.M.; Tasarım – C.T.M.; Denetleme – M.L.S.; Kaynaklar – C.T.M.; Malzemeler – C.T.M.; Veri Toplanması ve/veya İşlemesi – M.L.S.; Analiz ve/veya Yorum – M.L.S.; Literatür Taraması – M.L.S.; Yazıyı Yazan – C.T.M.; Eleştirel İnceleme – M.L.S.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

REFERENCES

1. Budras D, Fricke W, Richter R. *Veteriner anatomi atlası köpek*. Malatya, Türkiye: Medipres Yayıncılık; 2009;1:8-174.
2. König HE, Liebich HG. *Veteriner Anatomi (Evcil Memeli Hayvanlar)*. 6 th ed. Malatya, Türkiye: Medipres Yayıncılık; 2015:150-556.
3. Evans HE, Lahunta A. *Miller's Anatomy of the Dog*. 4 th ed. Philadelphia: Elseiver Saunders; 2013:158-181.
4. Takci I, Ozcan S. Sacrotuberal ligament in the Kars Dog. *J Anim Vet Adv*. 2009;8(1):199-201.
5. Ozaydın I, Kılıç E, Baran V, Demirkan I, Kamiloglu A, Vural S. Reduction and stabilization of hip luxation by the transposition of the ligamentum sacrotuberale in dogs. An in vivo study. *Vet Surg*. 2003;32(1):46-51. [CrossRef]
6. Venturini A, Pinna S, Tamburro R. Combined intra-extra-articular technique for stabilisation of coxofemoral luxation. Preliminary results in two dogs. *Vet Comp Orthop Traumatol*. 2010;23(3):182-185. [CrossRef]
7. Khatri-Chhetri N, Khatri-Chhetri R, Chung CS, Chern RS, Chien CH. The spatial relationship and surface projection of canine sciatic nerve and sacrotuberous ligament: a perineal hernia repair perspective. *PLOS ONE*. 2016;11(3):e0152078. [CrossRef]
8. Yoon HY, Jeong SW. Coexistence of sciatic, dorsal, and caudal perineal hernias in a dog. *J Vet Clin*. 2010;27(5):584-587.
9. Dyce KM, Sack WO, Wensing CJ. *Veteriner Anatomi Konu Anlatımı ve Atlası*. 4th ed. Ankara, Türkiye: Elsevier; 2018:3-475.
10. Anderson WD, Anderson GB. *Atlas of Canıne Anatomy. Chester Field Parkway Malvern, Pensilvanya*. USA: Lea & Febiger; 1994:945-947.
11. Schaller O. *Illustrated Veterinary Anatomical Nomenclature*. 2 nd ed. Germany: MVS Medizinverlage Stutgart; 2007:1-265.
12. Cinti F, Rossanese M, Pisani G. A novel tecnique to incorporate the sacrotuberous ligament in perineal herniorraphy in 47 dogs. *Vet Surg*. 2021;50(5):1023-1031. [CrossRef]
13. Klc E, Ozaydın I, Atalan G, Baran V. Transposition of the sacrotuberous ligament fort he treatment of coxofemoral luxation in dogs. *J Small Anim Pract*. 2006;43(8):341-344. [CrossRef]
14. Bahar S, Dayan MO. Two and three-dimensional computed tomographic anatomy of the guttural pouch in Arabian foals. *J Anim Vet Adv*. 2014;13(12):694-701. [CrossRef]
15. Yilmaz O, Demircioğlu İ. Morphometric analysis and three-dimensional computed tomography reconstruction of the long bones of femoral and crural regions in Van cats. *Folia Morphol*. 2021;80(1):186-195. [CrossRef]
16. Dobak TP, Voorhout G, Vernooij JCM, Boroffka SAEB. Computed tomographic pelvimetry in English bulldogs. *Theriogenology*. 2018;118:144-149. [CrossRef]
17. El-Gendy S, Alsafy M, Hanafy B, Karkoura A, Enany ES. Morphology, ultrasonographic and computed tomography configuration of the dog pelvis and perineum. *Anat Histol Embryol*. 2021;50(1):114-127. [CrossRef]
18. Tisnado J, Amendola MA, Walsh JW, Jordan RL, Turner MA, Krempa J. Computed tomography of the perineum. *AJR Am J Roentgenol*. 1981;136(3):475-481. [CrossRef]
19. Feeney DA, Fletcher TF, Hardy RM. Atlas of correlative imaging anatomy of the normal dog, ultrasound and computed tomography. *Can Vet J*. 1992;33(8):546-552.
20. Dayan MO. *Yeni Zelanda tavşanlarında mide ve bağırsakların bilgisayarlı tomografi görüntülerinden üç boyutlu görüntü elde edilmesi* [Tez]. Selçuk Üniversitesi Veterinerlik Fakültesi; 2009. [CrossRef]
21. Gezici M, Maviş CT. Macro-anatomic, cross-sectional anatomic, and computerized tomographic examination of anal region in dogs. *EurasianJVetSci*. 2019;35(4):186-198. [CrossRef]
22. Mavis CT, Gezici M. Macro-anatomic, cross-sectional anatomic, and computerized tomographic examination of the urogenital region in dogs. *ActaVetEurasia*. 2022;48(1):4-11. [CrossRef]
23. Gill SS, Barstad RD. A review of the surgical management of perineal hernias in dogs. *J Am Anim Hosp Assoc*. 2018;54(4):179-187. [CrossRef]
24. Jones GM, Pitsillides AA, Meeson RL. Moving beyond the limits of detection: the past, the present, and the future of diagnostic imaging in canine osteoarthritis. *Front Vet Sci*. 2022;15(9):1-15. [CrossRef]
25. Pasquini C, Spurgeon T, Pasquini S. *Anatomy of Domestic Animals Systemic & Regional Approch*. 11th ed. USA: Sudz Publishing; 2003:220-520.
26. Shahar R, Shamir MH, Niebauer GW, Johnston DE. A possible association between acquired nontraumatic inguinal and perineal hernia in adult male dogs. *Can Vet J*. 1996;37(10):614-616.
27. Bitton E, Keinan Y, Shipov A, Joseph R, Milgram J. Use of bilateral superficial gluteal muscle flaps for the repair of ventral perineal hernia in dogs: a cadaveric study and short case series. *Vet Surg*. 2020;49(8):1536-1544. [CrossRef]
28. Miller ME. *Miller's Guide to the Dissection of the Dog*. 4 th ed. Philadelphia: Elseiver Saunders; 1993:211-243.

ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Reviewers List

Acknowledgment of Reviewers

Abuzer Acar
Adem Kaya
Ademola Clement Famurewa
Ahmet Uyar
Ali Cihan Taşkın
Ali Doğan Ömür
Alper Kürşat Demirkaya
Barış Atalay Uslu
Barış Sarı
Bekir Oğuz
Cüneyt Çağlayan
Cüneyt Çağlayan
Çiğdem Sevim
Dilek Güvenç
Dina Mostafa Mohammed
Dursunali Çınar
Ebubekir Ceylan
Elif Doğan
Faruk Pehlivanoğlu
Gamze Çakmak
Gürkan Uçar
Güzin İplikçioğlu Çil
Güzin Kaban
Hasan Altan Akkan
Hülya Kara
Hüseyin Erdem
Hüseyin Serkan Erol
Mehmet Cemal Adıgüzel
Mehmet Özkan Timurkan
Miroslav Kjosevski
Mohammed Shoaib
Muhammed Bahaeddin Dörtbudak
Mustafa Gül
Mustafa Sönmez
Mutlu Yağanoğlu
Nazmi Atasoy
Nergis Ulas
Nurcan Dönmez
Ömer Faruk Keleş
Sefa Küçükler
Selim Sekkin
Serap Kılıç Altun
Serkan Ali Akarsu
Sevda Urçar Gelen
Seyda Cengiz
Sıtkıcan Okur
Sinan Kandır
Sivashankar Ramakrishnan
Şahin Aslan
Şebnem Pamuk
Tuğrul Ertuğrul
Uğur Uslu
Uğur Ersöz
Yeliz Yıldırım
Yusuf Sinan Şirin
Zeynep Karapınar