مجله دامپزشکی ایران

آدرس پستی سردبیر
اهواز، دانشگاه شهید چمران اهواز، دانشکده دامپزشکی، سردبیر مجله علمی، کد پستی ۶۱۳۵۵، صندوق پستی ۱۴۵
تلفن و دورنگار: ۳۳۳۳۶۳۱۲ (۰۶۱)
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- این مجله به استناد نامه شماره ۳/۲۹۱۰/۵۴۵ مورخ ۸۴/۵/۸ کمیسیون بررسی نشریات وزارت علوم، تحقیقات و فناوری دارای **درجه علمی ـ پژوهشی** می‌باشد.
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Vol. 21, No. 3, Autumn, 2025, 5-10 *Original Article*
DOI: 10.22055/ivj.2025.513762.2786
Journal Homepage: http://www.ivj.ir/

Persistence of Maternal-Derived Antibody against *Clostridium Perfringens* Type D Epsilon Toxin in Goat Kids

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Received: 24.03.2025 Accepted: 02.06.2025

Abstract

Enterotoxemia occurs in goats as a common disease with high mortality. The present study aimed to investigate maternal-derived antibodies against epsilon toxin to improve vaccination strategies for enterotoxemia in goats in Iran. Kids born from 12 vaccinated goats and kids born from 7 unvaccinated goats were sampled at 0, 3, 14, 28, 42, 56, 70, and 86 days of age. A commercial blocking ELISA kite was used for serological analysis of samples. The percentage of inhibition was significantly higher in vaccinated dams compared to unvaccinated ones. In kids, the percentage of inhibition in both groups was statically similar until 42 days of age. After this point, the percentage of inhibition in the control group decreased significantly, becoming notably lower compared to the treatment group at 56 and 70 days old. At 86 days old, the percentage of inhibition in both groups was statically indifferent. In the unvaccinated group, the percentage of inhibition at 56 days of age is significantly lower than the percentage of inhibition at 42 days of age. Moreover, in the vaccinated group, the percentage of inhibition at 70 days of age was significantly lower than at 56 days of age. In conclusion, vaccinating dams during the last month of pregnancy is effective in enhancing maternally derived antibody titers against epsilon toxin in kids. In goat kids born to vaccinated and non-vaccinated dams, 56–70 and 42–56 days of age, respectively, seem to be the proper times for the administration of the first dose of the enterotoxemia vaccine.

Key words: *Clostridium perfringens*, Entrotoxemia, Epsilon toxin, Kids, Vaccine

Introduction

Enterotoxemia occurs in goats as a common disease with high mortality (Rajamohan and Rajasekaran, 2025). Depending on their ability to produce the four typing toxins (α, β, ε, ι toxins), *Clostridium perfringens* is classified into five toxinotypes (A, B, C, D, E) (Sumithra et al, 2013). *Clostridium. perfringens* type D is the principal organism involved in caprine enterotoxemia (Rajamohan and Rajasekaran, 2025; Sumithra et al, 2013). However, types B and C have also been

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mentioned as its etiology (Sumithra et al, 2013). Epsilon toxin is considered to be the most important toxin in *Clostridium perfringens* type B and D enterotoxemia (Sumithra et al, 2013).

Clostridium perfringens, considered part of the normal flora in goats, proliferates rapidly and produces large amounts of toxins when the intestines are altered by sudden changes in diet (Sumithra et al, 2013). Consequently, overfeeding is the main predisposing factor (Rajamohan and Rajasekaran, 2025). Epsilon toxin induces an increase in the permeability of intestinal mucosa, facilitating the passage of toxins into blood, and their spread into various organs is considered the causative virulence factor of all symptoms and lesions caused by *Clostridium perfringens* type D infection (Sumithra et al, 2013).

Enterotoxemia occurs commonly in goat farms in Iran and leads to significant economic losses for farmers (Alimolaei and Ezatkhah, 2022; Hayati et al, 2020; Hussain et al, 2022). Peracute enterotoxemia has been reported in two goat herds with high mortality in Iran (Esmaeili and Joghataei, 2024). The report states that the disease has been well controlled by vaccination (Esmaeili and Joghataei, 2024). Many studies have been published on the importance of vaccination in controlling enterotoxemia in goats (Asadi, et al, 2023; Naz et al, 2012; Veschi et al, 2006). However, many aspects of vaccination against enterotoxemia in goats are not fully understood. For example, it is not yet fully clear when maternal derived antibodies against enterotoxemia in kids decay or, more precisely, when kids should be vaccinated against enterotoxemia so that these maternal-derived antibodies are sufficiently reduced and probably do not neutralize the active antibodies induced by vaccination. The present study aims to investigate these issues to improve vaccination strategies against enterotoxemia in goats in Iran.

Material and method

Animals

This study was conducted on a dairy goat farm (housing nearly 5000 Murciana goats). Goats are kept indoors all year round.

A total of 30 maiden goats, approximately 11 months old, and carrying one fetus at 6 weeks before the expected parturition date, were selected for the study. The number of fetuses in each goat and the pregnancy dates were determined based on ultrasound examination findings and mating records. The selected goats were divided into two equals: treatment and control. The treatment group was vaccinated at 6 and 4 weeks before the expected parturition, while the control group was not vaccinated.

Sufficient colostrum (20% of body weight) was administered to the kids via bottle feeding. The first feeding occurred within 30 minutes after birth, followed by three colostrum feedings every four hours. After that, milk feedings were started 3 times a day. Water and starter were made available to the kids at 3 days of age. The kids were weaned at approximately 90 days of age. Milk and colostrum were pasteurized before being given to the kids.

The enterotoxemia vaccine used in this study was Polivac cl 7® (Vetal com, Turkey).

Blood sampling

Blood sampling was performed at different times, as shown in Figure 1. Blood was collected from the dams at one week before parturition (3 weeks after the second dose of vaccination). Kids were sampled at 0 (before colostrum intake), 3, 14, 28, 42, 56, 70, and 86 days old.

Exclusion criteria

Dams

Goats affected by any disease (such as pregnancy toxemia) during pregnancy, those with a low body condition score, udder problems, mastitis, with low colostrum production, or those that experienced abortion or stillbirth were excluded from the study.

Kids

Kids that did not receive sufficient colostrum at a proper time, those that died during the study, those with low birth weight (weak kid), those born with dystocia, and those with congenital defects were excluded from the study.

As a result of applying these exclusion criteria, some goats and their kids were removed from the study, leaving samples from 12 goats in the treatment group and 7 goats in the control group for serological analysis.

ELISA testing

Blocking ELISA was used to measure antibody titers against *Clostridium perfringens* epsilon toxin in samples, as previously mentioned (Rousselet et al, 2021) and according to the kit manufacturer's instructions [*Monoscreen* ELISA Kit (BioX Diagnostics, BIO K 222 - Monoscreen AbELISA *Clostridium perfringens* epsilon toxin / blocking)].

Inhibition percentage was calculated using the following formulas:

% Inhibition sample = [($OD_{450\,nm}$ negative sera − OD sample)/$OD_{450\,nm}$ negative sera] × 100,

% Inhibition positive = [($OD_{450\,nm}$ negative sera −OD positive sera)/$OO_{450\,nm}$ negative sera] × 100.

The test was validated only if the OD negative − OD positive was >0.7 and inhibition of the positive control was >30%.

Data analysis

The normal distribution of data in each group was checked using the Shapiro–Wilk test. After confirming normal distribution, independent t-test was used to compare the groups. A *P*-value of less than 0.05 was considered statistically significant.

Results

During the study, no clinical disease was observed on the studied farm. The percentage of inhibition was significantly higher in vaccinated dams than in unvaccinated ones; however, unvaccinated dams also exhibited high percentages of inhibition.

In kids, the percentage of inhibition in both groups was statically similar until 42 days of age. After that, percentage of inhibition in the control group decreased significantly, becoming notably lower than that in the treatment group at 56 and 70 days of age. At 86 days old, the percentage of inhibition in both groups was statically comparable.

Additionally, the percentage of inhibition in the unvaccinated group at 56 days of age was significantly lower than at 42 days of age.

In the vaccinated group, the percentage of inhibition at 70 days of age was significantly lower than at 56 days of age.

Table 1: The percentage of inhibition in the control and treatment groups at different times

	BP	BC	3-d	14-d	28-d	42-d	56-d	70-d	86-d
Treatment (mean±sem)	96.2±0.28	13.2±2.4	79.69±5.4	91.3±1.43	92.3±0.61	92.2±1.06	90.8±1.58	78.3±4.63	57.7±5.62
Control (mean±sem)	75.4±7.8	17.6±4.2	86.35±5.1	94.1±1.49	90.12±1.97	85.9±2.8	71.7±4.55	56.4±10.29	52.9±12.53
P Value	0.038*	0.339	0.439	0.223	0.192	0.085	0.005*	0.043*	0.737

**P*<0.05, BP: before parturition, BC: before colostrum intake

Table 2: Comparison between percentage of inhibition in the treatment and control groups in different times after birth

Days		0 , 3	3 , 14	14 , 28	28 , 42	42 , 56	56 , 70	70 , 86
P value	Treatment	0.000*	0.064	0.952	0.881	0.493	0.025*	0.07
	Control	0.000*	0.194	0.134	0.257	0.028*	0.197	0.836

*$P<0.05$

Discussion

Enterotoxemia associated with C. perfringens type D is a disease of ruminant animals, primarily of lambs and kids, and is worldwide in its distribution (Constable et al, 2017). Also, one of the most important diseases in kids is enterotoxemia associated with C. perfringens type D and the highest incidence of the disease is in suckling kids between 3 and 10 weeks of age (Constable et al, 2017; Rajamohan and Rajasekaran, 2025); the most important strategy for controlling this disease in goat kids is dam vaccination (Rajamohan and Rajasekaran, 2025). Dam vaccination is an effective way to protect kids from diseases, but the challenge is determining when this immunity ends. Research has been conducted in this field for some diseases like Contagious agalactia (Abdollahi et al, 2022) Pox, (Abdollahi et al, 2024), PPR (Abdollahi et al, 2023) in kids in Iran. However, despite the importance of enterotoxemia in goat kids, few studies have been conducted on maternally acquired immunity against epsilon toxin in kids in Iran and, to the best of our knowledge, this is the first study on this topic.

The roles of β- and ε-toxins in dysentery and enterotoxemia in lambs and kids have been well established (Alimolaei and Shamsaddini Bafti, 2023). The β-toxin produced by type B strains causes dysentery in young lambs and kids (<14 days old), while the ε-toxin produced by type D strains is a common cause of enterotoxemia in older lambs and kids (Alimolaei and Shamsaddini Bafti, 2023). Despite the recognized importance of type B in postnatal enterotoxemia, a study conducted in Iran found that the second most prevalent *Clostridium perfringens* toxinotype was type D (Alimolaei and Shamsaddini Bafti, 2023). Additionally, another study in Iran reported that type B isolates were the least frequent among *C. perfringens* strains collected from sheep and goats (Hayati et al, 2020). Therefore, we focused our study on type D. Nevertheless, investigating β-toxins remains a promising direction for future research.

In our study, all kids born to vaccinated dams had a percentage of inhibition above 50% at 70 days of age. At 86 days of age, approximately 60% of kids born to vaccinated dams had a percentage of inhibition above 50%, with the lowest percentage of inhibition recorded at 37%. In contrast, at 70 days of age, approximately 60% of kids born to unvaccinated dams had no titer and at 86 days of age, this proportion decreased to about 30%. Therefore, we can conclude that dam vaccination with the enterotoxemia vaccine can protect kids against enterotoxemia up to 86 days of age. Conversely, kids born to mothers who were not vaccinated in the last months of pregnancy but had previously undergone a regular and repeated vaccination program were protected against enterotoxemia up to 56 days of age. The significantly higher percentage of inhibition at 56 and 70 days of age in kids born to vaccinated dams compared to those born to unvaccinated dams, could be another reason for this conclusion. However, the percentage of inhibition in the vaccinated group begins to decline at 56 days of age, and by 70 days of age, it is significantly lower than at 56 days. Similarly, in the unvaccinated group, the percentage of inhibition begins to decline at 42 days of age, and at 56 days of age, it is significantly lower than at 42 days. It has been mentioned

that vaccination of maiden ewes twice-at an interval of at least 1 month, with the last vaccination approximately 4 weeks before lambing-results in good passive immunity in young lambs, with 97% of lambs having protective antibody levels at 8 weeks of age, with a significant proportion still protected at 12 to 16 weeks of age (Constable et al, 2017). Additionally, one study showed that vaccination of ewes before lambing provides passive protection to lambs up to 84 days of age (de la Rosa et al, 1997). Although these findings are consistent with our study, some research suggests that the antibody titer induced by the enterotoxemia vaccine in goats is lower and has a shorter duration compared to that in sheep (Asadi et al, 2023; Asadi et al, 2023; Biotech et al, 2012). To our knowledge, there are few studies on the persistence of maternal antibodies against epsilon toxin in goat kids. Only a few textbooks and review articles recommend that kids be vaccinated initially at four to six weeks of age (Constable et al, 2017); (Sumithra et al, 2013). Since the maternal-derived antibody against epsilon toxin can be affected by the type of vaccine used in dam, husbandry and management conditions, and the frequency of previous vaccinations administered to the dam, further studies are needed to conclude definitively about maternally derived immunity against epsilon toxin in kids.

A study conducted in Iran on the humoral immune response against *Clostridium perfringens* epsilon toxin in goats and sheep showed that the duration of the humoral immune response in goats upon administration of the clostridial vaccine was relatively brief, requiring multiple booster injections (Asadi et al, 2023). The goats in our study had received multiple booster injections and they had been vaccinated 4 times (two doses at 45 days old and then two doses at 6 months old) until they reached 11 months (the time selected for the study). This routine vaccination regimen and the multiple booster injections may explain the high percentage of inhibition in the unvaccinated dams, as well as the insignificant differences in percentage of inhibition in both groups until 42 days of age. On the other hand, another study states that natural acquired antibodies against *Clostridium perfringens* epsilon toxin can appear in young goats and increase with age, without any evidence of clinical disease (Veschi et al, 2008). The presence of these naturally acquired antibodies could also explain the high percentage of inhibition in unvaccinated goats. However, the percentage of inhibition was significantly higher in vaccinated dams than in unvaccinated ones, underscoring the importance of vaccination in late pregnancy. Later significant decrease of the percentage of inhibition in goat kids born to vaccinated dams compared to unvaccinated ones also highlights the importance of vaccination in late pregnancy.

In conclusion, vaccinating dams during the last month of pregnancy is effective in enhancing maternally derived antibody titers against epsilon toxin in kids. In goat kids born to vaccinated and non-vaccinated dams, 56–70 and 42–56 days of age, respectively, seem to be the proper times for the administration of the first dose of the enterotoxemia vaccine.

Acknowledgements

The authors wish to thank everyone who contributed their time and experience to this study.

Conflict of Interest

The authors declare no conflict of interest.

Funding

No financial aid was received for this study.

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Received: 24.03.2025
Accepted: 02.06.2025

Vol. 21, No. 3, Autumn, 2025, 11-21 *Original Article*
DOI: 10.22055/ivj.2025.512793.2783
Journal Homepage: http://www.ivj.ir/

Presence of certain extended-spectrum beta-lactamase (ESBL) genes in fecal strains of *Escherichia coli* from dogs and the antibiotic sensitivity of the isolates

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Received: 16.03.2025 Accepted: 24.06.2025

Abstract

Escherichia coli (*E. coli*) is a member of the *Enterobacteriaceae* and part of the normal flora in the intestines of both humans and warm-blooded animals. It causes a wide range of infections, both gastrointestinal and extra-intestinal, in humans and animals, including dogs and cats. Dogs and cats are often considered potential reservoirs of *E. coli* strains that can cause intestinal or extra-intestinal infections in humans. Therefore, zoonotic transmission aspects of infection are highly important. The use of certain antibiotics and the selective pressure in the environment contribute to the selection and spread of resistance genes to similar antibiotics, complicating the treatment of many bacterial infections. This study aimed to investigate the presence of certain extended-spectrum beta-lactamase (ESBL) genes in *E. coli* strains isolated from feces of both healthy and diarrheal dogs, and to examine their antibiotic sensitivity. A total of 100 *E. coli* isolates were screened phenotypically for the production of ESBL enzymes using cefotaxime and cefotaxime/clavulanic acid combination disks. The antibiotic sensitivity of the ESBL-producing strains to 12 antibiotics from various classes was evaluated. Furthermore, the presence of the genes *blaTEM*, *blaSHV*, *blaCTX-M-1*, *blaCTX-M-9*, and *blaOXA-1* in the ESBL-producing isolates was assessed using multiplex PCR. The results showed that 31 out of 100 *E. coli* isolates were phenotypically ESBL producers. The *blaTEM* gene was identified as the predominant ESBL gene in 45.2% of the isolates, while the *blaCTX-M-1* gene was found in 25.8%. The highest antibiotic resistance was observed against erythromycin, while the lowest was against meropenem. Additionally, 20 different antibiotic resistance patterns were identified in the isolates. Given the zoonotic aspects of *E. coli* transmission, further epidemiological studies and pre-treatment antibiotic sensitivity profiling are recommended to ensure successful treatment and prevent the spread of ESBL-producing strains.

Key words: Extended, Spectrum Beta, Lactamase, *E. coli*, Antibiotic Sensitivity, Dogs

Introduction

E. coli is a member of the *Enterobacteriaceae* and a part of the normal intestinal flora in humans and warm-blooded animals. It causes a broad spectrum of infections, both gastrointestinal and extra-intestinal, in humans and animals,

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including dogs and cats (Kaper et al, 2004). Dogs and cats are often considered potential reservoirs of *E. coli* strains, which can cause intestinal or extra-intestinal infections in humans, making the zoonotic transmission of infection a crucial concern. Poor hygienic practices in keeping animals may increase the risk of colonization of these pathogens in humans (Sevilla et al, 2020; Yasugi et al, 2021).

The use of antibiotics within similar classes in both human and veterinary medicine, combined with environmental selective pressure, plays a significant role in the selection and spread of resistance genes to similar antibiotics, complicating the treatment of many bacterial infections. Inappropriate use and incorrect prescription of antibiotics, underdosing, irregular administration of antimicrobial drugs, and the evolving role of dogs in society today are key risk factors for the emergence of antimicrobial resistance and the transmission of bacteria. More than 200 types of ESBLs are found worldwide, mostly within the *Enterobacteriaceae*. The *blaTEM*, *blaSHV*, and *blaCTX-M* groups are examples of common ESBLs (Huang et al, 2020; Yasugi et al, 2021; Pathak et al, 2017). Carrying ESBL genes on plasmids facilitates their transfer to other bacteria through conjugation. These plasmids also carry genes responsible for resistance to various antimicrobial classes, including fluoroquinolones, aminoglycosides, sulfonamides, and trimethoprim, thus limiting the treatment options for infections caused by ESBL-producing bacteria (Pitout and Laupland, 2008).

Detecting extended-spectrum beta-lactamases (ESBLs) and monitoring antibiotic resistance in *E. coli* as a sentinel bacterium is an essential tool in both preventing the spread of antibiotic resistance and controlling antibiotic usage. The availability and consumption of antibiotics, along with hygiene practices and disease prevalence, can influence the frequency and types of ESBL enzymes in bacterial strains within any given geographic area (Marchetti et al, 2021; Salgado-Caxito et al, 2021). Direct and indirect contact with pets carrying bacteria with genetic elements such as plasmids and integrons is one of the routes for antibiotic resistance transmission from animals to humans, complicating the treatment of infections caused by *E. coli* strains (Vinue et al, 2008). Formenti et al. demonstrated the role of household dogs as carriers of ESBL-producing *E. coli* strains (Formenti et al, 2021). Given reports on the spread of ESBL-producing *E. coli* strains in both animal and human infections, controlling and preventing these infections is vital. Furthermore, considering the importance of *E. coli* infections in small animals and its zoonotic potential, the goal of this study was to determine the antibiotic resistance patterns of *E. coli* isolates from dogs and assess the prevalence of extended-spectrum beta-lactamases (*blaSHV*, *blaTEM*, *blaCTX-M-1*, *blaCTX-M-9*, and *blaOXA-1*) among the isolates. The results of this study can contribute to identifying antibiotic sensitivity patterns in *E. coli* and the potential resistance in dog populations, thus aiding effective control and preventive measures.

Materials and Methods

E. coli isolates

The *E. coli* isolates used in this study were archived isolates collected from both apparently healthy dogs and dogs with diarrhea. A total of 75 isolates were from apparently healthy dogs, and 25 isolates were from dogs with diarrhea. The isolates were stored in skimmed milk at -70°C. After removal from the freezer and thawing, they were cultured onto nutrient agar plates using a sterile loop and incubated for 18–24 hours at 37°C. All *E. coli* isolates were identified based on Gram staining, catalase and oxidase tests, triple sugar iron (TSI) agar reactions, IMViC tests, urease test, phenylalanine deaminase test, lysine decarboxylase test, and the characteristic

metallic sheen on eosin methylene blue (EMB) agar (Markey et al, 2013).

Initial Screening of Isolates Using Ceftriaxone Disc Diffusion Method

According to the CLSI protocol, *E. coli* isolates were first screened using ceftriaxone (30 µg) discs and the Kirby-Bauer disk diffusion method. Isolates with a zone of inhibition equal to or smaller than 27 mm were considered suspected ESBL producers (CLSI, 2023).

Phenotypic Evaluation and Confirmation of ESBL Production by the Combined Disk Method

After the initial screening, the presence of ESBLs in the isolates was confirmed using the combined disk method. For this, a 5 mL suspension of each isolate, equivalent to a 0.5 McFarland standard, was prepared in physiological saline and plated onto Mueller-Hinton agar. Cefotaxime and cefotaxime with clavulanic acid antibiotic discs were then placed on the agar plates and incubated at 37°C for 18–24 hours. After incubation, the zone of inhibition was measured, and isolates that showed an increase of 5 mm or more in the diameter of the zone around the cefotaxime + clavulanic acid disc compared to the cefotaxime-only disc were confirmed as ESBL-producing *E. coli* isolates (CLSI, 2023).

Phenotypic Evaluation of Antibiotic Resistance Patterns in ESBL-Producing *E. coli* Isolates

After identifying the *E. coli* isolates that produce extended-spectrum beta-lactamases (ESBLs), the resistance or susceptibility of each of the 31 isolates to 12 antibiotics (ciprofloxacin, erythromycin, neomycin, nitrofurantoin, cefoxitin, furazolidone, trimethoprim-sulfamethoxazole, ampicillin, gentamicin, tetracycline, meropenem, and nalidixic acid) was individually tested. These antibiotics belong to different groups, including fluoroquinolones, macrolides, aminoglycosides, nitrofurans, cephalosporins, sulfonamides, beta-lactams, tetracyclines, carbapenems, and quinolones, following standard conditions. For this purpose, a 5 mL suspension of each confirmed ESBL-producing *E. coli* isolate was prepared in sterile physiological saline to match the turbidity of the 0.5 McFarland standard. Using a sterile swab, the suspension was cultured onto Mueller-Hinton agar plates, and antibiotic discs were placed on the surface of the agar. The plates were then incubated at 37°C for 18–24 hours. After incubation, the zone of inhibition around each antibiotic disc was measured, and the susceptibility or resistance of each isolate to the tested antibiotics was evaluated based on standard tables (CLSI, 2023).

Detection of *blaTEM, blaSHV, blaCTX-M-1, blaCTX-M-9,* and *blaOXA-1* Genes in ESBL-Producing *E. coli* Isolates by Multiplex PCR

For PCR testing, the DNA of each isolate was extracted by the boiling method. Then, multiplex PCR and specific primers for the genes *blaTEM*, *blaSHV*, *blaCTX-M-1*, *blaCTX-M-9*, and *blaOXA-1* were used to identify the types of extended-spectrum beta-lactamases (ESBLs) (Table 1). For each ESBL-producing *E. coli* isolate, the following reaction mixture was prepared: 15 µL of master mix, 5 µL of extracted DNA from each isolate, 0.6 µL of forward primer and 0.6 µL of reverse primer for each of the *blaTEM*, *blaSHV*, *blaCTX-M-1*, and *blaOXA-1* genes, 1.2 µL of forward primer and 1.2 µL of reverse primer for the blaCTX-M-9 gene, and 2.8 µL of distilled water, giving a final volume of 30 µL. The thermal cycling program for amplifying the fragments is shown in Table 2 (Ogutu et al., 2015).

PCR Product Analysis by Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis in a 1.5% agarose gel. Seven microliters of PCR products were loaded into the wells of the agarose gel alongside a molecular weight marker and

electrophoresed at 80 volts. After the electrophoresis was completed, the gel was placed in a transilluminator. By exposing the gel to UV light, the amplified DNA fragments were examined for the correct fragment size by comparing them with the molecular marker.

Statistical Analysis

The data obtained from each step were analyzed using SPSS software and the Chi-square statistical test.

Table 1: Primer sequences used to determine the type of extended-spectrum beta-lactamase (ESBL) enzymes in *E. coli* ESBL-producing isolates (Ogutu et al, 2015)

Target Gene	Primer Sequence (5' to 3')	Product Length (bp)
blaTEM	F: 5'-CATTTCCGTGTCGCCCTTATTC-3' R: 5'-CGTTCATCCATAGTTGCCTGAC-3'	800
blaSHV	F: 5'-AGCCGCTTGAGCAAATTAAAC-3' R: 5'-ATCCCGCAGATAAATCACCAC-3'	713
blaCTX-M-1	F: 5'-TTAGGAAGTGTGCCGCTGTA-3' R: 5'-CGGTTTTATCCCCCACAAC-3'	655
blaOXA-1	F: 5'-GCCCTTTACCAAACCAATAC-3' R: 5'-ACTTGATTGAAGGGTTGGGC-3'	564
blaCTX-M-9	F: 5'-GGTGATGAACGCTTTCCAAT-3' R: 5'-TTATCACCYRCAGTCCACGA-3'	518

Table 2: PCR thermal program for determining the type of extended-spectrum beta-lactamase (ESBL) enzymes in *E. coli* ESBL-producing isolates (Ogutu et al, 2015)

Cycle Step	Temperature (°C)	Time (Seconds)	Number of Cycles
Initial Denaturation	94	300	1
Denaturation	94	30	30
Annealing	61	30	
Extension	72	40	
Final Extension	72	600	1

Results

Initial Screening of *E. coli* Isolates Suspected of Producing Extended-Spectrum Beta-Lactamase (ESBL)

Out of 100 *E. coli* isolates (25 from diarrheic dogs and 75 from clinically healthy dogs), 36 isolates (including 13 diarrheic and 23 clinically healthy isolates) showed a zone of inhibition of 27 mm or less in the initial screening, indicating suspicion of ESBL production.

Phenotypic Confirmation of ESBL Production in Isolates Using the Combined Disk Method

The results obtained using the combined disk method indicated that 31 isolates (including 12 diarrheic and 19 clinically healthy isolates) were capable of producing ESBL enzymes. The frequency and percentage of isolates confirmed to produce ESBL enzymes are summarized in Table 3.

Table 3: Frequency of *E. coli* isolates confirmed to produce ESBL enzymes

Origin of Isolate	ESBL-Producing Isolates	Non-ESBL-Producing Isolates
Healthy Dog (75)	19 (25.3%)	56 (74.6%)
Diarrheic Dog (25)	12 (48%)	13 (52%)
Total (100)	31 (31%)	69 (69%)

Results of Antibiotic Sensitivity and Resistance Patterns of *E. coli* Isolates Producing ESBL Enzymes

The results showed that 91% of the isolates were resistant to the antibiotic erythromycin, while resistance to meropenem was 3% (1 isolate) and to gentamicin was 6% (2 isolates). The number and percentage of resistance and sensitivity of the isolates to each antibiotic are presented in Table 4. The resistance patterns of each isolate are shown in Table 5. A total of 20 resistance patterns were observed, with the most common multidrug resistance pattern being simultaneous resistance to four antibiotics: ciprofloxacin, trimethoprim-sulfamethoxazole, erythromycin, and nalidixic acid.

Table 4: Antibiotic resistance results of *E. coli* isolates producing ESBL enzymes

Antibiotic	Abbreviation	Antibiotic Concentration (µg)	Resistant (Number)	Resistant (%)		Intermediate (Number)	Intermediate (%)	Sensitive (Number)	Sensitive (%)
Ciprofloxacin	CP	5	8	25.8%		1	3.2%	22	71%
Erythromycin	E	15	28	90.3%		2	6.4%	1	3.2%
Neomycin	N	30	3	9.7%		2	6.4%	26	83.9%
Nitrofurantoin	FM	300	3	9.7%		1	3.2%	27	87.1%
Cefoxitin	FOX	30	8	25.8%		0	0%	23	74.2%
Furazolidone	FX	100	2	6.4%		4	12.9%	25	80.7%
Trimethoprim-Sulfamethoxazole	SXT	25/1	21	67.8%		1	3.2%	9	29%
Ampicillin	AM	10	22	71%		3	9.7%	6	19.3%
Gentamicin	GM	10	2	6.4%		0	0%	29	93.5%
Tetracycline	TE	30	20	64.5%		1	3.2%	10	32.2%
Meropenem	MEN	10	1	3.2%		0	0%	30	96.8%
Nalidixic Acid	NA	30	15	48.4%		3	9.7%	13	41.9%

Table 5: Antibiotic resistance patterns and number of *E. coli* strains producing ESBL enzymes with similar antibiotic resistance patterns

Obtained Patterns	Resistant Antibiotics in Each Pattern	Number of Strains with Similar Pattern
1	E	2
2	E and AM	1
3	E and TE	1
4	TE, SXT, and AM	1
5	TE, SXT, and E	3
6	E, AM, and TE	2
7	E, SXT, and AM	3
8	E, AM, GM, and NA	1
9	E, FOX, and AM	1
10	E, SXT, AM, and TE	2
11	E, AM, FM, and NA	1
12	E, SXT, AM, TE, FOX, and NA	1
13	E, SXT, AM, TE, FOX, NA, and MEN	1
14	CP, SXT, E, and NA	4
15	CP, SXT, N, TE, AM, and NA	2
16	CP, E, N, TE, AM, and NA	1
17	CP, SXT, E, FM, and NA	1
18	SXT, AM, TE, FOX, and FM	1
19	CP, E, FOX, SXT, TE, AM, GM, and NA	1
20	E, FM, FOX, SXT, AM, FX, and NA	1

Results of Multiplex PCR Test for Detecting Genes Related to the Production of Extended-Spectrum Beta-Lactamases (ESBLs) in *E. coli* Strains Producing ESBLs

Out of 31 ESBL-producing strains identified by phenotypic methods, 20 strains were found to carry the target genes in the multiplex PCR assay. Fourteen strains (45.2%) had the *blaTEM* gene, and 8 strains (25.8%) had the *blaCTX-M-1* gene. Additionally, 2 strains (6.5%) carried both the *blaCTX-M-1* and *blaTEM* genes. All the strains tested negative for the *blaSHV*, *blaCTX-M-9*, and *blaOXA-1* genes (Figure 1).

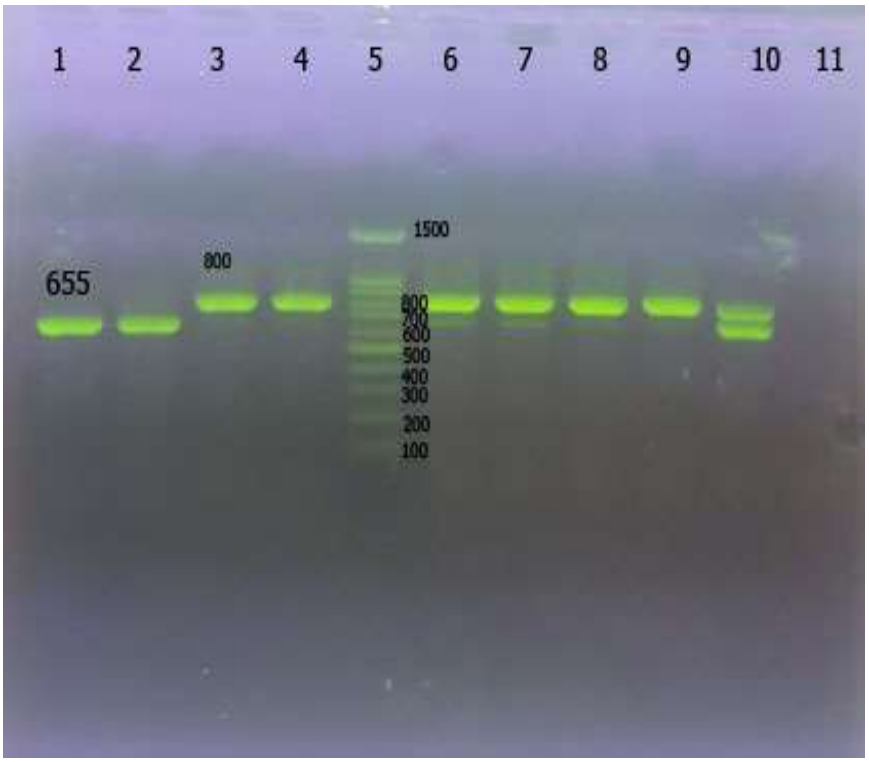

Figure 1 - PCR results for *blaCTX-M-1* and *blaTEM* genes in *E. coli* strains producing ESBLs.1-positive control for the *blaCTX-M-1* gene (655 bp).2-strain containing the *blaCTX-M-1* gene.3-positive control for the *blaTEM* gene (800 bp). 4, 6, 7, 8, 9. strains containing the *blaTEM* gene.5-marker (100 bp).6-strain containing both *blaTEM* and *blaCTX-M-1* genes.11-negative control

Discussion

Although the correct use of antibiotics for the prevention and treatment of infectious diseases leads to the eradication or inhibition of pathogenic microorganisms, inappropriate use can result in unintended consequences. According to available statistics, the per capita drug consumption in Iran is significantly high due to cultural factors and the relatively low cost of medications, which is four times the global average (Hosseinzadeh et al, 2016). In the recent years, there has been a widespread increase in infections caused by antibiotic-resistant bacteria commonly used in treatment. Therefore, monitoring antibiotic resistance patterns is essential for public health surveillance and preventive measures (Miriagou et al, 2003). Like pathogenic bacteria, commensal bacteria are also exposed to antimicrobial agents, and *E. coli* is often used as an indicator for the spread of resistance genes (Bartoloni et al, 2006). The molecular characteristics of antimicrobial resistance can be valuable not only in surveillance studies and tracking multi-drug-resistant strains but also in gaining insights into the relationships between human and animal bacterial strains

(Carvalho et al, 2016). Specifically, attention should be given to bacteria like *E. coli*, which are well adapted for use in both humans and animals, as shared environments create opportunities for the rapid transmission of these strains between hosts (Formenti et al, 2021).

One of the most important mechanisms of resistance in *E. coli* is the production of enzymes, particularly extended-spectrum beta-lactamases (ESBLs), which inactivate beta-lactams by hydrolyzing their beta-lactam rings (Liu et al, 2016). ESBL producers typically exhibit a multidrug-resistant phenotype. Additionally, the genes encoding ESBLs are mostly plasmid-mediated, which facilitates the transfer of antibiotic resistance genes to other bacteria (Huang et al, 2020). Recent studies have raised concerns about the widespread presence of ESBLs and integrons in *E. coli* strains isolated from humans and healthy animals as reservoirs of antibiotic resistance. Dogs and their feces can serve as a source of *E. coli* strains, which may pose a potential threat to humans through virulence factors or multidrug resistance in these bacteria (Sevilla et al, 2020). Since companion animals like dogs and cats are in close contact with humans, they can acquire ESBL-producing microorganisms from humans and potentially transmit them back to humans, which raises a public health concern (Huang et al, 2020).

Various studies have been conducted on ESBL-producing bacteria, especially *E. coli*, in different animals. However, based on the research conducted so far, it appears that no study with this focus has been carried out on household dogs in Khuzestan province.

In the present study, out of 100 *E. coli* isolates, 31 isolates (31%) were phenotypically identified as ESBL producers. Of these, 12 isolates (38.7%) were from diarrheal samples, and 19 isolates (61.3%) were from healthy samples. In other studies, the phenotypic frequency of ESBL-producing *E. coli* isolates ranged from 1.9% to 28.5%. Studies conducted in other parts of the world have also shown variable prevalence of ESBL-producing isolates, ranging from 5% to 28% in both healthy and diarrheal dogs (Marchetti et al, 2021; Aslantas et al, 2017; Formenti et al, 2021; Carvalho et al, 2021; Sevilla et al, 2020; Huang et al, 2020; Carvalho et al, 2016; Liu et al, 2016; Wang et al, 2020; Tamang et al, 2012). Differences in the frequency of ESBL-positive *E. coli* isolates may be due to variations in the antibiotic consumption rates in the studied animals, the origin of the isolates (stray dogs, cats, chickens, pigs, and cows), the prevalence of disease among the animals in the mentioned studies, and the management practices in animal husbandry. The high prevalence of ESBL-producing *E. coli* isolates in the studied area could be attributed to the health status of the animals (both diarrheal and seemingly healthy), the excessive use of beta-lactam antibiotics, and consequently the increased selective pressure on the animal strains in this region. This could potentially raise the risk of the spread and transmission of ESBL-producing strains to the human population in the area.

Another key aspect addressed in this study was the genotypic analysis of *E. coli* isolates producing ESBL enzymes and the genes related to their production. The analyses conducted in this study revealed that, out of 31 *E. coli* isolates producing ESBL enzymes, 20 isolates (64.5%) contained the enzymes of interest. Fourteen isolates (45.2%) harbored the *blaTEM* gene, and eight isolates (25.8%) carried the *blaCTX-M-1* gene. Additionally, two isolates (6.5%) contained both the *blaCTX-M-1* and *blaTEM* genes, and all isolates tested negative for the *blaSHV*, *blaCTX-M-9*, and *blaOXA-1* genes. In studies conducted by other researchers on companion animals, including dogs, the frequency of these genes in *E. coli* isolates varied. In the study by Formenti et al. (2021) in dogs, the *blaCTX-M* gene (79.7%)

was the predominant ESBL gene. *blaTEM* (47.8%), *blaSHV* (5.8%), and *blaCMY* (13%) genes were also identified. In the study by Aslantas et al. (2017), *blaCTX-M-15* (86.1%) was the dominant ESBL gene. Liu et al. (2016) in China found that the predominant ESBL genes were *blaCTX-M* (87.5%) and *blaTEM* (87.5%). Tamang et al. (2012) in South Korea reported that all *E. coli* isolates contained the *blaCTX-M* gene (100%). *blaCTX-M-1* was found in two isolates (16.6%), and *blaCTX-M-9* was present in ten isolates (83.3%). In the study by Carvalho et al. (2016), *blaCTX-M* (75%) was the predominant ESBL gene, with *blaTEM* (66.7%) and *blaSHV* (38.8%) was also identified. Among the 8 ESBL-producing isolates from dog owners, the percentages of *blaCTX-M*, *blaTEM*, and *blaSHV* genes were 50%, 75%, and 12.5%, respectively. Huang et al. (2020) in Taiwan identified *blaCTX-M-1* as the predominant gene (54%) in 65 *E. coli* isolates, followed by *blaCTX-M-9* (32%), *blaTEM* (38%), *blaSHV* (6%), and *blaCTX-M-2* (18.5%). In the study by Carvalho et al. (2021) in Portugal, involving 361 dogs, the *blaCTX-M* gene was found in 95.7% of the isolates, with 21.3% of these related to *blaCTX-M-1*. Four other types of *blaCTX-M* enzymes were identified: *blaCTX-M-15* (55.3%), *blaCTX-M-32* (6.4%), *blaCTX-M-55* (6.4%), and *blaCTX-M-14* (4.2%). As mentioned earlier, it seems that sanitary conditions, the frequency of different infections, and the accessibility and consumption of antibiotics, which affect the level of selective environmental pressure, may be factors contributing to the differences in the prevalence and types of ESBL enzymes in bacterial strains in different geographical regions.

Another aspect investigated in this study was the antibiotic susceptibility and resistance patterns of the ESBL-producing isolates. In the present study, the antibiotic susceptibility of 31 *E. coli* isolates producing ESBL enzymes was investigated. The highest antibiotic resistance was observed against erythromycin (91%), while the lowest resistance was seen with meropenem (3%). Resistance to ciprofloxacin (26%), neomycin (10%), nitrofurantoin (10%), cefoxitin (26%), furazolidone (6%), trimethoprim-sulfamethoxazole (68%), ampicillin (71%), gentamicin (6%), tetracycline (65%), and nalidixic acid (48%) was also measured. Several studies have evaluated the sensitivity and resistance of *E. coli* isolates from dogs, with varying resistance or susceptibility patterns to different antibiotics. In the study by Aslantas et al. (2017), the highest resistance was observed against cephalothin (98.9%), and the lowest against tobramycin (20%). Liu et al. (2016) reported, in their study of 40 *E. coli* isolates producing ESBL enzymes, that the highest resistance was to doxycycline (95%), while the lowest resistance was found for imipenem and meropenem (25%). In Tamang et al.'s study (2012) in South Korea, the highest antibiotic resistance among ESBL-producing *E. coli* isolates was observed against tetracycline (75%), with the lowest resistance against amikacin (3.8%). In the study by Wang et al. (2020) in China, involving 400 *E. coli* isolates from chickens, dogs, pigs, and cows, the highest antibiotic resistance in 100 *E. coli* isolates from dogs was against ampicillin (70%), while the lowest resistance was seen against meropenem (5%). In the study by Marchetti et al. (2021) in Argentina, involving 95 dogs, the highest antibiotic resistance was against tetracycline (50.5%), and the lowest resistance was observed against imipenem, amikacin, and nitrofurantoin (0%). In the study by Formenti et al. (2021) on 266 dogs, the antibiotic susceptibility of 69 *E. coli* isolates producing ESBL enzymes was measured, and all isolates were resistant to at least one antibiotic. The highest resistance was observed against cefotaxime (100%), while the lowest resistance was against imipenem (0%). In the study by Huang et al. (2020) in Taiwan, involving 283 dogs and cats (224 dogs and 59 cats),

the highest resistance was observed against ampicillin (100%), and the lowest resistance was against imipenem (0%). Similarly, in the study by Carvalho et al. (2021) in Portugal, involving 361 dogs, the highest resistance was against ampicillin (100%), and the lowest was against imipenem (0%).

The analysis conducted in this study showed that out of the 31 *E. coli* isolates confirmed to produce ESBL enzymes, only 2 isolates (6%) were resistant to a single antibiotic (erythromycin), 2 isolates (6%) showed resistance to two antibiotics, and 27 other isolates (88%) demonstrated resistance to at least three antibiotics or more, and were identified as multi-drug resistant (MDR) isolates. In this study, no isolate was found to be sensitive to all the antibiotics tested. In the study by Aslantas et al. (2017), 17.9% of isolates were resistant to one antibiotic, 14.7% to two antibiotics, and 67.4% of isolates were resistant to three or more antibiotics, identifying them as MDR strains. Furthermore, in the study by Carvalho et al. (2021) in Portugal, conducted on 361 dogs, it was shown that out of 47 *E. coli* isolates producing ESBLs, all isolates were resistant to at least one antibiotic. Only 1 isolate (2.1%) was resistant to one antibiotic, 3 isolates (6.4%) were resistant to two antibiotics, and 41 isolates (91.5%) were resistant to three or more antibiotics, classifying them as MDR strains. In the study by Liu et al. (2016) in China, 165 *ExPEC* isolates were examined, and it was shown that out of 40 *E. coli* isolates producing ESBLs, only 1 isolate (2.5%) was resistant to two antibiotics, while 39 isolates (97.5%) exhibited an MDR phenotype. In the retrospective study by Tamang et al, (2012) in South Korea, conducted between 2006 and 2007 on 628 dogs, 12 *E. coli* isolates producing ESBLs were analyzed. Of these, 2 isolates (16.6%) were not resistant to any antibiotics, 1 isolate (8.3%) was resistant to two antibiotics, and 9 isolates (75%) showed an MDR phenotype. In the study by Wang et al. (2020) in China, involving 400 *E. coli* isolates from chickens, dogs, pigs, and cows, it was shown that 267 isolates (66.8%) were resistant to three or more antibiotic classes. Additionally, in the study by Marchetti et al. (2021) in Argentina, involving 95 dogs, it was found that 17 isolates were resistant to one antibiotic, 10 isolates were resistant to two antibiotics, and the remaining 41 isolates were resistant to three or more antibiotic classes. The differences in the level of antibiotic resistance observed in the present study compared to other studies can be explained by factors such as the availability and use of antibiotics, hygiene levels, the occurrence of infections in animals, living environments, the presence of specific traits such as broad-spectrum beta-lactamase production by strains, and their origin (apparently healthy dogs, stray dogs, out-of-town dogs, hospital samples, livestock, and poultry). The high prevalence of MDR *E. coli* isolates and the high resistance rates to various antibiotic groups suggest the need for further studies on controlling antibiotic resistance and appropriate antibiotic prescription in treating infections.

Considering the diversity in the characteristics of *E. coli* strains from different geographic regions and animal species, the high prevalence of ESBL-producing isolates, and the presence of multi-drug resistance (MDR) patterns in these isolates in this study, the zoonotic transmission aspects of *E. coli* and the potential transfer of this bacterium from companion animals, such as dogs and cats, to the human population are significant. To ensure successful treatment and prevent the spread of ESBL-producing strains, further epidemiological studies are recommended, along with the determination of antibiotic susceptibility patterns of *E. coli* isolates over various time periods and locations, as well as prior to treatment.

Acknowledgments

The authors gratefully acknowledge Shahid Chamran University of Ahvaz (Khuzestan Province, Iran) for providing financial support, laboratory space, and facilities, and covering associated costs..

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research was funded by the research grant number (SCU.VP1401.261) from Shahid Chamran University of Ahvaz.

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Received: 16.03.2025
Accepted: 24.06.2025

Vol. 21, No. 3, Autumn, 2025, 22-29 *Original Article*
DOI: 10.22055/ivj.2025.551126.2854
Journal Homepage: http://www.ivj.ir/

Molecular Detection of Tick-Borne Hemoparasites (Theileria, Babesia and Anaplasma) in Stray Dogs using Nested PCR

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Received: 04.10.2025 Accepted: 07.12.2025

Abstract

Haemoparasitic infections are frequently observed in dogs from tropical regions, including Iraq. Numerous dogs become infected with several blood parasites, resulting in more serious diseases than a singular infection. This investigation was designed in Wasit Province, Iraq to conduct a comprehensive molecular detection and characterization of haemoparasites in Infested Dogs. This cross-sectional study was performed from the beginning of May 2024 to the end of December of 2024. Totally 280 stray dogs were examined in different areas in waist, Iraq. The blood sample were obtained from the jugular vein of infested dogs was used for both microscopic and molecular analysis. Thin blood smears were prepared, detected by giemsa staining and screened for piroplasms Babesia, Theileria and Anaplasma. Total DNA was extracted followed by nested PCR using primer targeting16S *rRNA* gene to detect for Anaplasma spp. and 18SrRNA for Babesia spp and Theilleria spp. PCR products were confirmed by agarose gel electrophoresis. Nucleotide sequencing verified the authenticity of the amplified genes, whose sequences were compared with reference sequences of the *16S rRNA* and *18S rRNA* genes, and the isolate sequences from this work were posted in GenBank. A microscopic analysis of thin Diff-quick-stained blood smears identified big intra-erythrocytic *Babesia sp*., *Theileria sp*., and *Anaplasma sp*. in thirty four dogs. The PCR investigation revealed *Anaplasma sp*. in 77 dogs (27.5%), *Babesia sp*. in 55 dogs (19.6%), and *Theileria sp*. in 63 dogs (22.5%). The identification and similarity scores between the isolates of this investigation and the reference strains were 100% identical. The findings of this study indicate that stray dogs are reservoirs of *Anaplasma spp*., *Babesia spp.*, and *Theileria spp.*, potentially playing a significant role in the epidemiology and dissemination of blood parasites, hence posing a substantial threat to the cattle industry.

Key words: Iraq, Blood-Borne Parasites, Stray dog, Nested PCR assay

Introduction

Ticks are ubiquitous obligate blood-sucking ectoparasites within the class Arachnida, non-permanent arthropods that infest both animals and humans. They are responsible for various diseases that lead to economic repercussions through morbidity or mortality, as they serve as vectors for multiple infections, including haemoprotozoa, bacteria and viruses (Eisen, 2022). Infections with blood-borne parasites have long been recognized as a major health threat for dogs and other animals, as they affect the vascular system and can occur intraerythrocytically,

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intraleukocytically, or in free forms. Among the most common disorders are babesiosis, ehrlichiosis, and anaplasmosis, which are transmitted mainly by ticks and occasionally through blood transfusion, biting, or transplacental transmission (Phuyal et al, 2017; Tamang, 2023). Canine babesiosis is caused by different intraerythrocytic protozoa such as *Babesia canis*, *B. gibsoni*, and *B. vogeli*, with dogs acting as both hosts and reservoirs, representing a risk for other domestic and wild animals (Baneth, 2018; Li et al, 2020). Theileria species such as *T. annae* and *T. equi* have also been reported in dogs, reflecting the close overlap between small Babesia-like organisms and *Theileria* spp. (Baneth et al, 2015; Bahrami et al, 2018). *Anaplasma* species, belonging to the family Anaplasmataceae, represent another group of emerging tick-borne pathogens, including A. phagocytophilum and A. platys, with zoonotic potential and considerable genetic variability that influence host specificity and pathogenicity (Rar et al, 2021). These blood parasites are associated with significant hematological and biochemical alterations, including anemia, thrombocytopenia, leukocyte abnormalities, and biochemical imbalances that can threaten animal survival (Sykes, 2022). With the increasing prevalence of these pathogens and their role as reservoirs of infection, molecular tools such as nested PCR have become crucial for detection and epidemiological studies (Weese and Evason, 2019). The present study, therefore, aimed to investigate the blood-borne parasites, including hemoparasites such as anaplasmosis and babesiosis, by using conventional and nested PCR approaches, and to provide an insight into their occurrence and potential impact.

Materials and methods

Description of the study sampling, period and location

A total of 280 stray dogs (137 male and 143 female) were collected randomly from the beginning of May to the end of December of (2024) and different regions of Wasit (Al-kut, AL-sweara, Al- Aziziah, Al-Numaniyah, Al-Hay and Badra). Each dog was examined systemically, and then the information about gender and age was recorded.

Blood sample collection

Blood samples were obtained from infected dogs. All dogs exhibited no symptoms at the time of sample collection. Blood samples were collected from the animals' jugular veins under aseptic circumstances and stored in 10ml ethylenediamine tetraacetic acid (EDTA) tubes.

Laboratory Techniques

The blood samples collected were analyzed in the Microbiology and Molecular Biology laboratories of Faculty of Veterinary Medicine Urmia University.

Microscopic examination

Immediately following the sample collection, thin blood smears were prepared for microscopic analysis. The smears were preserved in methanol for 5 minutes and thereafter stained with a 5% May-Grunwald Giemsa solution in buffer for 30 minutes. The dyed slides were analyzed under oil immersion at 1000× magnification using a Nikon microscope to detect piroplasms (Babesia, Theileria) and inclusion bodies (Anaplasma).

Molecular Assays

DNA isolation and PCR amplification

The genomic DNA was extracted from blood samples collected from both adult male and female stray dogs using the gSYAN Genomic DNA Extraction Kit (Geneaid, USA), following the guidelines provided by the manufacturer. A total of 111 DNA samples from infested dogs were selected for quantification. DNA yield (ng/µL) and purity ratios (A260/A280) were assessed using a NanoDrop® ND-2000 UV/Vis Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA). To reduce the risk of cross-contamination, DNA extraction, PCR setup,

nested PCR amplification, and agarose gel electrophoresis were carried out in separate laboratory rooms. All DNA samples were subjected to nested PCR analysis using specific primers designed to identify the target blood parasites. The amplification targeted the 18S rRNA gene for *Babesia* spp. or *Theileria* spp., and the 16S rRNA gene for *Anaplasma* spp., as shown in Table 1.

Table 1: Nested PCR primers for *Babesia sp*., *Theileria sp.* and *Anaplasma sp.* detection

Microorganism	gene	PCR	primer	Sequence 5'-3'	Product size
Babesia	18S rRNA	Normal	F	ATTGGAGGGCAAGTCTGGTG	697bp
			R	TCCACCAACTAAGAACGGCC	
		Nested	F	ATTGGTCGCGTCGCTTCTAA	429bp
			R	GACTTGCGACCATACTCCCC	
Theileria	18S rRNA	Normal	F	ATTGGAGGGCAAGTCTGGTG	740bp
			R	TCCACCAACTAAGAACGGCC	
		Nested	F	TTCCGGCCCATTTTTCCAGA	556bp
			R	TGCACCACCACCCAAAGAAT	
Anaplasma	*16S rRNA*	Normal	F	GGCAAGCGTTGTTCGGAATT	861bp
			R	GCAGTGTGTACAAGACCCGA	
		Nested	F	AGGGCATGTAGGTGGTTTGG	583bp
			R	CCCTTAAAGTCCCCGGCATT	
			R	GACTTGCGACCATACTCCCC	

The PCR reaction mixture consisted of 12.5µL of Taq 2x Master mix (containing Taq DNA polymerase, dNTPs, MgCl2, KCl and stabilizers), 2µL of Forward Primer, 2µL of Reverse Primer, 0.8µ of MgCl, 5µL of DNA template and 3.5 µL of nuclease free water to a total volume of 25µ.A positive and negative controls (master mix without DNA template) were included to monitor false positive and false negative results. An initial denaturation step at 95°C for five minutes, succeeded by 35 cycles of denaturation at 95°C for 30 sec, followed by an annealing step temperature at 58°C for 30 sec. A subsequent extension step was conducted at 72°C for 2 min. (repeated for 35 cycles), followed by a final extension at 72°C for 5 min.

Gel Electrophoresis

Gel electrophoresis was done for all DNA sample amplicons including 100-3000 bp molecular ladder on first lanes on 2% agarose, all the amplicons were stained with an ethedium bromide dye. The gel was run at 100V, 300MA for 45 minutes and viewed under UV light.

Statistical analysis

Data were input and analyzed using Excel, while the significant relationships between variables were assessed by chi-square tests or Fisher's exact tests, conducted with SPSS 20.0 statistical software. A statistical significance criterion of p-value < 0.05 was established.

Results

Giemsa staining for parasites Identification in infested stray dog

Identification of parasites was based on the presence of intra- erythrocytic bodies in blood smears when they were viewed under a light microscope. *Anaplasma*, *Babesia*, and *Theileria spp.* were identified using physical characteristics of the merozoite in blood smear results. In the present work, out of the 280 blood samples examined by microscopic examination, 34 tested positive for blood parasites, resulting in an overall prevalence of 12.14% in Waist City. The individual infection rates for *Anaplasma spp.* were 5.35%, with 15 positive cases through the presence of pairs of merozoites in blood smears. for *Babesia spp*., 3.92%,

with 11 positive cases where the intra-erythrocytic piroplasms are typically rounded or double pear-shaped, located at the periphery of the infected erythrocytes, and for *Theileria spp*., lower at 2.85%, with 8 positive cases that exhibit small rod-shaped, ring, and rounded forms, which can be found within lymphocytes, as illustrated in Table (2), (Figure 1).

Anaplasma spp was the most common blood parasite infecting dogs in waste. These results indicate that *Anaplasma* is the most prevalent among the three parasites, highlighting the importance of implementing preventive and therapeutic measures against this species. Additionally, the presence of *Babesia* and *Theileria*, although lower, remains a significant health concern, especially in stray dogs living in environments prone to infection. Similar studies have been conducted in various provinces of Iraq, including Baghdad (Badawi et al, 2020). Furthermore, research has also been carried out in neighboring countries (Khanmohammadi et al, 2021; Hosseini et al, 2022). In addition, several studies have taken place in European countries, such as France (René-Martellet et al, 2015), Italy (Solano-Gallego et al, 2008), and Romania (Imre et al, 2013).

Molecular screening PCR amplification of blood parasites in infested stray dogs

The incidence of blood parasites spp. was detected in the peripheral blood samples from a total of 280 stray dogs, including both males and females, tested by nested PCR. In this study, 111 (39.64%) were infected with one or more parasitic species, including *Anaplasma spp*., *Babesia spp*., and *Theileria spp*. Analysis of PCR products after agarose gel electrophoresis of the DNA extracted from blood samples showed specific amplifications with *Anaplasma sp*. (383bp), *Babesia spp.* (429bp), and *Theileria spp.* (556bp) at the annealing temperature of 55°C as shown in Figure (2).

Prevalence of blood parasites (*Anaplasma, Babesia*, and *Theileria*) Species in stray Dogs using nested PCR molecular identification

Of all examined dogs, 111 dogs were infected with *Anaplasma* spp. (27.5%)., *Babesia* spp (19.6%) and *Theileria* spp. (22.5%) (Table 3).

Table 2: Microscopic examination of blood parasites prevalence in infested stray dog

Experiment dog	*Anaplasma*		*Babesia*		*Theileria*	
280	+	%	+	%	+	%
	15	5.35	11	3.92	8	2.85

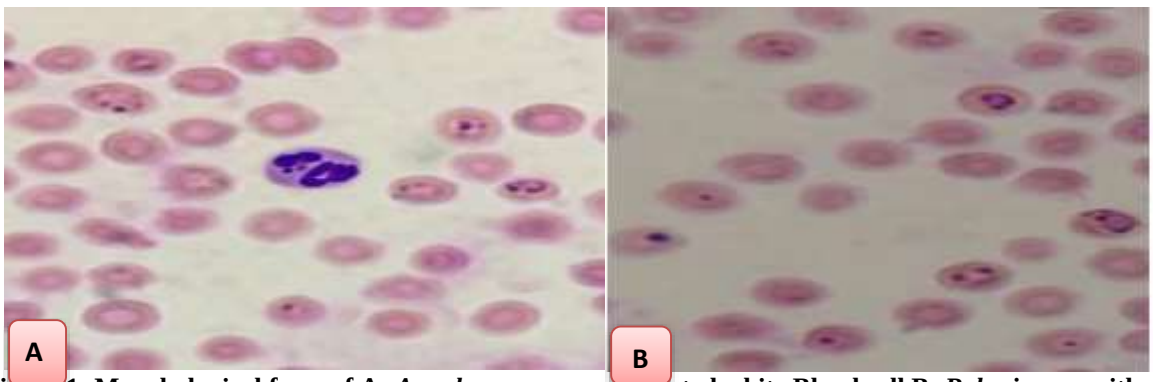

Figure 1: Morphological form of A: *Anaplasma spp* spp. Infected white Blood cell B: *Babesia spp*. with an infected erythrocyte stained with Giemsa examined under an oils immersion lens (100X).

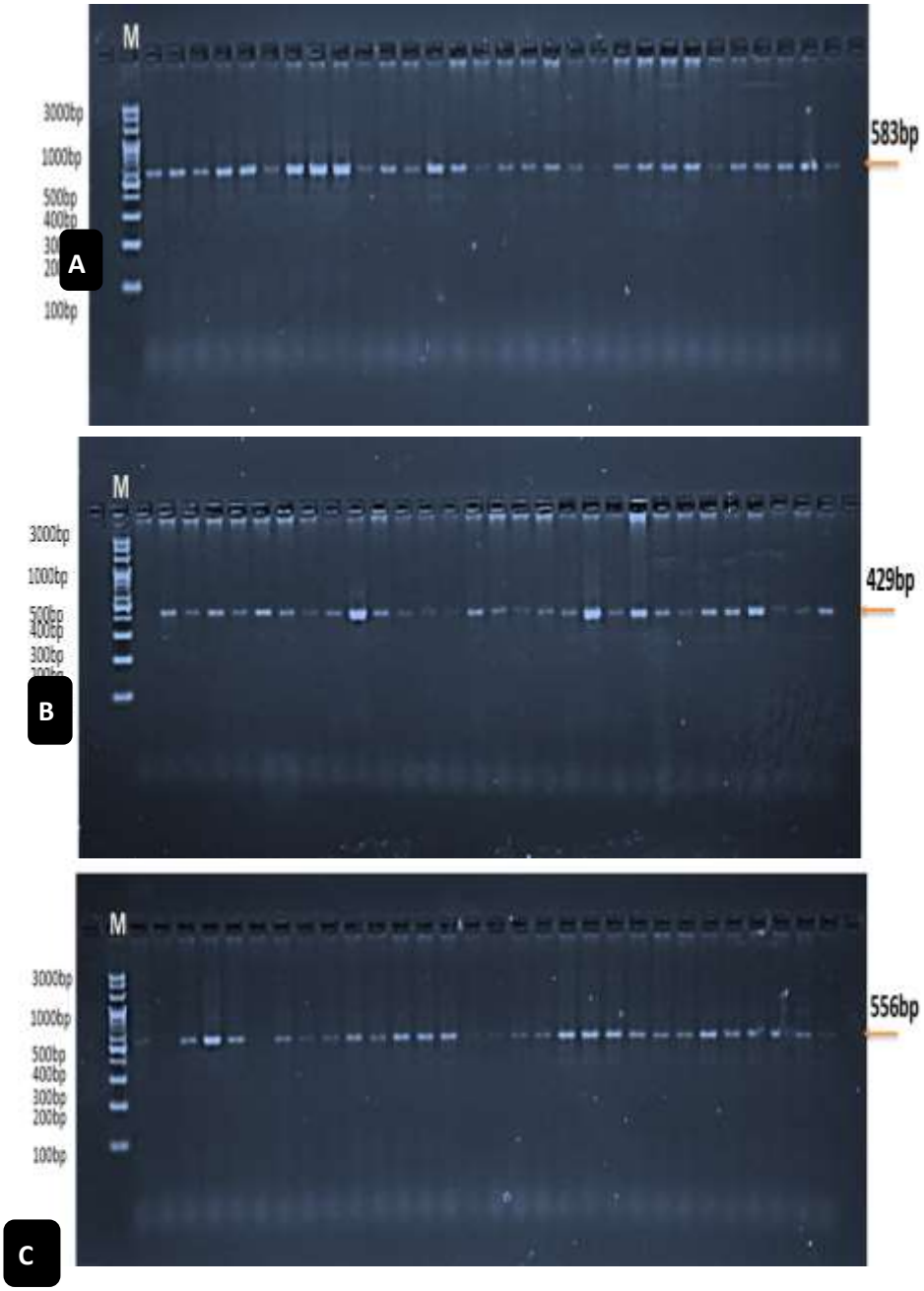

Figure 2: Nested PCR agarose gel electrophoresis products of some positive amplification small subunit ribosomal RNA gene in A: *Anaplasma spp.* **at (53bp) product size B:** *Babesia spp.* **at (429bp) product size C:** *Theileria spp.* **from stray dog blood samples. Where M: marker (100-3000bp) and positive Nested PCR was show at (556bp) product size.**

Table 3: Prevalence of (*Anaplasma Theileria***, and** *Babesia***) in stray dog's blood samples**

Parasitic Species	Number of Infected Dogs	Percentage (%)
Anaplasma	77	27.5
Theileria spp.	55	19.6
Babesia spp.	63	22.5
Total Infected	111	39.64
Total Examined	280	100

Infestation of blood parasite relation to dog's sex factor

According to sex, as illustrated in table (4), the prevalence of blood parasites infestation by a nested PCR demonstrated that in male dogs (137 total), *Anaplasma* was the most common infection (41 cases, ~29.9%), followed by *Babesia* (29 cases, ~21.2%), and then Theileria (26 cases, ~19.0%) while in female dogs (143 total): Anaplasma was also the most common infection (36 cases, ~25.2%), followed closely by Babesia (34 cases, ~23.8%), and finally Theileria (29 cases, ~20.3%).

Table 4: Prevalence of blood parasites accordance to the age factor in a Studied stray dogs

Category	Group	No. Infected dog	Positive of blood ticks infestation		
			Anaplasma	*Babesia*	*Theileria*
Sex	Male	137	41	29	26
	Female	143	36	34	29
Total No.		280	77	63	55

Discussion

The present study provides robust molecular evidence for the circulation of three clinically important blood-borne parasites *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. among stray dogs in Wasit Province. The detection of these haemoparasites at relatively high frequencies underscores the role of free-roaming dogs as potential reservoirs and sentinels for vector-borne diseases, with significant implications for both veterinary and public health surveillance systems.

Molecular screening revealed that 27.5% of the examined dogs were infected with Anaplasma spp., a prevalence comparable to the previous studies from Iraq and neighboring countries, where reported rates ranged from the 20–32% depending on the dog population and diagnostic methods used. This prevalence is also consistent with research highlighting the widespread distribution of the brown dog tick, *Rhipicephalus sanguineus*, the principal vector of *A. platys*, in central and southern Iraq (Reif, 2011). In contrast, lower prevalence levels (5–15%) have been recorded in colder regions such as parts of Turkey and northern Iran, where Ixodid tick populations are more seasonal, limiting transmission periods. Meanwhile, substantially higher rates—sometimes exceeding 40%—have been observed in tropical climates (e.g., India and Brazil), likely due to year-round vector activity and dense stray dog populations. The observed prevalence in our study aligns with the ecological conditions of Wasit Province, characterized by warm temperatures and sustained vector presence. Furthermore, the close coexistence of stray dogs with human communities may elevate zoonotic spillover risks, supporting their value as sentinel species for monitoring environmental pathogen circulation (Alhassan et al, 2021).

Babesia spp. infections were identified in 22.5% of sampled dogs. This result fits within the globally reported wide range of canine babesiosis prevalence. Low to moderate prevalence (3–15%) has been reported in parts of Iran, Iraq, and Turkey, while significantly higher rates—ranging from 25% to over 50%—have been observed in regions such as India, Egypt, and parts of Southeast Asia (Laha et al, 2014; Albakri et al, 2024). These regional differences can be attributed to several factors, including vector density, climatic stability, and levels of veterinary care and tick control. Our findings for *B. vogeli*, a species strongly associated with R.

sanguineus s.l., are consistent with studies from the Mediterranean and Middle East, where this tick species is dominant. Poor tick-control practices among stray dogs, environmental conditions favoring tick survival, and lack of regular veterinary treatment likely contribute to the relatively high Babesia prevalence observed in the current study.

The prevalence of Theileria spp. in this study was 19.56%, which aligns with molecular surveys from nearby regions confirming *T. annulata* infections in dogs. However, the prevalence is lower than that reported in South Africa (66.6%) (Rosa et al, 2014), Pakistan (~40%), and certain hyperendemic regions of Sudan. Differences in species of circulating Theileria, variation in vector competence, and ecological diversity primarily explain these discrepancies. Notably, in tropical countries where *Hyalomma* ticks thrive, canine theileriosis tends to be more common and often more severe. In contrast, the relatively moderate prevalence observed in Wasit may reflect lower densities of competent Hyalomma spp. or the possibility that dogs play a secondary or accidental host role in local transmission cycles. Importantly, many infected dogs in our study exhibited subclinical or mild infections, a pattern commonly reported in endemic regions where long-term host–parasite coadaptation may lead to reduced clinical severity.

Overall, among the 280 stray dogs examined, infection with at least one tick-borne haemoparasite was documented, with Anaplasma spp. being the most prevalent (77 cases, 27.5%), followed by Babesia spp. (63 cases, 22.5%) and Theileria spp. (55 cases, 19.6%). These distribution patterns correspond with the abundance of specific tick vectors and environmental factors such as temperature and humidity, which influence vector activity and pathogen transmission. Notably, female dogs had slightly higher infection rates (143 females vs. 137 males), potentially due to increased roaming behavior during reproductive cycles, greater exposure to tick habitats, or sex-related differences in immunity (Nasr and Ghafar, 2020). Comparable sex-associated patterns have been observed in studies from Iran, India, and Egypt.

Taken together, the findings emphasize the active transmission of multiple haemoparasites among stray dogs in Wasit Province. The data reflect the ecological suitability for vector proliferation and highlight the need for integrated tick control programs, public awareness campaigns, and sustained veterinary surveillance to reduce transmission risks to both animals and humans.

In conclusion, findings of this study suggest that, molecular detection confirmed the presence of three haemoparasites, *Anaplasma sp*, *Babesia sp* and *Theilleria sp* primarily transmitted by stray dogs in Wasit Province. These findings indicate potential public health risks and underline the importance of monitoring programs, preventive deworming, and improved management of stray dog populations to reduce the transmission of blood-borne parasites to domestic animals and humans.

Acknowledgments

The authors would like to thank the Urmia University and Iraqi expertise for their scientific assistance and supports.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

The vice- chancellor of research at Urmia University and University of Kut granted all financial supports**.**

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Received: 04.10.2025
Accepted: 07.12.2025

Vol. 21, No. 3, Autumn, 2025, 30-41
DOI: 10.22055/ivj.2025.545068.2841
Journal Homepage: http://www.ivj.ir/

Original Article

Protective effect of ellagic acid on epididymal sperm profile changes and testicular tissue apoptosis in male rats receiving nicotine

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Received: 04.09.2025 Accepted: 26.11.2025

Abstract

Nicotine (NC) is the major alkaloid in tobacco and has been strongly associated with male reproductive toxicity, mainly through mechanisms related to oxidative stress and apoptosis. Ellagic acid (EA), a naturally occurring polyphenol with well-documented antioxidant and anti-inflammatory properties, has been reported to counteract oxidative tissue damage in various experimental models. The present study investigated the protective effects of EA against NC-induced reproductive impairment in adult male Wistar rats. To do this, a total of twenty-four adult male Wistar rats were randomly divided into four groups (n = 6), including control group, EA group (60 mg/kg, orally), NC group (1 mg/kg, intraperitoneally), and NC combined with EA group. Following 30 days of treatment, the animals were euthanized, and testicular tissues were examined for gonadosomatic index (GSI), sperm quality parameters (count, motility, viability, and morphology), and expression levels of key apoptotic genes (Bcl-2, caspase-3, p53, and BAX). The administration of NC significantly reduced GSI, sperm count, motility, viability, and normal sperm morphology compared to the control group. Also, NC significantly increased the expression of p-53, caspase-3, and BAX genes and decreased Bcl-2 gene expression. Co-treatment with EA and NC significantly attenuated these adverse effects and improved reproductive parameters and apoptotic gene expression. In conclusion, these findings suggest that EA mitigates NC-induced reproductive toxicity in rats by reducing apoptosis, indicating its potential as a therapeutic agent for NC-related reproductive disorders.

Key words: Nicotine, Ellagic acid, Reproductive toxicity, Apoptosis, Testis

Introduction

Nicotine (NC) is the major alkaloid found predominantly in tobacco plants (Nicotiana tabacum), and is widely recognized as a key mediator of the adverse health effects associated with tobacco use (Sansone et al, 2023). As a psychoactive compound, nicotine exerts systemic effects by binding to nicotinic acetylcholine receptors and triggering a cascade of physiological responses (Benowitz et al, 2020). In the

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context of reproductive health, nicotine is a well-documented toxicant that impairs male fertility (Omolaoye et al, 2022). Studies have shown that nicotine exposure, whether through smoking or other forms of tobacco consumption, disrupts spermatogenesis, reduces sperm quality, and compromises testicular function (Harlev et al, 2015; Cui et al, 2025). These effects manifest as decreased sperm count, motility, and viability, as well as increased morphological abnormalities, which collectively contribute to male infertility (Aitken, 2020). Furthermore, nicotine induces oxidative stress in testicular tissue by increasing the production of reactive oxygen species (ROS), which overwhelms endogenous antioxidant defenses and leads to lipid peroxidation and cellular damage (Oztekin et al, 2020). This oxidative imbalance disrupts the integrity of the blood-testis barrier and impairs Leydig cell function, reducing testosterone levels and exacerbating reproductive dysfunction (Ukwenya et al, 2020).

The mechanisms underlying nicotine-induced reproductive toxicity are closely tied to oxidative stress and apoptosis. Nicotine promotes excessive ROS generation, which depletes antioxidant enzymes (Das et al, 2012; Mahmoud et al, 2021). This oxidative stress damages testicular germ cells and Sertoli cells, impairing spermatogenesis and sperm maturation (Budin et al, 2017). The previous studies have demonstrated that nicotine-induced apoptosis disrupts the balance between cell survival and death in the testes, leading to germ cell loss and reduced fertility (Paccola and Miraglia, 2016; Mosadegh et al, 2017). For instance, Madi et al. reported that nicotine administration in rats significantly increased caspase-3 activity, correlating with reduced sperm quality (Madi et al, 2021). Similarly, human studies have linked smoking-related nicotine exposure to elevated oxidative stress markers and sperm DNA fragmentation (Ni et al, 2016; Ribas-Maynou et al, 2021).

It is essential to identify natural compounds capable of counteracting nicotine-induced oxidative and apoptotic damage in testicular tissue. Ellagic acid (EA) is a naturally occurring polyphenolic compound widely distributed in various plant sources, including fruits such as pomegranates, strawberries, raspberries, blackberries, and grapes, as well as in nuts, seeds, and certain medicinal plants and oak species (Gupta et al, 2020; Li et al, 2025). Chemically, EA is a dimeric derivative of gallic acid with a well-documented ability to scavenge free radicals and enhance the activity of antioxidant enzymes (Evtyugin et al, 2020). In reproductive health, EA has demonstrated protective effects against chemically induced testicular damage. Experimental studies have shown improvements in sperm quality, testicular histoarchitecture, and steroidogenesis following EA administration (Rostami et al, 2022). Antioxidant effects of EA are mediated by increasing SOD, CAT, and GPx levels while reducing MDA, thereby mitigating lipid peroxidation (Bhattacharjee et al, 2021). Additionally, EA exerts anti-apoptotic effects by modulating the expression of Bcl-2, caspase-3, and BAX, promoting cell survival in various tissues (Aslan et al, 2020; Liu et al, 2024). Although the therapeutic potential of EA has been widely studied in conditions such as diabetes, oxidative organ injury, and chemotherapy-induced toxicity (ALTamimi et al, 2021), its role in mitigating nicotine-induced reproductive dysfunction remains largely unexplored. Therefore, this study aimed to evaluate the protective effects of ellagic acid against nicotine-induced reproductive failure in male rats.

Materials and Methods

Animals

Twenty-four adult male Wistar rats (8–10 weeks old, weighing 200–250 g) were selected from the Animal Research Center of Shahid Chamran University of Ahvaz. The rats were housed in polycarbonate cages under controlled conditions (22 ± 2°C, 50–60% humidity, 12:12 h light/dark cycle) with ad libitum access to standard rodent chow and filtered water. Animals were acclimatized for one week before the experiment to minimize stress. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Shahid Chamran University of Ahvaz (Approval No. IR.SCU.REC.1404.113).

Experimental Design

Twenty-four adult male Wistar rats were randomly divided into four groups (n=6 per group), including: (1) vehicle control, receiving 0.9% normal saline (1 ml/kg, intraperitoneally [IP]); (2) ellagic acid (EA) group, receiving EA (60 mg/kg/day, orally); (3) nicotine (NC) group, receiving nicotine (1 mg/kg/day, IP); and (4) nicotine + EA group, receiving nicotine (1 mg/kg/day, IP) and EA (60 mg/kg/day, orally). Treatments were administered daily between 8:00 and 9:00 AM for 30 days. The 30-day treatment duration was selected to cover at least one complete spermatogenic cycle in rats, which lasts approximately 48–52 days, allowing sufficient time to evaluate the sub-chronic effects of nicotine and the potential protective role of ellagic acid on reproductive parameters. This duration has also been widely used in the previous studies assessing nicotine-induced reproductive toxicity and antioxidant interventions (Aprioku and Ugwu, 2015). The doses were selected based on the previous studies demonstrating reproductive toxicity for nicotine (Zhang et al, 2022) and antioxidant efficacy for EA (Alkully et al, 2025).

Sampling and gonadosomatic index (GSI) measurement

On day 31, body weights were recorded using a digital scale (CAS CA, South Korea). Then, the rats were anesthetized with a combination of ketamine (10%, 80 mg/kg, intraperitoneally) and xylazine (2%, 10 mg/kg, intraperitoneally) before sample collection (Sadeghi et al, 2023). Both testes were excised, weighed using a precision balance, and the gonadosomatic index (GSI) was calculated as: GSI = (testicular weight/body weight) × 100. The left epididymis was minced in 5 ml of Dulbecco's Modified Eagle Medium (DMEM) at 37°C for 15 min to release sperm for analysis (Khazaeel et al, 2022). The left testis was snap-frozen in liquid nitrogen for molecular assays. All procedures were performed under sterile conditions to prevent contamination.

Assessment of sperm parameters

The epididymal sperms were analyzed for count, motility, viability, and morphology. Sperm suspensions from the left epididymis were diluted 1:20 in phosphate-buffered saline (PBS). The sperm count was determined using a Neubauer hemocytometer under a light microscope (400× magnification, Nikon, Tokyo, Japan), with counts expressed as sperm per milliliter (Sadeghi et al, 2020). Motility was assessed by classifying 200 sperm per sample as progressive, non-progressive, or immotile, expressed as a percentage of total motile sperm. Viability was evaluated using eosin-nigrosin staining, where 200 sperm were scored as live (unstained) or dead (stained). Morphology was examined by staining smears with Papanicolaou stain, assessing 200 sperm for normal versus abnormal head and tail structures under 1000× magnification. All assessments were

performed by a single trained technician to ensure consistency (Kalantari et al, 2014).

Assessment of apoptosis-related genes

Apoptosis in testicular tissue was evaluated by analyzing the expression of Bcl-2, caspase-3, p53, and BAX genes. Testicular tissues were homogenized, and total RNA was extracted for gene expression analysis. Gene expression levels were quantified using real-time quantitative polymerase chain reaction (qPCR). Primers were designed using Primer-BLAST and validated for specificity (Table 1). GAPDH was used as the housekeeping gene for normalization.

Table 1: List of primers employed for quantitative real-time RT-PCR in rat target genes

Gene name	Sequences	Product (bp)	GenBank
Caspase-3	F: CTATCCATGGAAGCAAGTCGATG R: TTGCGAGCTGACATTCCAGT	136	NM_014215
Bcl-2	F: ATCGCTCTGTGGATGACTGAGTAC R: AGAGACAGCCAGGAGAAATCAAAC	135	NM_017531
BAX	F: TGCTACAGGGTTTCATCCAG R: AAATGCAGACAGGCTTTGCAG	144	NM_017059
P-53	F: ATGCTGGTGCTGAGTATGTC R: AAATGCAGACAGGCTTTGCAG	122	NM_030989
GAPDH	F: ATGCTGGTGCTGAGTATGTC R: AGTTGTCATATTTCTCGTGG	162	NM_017008

RNA isolation and cDNA synthesis

Approximately 50 mg of snap-frozen testis was homogenized in 1 ml TRIzol (Yekta Tajhiz Azma, Tehran, Iran), followed by phase separation with chloroform and RNA precipitation with isopropanol. RNA pellets were washed with 75% ethanol, air-dried, and resuspended in RNase-free water. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), ensuring that A260/A280 ratios of 1.8–2.0. cDNA was synthesized from 1 µg of RNA using a reverse transcription kit (Takara Bio, Kusatsu, Japan) with oligo-dT primers in a 20 µl reaction volume. The reaction was incubated at 42°C for 60 min, followed by 70°C for 5 min to inactivate the enzyme. cDNA was stored at -20°C for qPCR analysis.

Real-time quantitative real-time polymerase chain reaction

Real-time qPCR was performed using a SYBR Green-based system on a StepOnePlus Real-Time PCR System. Reactions (20 µl) contained 10 µl SYBR Green Master Mix, 1 µl cDNA, 0.5 µM forward and reverse primers for Bcl-2, caspase-3, p53, BAX, and GAPDH, and nuclease-free water. Cycling conditions were initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Melt curve analysis confirmed the amplicon specificity. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to GAPDH.

Statistical Analysis

Data were analyzed using GraphPad Prism (Version 9, GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± standard deviation (SD). Differences between the groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Normality was confirmed using the Shapiro-Wilk test, and homogeneity of variances was verified with Levene's test. Statistical significance was set at $P<0.05$.

Results

Body and testicular weight

Nicotine administration significantly affected both body and testicular weights. The nicotine group exhibited a significant reduction in body weight, testicular weight, and gonadosomatic index (GSI) when compared with the control group ($P<0.01$). Notably, co-administration of EA with nicotine significantly increased these values compared to the nicotine-only group ($P<0.01$). Body and testis weights and GSI in the nicotine + EA group did not show significant differences compared to the control group ($P>0.05$; Figure 1).

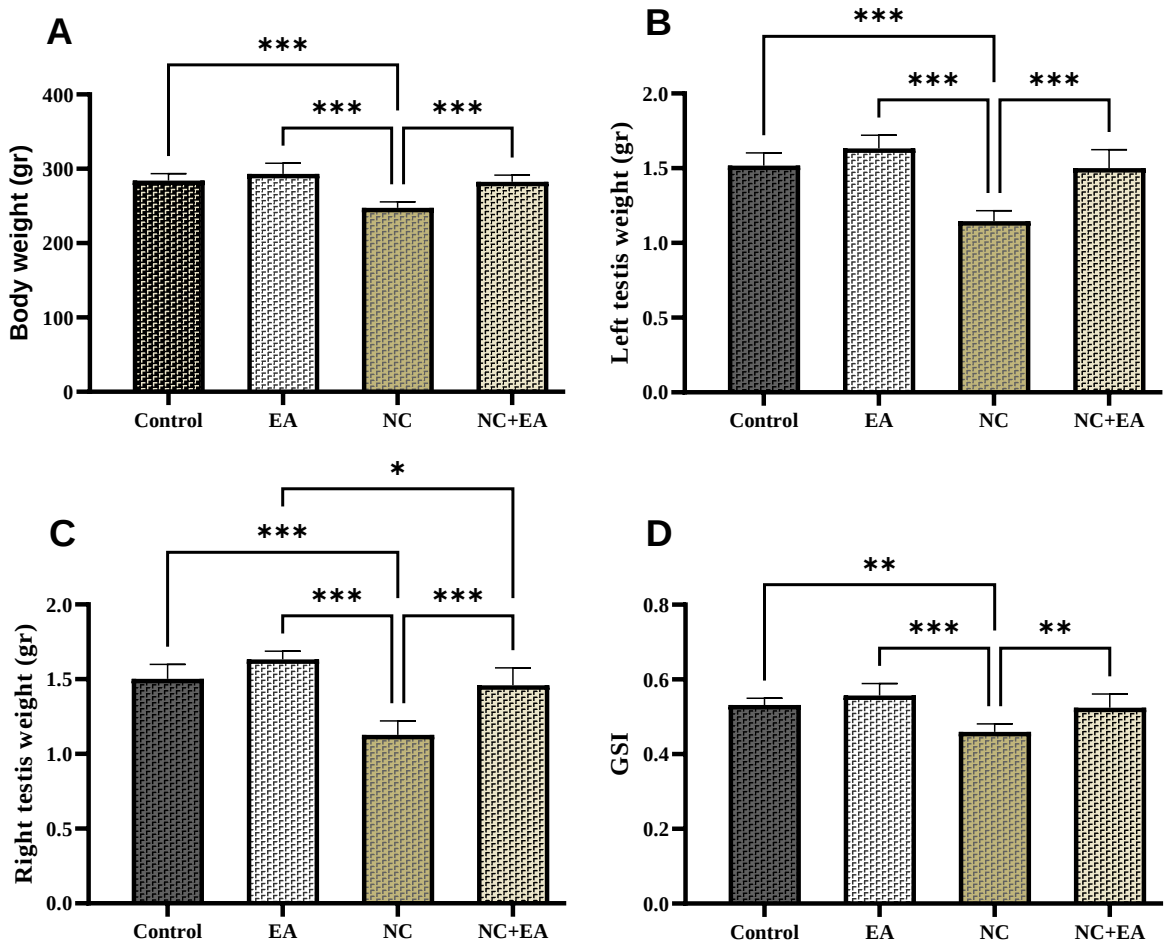

Figure 1. Effect of nicotine (NC) and ellagic acid (EA) on body weight (A), left (B) and right (C) testicular weight, and GSI (D) in rats. * P < 0.05, ** P < 0.01, *** P < 0.001 indicates statistically significant differences between groups.

Sperm parameters

The administration of nicotine significantly decreased the sperm count, motility, viability, and normal morphology compared to the control group ($P < 0.001$). The nicotine + EA group displayed significantly higher sperm count, motility, viability, and normal morphology compared to the nicotine group ($P<0.001$; Table 2; Figure 2).

Table 2: Effect of nicotine (NC) and ellagic acid (EA) on sperm parameters (count, motility, viability, and normal morphology) in rats

	Control	EA	NC	NC + EA
Mean of sperm count (10^6)	34.43±1.71	35.39±1.41	25.68±1.36***	31.14±1.51**###
Sperm motility (%)	88.50±3.27	90.33±3.44	65.67±3.77***	83.17±2.31*###
Sperm viability (%)	86.83±3.06	88.17±3.48	66.67±3.26***	79.17±2.63**###
Sperm normal morphology (%)	89.00±3.34	90.67±3.67	76.83±2.85***	83.17±2.31*###

* P<0.05, ** P<0.01, *** P<0.001 indicates statistically significant differences compared with the control group and # P<0.05, ## P<0.01, ### P<0.001 indicates statistically significant differences compared with NC group.

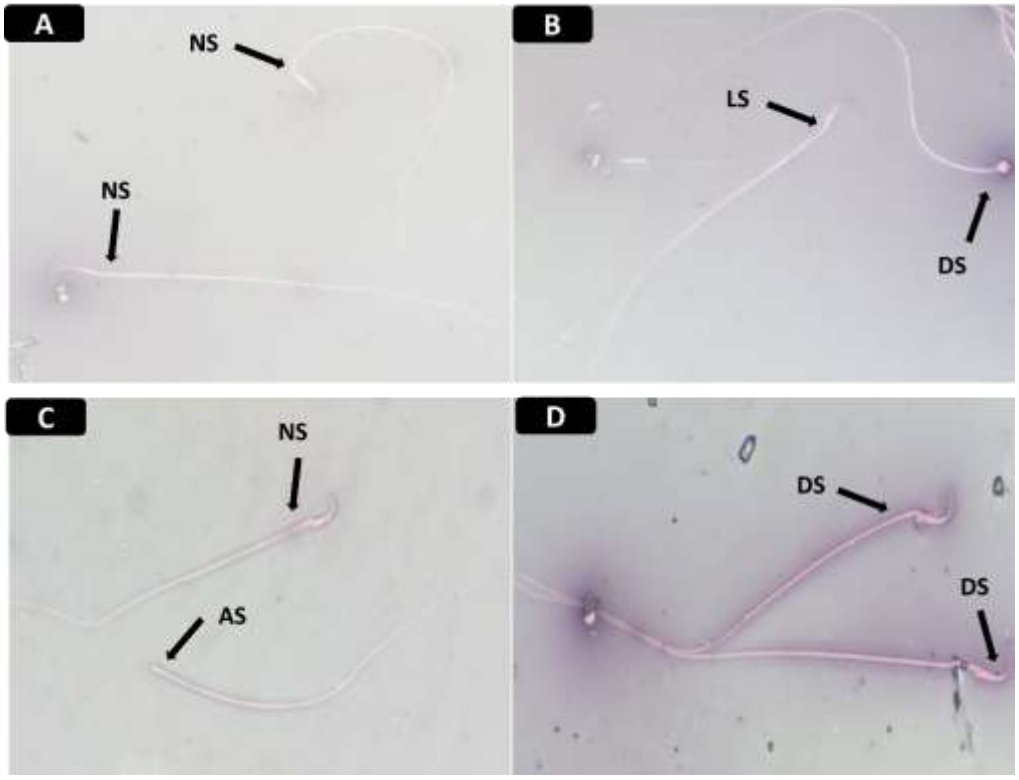

Figure 2: Photomicrograph of rat epididymal spermatozoa (A-D). NS: Normal sperm, AS: acephalic sperm (Headless sperm); DS: Dead sperm, LS: Live sperm (Eosin-Nigrosin staining; 400×).

Expression of apoptosis-related genes

Nicotine significantly altered testicular apoptosis-related gene expression. The nicotine group showed a significant increase in caspase-3, p53, and BAX expression, and a significant decrease in Bcl-2 expression compared to the control group (P<0.001). The EA group exhibited no significant changes in these genes compared to controls (P>0.05). The expression of caspase-3, p53, and BAX significantly (P<0.001) reduced in the nicotine + EA group, while Bcl-2 expression in the nicotine + EA group significantly (P<0.001) increased compared to the nicotine group (Figure 3).

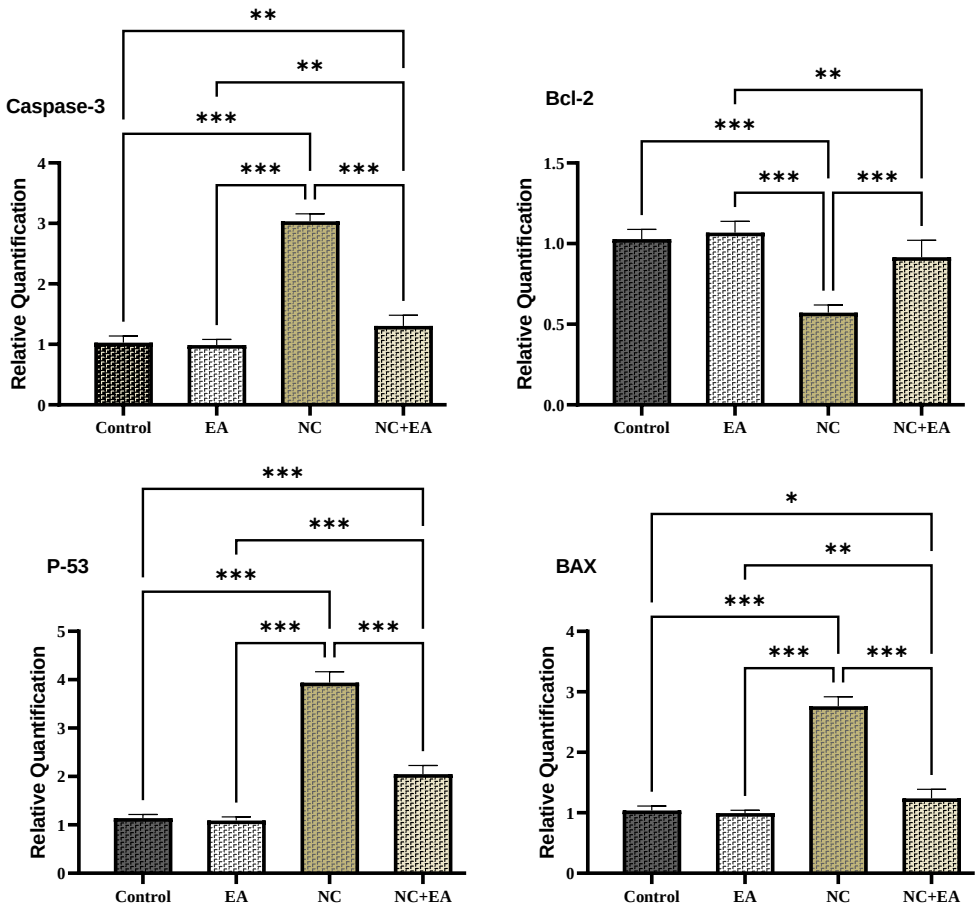

Figure 3. Effect of nicotine (NC) and ellagic acid (EA) on expression of Bcl-2, caspase-3, P-53, BAX genes. * P<0.05, ** P<0.01, *** P<0.001 indicates statistically significant differences between groups.

Discussion

The present study investigated the protective effects of ellagic acid (EA) on nicotine-induced reproductive failure in male Wistar rats, with a specific focus on its ability to alleviate apoptosis within testicular tissue. Findings revealed that nicotine administration of 1 mg/kg intraperitoneally for 30 days significantly impaired reproductive function, as evidenced by reduced gonadosomatic index (GSI), sperm count, motility, viability, and normal morphology. Moreover, molecular analyses indicated a downregulation of the anti-apoptotic protein Bcl-2, coupled with an upregulation of pro-apoptotic markers including caspase-3, p53, and BAX. Co-administration of EA at 60 mg/kg orally effectively reversed these alterations, restoring reproductive parameters to levels comparable to the control group. These findings suggest that EA confers reproductive protection, likely through mechanisms involving the suppression of apoptotic pathways within testicular tissue. Overall, EA demonstrates strong potential as a natural therapeutic candidate for alleviating nicotine-associated reproductive toxicity.

Our findings on the adverse effects of nicotine on male reproductive health are in agreement with a growing body of evidence demonstrating that tobacco-derived alkaloids exert testicular toxicity primarily through oxidative stress and apoptotic mechanisms (Cui et al, 2025). The previous studies have shown that nicotine disrupts spermatogenesis by increasing ROS production, which in turn triggers lipid

peroxidation and compromises sperm quality in animal models (Erfani et al, 2013; Oyeyipo et al, 2014). Consistent with these studies, Mosbah et al. (2015) reported that nicotine administration in male rats caused significant oxidative damage, histopathological changes in testicular tissue, and reductions in fertility indices (Mosbah et al., 2015). Similarly, our study revealed decreases in gonadosomatic index (GSI) and sperm parameters, suggesting a convergent mechanism in which nicotine impairs Leydig cell function and disrupts seminiferous tubule architecture, thereby interfering with testosterone synthesis and gametogenesis (Guo et al., 2017). However, variations across studies highlight that the effects of nicotine are not entirely uniform. Investigations utilizing lower doses have reported milder impairments, such as reduced sperm motility without significant changes in GSI. These discrepancies may be attributable to differences in dosage, duration of exposure, or strain-dependent susceptibility (Rahimi-Madiseh et al., 2020). Additionally, methodological factors such as the route of administration, intraperitoneal in our study compared to oral or inhalation routes in others, could account for discrepancies. Moreover, the absence of comorbid conditions such as diabetes, which are known to intensify nicotine's toxic effects, may further influence the observed outcomes (Kushwaha and Jena, 2014; Ansari et al, 2022).

At the molecular level, the present study demonstrates that nicotine induces significant apoptotic signaling in testicular tissue, as evidenced by the upregulation of pro-apoptotic genes (caspase-3, p53, BAX) and the downregulation of the anti-apoptotic marker Bcl-2. These findings are consistent with earlier reports linking nicotine exposure to enhanced apoptotic activity in male reproductive organs. For instance, Mosadegh et al. reported elevated expression of p-53 and caspase-3 mRNA in nicotine-treated rat testes, attributing these changes to oxidative stress-induced DNA damage that triggers mitochondrial apoptotic pathways (Mosadegh et al, 2017). Similarly, Jalili et al. reported altered apoptotic gene expression in several organs following nicotine administration, with a pronounced BAX/Bcl-2 imbalance that favored cell death (Jalili et al, 2017). Our study extends these observations by demonstrating that nicotine's pro-apoptotic effects are not only confined to germ cells but also disrupt Sertoli cell function, as evidenced by the significant reduction in Bcl-2 expression. Sertoli cells are essential for maintaining testicular microenvironmental homeostasis and supporting spermatogenesis (Zangene et al, 2025). Therefore, their impairment may accelerate spermatogenic arrest and contribute to infertility (Marinucci et al, 2020). The marked increase in p-53 expression observed in our nicotine-treated group further supports its role as a central regulator of DNA damage responses, potentially activating BAX translocation to mitochondria, thereby initiating cytochrome c release and caspase-3 activation (Azad et al, 2019). This mechanistic cascade aligns with the findings of Mohammadghasemi et al, 2021 who demonstrated that nicotine-induced oxidative stress amplifies p53-dependent apoptosis, thereby disrupting seminiferous tubule architecture and impairing sperm development.

The protective role of ellagic acid (EA) against nicotine-induced reproductive toxicity highlights a novel contribution, as limited research has investigated this polyphenol in the context of tobacco-related testicular damage. The present findings show that EA not only improves sperm parameters but also restores apoptotic gene expression, effects likely mediated by its strong free radical scavenging ability and anti-inflammatory action. By correcting the BAX/Bcl-2 imbalance and suppressing caspase-3 and p53 expression, EA appears to counteract the pro-apoptotic signaling

activated by nicotine exposure. This multifaceted protective action aligns with evidence from other toxicant models. For instance, EA has been reported to alleviate arsenic-induced testicular oxidative stress by activating the Nrf2 pathway and modulating key steroidogenic regulators such as Nfe2l2, Ppargc1a, and StAR, thereby preserving testosterone synthesis (Guvvala et al, 2019). Similarly, EA reduced testicular inflammation and apoptosis in phthalate-exposed mice by downregulating BAX and caspase-3 (Hosseinzadeh et al, 2021). Mechanistically, the efficacy of EA may involve activation of the Nrf2/HO-1 signaling axis, which enhances endogenous antioxidant defenses and attenuates nicotine-induced oxidative stress. This mechanism has also been demonstrated in neuronal cells, where EA enhanced Nrf2 nuclear translocation and increased expression of antioxidant enzymes such as superoxide dismutase (Liu et al, 2024). In the testicular context, this pathway likely preserves germ cell viability by stabilizing mitochondrial membrane potential, reducing lipid peroxidation, and preventing DNA strand breaks that would otherwise activate p53-dependent apoptosis (Naraki et al, 2022; Mehrzadi et al, 2018). Beyond oxidative stress regulation, EA may also exert epigenetic effects, such as inhibiting histone deacetylases (HDACs), thereby suppressing transcription of pro-apoptotic genes (Golmei et al, 2024).

In conclusion, the present study demonstrates that nicotine exposure impairs male reproductive function through oxidative stress and apoptosis, leading to significant declines in sperm quality, gonadosomatic index, and testicular integrity. Importantly, ellagic acid (EA) supplementation effectively counteracted these adverse effects by restoring antioxidant balance, modulating apoptotic gene expression, and preserving testicular architecture. The dual mechanisms of attenuating oxidative damage and suppressing apoptosis signaling by ellagic acid highlight its potential as a natural therapeutic agent against nicotine-induced reproductive toxicity. While these findings provide compelling experimental evidence, further investigations, including clinical trials, are warranted to validate the protective efficacy of EA and establish its translational relevance for managing tobacco-related male infertility.

Acknowledgments

The authors are grateful to the Vice Chancellor of Research of Shahid Chamran University of Ahvaz for providing financial support for this study through grant no. SCU, VB1404.293.

Conflict of interest

The authors declare no competing interests.

Funding

This study was funded by Shahid Chamran University of Ahvaz (Grant Number SCU, VB1404.293).

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Received: 04.09.2025
Accepted: 26.11.2025

Vol. 21, No. 3, Autumn, 2025, 42-53 *Original Article*
DOI: 10.22055/ivj.2025.535387.2818
Journal Homepage: http://www.ivj.ir/

Effect of Dietary Supplementation with Rosemary Essential Oil and Selenium on Fertility Gene Expression in Broiler Breeder Roosters

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Received: 18.07.2025 Accepted: 08.10.2025

Abstract

Advancing age in broiler roosters reduces reproductive potential. Natural antioxidants, such as rosemary oil and selenium, are expected to enhance reproductive function by reducing oxidative stress. This study aimed to investigate how these antioxidants affect the expression of fertility genes (StAR and PVRL3) in aging roosters. Forty-two Ross 308 broiler roosters, all over 50 weeks old, were used in a factorial experiment in a completely randomized design over 10 weeks. Treatments were: 1) Control diet, 2) Basic diet +100mg/kg rosemary essential oil, 3) +200mg/kg rosemary essential oil, 4) +0.3mg/kg selenium-enriched yeast, 5) +100mg/kg rosemary essential oil and 0.3mg/kg selenium-enriched yeast, and 6)+200mg/kg rosemary essential oil and 0.3mg/kg selenium-enriched yeast. At the end of the treatment, three samples of testicular tissue from each treatment were collected and stored at -80°C. The expression levels of the StAR and PVRL3 genes were measured using real-time quantitative PCR. The results indicated that rosemary oil did not significantly affect StAR gene expression. However, a dose of 200 mg/kg significantly reduced PVRL3 expression, whereas the 100 mg/kg dose did not show a significant effect. Selenium supplementation at a dosage of 0.3 mg/kg significantly increased the expression of the StAR and PVRL3 genes. Adding 100 mg/kg of rosemary along with 0.3 mg/kg of selenium significantly increased the expression of the PVRL-3 gene. However, when 200 mg of rosemary was added in the presence of selenium, a decrease in PVRL-3 gene expression was observed, and selenium did not prevent this decline. This suggests that using 200 mg of rosemary essential oil may be undesirable. Based on these results, adding 100 mg/kg of rosemary along with 0.3 mg of selenium-enriched yeast to the diet of older broiler breeder roosters is recommended.

Key words: Aged rooster, PVRL3, Rosemary, Selenium

Introduction

In broiler chickens, a rooster can successfully inseminate scores of hens; therefore, the reproductive performance of the roosters is one of the most significant

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production characteristics (Sun et al, 2019). Various factors such as body weight loss and oxidative stress, decrease in circulating testosterone, decrease in reproductive activity, low sperm rate, and impaired sperm quality are the causes of reproductive disorders in old flocks. Antioxidant function of rooster sperm reduces with age, and oxidative stress becomes evident when oxidant impact surpasses antioxidant defenses (Bansal and Bilaspuri, 2011). Oxidative stress intensifies the production of reactive oxygen species (ROS), causing lipid peroxidation (LPO), apoptosis, and DNA damage (Budai et al, 2014). ROS are highly reactive oxidants with free electrons and may participate in radical substitution reactions that form more radicals. Potent reactive radicals such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), peroxyl (ROO^-), and hydroxyl radicals (OH^-) are harmful to reproduction, but peroxynitrite anion ($ONOO^-$) and nitric oxide's nitrogen radicals (NO) possess physiological roles in fertilization and other animal reproduction determinants (Maneesh and Jayalekshmi, 2006). Sperm plasma membrane contains elevated levels of polyunsaturated fatty acids (PUFAs) (Khan, 2011), and the main rooster semen PUFA (22:4n-6, docosatetraenoic acid) is highly peroxidizable. Such sperm parameters, combined with age-related declines in seminal antioxidant function and activity of gonadal axis, render bird species susceptible to compromised fertility with aging (Surai et al, 2011).

Males' fertility peaks at around 37 weeks of age and declines with age above about 40 weeks of age (in reference to genetic strain). Low fertility leads to the reduction of egg production for hatch and whopping economic losses (Lagares et al, 2017). In this regard, it was found that supplements with higher levels of antioxidants will prove to be beneficial in an effort to improve the reproductive function in aged broiler breeder roosters. Over the recent decades, the issue has been of particular research interest into effective means of maintaining fertility within stress-charged roosters. One such popular technique is plant antioxidant compound, vitamins, and minerals supplementation that has been of critical significance to rooster breeding. It has been shown through experiments that antioxidant compound supplementation through diet or sperm extenders significantly reduces the adverse impact of oxidative stress while optimizing rooster fertility (Zhandi et al, 2020; Khalil-Khalili et al, 2021).

Medicinal plants have been widely used in the poultry industry due to their beneficial properties (such as antimicrobial, antioxidant, anti-inflammatory, and etc.) in the recent years (Shahraki Mojahed et al, 2024; Sabahi et al, 2020; Nazari et al, 2023; Rabieh et al, 2020; Mosavi et al, 2022; Nazari et al, 2024). Antioxidants are found in high concentrations within medicinal plants and can neutralize free radicals, converting them into harmless compounds (Parlet et al, 2005). Antioxidants within plants have also been researched highly for potential application in the treatment of sexual dysfunction and fertility enhancement within the recent years (Zarghi et al, 2015). Supplementing the diet of aged cocks with rosemary oil as an antioxidant appears to enhance fertility. The secondary metabolites of rosemary include flavonoids (genkwanin, isoscutellarein, and homoplantaginin), phenolic diterpenes (carnosic acid, carnosol, and rosmarinol), and triterpenes (ursolic acid) (Borras-Linares et al, 2014). Phenolic diterpenes are the major bioactive antioxidant compounds of rosemary extract (Rostami et al, 2017). They exhibit diverse biological activities such as antioxidant, anti-inflammatory, and anticancer activity (Borghei-Rad et al, 2017).

Selenium (Se) is an animal- and human-required trace element and serves a special

role in the preservation of spermatogenesis and male fertility and has structural and enzymatic functions and is exceptionally well known to carry out catalytic and antioxidant function (Qazi et al, 2019). Selenium is incorporated into enzymes like glutathione peroxidases (GPx) and Selenoproteins that are responsible for the antioxidant defense (Pappas et al, 2008). The principal role of glutathione peroxidases is the elimination and detoxication of hydrogen peroxide and lipid hydroperoxides to avoid oxidative damage to the sperm (Lemoine et al, 2011). Glutathione peroxidase occurs in rooster seminal plasma and semen (Bréque et al, 2003). Selenium plays a vital role in supporting healthy testicular function and structure, as well as ensuring sperm to move properly and work effectively. It has been postulated in some studies that selenium supplementation enhances the probability of fertility success and Sertoli cell count (Shi et al, 2010).

One such fertility-related gene is PVRL3 (PoliovirusReceptor-Related3). As a member of the family of cell adhesion proteins, the PVRL3 gene is of great relevance in mechanisms of spermatogenesis and fertility. It is particularly pertinent to sperm development and Sertoli cell-spermatid adhesion formation. Research has proven that the lack of expression or dysfunction of PVRL3 can lead to defects in sperm morphology, which ultimately leads to male infertility. PVRL3 not only contributes to sperm production but also sperm function and quality (Adeldust et al, 2021). Another key gene that impacts fertility is StAR (Steroidogenic Acute Regulatory Protein). This gene is responsible for spermatogenesis and fertility. StAR is responsible for cholesterol transport into the mitochondria, a critical step of steroid hormone biosynthesis such as testosterone and progesterone, which are crucial to spermatogenesis and fertility (Stocco et al, 2001).

According to the above-mentioned properties of rosemary oil and selenium, this study aimed to establish the effect of rosemary essential oil and selenium as antioxidants on the fertility-associated StAR and PVRL3 gene expression in aged broiler roosters. We hypothesized that rosemary oil and selenium would modulate StAR and PVRL3 expression and improve reproductive potential in aged roosters. The aim of this study is therefore to understand potential nutritional treatments for stimulating reproductive function by observing changes in gene expression.

Materials and methods

Farming Management

Forty-two Ross 308 broiler roosters, each over 50 weeks of age, were used in a 10-week factorial experiment (3 × 2) in a completely randomized design including rosemary oil at three levels (0, 100, 200 mg/kg) and selenium at two levels (0, 0.3 mg/kg).The experiment included 6 treatments with 7 birds per treatment group in were kept in single wood shavings-lined cages with one feeder and one drinker per cage. The condition in which they were reared was under strict control, where temperature was maintained at 21°C throughout, humidity at 60%and a light and dark regime of 14 hours light and 10 hours darkness. The feeding regimen was structured based on the Ross 308 management guide (2016) (Table 1). The complete design and description of the treatments are provided in Table 2.

Table 1: Composition and nutrients of experimental diets for roosters

Ingredient (g)	Amount kg/100 kg
Maize (8/5 % crude protein)	65.43
Wheat bran	22.88
Soybean meal (49% Crude Protein)	8.00
Calcium hydrophsphate	1.25
Calcium Carbonate	0.95
Nacl	0.37
Methionine	0.09
Lysine	0.03
Soybean oil	0.5
Vitamin[1]	0.25
Mineral[2]	0.25
Composition	
ME(Kcal/kg)	2850
CP (%)	12.2
Ca (%)	0.76
L-Lysine (%)	0.44
Methionine + Cystine (%)	0.47
Theronine(%)	0.36
Arginine	0.65
Sodium (%)	0.15
Availablephosphorus (%)	0.33

*1. Supplied per kilogram of diet: vitamin A, 15,000 IU; vitamin E,100 IU; vitamin K3, 4 mg; vitamin B12, 25 mg; vitamin D, 3000 IU; riboflavin, 7.5 mg; niacin, 50 mg; pantothenic acid, 18 mg; pyridoxine, 5.5 mg; biotin, 50 mg and folic acid, 1.5 mg.
2. Supplied per kilogram of diet: Fe, 90 mg; Mn, 120 mg; Zn, 110 mg; I, 2mg and Se, 0.3 mg

Table 2: Structure of the dietary treatments in a 3 × 2 factorial arrangement

Treatment Group	Rosemary Essential Oil (mg/kg)	Selenium Enriched Yeast (mg/kg)
1 (Control)	0	0
2	100	0
3	200	0
4	0	0.3
5	100	0.3
6	200	0.3

Testicular Tissue Sampling

At the end of the experiment, three animals from each treatment group were sacrificed to collect testicular tissue samples in order to explore the StAR and PVRL3 genes. To achieve this, a portion of the testis was quickly excised using a sterile knife and placed in 1.5 ml RNase-free microtubes. The samples were sent to the labratory in liquid nitrogen and stored at –80°C.

RNAextractionandcDNAsynthesis

The RNA isolated from the tissues was purified with the Denazist kit according to the manufacturer's instructions. The quality of the isolated RNA was assessed by electrophoresis on a 2% agarose gel and Its purity and concentration by the ratios A260/A280 and A260/A230 was determined quantified by NanoDrop spectrophotometer (Thermo Scientific NanOdrOp. 2000C. USA). The purified RNA was kept at -80°C for future use. Finally, the reverse transcription reaction was carried out with Sinaclon's cDNA Synthesis Kit. And for each sample, about 8 high-quality microRNAs were used using random primers included in the kit. The cDNA products were stored at -20°C for subsequent analysis.

Real-Time PCR Using the SYBR Green Method

To analyze the expression of target genes, primer sequences for the StAR, β-actin (Amin Altawash et al, 2019) and PVRL3 (Adeldust et al, 2022) genes were considered according to the specifications presented in Table 3 and synthesized by Sinaclone Company (Iran).

Table 3: List of sequences and properties of primers used in this study

Gene name	Sequence	Piece length (bp)	Annealing temperature	Accession number
Star	F: 5'- TTCAGCGAGATGGAGATGTCC-3' R: 5'- GGAACACCTTACCCACGTCC-3'	160	60	NN_204686.2
PVRL3	F: 5'- CATGTGGACCAGGCTGGATG-3' R: 5'- GTCTTCTGATCACTCCTCTGACC-3'	150	60	XM_416630.5
β-actin	F: 5'- ACGTCGCACTGGATTTCGAG-3' R: 5'- AAAGATGGCTGGAAGAGGGC-3'	145	60	X00182

Before performing the first PCR reactions with the designed primers, primer specificity to the target genes was ensured by using a Thermalcycler Mini (Germany). Subsequently, real-time PCR was done in duplicate for genes β-actin, StAR and PVRL3, where each gene was done in separate plates using the StepOnePlus Real-Time PCR System (USA). Table 3 illustrates the step-by-step details of the real-time PCR process.

To confirm the target gene specific amplification, PCR reaction was carried out first using the designed primers in thermal cycler (Thermalcycler Mini, Germany). Real-time PCR was carried out afterwards in the samples in two technical replicates for StAR, PVRL3 and reference gene β-actin. The reactions were also carried out in single plates in the Step One Plus Real-Time PCR System (USA). The reaction in a volume of 25 µL contained 12.5 µL of Master Mix Green, 1 µL of each of the forward and reverse primers (Concentration 10 µmolar), 2 µL of template cDNA (50 ng), and 8.5 µL of double-distilled water. The samples were then cycled on a Bio-Rad thermocycler using the following thermal cycling profile for gene expression analysis: the initial step was 95°C for 10 minutes of denaturation followed by 40 cycles of 95°C for 30 seconds of denaturation, 60°C for 30 seconds of annealing, and 72°C for 45 seconds of extension. The final extension of 72°C for 5 minutes was performed. Melting curve analysis was performed to verify primer specificity.

To compare the data of Real-time PCR, an initial report was initially made utilizing the Step One ABI software and exported in Excel (v 2016). Second, the relative expression levels of the target gene were normalized to the reference gene β-actin. Relative gene expression was calculated using Pfaffl method (2004) by the formula $2^{-\Delta\Delta CT}$. After the Calculation of fold change values, all experimental values were obtained by using SAS software (v 9.4) with a factorial 2×3 design of the complete randomized design with the help of the GLM procedure. Treatment means were tested at a significance level of 5% by using the LSD test based on the given equation.

$y_{ijk}= \mu + C_i + T_j + (CT)_{ij} + e_{ijk}$

In this equation: y_{ijk} represents the value of each observation, µ is the overall mean, C_i denotes the effect of the different rosemary levels, T_j indicates the effect of the different selenium levels, $(CT)_{ij}$ corresponds to the interaction effect between rosemary and selenium and e_{ijk} is the experimental error.

Result

Results of RNA quantity and quality assessment

The RNA integrity and yield of purified RNA were accurately ascertained by gel electrophoresis and Nanodrop spectrophotometry. Pureness was revealed in Nanodrop absorbance readings with A260/A280 values ranging consistently between 1.8–2.2 denoting negligible protein contaminations. RNA integrity was also claimed by clear sharp 28S and 18S rRNA bands on agarose gel with not even a trace of degradation. These assays ensured the quality of the RNA samples and suitability for downstream investigations. Successful cDNA synthesis was subsequently guaranteed, with sufficient templates for subsequent gene expression investigation.

Evaluation of the replication efficiency of the studied genes

The PCR products were observable as sharp, well-defined bands of the defined size on the agarose gel following electrophoresis (Figure 1). As you can see, the StAR gene has a band of 160pb, the PVRL3 gene has a band of 150pb, and the β-actin gene has resulted in a band of 145 base pair on the agarose gel. The sharpness and intensity of the bands were evidence of the specificity and efficiency of the amplification, indicating high-quality PCR output in the absence of non-specific amplification.

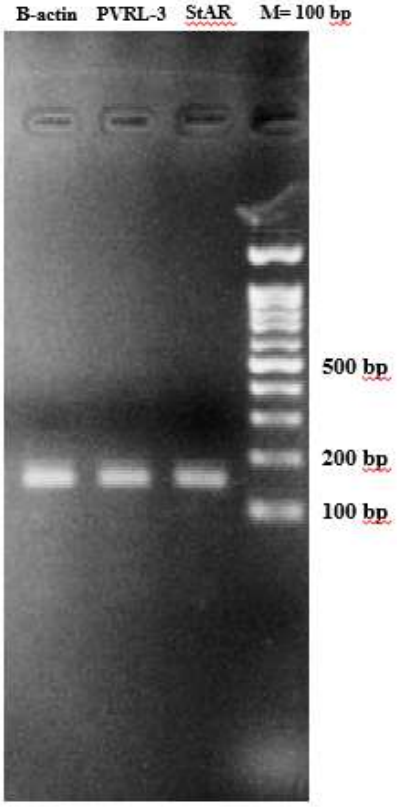

Figure 1. Agarose gel electrophoresis showing PCR products of β actin, PVRL3 and StAR genes with band lengths of 150pb, 145pb and 160pb respectively. The molecular marker (Ladder) is 100 bp.

Reviewing the results of the Real-time PCR reaction

Melting Curves of the StAR, PVRL3, and β-actin genes are shown in sections A, B, and C of Figure 2, respectively. Specificity of PCR amplification was also confirmed through the presence of single sharp peaks during melting curve analysis of Real-time PCR reactions. There are no additional peaks and no signs of nonspecific product or primer dimers. These results confirm that the primers actually designed had successfully achieved specific amplification, and the Real-time PCR assays were extremely reliable for additional gene expression analysis.

The effect of adding rosemary essential oil and selenium on the expression of StAR and PVRL-3 genes

Results on the effect of rosemary essential oil and selenium supplementation on StAR and PVRL-3 gene expression are presented in Table 4. Adding rosemary essential oil to the maternal rooster's diet had no significant effect on the expression of the StAR gene ($P>0.05$). However, the inclusion of 200 mg rosemary essential oil significantly reduced PVRL-3 gene expression ($P<0.05$), but 100 mg did not influence this gene ($P>0.05$). In addition, real-time PCR indicated that the inclusion of 0.3 mg selenium-enriched yeast significantly increased the expression of both genes ($P<0.05$). Overall, the impact of selenium and rosemary essential oil was leaded to a significant increase in the expression of both genes ($P<0.05$).

Co-treatment with 100 or 200 mg rosemary essential oil and selenium caused significant increase StAR gene expression ($P<0.05$). Co-treatment with 100 mg rosemary essential oil and 0.3 mg selenium-enriched yeast caused significant increase PVRL-3 expression ($P<0.05$), while 200 mg rosemary essential oil together with selenium caused decreased PVRL-3 expression, and selenium could not abrogate this decrease. It appears that the addition of rosemary essential oil at 200 mg/kg is not favorable, and even selenium, which was favorable, could not overcome this adverse effect.

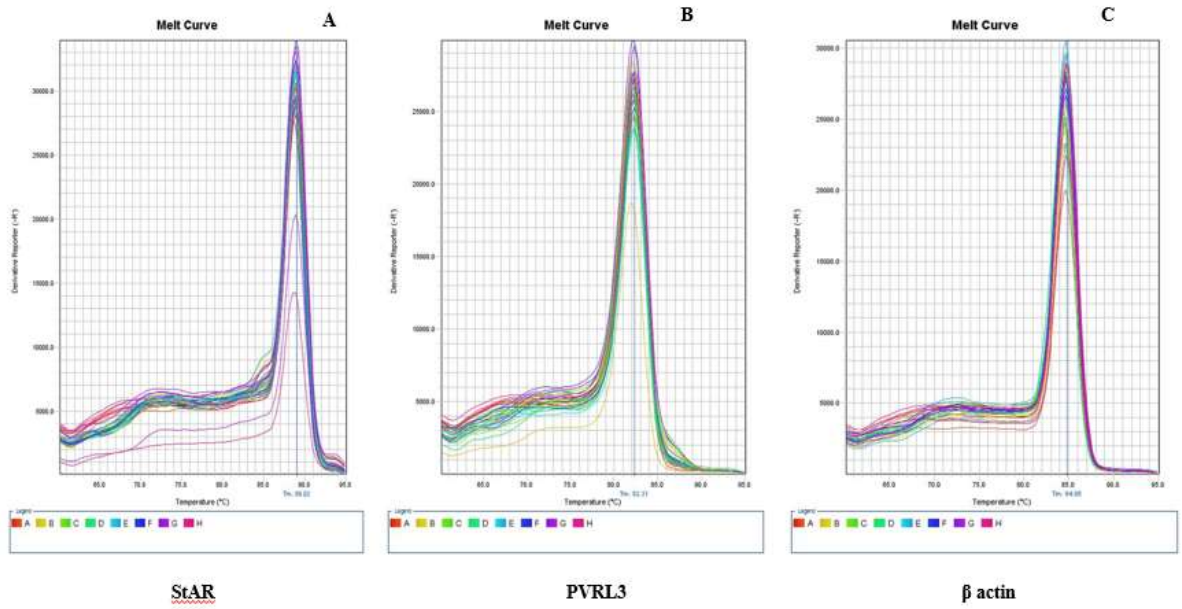

Figure 2. Melting curves of StAR (A), PVRL3 (B) and β actin (C) genes

Table 4: Effect of rosemary essential oil and selenium on the expression of StAR and PVRL-3 genes (Alpha = 0.05)

Treatments Group	Main effects		StAR	PVRL-3
	0		1.48	1.30[a]
Rosemary essential oil	100 200		1.30 1.29	1.22 [a] 0.76 [b]
SEM			0.07	0.18
Selenium	0		0.68 [b]	0.85 [b]
	0.3		2.03 [a]	1.34 [a]
SEM			0.14	0.06
Interactions				
Basal diet (control)	0	0	1 [b]	1 [b]
Basal diet + 100 mg/kg rosemary essential oil	0	100	0.49 [b]	0.75 [b]
Basal diet + 200 mg/kg rosemary essential oil	0	200	0.56 [b]	0.81 [b]
Basal diet + 0.3 mg/kg selenium	0.3	0	1.96 [a]	1.61 [a]
Basal diet + 100 mg/kg rosemary essential oil + 0.3 mg/kg selenium	0.3	100	2.11 [a]	1.70 [a]
Basal diet + 200 mg/kg rosemary essential oil + 0.3 mg/kg selenium	0.3	200	2.02 [a]	0.72 [b]

*Different letters in each column indicate significant differences($P<0.05$).

Discussion

Fertility is one of the key determinants of the economic profitability in poultry flocks and is determined by several factors such as breed, quality of nutrition, age of flock, and quality of semen (Miazi et al, 2012). During the collection of eggs, producers will observe a decline in fertility (Khalil-Khalili et al, 2021), which is primarily due to cocks aging and the consequent loss of reproductive ability. Current studies have shown that natural antioxidant compounds present in dietary supplements reverse the negative effect of aging on fertility in roosters and affect gene expression related to fertility.

Our research revealed that supplementation with selenium increased the expression of the StAR and PVRL-3 genes. Other studies have repeatedly demonstrated that organic selenium is of higher bioavailability compared to the inorganic substances (Hadrup and Ravn-Haren, 2021). Selenium yeast contains mostly organic selenium compounds such as seleno-methionine and analogs (Schrauzer, 2001). It is worth noting that the selenium yeast lowered the production of reactive oxygen species (ROS) and inhibited oxidative cellular injury among various animal species (Yang et al, 2022; Liu et al, 2020; Samo et al, 2020). Generally, selenium yeast significantly lessens oxidative damage to testis and maintains regular reproductive function in roosters under oxidative stress (Xiong et al, 2025). The results of adding selenium nanoparticles to the diet demonstrated improvements in sperm quality and reproductive performance in male rainbow trout breeders. In fact, dietary selenium enhanced sperm volume, motility time, and concentration in males. Furthermore, the antioxidant character of the selenium nanoparticles in diet was the determining factor for the enhanced fertilization and hatching rates (Jahaabad et al, 2020).

One important consideration in this case is the fact that selenium is a fundamental component of the glutathione peroxidase enzyme, which plays an important role in detoxifying lipid peroxides and protecting cells against oxidative damage from reactive oxygen species (ROS) (Zachara, 1992). It has been determined from the previous research that selenium-supplemented yeast is able to counteract testicular toxicity accumulated via oxidative stress (Cao et al, 2020). Results indicate that selenium yeast can reverse oxidative damage to testicles in roosters caused by oxidative stress and activate the Nrf2/HO-1 signaling pathway (Xiong et al, 2025).

Healthy production of spermatozoa and androgens by the testis is crucial to male reproductive function (McBride and Coward, 2016). In addition, the function of molecules involved in testosterone generation, such as StAR, has already been highlighted previously in the regulation of steroidogenesis (Heng et al, 2017). In a research study, main markers in terms of testosterone generation, such as StAR, were explored. The western blot examination revealed that testis from rooster exposed to oxidative agents had significantly lower StAR expression levels compared to controls. This was in concordance with previously mentioned mRNA expression patterns for StAR molecules. Importantly, pretreatment of cells with selenium yeast avoided such changes. These findings strongly suggest that selenium yeast restores impaired expression of molecules associated with testosterone production and supports normal reproductive performance in roosters. Therefore, maintenance of testosterone production is of utmost significance in the preservation of male reproductive health (Xiong et al, 2025).

The results of the present study indicated that the addition of rosemary essential oil did not have an impact on the StAR gene expression, but with a high concentration (200 mg), the PVRL-3 gene expression was significantly reduced. Researchers have

explored the antioxidant effect of rosemary in the preservation of testicular integrity and fertility and the potential role in the improvement of reproductive performance and in the reduction of oxidative stress. Rosemary has potent antioxidant activity responsible for lowering oxidative stress in testicular tissues. Rosemary supplementation has been shown in studies to boost total antioxidant capacity, leading to sperm quality and testosterone enhancement in animal models (Mansouri Torghabeh et al, 2022; Alahmadi and Alahmadi, 2024). In diabetic mice, rosemary extract also lowered malondialdehyde (MDA) content, a sign of oxidative stress, and increased glutathione and superoxide dismutase contents, signs of protection against oxidative damage (Alahmadi and Alahmadi, 2024). In addition, supplementation with rosemary essential oil has also been associated with semen quality improvement in semen parameters like an improvement in the concentration and motility of sperm and an increase in testosterone levels in rams (Ali et al, 2024).

Conversely, some research reveals that rosemary in high concentrations may be antifertility as, in male rats, spectacular morphological changes in testicular morphology were observed with increasing dosage (El-Din et al, 2012). This is to imply that in spite of the beneficial antioxidant effect of rosemary, its effect on fertility is dose- and context-dependent.

This study found no significant effect of rosemary on fertility genes. However, when an optimal dosage of rosemary essential oil was administered alongside selenium yeast, there was an enhanced expression of fertility genes. This suggests a synergistic action between the two compounds. In summary, selenium is known to support testicular fertility and health, while rosemary may produce adverse effects at higher dosages. Therefore, caution should be exercised when using both of these substances simultaneously. More research is necessary to confirm their potential synergy.

Acknowledgments

We acknowledge the Research deputy of Agricultural Sciences and the Natural Resources University of Khuzestan for financial support.

Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Funding

This research was financially supported by the Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

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Received: 18.07.2025
Accepted: 08.10.2025

Vol. 21, No. 3, Autumn, 2025, 54-67 *Original Article*
DOI: 10.22055/ivj.2025.535198.2817
Journal Homepage: http://www.ivj.ir/

Comparative Evaluation of Sedative and Hematologic and Biochemical Effects of Intravenous Administration of Xylazine, Detomidine, Medetomidine, and Dexmedetomidine in Caspian Miniature Horses

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Received: 20.07.2025 Accepted: 21.09.2025

Abstract

Pharmaceutical research on α2-adrenergic agonists in Caspian miniature horses, a breed native to Iran, is currently limited. So, the objective of the present study was to evaluate sedative and hematobiochemical effects of intravenous administration of xylazine, detomidine, medetomidine, and dexmedetomidine in Caspian miniature horses. The study involved the random assignment of six Caspian miniature horses (by drawing of lots), crossover design into five groups. Each horse received one of four α2-adrenoreceptor agonists or saline. The study employed a randomized crossover design with a minimum washout period of seven days. The horses received either intravenous treatments of 1 mg/kg xylazine, 20 µg/kg detomidine, 10 µg/kg medetomidine, 5 µg/kg dexmedetomidine, or 5 mL of 0.9% saline. The sedation scores and physiological responses, including heart rate, respiration rate, digestive motility, and rectal temperature, were assessed immediately prior to drug administration (0 min) and subsequently at intervals of 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min post-administration. The results indicated that there were no significant differences in some mean sedation scores and incoordination (impairment of the ability to coordinate muscle movements) among the treatments examined. The findings showed that there were no significant differences in the mean heart rate among the treatments and control assessed at any of the time points; however, significant differences in the mean respiration rate, digestive motility, rectal temperature, and selected hematobiochemical parameters were observed at some measurement time points post-injection. In conclusion, these agents demonstrated potential for effective sedation in healthy Caspian miniature horses, though further studies are recommended.

Key words: Caspian miniature horse, Alpha 2 agonist, Sedation, Hematobiochemical effects

Introduction

The Caspian miniature horse is a unique equine breed known for its small stature and historical significance. This breed has endured through centuries of tumultuous historical events, maintaining its existence despite its limited population (Dalton, 2000). The Caspian miniature horse is recognized for its ancestral significance in the broader context of equine history. The average height of a Caspian miniature horse

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ranges from 100 to 120 cm at the withers. While they are comparable in size to small pony breeds, Caspian miniature horses are classified as small horses and exhibit a conformation similar to that of full-sized equine breeds. Their average body weight generally falls between 180 and 270 kg, influenced by factors such as age, gender, health, and overall physical condition (Dalton, 2000).

Xylazine, detomidine, medetomidine, and dexmedetomidine are classified as α_2 -adrenergic agonists, which are non-narcotic agents exhibiting both sedative and analgesic properties. The sedative and anti-nociceptive effects of these α_2 -adrenergic agonists have been documented in some species of Equidae (Gozalo-Marcilla et al, 2018). These pharmacological agents primarily exert their sedative effects by modulating noradrenergic neuron activity, leading to decreased sympathetic outflow and enhanced sedation. Furthermore, their analgesic effects are mediated through the activation of spinal α_2 -adrenergic receptors, which inhibit nociceptive transmission, thus providing effective pain relief without the associated risks of opioid use (Zeiler, 2015; Sajjadi et al, 2025). The most dependable sedative medications approved for use in horses are α_2 - agonists. These drugs also offer several additional advantages, such as pain relief, muscle relaxation, decreased need for anesthetic drugs during induction and maintenance, and a reduction in the stress response associated with pain and surgery. Furthermore, α_2 -agonists are utilized to sedate horses during transport, as part of pre-anesthetic medication for both surgical and non-surgical standing procedures, and as elements of pain management protocols (Vigani and Garcia-Pereira, 2014).

To the best of our knowledge, no published studies have evaluated the effects of α2-adrenergic agonists in Caspian miniature horses. Therefore, the present study aimed to assess the sedative and hematobiochemical effects of intravenous administration of xylazine, detomidine, medetomidine, and dexmedetomidine in this breed.

Materials and Methods

Animals

In the present study, six miniature Caspian horses were selected based on their height at the withers, approximately five years of age and representing both sexes (3 males and 3 females). Each horse had an estimated weight of 150 ± 25 kg and exhibited a moderate body condition score at the commencement of the experiment. All animals were maintained under uniform management conditions, receiving a diet tailored to meet their maintenance and physiological requirements. The composition of the diet remained consistent throughout the duration of the experiment. To enhance nutritional intake, rare mineral salts were incorporated into the feed at a concentration of 0.3% by weight. Food was provided ad libitum in two daily meals. Prior to the initiation of the experiment, all horses underwent a comprehensive broad-spectrum anti-parasitic treatment to mitigate any potential contamination from internal and external parasites. Furthermore, before the commencement of the research, a thorough clinical and laboratory evaluation was conducted on each animal. Only those horses that were determined to be clinically and laboratory healthy were included in the study. The animals were deprived of food for a duration of 12 hours and water for 8 hours.

Experimental design

Prior to the experiment, the approval were received from the animal welfare committee at the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran (institutional approval number: IR.UK.VETMED. REC. 1401. 022). The horses underwent a behavioral assessment and were trained to adapt to physical restraint using a halter. Their health was evaluated through clinical

assessments and para-clinical (hematological, biochemical, and fecal parasitological) tests. The experiment was conducted under controlled environmental conditions (The temperature and relative humidity in the environment during the experiment ranged from 18 to 24 ºC and 12 to 18%, respectively) and each horse was individually restrained in a quiet, and covered area, equipped with a soft pad.

The study involved the random assignment of horses (by drawing lots with random number table), into five groups, each receiving one of four distinct α_2 -adrenoreceptor agonists or saline, utilizing a randomized crossover design with a minimum washout period of seven days, as detailed in Table 1. The horses were administered intravenous (IV) treatments consisting of 1 mg/kg xylazine (Xyla; Interchemie Werken "De Adelaar" B.V., Venray, Holland), 20 µg/kg detomidine (Domosedan, Orion Corporation, Espoo, Finland), 10 µg/kg medetomidine (DorbeneVet; N-Vet AB, Uppsala, Sweden), 5 µg/kg dexmedetomidine (Dexdomitor, Orion Corporation), or 5 mL of 0.9% saline (Shahid Ghazi Pharmaceutical Company, Tabriz, Iran). Prior to administration, all sedatives were diluted with 0.9% saline to achieve a final volume of 5 mL, following aseptic preparation of the left jugular vein. The drugs were administered to standing horses using a 16-gauge needle. Following the administration of the sedative agents, the halter was removed from each horse to facilitate observation.

To enhance the reliability and validity of the observations (without blind assessment), three independent observers evaluated the level of sedation in each horse using a standardized 4-point sedation scale, as described by the previous researchers (Deupree et al, 2008; Dalton, 2000). The sedation scores were defined as follows: Score 1: No sedation (the horse is alert, sensitive to noise and environmental stimuli). Score 2: Mild sedation (characterized by reduced alertness with slight reactions to external stimuli, occasional stumbling, and easily able to continue walking). Score 3: Moderate sedation (indicated by drowsiness and lethargy, sporadic responses to external stimuli, a minor drop in the position of the head, lips, and upper eyelids, along with marked stumbling and significant ataxia during ambulation). Score 4: Deep sedation (evident lethargy, a pronounced drop in head position, lack of response to external stimuli, and potential recumbency or falling while walking). The assessment of sedation and other clinical signs was conducted immediately prior to drug administration (0 min) and subsequently at intervals of 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 min post-administration. Sedation scores were consistently evaluated before measuring other clinical variables to ensure accurate monitoring of the horses' responses

Table 1: The order in which six miniature Caspian horses were assigned to receive intravenous treatments consisting of either 1 mg/kg xylazine (Xyl), 20 µg/kg detomidine (Det), 10 µg/kg medetomidine (Med), 5 µg/kg dexmedetomidine (Dex), or saline (Sal), over five distinct testing days, ensuring a washout period of no less than seven days between each treatment. The study involved the random assignment of horses (by drawing of lots with random number table), into five groups, with a minimum washout period of seven days

Testing days	Horses No					
	1	2	3	4	5	6
1	Sal	Xyl	Det	Med	Dex	Sal
2	Dex	Sal	Xyl	Det	Med	Dex
3	Med	Dex	Sal	Xyl	Det	Med
4	Det	Med	Dex	Sal	Xyl	Det
5	Xyl	Det	Med	Dex	Sal	Xyl

Sedation scores and clinical signs

Two large animal internists conducted an assessment of the physiological responses, including heart rate (HR), respiration rate (RR), digestive motility (DM), and rectal temperature (RT). The HR was measured using a stethoscope (Classic II SE, Littmann Co, USA), with the bell positioned on the left side of the chest wall at the fourth intercostal space, behind the olecranon, for a duration of one min. The RR was determined through direct observation of

thoraco-abdominal movements over one min. The RT was recorded using a medical digital thermometer (FT09, Beurer GmbH, Ulm, Germany), which was inserted into the rectum. The DM was assessed by auscultating the four abdominal quadrants, left upper, left lower, right upper, and right lower, using the same stethoscope for four min on each quadrant (Samimi, 2020).

Hematologic and biochemical parameters

Blood samples (5 ml) were collected from each subject at three time points: before injection (baseline), 2 hours after injection, and 24 hours after injection. For serum biochemical analysis, blood was drawn into plain tubes (without anticoagulant). The samples were allowed to clot at room temperature and then centrifuged at 3000 g for 15 minutes to separate the serum. The serum was aliquoted and stored at - 20°C until analysis. For hematological analysis, blood was collected into tubes containing EDTA as an anticoagulant and was analyzed immediately to ensure accuracy.

Serum levels of blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transaminase (GGT), cholesterol, triglyceride, calcium, phosphorus, sodium, potassium, albumin, and total protein were measured using an automated clinical chemistry analyzer (Roche Diagnostics, Germany) according to the manufacturer's instructions. Hematocrit (HCT) and white blood cell (WBC) count was determined using an automated hematology analyzer (Sysmex XE-2100, Japan). For WBC differential, a manual count was performed on stained blood smears if required

Statistical analysis

The statistical analysis of the collected data was conducted using IBM SPSS software, version 27. Initially, descriptive statistics including indices such as mean, standard deviation, and standard error were calculated and reported for the studied variables. The normality of the data was assessed using the Kolmogorov-Smirnov test. For normally distributed data, repeated measures ANOVA was utilized to examine the effect of measurement times on the mean indices before and after drug administration, categorized by treatment groups. For the data that did not meet normality assumptions and non-parametric data, the Friedman test was applied. In cases where significant differences were detected, the Student's t-test for dependent groups was used for parametric data, while the Wilcoxon signed-rank test was employed for non-parametric data to determine differences between measurement times. Additionally, to compare parametric data with normal distribution among treatment groups at identical measurement times, one-way ANOVA and Tukey's post-hoc test were utilized. It is worth noting that throughout all stages of the analysis, a significance level of 0.05 was adopted for rejecting the null hypothesis (H0).

Results

Sedation scores and clinical signs

All animals successfully recovered following the sedation period, and no injuries were observed in any of the cases. The agreement among the observers was very good (k >0.81).

The results indicated that there were no significant differences (P>0.05) in the mean sedation scores and incoordination among the treatments examined at the time points prior to injection, as well as at 75, 90, 105, and 120 minutes post-injection. In contrast, significant differences (P<0.05) in the mean sedation scores among the treatments were noted at other measurement intervals (Figures 1 a and b)

Physiological signs

The test results revealed that there were no statistically significant differences (P>0.05) in the mean HH among the treatments assessed at the time points prior to injection, as well as at 105 and 120 minutes after injection. In contrast,

significant differences (P<0.05) in the mean HH among the treatments were noted at other measurement intervals (Figure 1 c).

Prior to conducting the statistical analysis of the results obtained, descriptive statistics, including count, mean, standard deviation, standard error, minimum, and maximum values, were calculated for the HR, RR, RT, and DM variables. These statistics were organized by the groups under investigation at both pre-injection and post-injection time points.

No statistically meaningful variation was observed (P>0.05) in the mean HR among the treatments and control assessed at any of the time points (Figure 1 d).

The results of the RR assessment indicated that there were no significant differences in the mean of RR among the treatments evaluated both prior to injection and at 5 minutes post-injection (P>0.05). However, marked differences (P<0.05) in the mean RR were observed at other time points, specifically at 10, 15, 30, 45, 60, 75, 90, 105, and 120 minutes (Figure 1 e).

No statistically meaningful variation was observed (P>0.05) in the average DM among the treatments assessed prior to injection. However, significant differences (P<0.05) among the treatments were observed at other measurement time points (Figure 1 f).

No statistically meaningful variation was observed (P>0.05) in the average RT across the various treatments at the time points prior to injection, as well as at 5, 15, 30, 45, 60, and 75 minutes post-injection. Conversely, significant differences (P<0.05) in average RT were observed at other measurement intervals (Figure 1 g).

Biochemical parameters

Marked differences were detected in mean BUN levels among the treatment groups: prior to injection (P<0.01), 2 hours post-injection (P<0.01), and 24 hours post-injection (P<0.01) (Figure 2 a). According to Tukey's post-hoc test, the Medetomidine and Dexmedetomidine groups exhibited significantly lower mean BUN values compared to the other groups. For creatinine, the results indicated no significant differences among groups before injection (P>0.05) or 2 hours after injection (P>0.05). However, a significant difference emerged at 24 hours post-injection (P<0.01) (Figure 2 b). Tukey's test showed that, at 24 hours, the Dexmedetomidine, Detomidine, and Xylazine groups had significantly higher mean creatinine levels than the Normal saline group.

No significant differences were found in the mean cholesterol levels among groups at any time point: before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 c). Similarly, the mean triglyceride levels did not differ significantly among groups before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 d).

Marked differences were detected in the mean AST levels among groups at all time points: before injection (P<0.05), 2 hours after (P<0.05), and 24 hours after injection (P<0.01) (Figure 2 e). Tukey's post-hoc analysis indicated that the Dexmedetomidine group had significantly higher mean AST levels at all time points compared to the Medetomidine group. No significant differences were observed in the mean ALT levels among groups at any time point: before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 f). For GGT, there were no significant differences before injection (P>0.05). However, significant differences were observed at 2 hours (P<0.05) and 24 hours post-injection (P<0.05) (Figure 2 j). Tukey's post-hoc test indicated that at 2 hours, the Normal saline, Medetomidine, and Detomidine groups had significantly lower mean GGT levels than Dexmedetomidine (P<0.05), and at 24 hours, the Normal saline group was significantly lower than Dexmedetomidine (P<0.05).

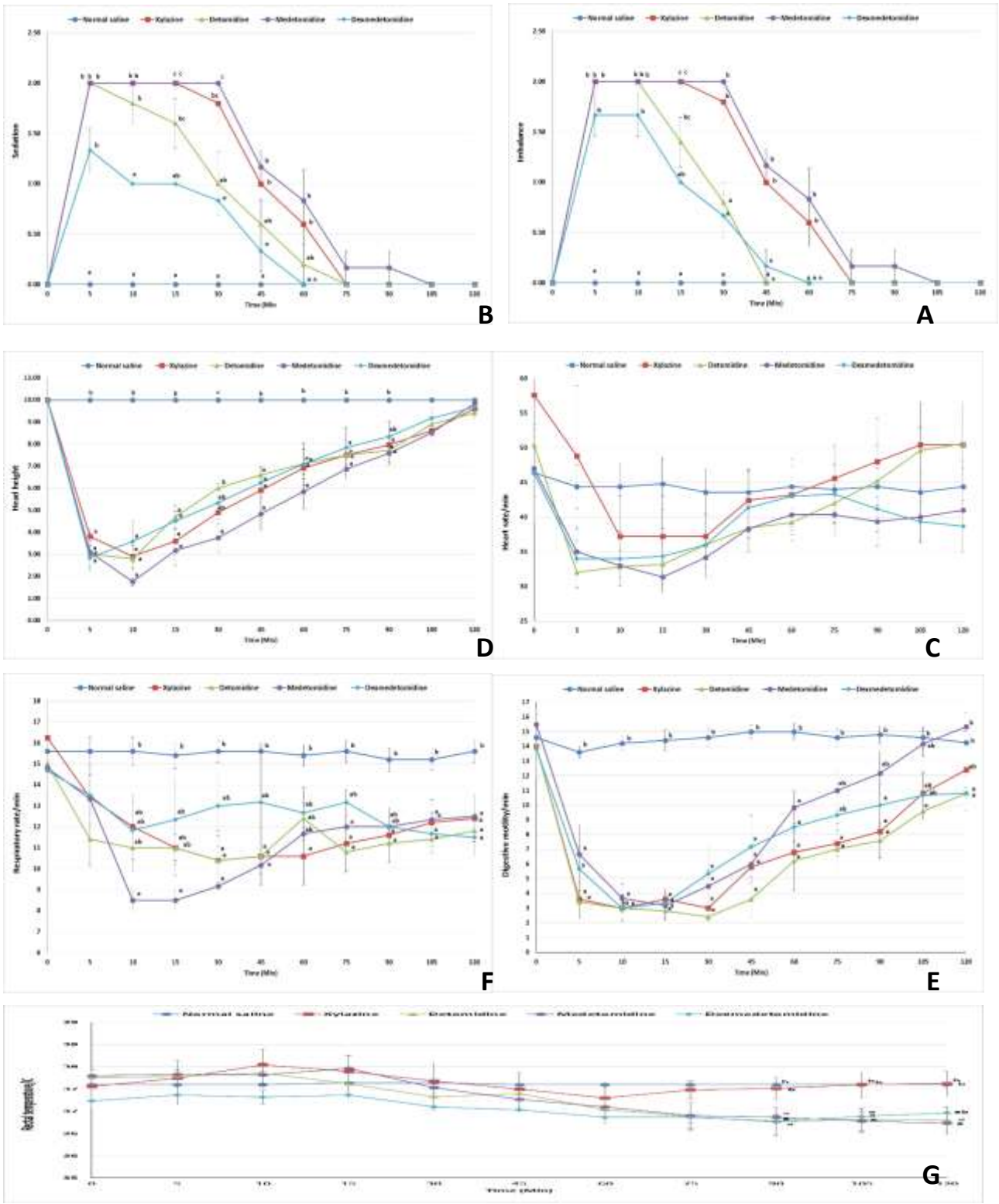


Figure 1: A: Sedation, B: incoordination, C: head height, D: hear rate, E: respiratory rate, F: digestive motility, G: rectal temprature (Mean ± standard error) before and after drug administration in treatment group (Red: Xylazine, green: Detomidine, purple: Medetomodine, light blue: Dexmedetomidine, and dark blue: control). One-way ANOVA and Tukey's post-hoc test were utilized Treatments that share similar letters do not have a statistically significant difference at the 95% probability level ($p<0.05$).

The observed significant changes in AST and GGT levels may indicate liver involvement or hepatocellular stress, which could be related to the pharmacological effects or toxicity of the administered drug. Understanding these alterations helps to better evaluate the safety profile and physiological impact of the treatment.

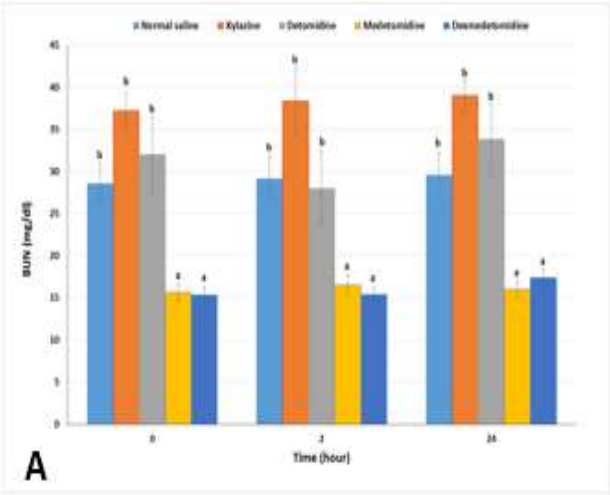
For calcium, no significant differences were found before injection (P>0.05) or 2 hours after (P>0.05). However, at 24 hours post-injection, a significant difference was detected (P<0.01) (Figure 2 g). Tukey's test showed that mean calcium levels at 24 hours were significantly lower in the Medetomidine and Detomidine groups compared to the Normal saline and Dexmedetomidine groups. Significant differences in the mean phosphorus levels were observed at all time points: before injection (P<0.01), 2 hours after (P<0.01), and 24 hours after injection (P<0.01) (Figure 2 h). Tukey's analysis revealed that before injection, the Medetomidine and Detomidine groups had significantly lower mean phosphorus levels than the Normal saline and Dexmedetomidine groups. At 2 hours post-injection, the Medetomidine, Detomidine, and Xylazine groups had significantly lower values than the Normal saline and Dexmedetomidine groups, and the Normal saline group was also significantly lower than Dexmedetomidine (P<0.05). At 24 hours, the Normal saline, Medetomidine, Detomidine, and Xylazine groups had significantly lower phosphorus levels than Dexmedetomidine, with the Detomidine group also significantly lower than the Normal saline (P<0.05).

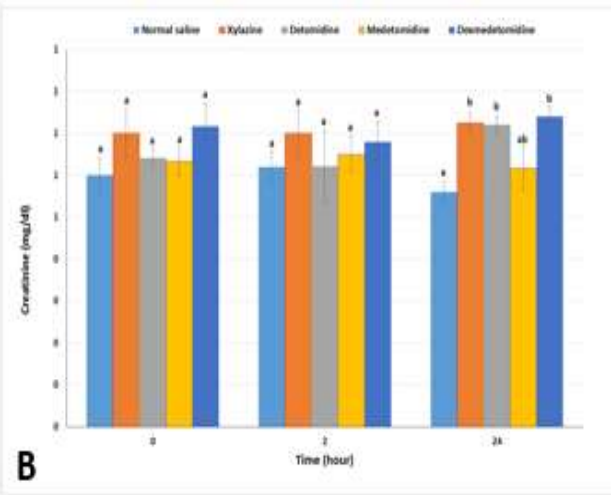
No statistically meaningful variation was observed in the mean albumin levels among groups at any time point: before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 i). Similarly, no significant differences were found in mean total protein levels among groups before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 m).

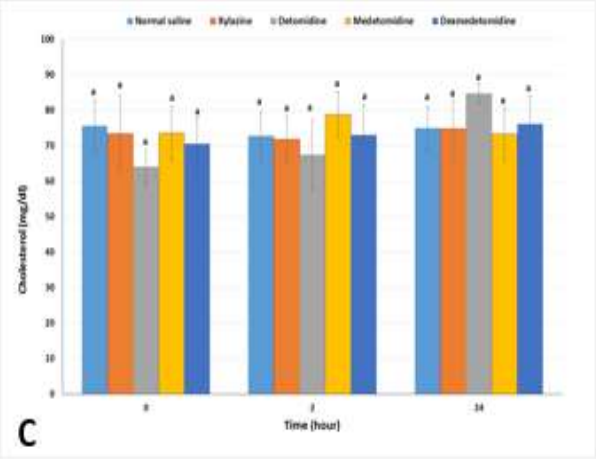
No statistically meaningful variation was observed in the mean sodium levels before injection (P>0.05) or 24 hours after (P>0.05), but a significant difference was found at 2 hours post-injection (P<0.05) (Figure 2 k). Tukey's test showed that at 2 hours, the Normal saline, Dexmedetomidine, and Detomidine groups had significantly lower sodium levels than the Medetomidine group. No significant differences were observed in the mean potassium levels among groups at any time point: before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 l).

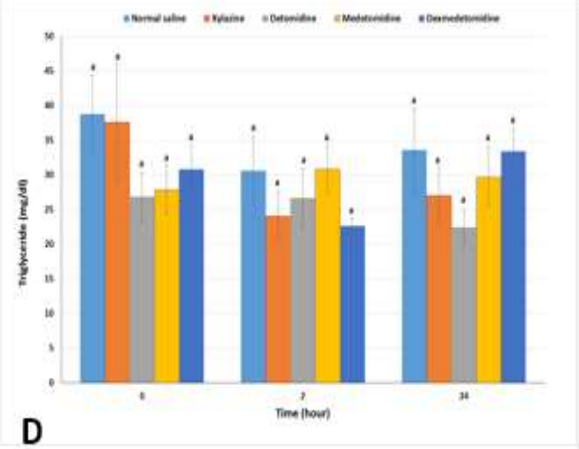
Hematological parameters

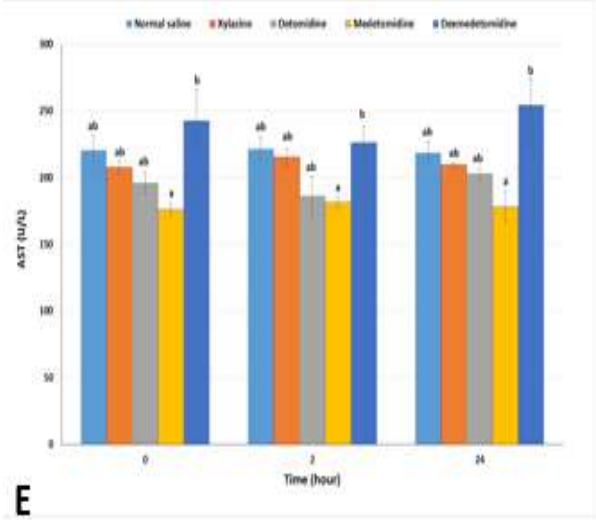
For WBC, no significant differences were observed at 2 hours (P>0.05) or 24 hours post-injection (P>0.05). However, a significant difference was found before injection (P<0.05) (Figure 2 n). Tukey's post-hoc analysis indicated that, prior to injection, the Detomidine group had significantly higher mean WBC counts than the Medetomidine group (P<0.05). Finally, no significant differences in the mean PCV were noted among groups at any time point: before injection (P>0.05), 2 hours after (P>0.05), or 24 hours after injection (P>0.05) (Figure 2 o).

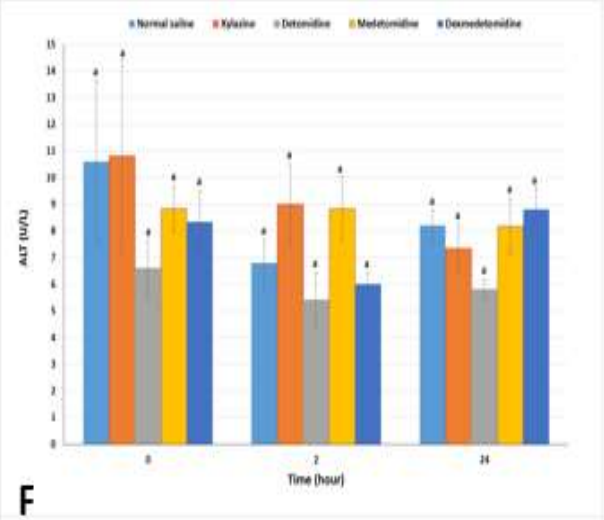

Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
BUN (mg/dl)
Time (hour)
A

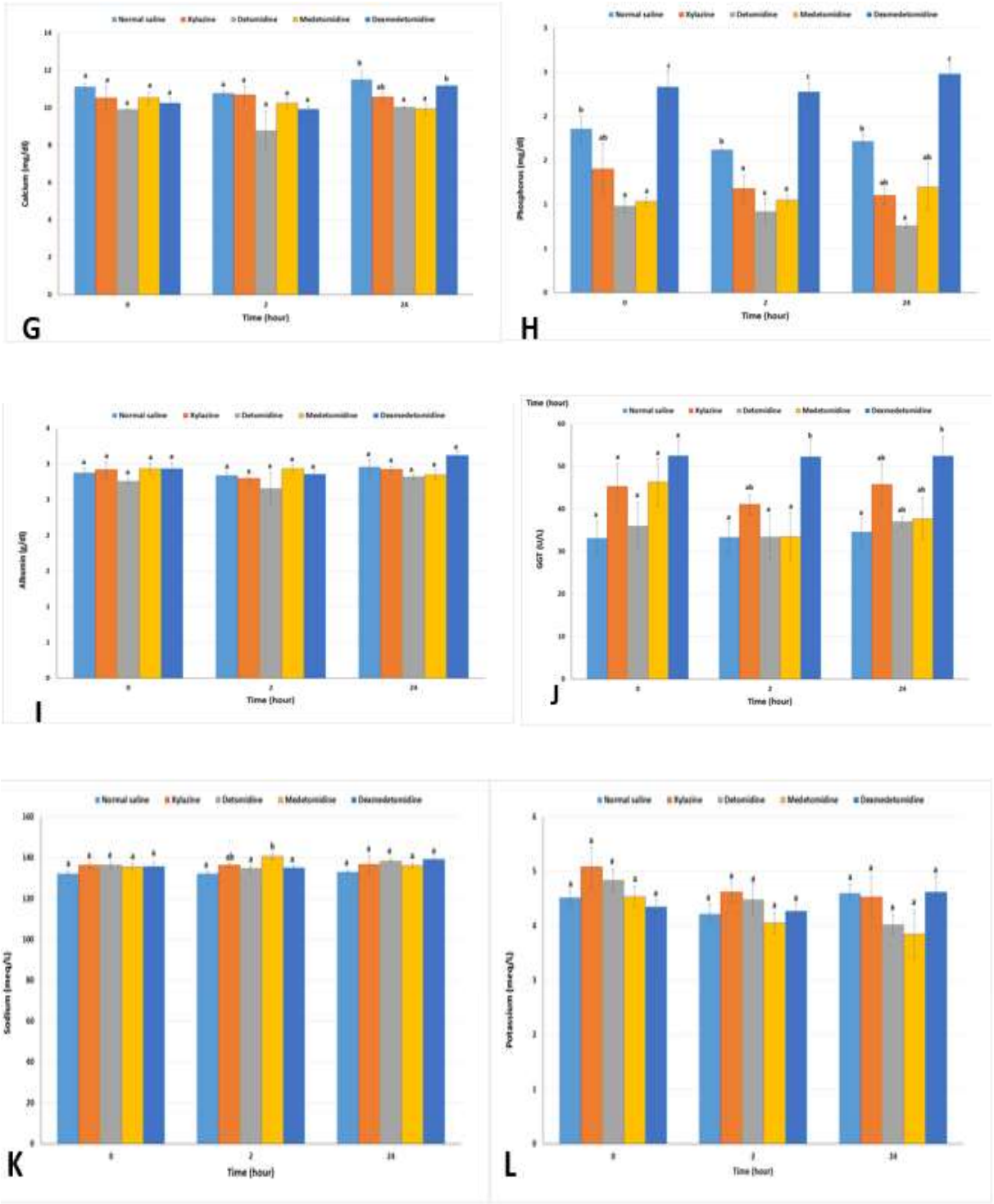

Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
Creatinine (mg/dl)
Time (hour)
B


Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
Cholesterol (mg/dl)
Time (hour)
C


Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
Triglyceride (mg/dl)
Time (hour)
D


Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
AST (U/L)
Time (hour)
E


Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
ALT (U/L)
Time (hour)
F


Normal saline
Xylazine
Detomidine
Medetomidine
Dexmedetomidine
Calcium (mg/dl)
Phosphorus (mg/dl)
Albumin (g/dl)
GGT (U/L)
Sodium (meq/L)
Potassium (meq/L)
Time (hour)
G
H
I
J
K
L

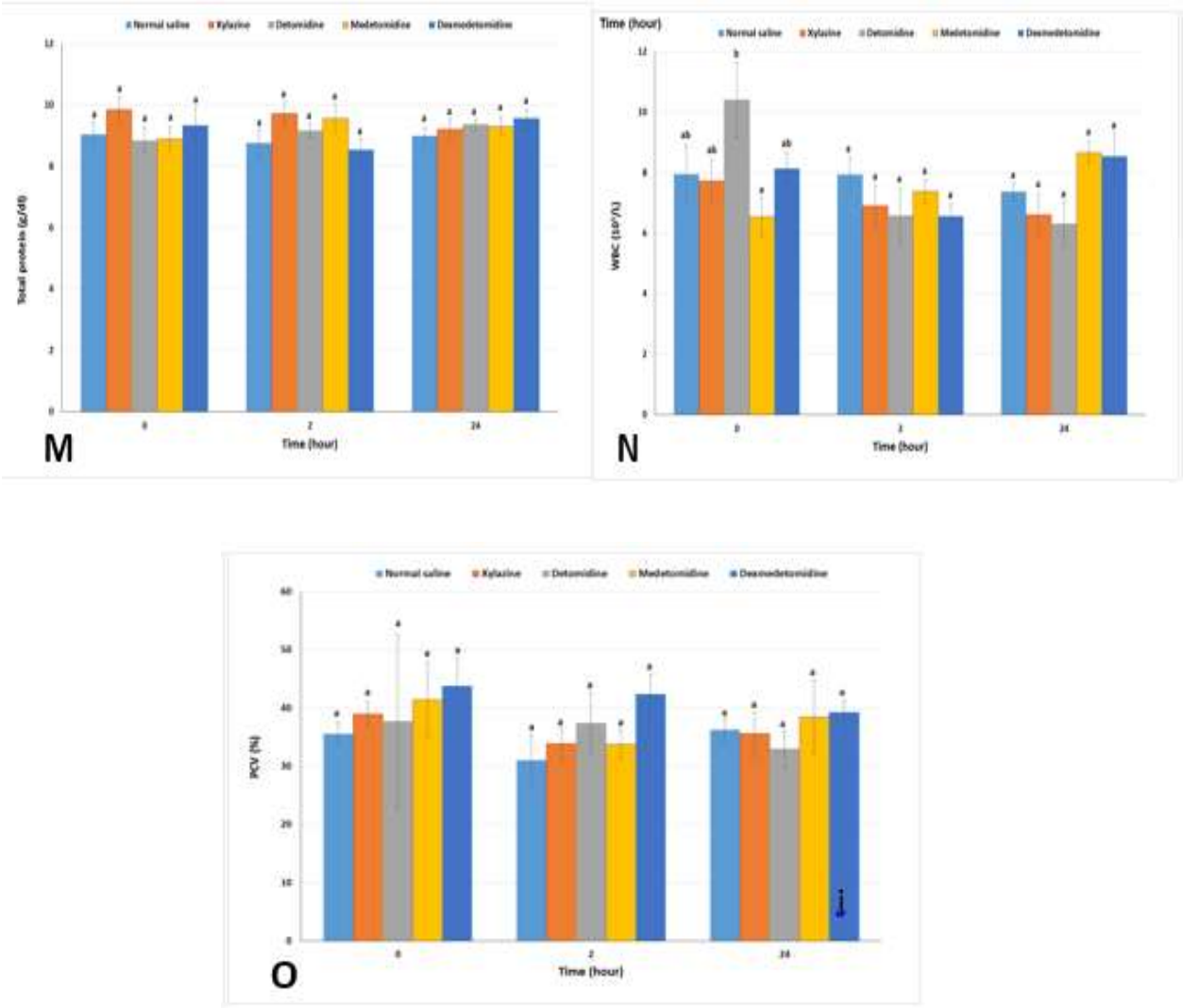


Figure 2. Trends in a: Blood Urea Nitrogen (BUN) levels, b: Serum Creatinine (Cr) levels, c: Serum Cholesterol levels, d: Serum Triglyceride levels, e: Serum AST levels, f: Serum ALT levels, g: Serum Calcium levels, h: Serum Phosphorus levels, i: Serum Albumin levels, j: Serum GGT levels, k: Serum Sodium levels, l: Serum Potassium levels, m: Serum Total Protein levels, n: WBC levels, and o: PCV levels (mean ± standard error) across experimental groups at pre- and post-drug injection time points. One-way ANOVA and Tukey's post-hoc test were utilized. At each time point, groups annotated with the same letter are not significantly different at the 95% confidence level ($p<0.05$).

Discussion

Research has extensively explored the effects of α_2-adrenergic drugs in horses; however, there is a notable absence of studies focusing on the Caspian miniature horse, a breed indigenous to Iran. This gap in the literature underscores the need for targeted research to elucidate the pharmacological effects of these agents within this specific equine population. Given that the Caspian miniature horse is a unique breed with distinct physiological characteristics, it is imperative to investigate how α_2-adrenergic agonists might influence its behavioral and physiological responses.

The findings within the designated time frame revealed that all four drugs administered significantly influenced HR, RR, RT and DM, thereby demonstrating their sedative effects on the equine subjects. Although certain signs of sedation were not consistently observed or were diminished at

specific time intervals throughout the study, overall, there was no statistically significant difference in the effectiveness of these drugs in eliciting sedative signs among the study groups (Gozalo-Marcilla et al, 2018, Yamashita et al, 2000).

α_2 - adrenergic receptor agonists have become integral components of contemporary anesthetic practices due to their capacity to induce sedation without significant respiratory depression. These agents contribute to cardiovascular stability and facilitate a reduction in the overall requirements for anesthetic agents. Their unique pharmacological profile allows for effective management of patients in various clinical settings, particularly in anesthesia, where maintaining hemodynamic stability is crucial while minimizing adverse effects associated with traditional anesthetics (Giovannitti et al, 2015).

The analysis of variance results with repeated measures indicated that the mean HR among the four groups exhibited statistically significant differences at various time points following drug administration. It was hypothesized that, due to the sedative properties of the administered drugs, the HR would decrease shortly after injection and subsequently return to baseline levels approximately one hour post-injection, contingent upon the specific characteristics of the drug utilized.

The stimulation of α_2 - adrenergic receptors may play a crucial role in mediating sedation, analgesia, and sympatholytic effects (Buerkle and Yaksh, 1998). Research has demonstrated that activation of these receptors inhibits adenylyl cyclase, leading to a reduction in cyclic adenosine monophosphate (cAMP) levels. This process results in hyperpolarization of noradrenergic neurons located in the medial dorsal pons, particularly within the locus ceruleus. As cAMP levels decline, potassium efflux through calcium-activated channels is facilitated, preventing calcium ions from entering the nerve terminal, which ultimately suppresses neural firing. This suppression inhibits norepinephrine release and diminishes the activity of ascending noradrenergic pathways, culminating in hypnosis and sedation. Furthermore, activation of this negative feedback loop may contribute to reductions in heart rate and blood pressure, as well as attenuation of the sympathetic stress response (Pichot et al, 2012).

One of the primary benefits of dexmedetomidine in comparison to other anesthetic agents is its negligible impact on respiratory function. This characteristic is particularly advantageous for patients who may have compromised airway patency, obesity, or restricted mobility, as dexmedetomidine facilitates effective sedation without endangering airway integrity or causing respiratory depression (Kaur and Singh, 2011).

The results of the present study indicate that the mean DM and RT across the four studied groups exhibited a statistically significant decrease at all-time points following injection, when compared to pre-injection measurements.

The previous research has shown that α_2-adrenoceptors play a complex role in regulating intestinal motility by modulating neurotransmitter release. Studies have demonstrated that α_2-agonists can reduce contractions in the equine jejunum, suggesting a potential therapeutic use for treating hyper motility in horses (Zullian, 2011).

Activation of α2-adrenoceptors in rodents induces hypothermia (Deupree et al, 2008). In humans, α2-adrenergic receptor agonists reduce energy expenditure and body temperature, promoting a drop in core temperature. Intravenous dexmedetomidine effectively lowers oxygen consumption, induces sedation, and suppresses shivering responses to induce hypothermia in healthy individuals (Callaway et al, 2024).

α_2 - adrenergic agonists are known to inhibit the activation of the brainstem

vasomotor center within the central nervous system (CNS). The sedative effects of these agents are linked to a reduction in sympathetic outflow from the CNS (Zullian, 2011). Our findings indicate that sedation levels were significantly higher at 15 minutes post-administration in Caspian miniature horses treated with various α_2 - adrenoreceptor agonists, as opposed to those receiving saline and dexmedetomidine shows a faster sedative effect compared to other medications.

The findings of this study reveal that symptoms such as head height (by an index), this measurement helps monitor the horse's response to sedative drugs by quantifying how much the head height changes during the sedation process, and incoordination, which are primary clinical indicators of sedation induced by the sedative agents under investigation, can present within 5 to 10 minutes following administration. The onset of these symptoms is contingent upon the specific sedative drug administered to the examined animals. Notably, there is no statistically significant difference in the onset timing of these symptoms across the various sedative agents evaluated. It is particularly noteworthy that both xylazine and dexmedetomidine demonstrated signs of incoordination slightly later than the other medications assessed.

The previous studies showed that following administration of α_2 - adrenergic agonists, responses such as HH and HR has been characterized as having a ceiling effect, where increasing the dose increases the duration of response but does not increase the magnitude. This ceiling effect is apparent when doses of 5, 20, 80, and 160 µg/kg of intravenous of detomidine were given to horses demonstrating that the HR decreased to values of low to mid-20 s for all three of the higher doses (Jochle & Hamm, 1986). The ceiling effect was also demonstrated for sedation, where the magnitude of sedation was similar for the three higher doses; however, the duration was longer with increasing doses, and animals remained standing even at the higher doses (Grimsrud et al, 2024).

So, it is recommended that future studies investigate the sedative effects of varying doses of the medications utilized in the present study on Caspian miniature horses. Furthermore, it would be beneficial to compare the results obtained from these investigations with those derived from administering the same drugs to horses.

Additional research is essential to comprehensively assess the cardiopulmonary effects associated with the doses utilized in this study. Specifically, investigations should focus on parameters such as stroke volume, blood pressure, pulmonary arterial pressure, and the partial pressures of arterial oxygen and carbon dioxide.

Significant alterations in blood urea nitrogen (BUN) and creatinine levels were observed following administration of the different agents. Both Medetomidine and Dexmedetomidine groups consistently exhibited lower BUN levels compared to other treatments at all measured time points, suggesting a potential renoprotective effect or altered renal handling of nitrogenous waste products. In contrast, at 24 hours post-injection, creatinine levels were significantly elevated in the Dexmedetomidine, Detomidine, and Xylazine groups relative to the Normal saline group. This delayed increase may indicate transient changes in glomerular filtration or altered muscle metabolism, which aligns with previous reports of alpha-2 agonists affecting renal hemodynamics.

The study also revealed significant changes in aspartate aminotransferase (AST) activity, particularly in the Dexmedetomidine group, which showed higher AST values compared to Medetomidine at all time points. This elevation could reflect mild hepatic stress or muscle enzyme leakage, a phenomenon occasionally reported with sedative administration, although additional

investigation is required to fully elucidate this issue. In contrast, alanine aminotransferase (ALT) levels remained stable across all groups, suggesting the absence of significant hepatocellular injury.

Alterations in calcium and phosphorus concentrations were also observed. Notably, at 24 hours post-injection, calcium levels were significantly lower in the Medetomidine and Detomidine groups compared to both Normal saline and Dexmedetomidine groups. This finding may be attributed to the pharmacodynamic effects of these agents on calcium homeostasis or renal excretion. Phosphorus levels exhibited a complex pattern, with Dexmedetomidine generally associated with higher values, suggesting a possible impact on phosphate metabolism or renal tubular handling.

Sodium levels showed a transient decrease at 2 hours post-injection in the Normal saline, Dexmedetomidine, and Detomidine groups compared to Medetomidine, but these differences were not sustained at 24 hours. Potassium and total protein levels, as well as albumin, remained unaffected, indicating that the observed effects were specific rather than generalized disturbances of fluid or protein balance. It seems that, changes in macro elements are temporary.

No significant changes were detected in cholesterol or triglyceride levels among the groups at any time point. This suggests that the acute administration of these sedatives does not markedly impact lipid metabolism in the short term, which is consistent with the previous studies.

White blood cell (WBC) counts and packed cell volume (PCV) were largely unaffected by the treatments, except for a higher baseline WBC in the Detomidine group compared to Medetomidine. This may reflect individual variability or a mild stress response, rather than a direct pharmacological effect. The stability of PCV across groups suggests that these agents do not induce significant hemoconcentration or hemodilution under the experimental conditions.

In conclusion, the intravenous administration of xylazine at a dosage of 1 mg/kg, detomidine at 20 µg/kg, medetomidine at 10 µg/kg, and dexmedetomidine at 5 µg/kg produced comparable levels of sedation in Caspian miniature horses. Consequently, any of the evaluated sedative agents may be considered effective options for sedation in healthy Caspian miniature horses, though further studies are warranted to confirm long-term safety and efficacy.

The study has several limitations, including a small sample size, the use of a specific breed, the lack of blinding procedures, and the absence of evaluation of long-term effects. These factors should be considered when interpreting the results.

Acknowledgements

We would like to express our sincere gratitude to the Vice Chancellor of Research and Technology at Shahid Bahonar University of Kerman, Kerman, Iran, for their invaluable support and guidance throughout the development of the research. The authors appreciate the resources and opportunities provided by the university.

Conflict of interest

None of the authors of this paper possess any financial or personal affiliations with individuals or organizations that could potentially unduly influence or bias the content presented in this paper.

Funding

This research was funded by a grant awarded by the Deputy of Research at Shahid Bahonar University of Kerman, Kerman, Iran

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Received: 20.07.2025
Accepted: 21.09.2025

Vol. 21, No. 3, Autumn, 2025, 68-77 *Original Article*
DOI: 10.22055/ivj.2025.534196.2837
Journal Homepage: http://www.ivj.ir/

Evaluation of hematology and some biochemical serum factors following experimental unilateral ureteral anastomosis in dogs

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Received: 18.08.2025 Accepted: 28.09.2025

Abstract

Ureteral obstruction is a clinically important condition in dogs that often requires surgical intervention. Such procedures can lead to significant physiological changes, particularly in hematological and biochemical parameters. This study aimed to evaluate hematological alterations and selected serum biochemical factors following experimental ureteral anastomosis in dogs. Five healthy mixed-breed dogs (15–30 kg, aged 1.5–4.5 years) underwent left ureteral transection and end-to-end anastomosis using a simple interrupted suture pattern. Anesthesia was induced with thiopental sodium and maintained with halothane after premedication with acepromazine (Schwartz, 2022; Hardie & Kyles, 2004). Urine and venous blood samples were collected pre-operatively and on days 1, 3, 7, 14, 21, 28, 35, 42, and 90 post-surgery for urinalysis, complete blood count, hematocrit, and measurement of blood urea nitrogen (BUN), creatinine, uric acid, calcium, phosphorus, chloride, sodium, and potassium. Data were analyzed using repeated measures ANOVA and Tukey's post hoc test. Significant increases in BUN, creatinine, and neutrophil counts were observed at all post-operative time points. BUN and creatinine exceeded normal ranges on days 1 and 3. Phosphorus levels were significantly elevated on days 1, 3, 7, 14, 21, 28, and 35, while potassium levels increased significantly on days 1, 3, 7, and 14. Uric acid, calcium, chloride, and sodium changes were not statistically significant. Despite compensatory mechanisms by the kidney and contralateral ureter, certain biochemical changes are inevitable following unilateral ureteral surgery, highlighting the need for careful postoperative monitoring to prevent complications.

Key words: Anastomosis, Biochemical changes, Dog, ureter, Urinalysis

Introduction

Ureteral obstruction in dogs and cats, primarily caused by ureteral calculi, manifests through symptoms such as abnormal urination, persistent urinary tract infections, abdominal pain, vomiting, anorexia, weight loss, and lethargy (Schwartz, 2022; Hardie and Kyles, 2004). It is important to distinguish ureteral obstruction, which involves blockage of the ureter between the kidney and bladder, from urethral obstruction, which occurs at the distal urinary tract. This study focuses exclusively on ureteral obstruction in small animals.

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Medical management of ureteral obstruction aims to restore urine flow, relieve discomfort, and preserve renal function until definitive resolution of the obstruction is achieved. As described in the previous studies (Schwartz, 2022; Hardie and Kyles, 2004), the initial therapy often includes aggressive fluid diuresis to promote stone passage, administration of smooth muscle relaxants to reduce ureteral spasm, analgesics for pain control, antibiotic therapy when urinary tract infection is present, and close monitoring of renal biochemical parameters such as blood urea nitrogen (BUN), creatinine, and electrolytes.

In cases of complete obstruction, failure of medical therapy, or presence of large calculi, surgical intervention becomes necessary. Surgical options include ureterotomy, ureteroneocystostomy, and, in specialized centers, minimally invasive techniques such as ureteral stenting or subcutaneous ureteral bypass (SUB). Prompt alleviation of complete ureteral obstruction is crucial for preserving renal function and preventing irreversible loss of glomerular filtration rate (Schwartz, 2022; Hardie and Kyles, 2004).

Ureteral surgeries, especially those involving ureteral re-anastomosis, are critical procedures necessitated by conditions like ectopic ureter and direct trauma to the ureter. These surgeries pose significant challenges in small animals due to the delicate nature of the structures involved. Potential complications from these procedures include hydronephrosis, hydroureter, stricture, or obstruction at the anastomosis site, which can significantly alter serum biochemical factors and impact the animal's overall health (Mayhew and Berent, 2023; Siddighi et al, 2017; Wormser et al, 2015; Hardie and Kyles, 2004).

Despite advancements in surgical techniques and tools (Pavia et al, 2018), ureteral anastomosis remains susceptible to complications. Functional or structural obstructions can still occur at the anastomosis site, interfering with ureteral peristaltic movements and reducing urine flow. This reduction can subsequently decrease the glomerular filtration rate in the associated kidney, leading to changes in serum biochemical factors (Hooi et al, 2021).

Although compensatory mechanisms in the healthy kidney may eventually restore electrolyte balance, these mechanisms are often not fully activated in the immediate postoperative period, resulting in temporary changes in blood electrolyte levels. Understanding these changes and the underlying mechanisms is crucial for improving postoperative outcomes and managing potential complications (Johnson et al, 2024; Wilson, 1977).

The previous studies have documented significant changes in blood biochemical factors following ureteral surgeries. Elevated levels of BUN and creatinine, for instance, are common indicators of impaired renal clearance and urine leakage into the abdominal cavity (Saygin et al, 2021). These biochemical markers are essential for assessing the kidney function and detecting postoperative complications.

This study aims to investigate the changes in serum biochemical factors, particularly BUN and creatinine, following ureteral re-anastomosis in dogs. By understanding these changes and their causes, we can develop better strategies for managing postoperative complications and improving the overall health and recovery of affected animals.

Materials and Methods

Selection of Animals: Five healthy adult mixed-breed dogs (three males and two females), weighing 15–30 kg and aged 1.5–4.5 years, were included. Age was determined by a board-certified small animal internal medicine specialist through dental examination (tooth eruption patterns, wear, and tartar accumulation) in accordance with standard veterinary guidelines. All dogs underwent clinical

examination to confirm normal appetite, urination, and defecation, and were dewormed using conventional antiparasitic drugs. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Iran. Although no specific approval code was issued, the study was conducted under the committee's supervision in accordance with institutional animal care and use guidelines.

This study aimed to monitor hematological and biochemical changes following unilateral ureteral anastomosis and to provide a detailed 90-day temporal profile of these parameters. While some changes in renal function markers were anticipated due to surgery, the innovative aspect lies in the longitudinal evaluation of multiple hematological and biochemical variables at frequent intervals, offering a clearer understanding of contralateral kidney compensatory mechanisms.

Sampling Protocol

Blood and urine samples were collected preoperatively and on postoperative days 1, 3, 7, 14, 21, 28, 35, 42, and 90. For blood collection, 5 mL was drawn from the cephalic vein into heparinized tubes. A complete blood count, including hematocrit and differential leukocyte count, was performed. Samples were centrifuged at 2500 rpm for 5 minutes, and plasma was stored at –20 °C until biochemical analysis. Urine samples were obtained via urinary catheter, and physical characteristics (color, clarity, specific gravity) were recorded before centrifugation and microscopic sediment examination.

Preoperative Preparation

Dogs were fasted for approximately 12 hours before the surgery, with free access to water. Sedation was achieved with intramuscular acepromazine (0.03 mg/kg). Anesthesia was induced intravenously with 2.5% thiopental sodium (6–10 mg/kg) and maintained with oxygen and halothane (1–1.5%) in a closed inhalation system, following standard veterinary anesthesia protocols (Hall, Clarke, & Trim, 2001). Animals were positioned in dorsal recumbency, and the area from the umbilicus to the pubis was shaved and aseptically prepared.

Surgical Procedure

A 10 cm midline incision was made from the umbilicus to the cranial pubis. After entering the abdominal cavity, the bladder and left ureter were exposed. The ureter was transected obliquely approximately 5 cm from its insertion into the bladder to increase the luminal cross-section. End-to-end anastomosis was performed with simple interrupted 6-0 polydioxanone sutures. The anastomosis site was checked for leakage before returning the ureter to the abdominal cavity. The laparotomy was closed routinely. Cefazolin (20 mg/kg IV) and tramadol (3 mg/kg IV) were administered intraoperatively.

Postoperative Care

Cefazolin (20 mg/kg IV, twice daily) was continued for 5 days after post-surgery. Tramadol was administered for up to 3 days, depending on pain sensitivity at the surgical site. Skin sutures were removed 14 days postoperatively.

Biochemical Assays

Plasma urea was measured by the urease method, creatinine by the Jaffe method, uric acid by the PAP method, calcium and phosphorus by direct colorimetry, chloride by the thiocyanate colorimetric method, and sodium and potassium by flame photometry.

Statistical Analysis

Data were analyzed using SPSS software. Changes over time were assessed by repeated measures ANOVA, with $P < 0.05$ considered statistically significant. Tukey's post hoc test was used for pairwise comparisons.

Results

Blood Urea Nitrogen (BUN): Mean BUN values showed a highly significant difference over time ($P \leq 0.001$). According to Tukey's test, preoperative BUN levels differed significantly from all postoperative time points. Given the wide normal range (10–28 mg/dl), the most notable increases occurred on days 1 and 3, when values exceeded the upper limit. The minimum preoperative BUN was 16.8 mg/dl, peaking at 31 mg/dl on day 1, then gradually declining but remaining above baseline (21.2 mg/dl) on day 90.

Creatinine: Mean serum creatinine increased immediately after surgery, reaching a maximum of 1.85 mg/dl on day 3, then gradually decreased toward normal. Differences between preoperative and all postoperative values were highly significant ($P \leq 0.001$). The most pronounced elevations were on days 1 and 3, exceeding the normal range (0.5–1.5 mg/dl). The minimum preoperative value was 0.97 mg/dl, and by day 90, creatinine stabilized at 1.27 mg/dl, still slightly above baseline.

Uric Acid: Changes in mean uric acid levels were not statistically significant ($P > 0.05$). Values showed a mild upward trend post-surgery, from 4.34 mg/dl preoperatively to a maximum of 4.52 mg/dl on days 21 and 90.

Calcium: Although mean calcium levels decreased after surgery and returned to normal (9.64 mg/dl) by day 28, these changes were not statistically significant ($P > 0.05$).

Phosphorus: Mean phosphorus levels increased after surgery, peaking at 4.68 mg/dl on day 3, then gradually declined toward baseline. Differences between preoperative values and those on days 1, 3, 7, 14, 21, 28, and 35 were highly significant ($P \leq 0.001$).

Chloride: Mean chloride levels rose after surgery, reaching 116 mg/dl on day 7, then declined. These changes were not statistically significant ($P > 0.05$).

Sodium: No statistically significant changes in mean sodium levels were observed over time ($P > 0.05$).

Potassium: Preoperative potassium levels differed significantly from those on days 1, 3, 7, and 14 ($P \leq 0.001$). Values increased from 4.50 mEq/dl preoperatively to a maximum of 4.96 mEq/dl on day 3, then decreased thereafter.

Table 1 presents the mean ± standard error for all biochemical parameters before and after surgery, with statistical significance indicated.

Hematology: Mean neutrophil counts before surgery differed significantly from all postoperative values ($P \leq 0.01$). The most marked increase occurred on day 1. Although subsequent values remained significantly different from baseline, they stayed within the normal range. Table 2 shows the mean ± standard error for hematological parameters, with significant changes indicated.

Table1: The mean and standard error of biochemical serum parameters following ureteral anastomosis surgery in five dogs

Potassium (mEq/l)	Sodium (mEq/l)	Chlorine (mg/dl)	Phosphorus (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine mg/dl	Blood urea nitrogen (BUN) mg/dl	Serum Parameters
4.52±0.280	146.4±1.82	114.4±1.82	4.24±0.330	9.44±0.240	4.34±0.220	0.97±0.130	16.8±0.84	Before surgery
4.72±0.180*	146.8±1.48	114.8±2.28	4.44±0.370*	9.06±0.330	4.38±0.230	1.68±0.250*	30.4±2.07*	1 day post surgery
4.96±0.250*	147.6±1.52	115.8±2.17	4.68±0.270*	9.06±0.560	4.40±0.200	1.85±0.210*	29.8±1.64*	3 days post surgery
4.78±0.300*	147.2±1.48	116±1.22	4.56±0.380*	9.42±0.620	4.30±0.160	1.58±0.280*	26.2±1.64*	7 days post surgery
4.78±0.250*	146.2±1.79	114.6±1.35	4.58±0.300*	9.26±0.350	4.42±0.280	1.42±0.200*	26±1.87*	14 days post surgery
4.70±0.200	146.8±1.83	115±2.12	4.46±0.290*	9.20±0.400	4.52±0.130	1.35±0.150*	23.4±2.30*	21 days post surgery
4.70±0.140	145.6±2.70	115±2.34	4.38±0.240*	9.46±0.190	4.42±0.290	1.32±0.100*	23.2±2.04*	28 days post surgery
4.56±0.280	144.6±2.07	113.4±1.14	4.42±0.290*	9.40±0.190	4.36±0.210	1.29±0.110*	22.6±1.52*	35 days post surgery
4.60±0.160	144.6±2.50	113.8±0.83	4.28±0.200	9.38±0.270	4.40±0.280	1.28±.0130*	21.4±0.55*	42 days post surgery
14.58±0.130	145.6±1.14	114.2±1.48	4.28±0.230	9.48±0.050	4.52±0.180	1.27±0.050*	21.2±1.92*	90 days post surgery

*The difference from the preoperative value was significant (P<0.001).

Table 2: The mean and standard error of hematology parameters following ureteral anastomosis surgery in five dogs

Hematocrit (%)	Monocyte (cell/µl)	Eosinophil (cell/µl)	Lymphocyte (cell/µl)	Neutrophil (cell/µl)	Blood parameters
42±1.30	368.2±86.50	982.6±355.30	2506±478.00	7293.2±624.70	Before surgery
43.8±1.50	476±148.90	613.8±156.50	1008.6±256.20	17293.6±2563.80*	1 day post surgery
40.2±1.30	672.9±301.30	627±210.30	1890.5±681.30	10086.1±2095.80*	3 days post surgery
41.6±0.90	525.3±175.40	831.7±110.60	1914.2±662.00	9778.8±1389.50*	7 days post surgery
49.2±2.20	442±74.40	404.5±189.50	2064.5±475.80	9489±1504.40*	14 days post surgery
44.2±0.80	316±113.40	784.9±165.10	1981.7±610.10	8377.4±717.10*	21 days post surgery
44.6±2.10	380.5±157.80	781.5±234.50	2220.5±291.3	8021.5±463.50*	28 days post surgery
46.6±2.20	166±116.50	810±178.70	2232±532.2	8592±1751.30*	35 days post surgery
48.4±1.90	370.5±148.20	692.5±251.40	2308±298.8	8229±1520.90*	42 days post surgery
48±2.10	240±185.90	825±202.70	1885±274.4	7350±380.30*	90 days post surgery

*The difference from the preoperative value was significant (*P<0.01*)

Urinalysis: Preoperative urinalysis confirmed normal physical, chemical, and cellular indices: amber-yellow, clear urine with mean specific gravity of 1.040, pH 7, and normal counts of red and white blood cells, epithelial cells, and transitional cells. Occasional uric acid and triple phosphate crystals were observed, consistent with normal carnivore urine.

On days 3 and 7 post-surgery, urinalysis revealed increased red and white blood cell counts. By day 14, these values began returning toward normal, and by day 90, they had stabilized. Mild proteinuria persisted throughout the sampling period but was not clinically or statistically significant. Overall, urinalysis indicated that inflammatory and hemorrhagic changes in the urinary tract gradually resolved during recovery.

Discussion

In certain ureteral surgeries, particularly in cases of ectopic ureter or direct trauma, ureteral re-anastomosis may be required (Dekerle et al, 2022; Brown, 2013). Performing this procedure in small animals is technically challenging, and postoperative alterations in the urinary tract are common. Among the most significant complications are hydronephrosis, hydroureter, stricture, or obstruction at the anastomosis site, all of which can alter serum biochemical parameters and adversely affect the animal's health (Degner et al, 2022).

Even with optimal surgical techniques and advanced instrumentation, ureteral anastomosis cannot fully prevent the occurrence of functional or structural obstruction at the anastomosis site (Cooper & Scansen, 2020). In some cases, without any visible structural obstruction, disruption of ureteral peristalsis—particularly in the early postoperative period—can reduce urine flow through the anastomosis. This reduction in flow decreases the glomerular filtration rate (GFR) of the affected kidney, leading to measurable changes in serum biochemical values (Mayhew and Berent, 2023; Barthez et al, 2004).

Although compensatory mechanisms in the contralateral kidney can eventually restore electrolyte balance, these mechanisms are not fully active in the immediate postoperative period. As a result, transient alterations in blood electrolyte levels are expected. In line with the previous literature, the present study also anticipated changes in serum biochemical parameters due to the potential for stricture or obstruction at the anastomosis site (Urie et al, 2007).

Blood Urea Nitrogen (BUN) and Creatinine (Cr): BUN and creatinine are key indicators of renal function. BUN reflects the concentration of nitrogen derived from urea, a protein metabolism byproduct, in the blood. Elevated BUN may indicate impaired renal clearance, dehydration, or high protein intake. Creatinine, a muscle metabolism byproduct, is excreted by the kidneys and serves as a reliable marker of GFR. Increases in creatinine typically indicate reduced renal function or obstruction (Hessels et al, 2018).

These two parameters are often measured together to provide a comprehensive assessment of kidney health. Both undergo significant changes in many urinary tract disorders, particularly in obstructive conditions. Creatinine is especially sensitive to reductions in renal clearance, making it a valuable marker for evaluating urinary tract obstruction, including postoperative cases (Inker and Titan, 2021).

Two main mechanisms may explain the marked increases in BUN and creatinine observed during the first three postoperative days. The first is either partial or complete obstruction at the anastomosis site, impairing clearance by the affected kidney (Aronson, 2020; Adin and Scansen, 2011). The second is temporary cessation of ureteral peristalsis, which reduces urine flow in the operated ureter (Geavlete,

2016). Both mechanisms are supported by the previous studies.

Clinical signs of ureteral obstruction in dogs include urinary incontinence, stranguria, dysuria, pollakiuria, polyuria, hematuria, persistent urinary tract infection, abdominal pain, vomiting, anorexia, lethargy, weight loss, fever or hypothermia, palpable abdominal mass, vaginal discharge, and prostatomegaly (Hardie and Kyles, 2004). In bilateral cases, clinical signs are often severe and acute. For example, in a 5-year-old Labrador Retriever with urethral obstruction secondary to bladder torsion, any impediment to urine outflow was associated with elevated BUN and creatinine (Pozzi et al, 2006).

Other reports describe similar findings. Nwadike et al, (2000) documented vomiting, lethargy, and anorexia in a 2-year-old Himalayan cat following ovariohysterectomy. Excretory urography revealed bilateral ureteral obstruction due to inadvertent ligation. Temporary nephrostomy tube placement normalized BUN and creatinine, and subsequent ureteral re-anastomosis resolved the obstruction. In a retrospective study, Kyles et al. (2005) identified elevated creatinine as a common laboratory abnormality in dogs with ureterolithiasis. Surgical removal of stones resulted in full recovery in many cases, though not all.

Interestingly, elevated BUN and creatinine can also occur without overt obstruction. For example, in ureterocolonic anastomosis, postoperative increases in these parameters during the first 2–3 days have been attributed to reabsorption of nitrogenous waste products from the colon (Saberi Afshar et al, 2007). Similarly, Sarin et al, (2006) and El-Leithy (2008) reported hydrourer and hydronephrosis without obstruction after ureteral surgeries, suggesting that reduced urine flow alone can elevate BUN and creatinine. Patil et al, (2008) also observed functional obstruction without physical blockage after psoas hitch ureteral reimplantation.

In the present study, necropsy confirmed the absence of complete obstruction, but stenosis at the anastomosis site was present in most cases. Therefore, the most plausible explanation for the early postoperative increases in BUN and creatinine is functional ureteral obstruction. The potential influence of the closed halothane anesthesia system cannot be entirely excluded. No severe postoperative complications such as wound infection, dehiscence, or persistent urinary abnormalities were observed. Only mild, transient signs—reduced appetite and mild discomfort at the surgical site were noted and resolved with supportive care.

Changes in Blood Electrolytes: Ureteral anastomosis can temporarily affect renal clearance and alter blood electrolyte levels. However, literature review reveals no consistent pattern for these changes. Obstructive uropathy, whether functional or physical, may cause various electrolyte disturbances before and after relief of obstruction, especially in high-grade chronic cases (Yaxley & Yaxley, 2023). Early post-renal obstruction often results in tubular solute wasting (sodium, potassium, bicarbonate, magnesium, calcium, phosphate), followed by retention of potassium, hydrogen, chloride, and ammonium as renal function declines.

Sodium and potassium disorders are the most clinically significant. Hyponatremia, often due to plasma dilution and tubulopathy, may take weeks to normalize after decompression (Moore, 1958). Hypernatremia can occur with prolonged diuresis, while hypokalemia is common post-obstruction and usually self-limiting. Hyperkalemia may arise from severe renal impairment or distal tubular injury (Yaxley and Yaxley, 2023; Yang et al, 2021).

Experimental and clinical studies support these findings. Chevalier et al, (2002) showed that even after relief of unilateral ureteral obstruction in rats, GFR, urine flow, and sodium/potassium excretion decreased, leading to retention of these ions.

Saberi Afshar et al. (2007) reported increased phosphorus and chloride and decreased calcium, sodium, and potassium after ureteral reconstruction with a colon graft. Bigham Sadegh et al, (2009) found no major electrolyte changes after ureteral replacement with fetal urachus, though potassium increase approached significance (P=0.06). Abbas et al, (2008) and Pozzi et al, (2006) documented variable potassium changes in bladder outlet obstruction cases.

Hematology: Postoperative leukocytosis can occur due to surgical stress or urinary tract stimulation from urine retention, even without infection (Pozzi et al, 2006). In this study, neutrophil counts were significantly elevated at all postoperative time points compared to baseline, but exceeded the normal range only during the first three days. Clinically, all animals remained healthy.

A limitation of this study is the absence of blood gas analysis, which could have provided additional insight into postoperative acid–base status. Future studies should incorporate such measurements.

Ureteral anastomosis, even when performed unilaterally with expected compensation from the contralateral kidney and ureter, can cause transient changes in parameters related to glomerular filtration rate, leading to alterations in serum biochemical values. These changes are generally temporary and most evident during the first few weeks of post-surgery.

Acknowledgments

We would like to express our sincere gratitude to Shahid Chamran University of Ahvaz for providing the facilities and resources necessary for conducting this research. Special thanks to the Veterinary Faculty for their invaluable support and guidance throughout the study.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this study.

Funding

This study was financially supported by the thesis budget of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz.

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Received: 18.08.2025
Accepted: 28.09.2025

Vol. 21, No. 3, Autumn, 2025, 78-88 *Original Article*
DOI: 10.22055/ivj.2025.542208.2838
Journal Homepage: http://www.ivj.ir/

A study of the Histogenesis and development of the pancreas of the Pheasant (*Phasianus colchicus*) embryos

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Received: 19.08.2025 Accepted: 15.10.2025

Abstract

The main objective of this study was to investigate the histogenesis and development of the pheasant's pancreas during embryonic stages. Twenty-five fertilized pheasant eggs were placed in an incubator at 37.5°C and a humidity of 58 to 62%. Three pheasant embryos at ages 13, 15, 17, 19, 21 as well as three one-day-old chicks were collected. After tissue processing including dehydration, clearing and impregnation with melted paraffin samples were sectioned (5 micrometer) and stained with Hematoxylin and Eosin, Masson's Trichrome and Gomori's trichrome stains. The pheasant's pancreas began to form between days 13 and 15 days of the embryonic period. In the 17-day-old pheasant embryo, in addition to the dorsal lobe, the formation of the ventral lobe had also begun. Similar to the 15-day-old pheasant embryo, the pancreas of the 17-day-old embryo consisted of undifferentiated epithelial cells, connective tissue, and underdeveloped ducts, but the number of acinar cells had increased. In the 19-day-old embryos, the acinus was formed and mainly organized. Also, the Langerhans islands were observed at this age. In the 21-day-old embryo, the interlobular ducts were identified, and the formation of the Langerhans Islands had increased. In the 1-day-old pheasant chick, the exocrine part of the pancreas, the acinus, was more developed. The islets of Langerhans were also clearly visible, as these islands in the splenic lobe were more numerous than in the other lobes. In conclusion, the histogenesis of the pheasant (*Phasianus colchicus*) pancreas begins to form between days 13 and 15 days of the embryonic period, and continues until after hatch. The dorsal lobe demonstrated primary by initiating development first. The definitive pancreatic architecture was established through the sequential differentiation of key components. The endocrine islets of Langerhans emerged on day 19, followed by the maturation of the exocrine acinar tissue and the ductal system on day 21, marking the culmination of embryonic organogenesis.

Key words: Pheasant embryo, Pancreas, Histogenesis, Development

Introduction

The pancreas is one of the important exocrine and endocrine glands in vertebrates, holding–a special place in the body's physiology, playing a key role in regulating metabolism and food digestion (Alkhatib, 2024; Ariyaan et al, 2023; Karpińska and Czauderna, 2022; Lee and Lee, 2024; Peyghan et al, 2023). In birds, particularly wild species such as pheasants (*Phasianus colchicus*), the pancreas exhibits unique structural and functional characteristics that distinguish it from other animal classes (Hollwarth and Prieto, 2025). A correct understanding of the

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developmental stages of the pancreas during embryonic development can provide valuable insights into the processes of cellular differentiation, tissue organization, and the evolution of the digestive system in birds, and provide a suitable basis for comparisons between species.

During the embryonic period, the differentiation and development of body tissues are influenced by genetic, hormonal, and environmental factors (Sirard, 2021). Genetic factors, such as the Hox and Pdx1 genes, control signaling pathways that direct the formation of early pancreatic structures and the differentiation of specialized cells (Jensen, 2004). Hormonal factors, including insulin and thyroid hormones, modulate pancreatic growth and differentiation at various stages by regulating cell proliferation and the expression of specific genes (Chen et al, 2018). Environmental factors, such as incubation temperature and maternal nutrition, also influence the speed and accuracy of pancreatic development by altering gene expression patterns and hormone levels (O'Dowd and Stocker, 2013; Reusens and Remacle, 2006).

The pancreas originates from the primary digestive bud. It gradually develops into its mature form at different stages of development, featuring specific structures such as lobes, islets of Langerhans, and excretory ducts (Slack, 1995). The development of the embryonic pancreas typically begins with the formation of primary ductal-endothelial projections from the propancreatic endoderm (Olaniru et al, 2023). These projections then differentiate into individual pancreatic ducts, and through processes of branching and differentiation, they create the tree-like structure of the gland. The initial cell population gradually differentiates into its final fates, namely exocrine (acinar), endocrine (islets of Langerhans), and ductal cells, through induction by neighboring tissues and the expression of specific gene sequences (Ornellas et al, 2020; Rooman and Real, 2012). A detailed study of these developments in a species such as the pheasant, which is of interest to biologists due to its physiological characteristics and high resistance in various habitats, can shed light on the new aspects of the tissue development process.

The pheasant, a native bird of many regions of Asia, including Iran, has high ecological and economic value. Despite its importance, few studies have been conducted on the developmental aspects of this bird's internal organs (Al-Shuwaili et al, 2022; Gheshlagh et al, 2020; Khodadadi et al, 2019; Khodaparast and Nabipour, 2024). Previous studies have focused on behavioral, ecological, and nutritional aspects of the topic. Detailed knowledge of the structure and tissue development of the pancreas can lead to improved breeding methods, improved digestive health and efficiency, and a deeper understanding of its physiology.

Extensive studies have been conducted on the development of the pancreas in various birds, including chickens (Vertiprakhov et al, 2023) and ducks (Pieler and Chen, 2006), which have helped clarify the different stages of this organ's development. Although the processes of pancreatic development and differentiation in mammals and some bird species, such as domestic chickens, have been well studied, there is very limited information on how these processes occur in wild birds, especially pheasants (*Phasianus colchicus*). This knowledge gap is clearly noticeable. There are no systematic histomorphological data on the early stages of pancreatic formation, including the differentiation of acinar, ductal, and islet of Langerhans cells, in pheasant embryos. Additionally, the correspondence or differences between this process and other bird model species have not been precisely determined. Therefore, the main objective of this study is to accurately and systematically characterize the stages of histogenesis and morphogenesis of the pancreas in the

pheasant embryo from the earliest stages of its formation until hatching and in 1-day-old pheasant chick.

Materials and methods

Separation of embryos and sampling

Twenty-five fertilized pheasant eggs were obtained from a pheasant egg production farm and transferred to the histology laboratory of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. The eggs were placed in an incubator at 37.5°C and 58-62% humidity.

The incubation period in pheasant is about 23 days. Three pheasant embryos at ages 13, 15, 17, 19, 21 as well as three one-day-old chicks, were collected. Eggs were opened from the air chamber side, and the calcareous shell and membrane shell attached to it were removed. All these membranes were cut into a circle with fine scissors. Then, all the egg contents were poured into a Petri dish, and the embryo was removed from the amniotic sac. Embryos were placed entirely in Bouin's solution. However, in the case of larger embryos, the pancreas was dissected and placed separately in the mentioned fixative.

Histological analysis

The embryos were dehydrated in a series of ascending ethanol concentrations, cleared in xylene, and then embedded in paraffin. 5 µm-thick paraffin sections were obtained with a rotary microtome (Leica RM 2145; Germany). The sections were stained using Hematoxylin and Eosin (H&E) for general tissue morphology, Masson's trichrome for collagen fiber differentiation, and Gomori's for reticular fiber visualization. Following staining, the prepared slides were thoroughly examined and imaged using an Olympus DP2-BSW light microscope equipped with a digital camera DP-12 (Olympus). The histogenesis and development of the pancreatic tissue of pheasant embryos at mentioned ages as well as three one-day-old chicks were studied.

Results

13-day embryo

The primitive gut was observed developing at this age (Figure 1).

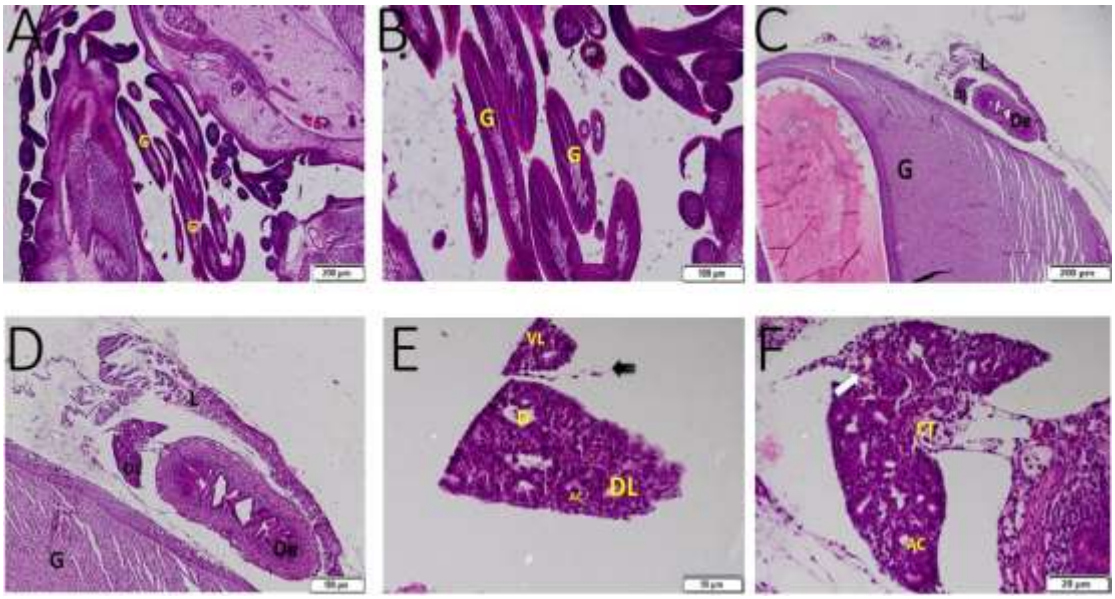

Figure 1: A) Showing the primary intestine in a 13-day-old pheasant embryo. Primary intestine (G). B) Showing the primary intestine (G) in a 13-day-old pheasant embryo. C and D) Showing the formation of the dorsal lobe of the pancreas in a 15-day-old pheasant embryo. Dorsal lobe (DL), duodenum (De), liver (L), gizzard (G). E) This figure shows the histological structure of the pancreas in a 17-day-old pheasant embryo. Dorsal lobe (DL), ventral lobe (VL), mesenchymal tissue separating the two lobes (arrow), duct (D), and acinar cells (AC). F) Showing the histological structure of the pancreas in a 15-day-old pheasant embryo. Connective tissue (CT), red blood cells (arrow), acini (AC). Hematoxylin and Eosin staining

15-day embryo

Early signs of pancreatic formation were observed at this age. Hence, it seems that the pheasant pancreas begins to form between days 13 and 15 of embryonic development. The pancreas was connected to the duodenum via a stalk, and it appears that the dorsal lobe of the pancreas is formed first, and the other lobes were not observed. The formation of the pancreas occurred near the liver. Regarding the histological structure of the pancreas in the 15-day embryo, the acini were not formed or were only partially formed in some areas, and arteries were observed. The pancreas consisted of undifferentiated epithelial cells, connective tissue, and undeveloped ducts. In addition, the islets of Langerhans were not formed in the 15-day embryo (Figure 1).

17-day embryo

In this age, in addition to the dorsal lobe of the pancreas, the formation of the ventral lobe had also begun. The dorsal lobe was noticeably larger than the ventral lobe. Moreover, the histological structure of the pancreas was similar to that of the 15-day embryo, with a decrease in the amount of connective and mesenchymal tissues and ducts. Still, the number of acinar cells had increased from the 15-day embryo. Therefore, the exocrine part of the pancreas develops more at this age. Blood vessels were also clear at this age. In addition, the islets of Langerhans were not observed at a 17-day embryo either (Figure 1).

19-day embryo

Concerning this age, all three lobes of the pancreas, including the dorsal, ventral, and splenic lobes, were observed. Moreover, the acini were largely organized and formed, and the exocrine part of the pancreas had grown. Ducts were observed. The connective tissue had fewer blood vessels compared to the 17-day-old age.

Due to the low connective tissue level, the lobules could not be distinguished. Acinar cells had spherical nuclei at the base of the cells. Signs of Langerhans islet formation were observed in the ventral lobe (Figure 2).

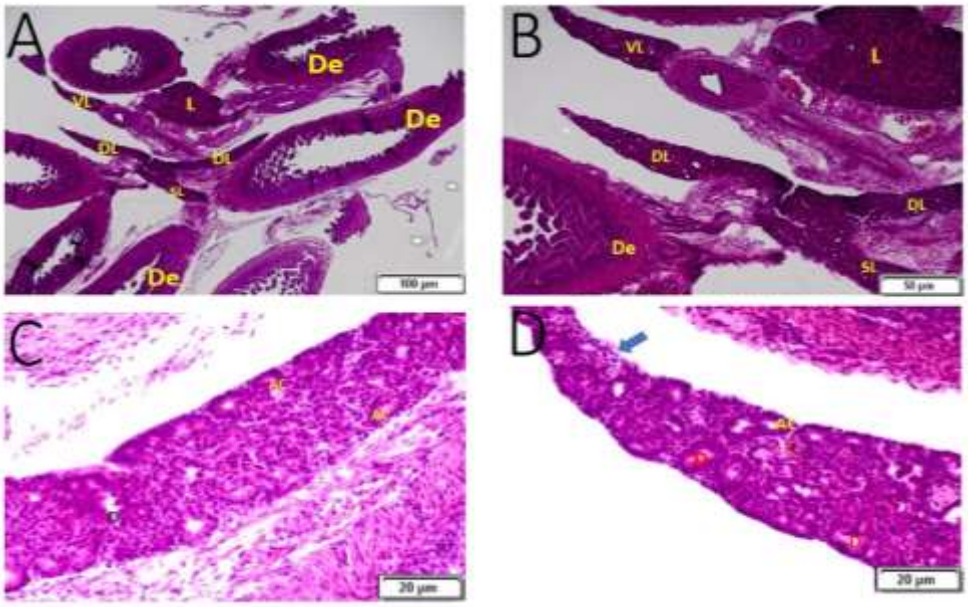

Figure 2: A and B) Represents the pancreas in a 19-day-old pheasant embryo. Dorsal lobe (DL), ventral lobe (VL), splenic lobe (SL), duodenum (De), and liver (L). C) Represents the histological structure of the dorsal lobe of the pancreas in a 19-day-old pheasant embryo. Acini (AC), duct (D). D) Represents the histological structure of the ventral lobe of the pancreas in a 19-day-old pheasant embryo. Acini (AC), ducts (D), islets of Langerhans (arrows), and erythrocytes (E) Hematoxylin and Eosin staining.

21-day-old embryo

In regard to this stage, the interlobular ducts were visible, and the formation of Langerhans islets had increased. However, intralobular ducts, such as intercalated ducts were not observed. The cytoplasm of the acinar cells was completely acidophilic, indicating the formation of zymogen granules containing enzymes. The centroacinar cell was not distinguishable at this age. Therefore, the exocrine part of the pancreas was fully developed (Figure 3).

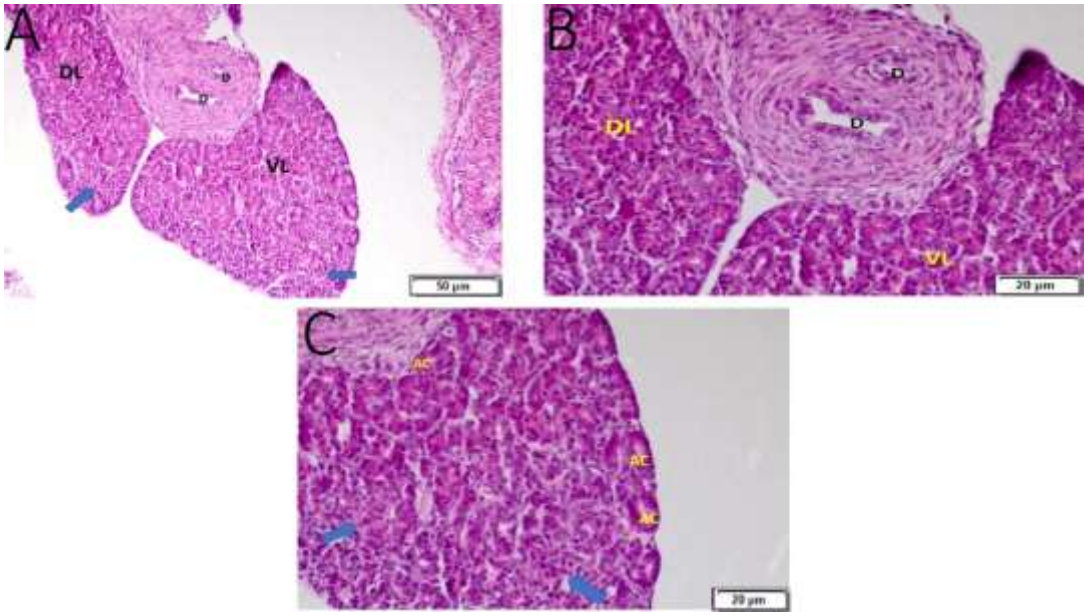

Figure 3: A) Shows the interlobular ducts of the pancreas in a 21-day-old pheasant embryo. Ducts (D), dorsal lobe (DL), ventral lobe (VL), and islets of Langerhans (arrows). B) Shows the interlobular ducts of the pancreas in a 21-day-old pheasant embryo. Ducts (D), dorsal lobe (DL), and ventral lobe (VL). Note the acidophilic cytoplasm of the acinar cells. C) Shows the histological structure of the pancreas in a 21-day-old pheasant embryo. Acini (AC) have cells with acidophilic cytoplasm, islets of Langerhans (arrows). Hematoxylin and Eosin staining.

1-day-old pheasant chick

In a one-day-old pheasant chick, the three pancreatic lobes, including the dorsal, ventral, and splenic lobes, were macroscopically distinct. The dorsal lobe was larger than the ventral lobe, and the splenic lobe was located anterior to the dorsal lobe. The splenic lobe was distinguished from the other two lobes by the greater concentration of Langerhans islets (Figure 4).

In relation to this age, the exocrine part of the pancreas had further developed. The islets of Langerhans were also clearly visible, with the number of these islets in the splenic lobe being higher than in the other lobes. Islets were also seen scattered in the ventral lobe. The amount of connective tissue and intralobular ducts was very low. The centroacinar cell was also not distinguishable at this age (Figure 5).

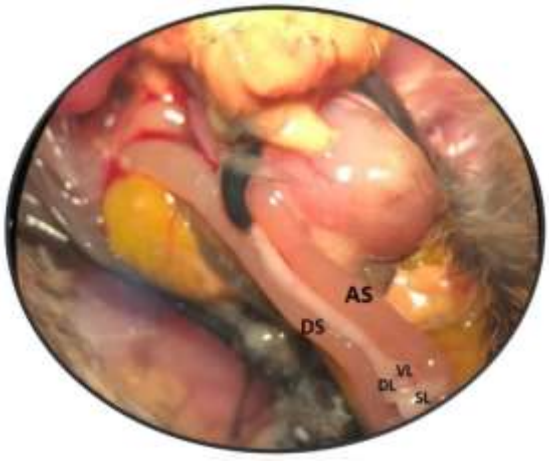

Figure 4: Pancreas in a one-day-old pheasant chick. Ascending duodenum (AS), descending duodenum (DS), dorsal lobe of the pancreas (DL), ventral lobe of the pancreas (VL), and splenic lobe of the pancreas (SL).

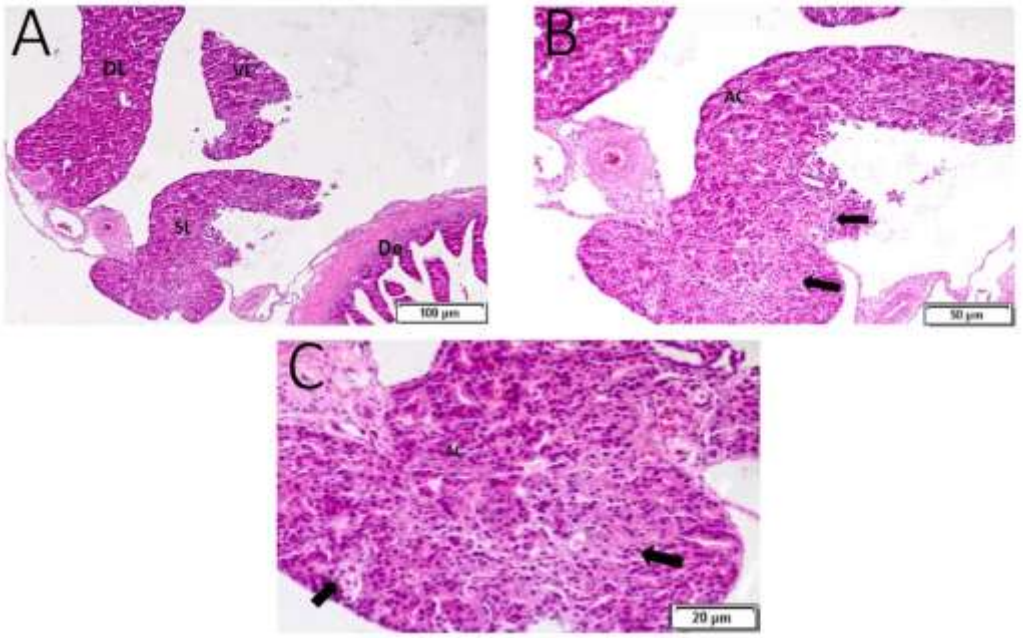

Figure 5: A) Represents the pancreas in a one-day-old pheasant chick. Dorsal lobe (DL), ventral lobe (VL), splenic lobe (SL), and duodenum (De). B and C) Histological structure of the splenic lobe in a one-day-old pheasant chick. Islets of Langerhans (arrows) and acini (AC). Hematoxylin and Eosin staining.

In Masson's Trichrome staining, the histological structure of the pancreas of the one-day-old chick was well differentiated and included dorsal, ventral, and splenic lobes. In the splenic lobe, acini, islet cells of Langerhans, and ducts were recognizable. Connective tissue and structural differentiation in the pancreas were observed. A one-day-old pheasant chick pancreas was histologically and functionally well-organized and active (Figure 6).

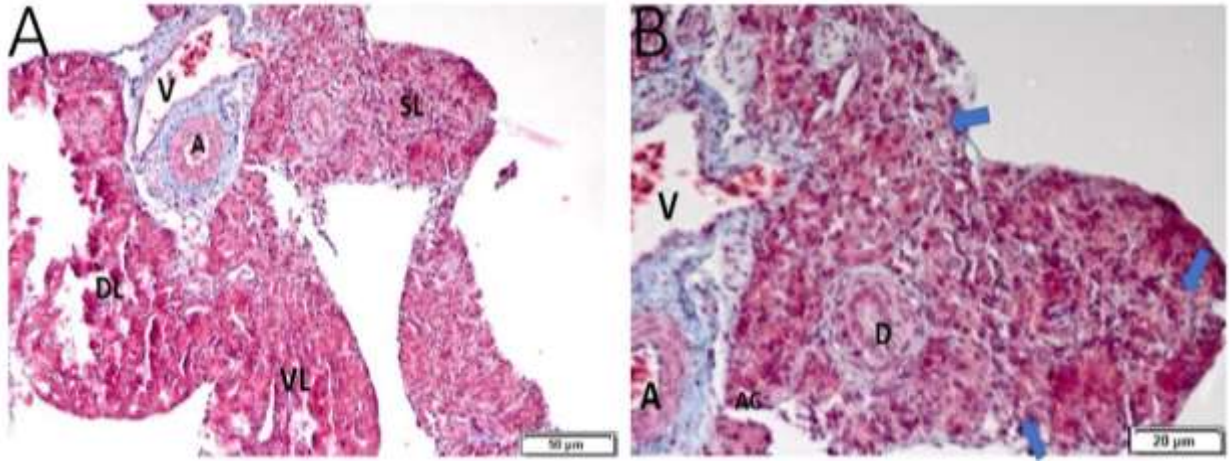

Figure 6: A and B) Shows the histological structure of the pancreas in a one-day-old pheasant chick. Dorsal lobe (DL), ventral lobe (VL), splenic lobe (SL), interlobular ducts (D), artery (A), and vein (V), Acini (AC), cells of the islets of Langerhans (arrows). A small amount of connective tissue is evident within the pancreas. Masson's Trichrome staining.

In Gomori's trichrome staining, the islets of Langerhans were observed in the pheasant embryo at 19 days. Langerhans islets were consisted of alpha and beta islets. The alpha islet cells had a slightly pale pink cytoplasm, and the beta cells were slightly basophilic or pale blue. Additionally, the alpha islets were larger than the beta ones. To ensure the correct functioning of the dye, staining was first performed on the pancreas of another species as a control sample (Figure 7).

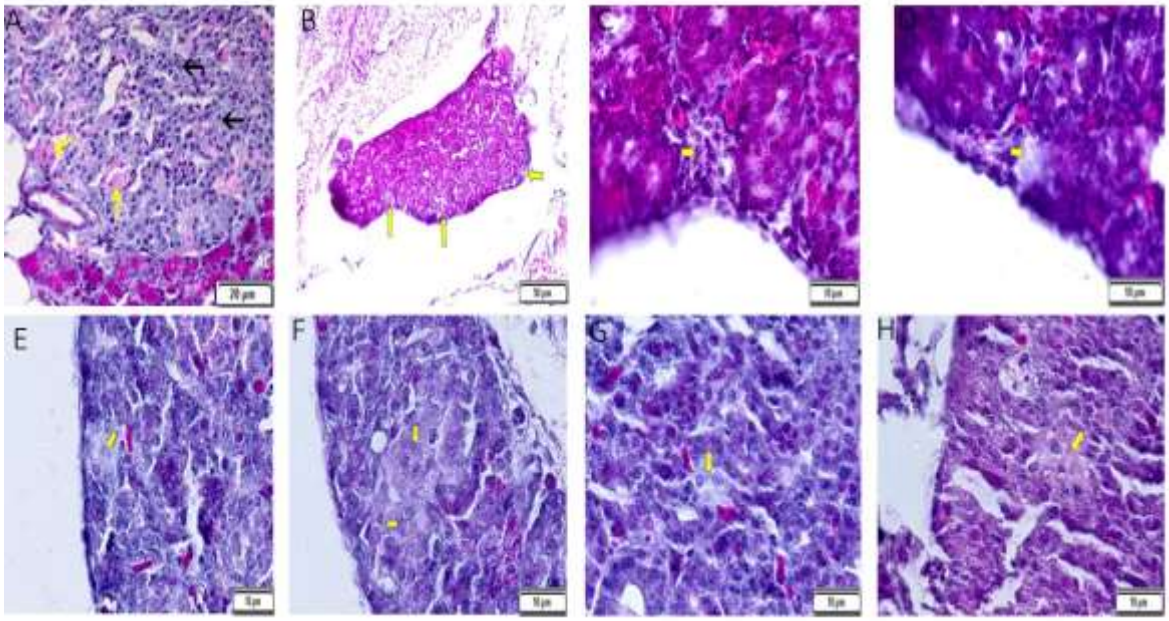

Figure 7: A) Shows the exocrine compartment and the islet of Langerhans in a guinea pig as a control. Acini (AC), alpha cells (yellow arrow), and beta cells (black arrow). B) Shows the islets of Langerhans (arrows) in the pancreas of a 19-day-old pheasant embryo. C) Shows the histological structure of the alpha islet (arrow) in the pancreas of a 19-day-old pheasant embryo. D) shows the beta islet (arrow) in the pancreas of a 19-day-old pheasant embryo. E) shows the beta islet (arrow) in the pancreas of a 21-day-old pheasant. F) Shows the alpha islet (arrow) in the pancreas of a 21-day-old pheasant embryo. G) Depicting the beta islet (arrow) in the pancreas of a one-day-old pheasant chick. H) Depicting the alpha islet (arrow) in the pancreas of a one-day-old pheasant chick. Gomori's trichrome staining.

Discussion

In the present study, the primitive pancreas was formed between days 13 and 15 of the embryonic period. The presence of blood vessels, such as arteries and veins, as well as limited connective tissue, suggested that this organ underwent early development and organization in the one-day-old chick. The pancreas of the pheasant had three lobes, including dorsal, ventral, and splenic, similar to that of the golden eagle (Al-Agele and Mohammed, 2012), guinea fowl (Pourhaji Motab et al, 2015), and ostrich (Stornelli et al, 2006). The pancreas of the goose (Beheiry et al, 2018), red jungle fowl (Kadhim et al, 2010), native chickens (Parchami and Kusha, 2015), crow (*Linnaecus corvus*), and Iraqi black partridge (*Melanoperdix niger*) have four lobes (Naser et al, 2024), including dorsal, ventral, splenic, and caudal. In the goose, the splenic lobe is connected to the ventral or dorsal lobes, or both (Beheiry et al, 2018). The splenic lobe is not connected to the other lobes in the Palam Dove (*Streptoplia selegalensis*) (Saadatfar et al, 2011). In the ostrich, the splenic lobe is not visible macroscopically but is microscopically seen in the anterior part of the dorsal lobe (Stornelli et al, 2006).

In our study, as well as in the red jungle fowl (Kadhim et al, 2010) and the ostrich (Stornelli et al, 2006), the pancreas

completely filled the space between the descending and ascending loops of the duodenum. However, the pancreas does not fill the space between the duodenal loops in the goose (Beheiry et al., 2018) and the golden eagle (Al-Agele and Mohammed, 2012). Moreover, the dorsal lobe was larger than the ventral lobe, and the splenic lobe was an extension of the dorsal lobe, located anterior to it. In the goose, the ventral lobe is larger than the dorsal lobe (Beheiry et al., 2018). In poultry, the ventral and dorsal lobes are of equal size, but in the guinea fowl, the ventral lobe is longer and narrower than the dorsal lobe (Pourhaji Motab et al., 2015). In the ostrich, the dorsal lobe is more developed and is located in the direction of the descending duodenal loop, and the ventral lobe is shorter. It is located adjacent to the ascending duodenal loop (Stornelli et al, 2006).

The exocrine part of the pheasant pancreas consisted of ducts and acini, and in our study, acini began to form on embryonic day 15, and ducts were formed on embryonic day 17. This indicates the early metabolic and secretory function at this stage of development. The acini cells were pyramidal, and the centeroacinar cell was not observed at any age. In the goose (Beheiry et al, 2018) and red fowl (Kadhim et al, 2010), the acini are pyramidal with a narrow lumen, and the acini's central cell, a slightly smaller and lighter cell than the acini, is located in the center of the acini's lumen. In the goose (Beheiry et al, 2018) and turkey (Mobini and Aghaabedi, 2009), the acini have two distinct regions, the apical part having basophilic cytoplasm and the basal part having acidophilic cytoplasm. In the turkey, the acini are pyramidal to cylindrical, with the nucleus at the base (Saadatfar and Asadian, 2009). In the Mynah (*Acridotheres tristis*), the acini are round to oval in shape with large granules, and the acini's central cell is absent (Saadatfar and Asadian, 2009).

Alpha, beta, and delta cells can be identified by immunohistochemistry in the 5-day-old chick embryo, but these cells cannot be identified by hematoxylin and eosin staining (Maňáková and Titlbach, 2007). In another study on the endocrine glands in the chick pancreas, on embryonic day 9, significant alpha and medium beta islets are identified in the splenic lobe and the third lobe (Rawdon and Larsson, 2000). Somatostatin cells are first observed at embryonic stage 26 H&H, and in older embryos, delta cells are located around the large alpha islets and are scattered in the exocrine parenchyma. In the present study, pancreatic islets were identified by hematoxylin and eosin staining and Gomori stains in the pheasant's embryonic stages, forming Langerhans' islets from embryonic day 19. Like other birds, Langerhans's islets of pheasant were composed of alpha and beta, with alpha islets appeared larger and more numerous than beta islets. The largest number of islets was concentrated in the dorsal and splenic lobes, and islands were rarely observed in the ventral lobe.

Most islets in the red jungle fowl are in the ventral lobe (Kadhim et al, 2010). In the mynah, alpha islets are more numerous than beta ones in the dorsal lobe and do not have a clear boundary with the exocrine part. Beta islets are spherical, brighter, and smaller than alpha islets, and are separated from the exocrine part by connective tissue. Beta islets are more numerous in the ventral lobe than alpha islets (Saadatfar and Asadian, 2009). The golden eagle has two types of islets. Alpha islets contain alpha, delta, and a few beta cells, and beta islets contain beta cells and a few delta cells located around the islet (Al-Agele and Mohammed, 2012). In the Palam Dove, beta islets are pale and separated from the exocrine part by a clear border. In the ventral lobe, beta islets are more numerous and smaller than alpha islets, but these islets are seen in all four lobes. Alpha islets are dark and have a more irregular shape. They occupy the periphery of the lobe, and in the third and dorsal lobes, the density of alpha islets is greater than that of beta islets.

Alpha islets are located in the splenic lobe. The islets are not distributed evenly throughout each lobe, so in the ventral lobe, they are concentrated in the central area, and in the dorsal lobe, they are concentrated around the central area (Saadatfar et al., 2011).

The islets of Langerhans in the ostrich are more concentrated in the splenic lobe and have two types of islets, alpha and beta (Stornelli et al, 2006). In ducks, the islets are not uniformly distributed throughout each lobe, with small islets around the ventral lobe and large islets in the central cylindrical region. In the splenic lobe, the islets are darker. In addition, the islets of the dorsal lobe are larger than those of the ventral lobe (McClish and Eglitis, 1969). In domestic fowl, three types of islets are found, with the endocrine part consisting mainly of beta islets, being more abundant in the splenic and third lobes. Alpha islets are absent in the ventral and dorsal lobes in both sexes (Parchami and Kusha, 2015). Mixed islets are not found in the goose (Beheiry et al, 2018), turkeys (Mobini and Aghaabedi, 2009), and golden eagles (Al-Agele and Mohammed, 2012).

Given the findings of this study on the progression of pancreatic histogenesis and histochemistry during the embryonic period of the pheasant, it is suggested that future studies should closely examine the molecular mechanisms and important cellular factors in the differentiation and organization of the endocrine and exocrine compartments of the adnexa, including the role of transcription factors-1, such as NPD. In addition, the use of advanced techniques such as electron microscopy to examine the ultrastructures of acinar cells and islets of Langerhans, as well as immunohistochemical methods to identify and quantify the types of main cells (insulin, glucagon, somatostatin) at different stages, can provide more effective insight into the functional maturation of the pancreas. In addition, comparison with other bird species, especially wild birds with different metabolic patterns, can help to understand the influence of experience and ecology on morphology and function. Investigating environmental factors, such as maternal diet and incubation conditions, on pancreatic development will also be valuable for future research. Ultimately, long-term studies are recommended to assess postnatal changes and their correlation with physiological adaptations in birds.

In conclusion, it seems that the pheasant pancreas begins to form between days 13 and 15 of embryonic development. The dorsal lobe demonstrates primacy by initiating development first. The definitive pancreatic architecture is established through the sequential differentiation of key components: the endocrine islets of Langerhans emerge on day 19, followed by the maturation of the exocrine acinar tissue and the ductal system on day 21, marking the culmination of embryonic organogenesis. It is recommended that the future studies focus on the molecular and hormonal mechanisms involved in cell differentiation and conduct comparative analyses with other bird species.

Acknowledgments

This research was supported by the Research Council of the Ferdowsi University of Mashhad (grant number 3.53795).

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

Ferdowsi University of Mashhad (Grant number: 3/53795)

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Received: 19.08.2025
Accepted: 15.10.2025

Vol. 21, No. 3, Autumn, 2025, 89-97 *Original Article*
DOI: 10.22055/ivj.2022.363499.2503
Journal Homepage: http://www.ivj.ir/

Evaluation of electrocardiogram changes in normovolemic hypotensive dogs treated with fluids alone or in combination with ephedrine or dobutamine

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Received: 25.09.2022 Accepted: 20.11.2022

Abstract

Normovolemic hypotension can occur due to vasodilation or loss of vascular tone sympathetic nervous system. The aim of the present study was to evaluate electrocardiographic changes and rhythm disorders in normotensive dogs with isoflurane-induced hypotension and treated with ephedrine, dobutamine and fluid therapy. Twenty-nine adult male and female dogs of Mixed breed, weighing 20.1 ± 4.3 kg and in the age range 1.5-2.5 years-old were selected. Anesthesia was induced and maintained with propofol and 1.5% isoflurane in 100% oxygen, respectively. Then, hypotension was induced by deep anesthesia provided with 3% isoflurane. The dogs were given one of five treatments of 1- Ringer's solution (1 ml/kg/min, n=5), 2- Ringer's solution (1 ml/kg/min) with intravenous administration of ephedrine (RE, 0.2 mg/kg, n=6, 3- Ringer's solution (1 ml/kg/min) with intravenous infusion of dobutamine (RD, 5 µg/kg/min, n=6), 4- Intravenous administration of ephedrine (E, 0.2 mg/kg, n=6) and 5- Intravenous infusion of dobutamine (D, 5 µg/kg/min, n=6). By the time the direct blood pressure reached above 60 mmHg after challenge, treatment was discountinued, and the amount of isoflurane was reduced. If there were no responses, the treatment was repeated once again. Electrocardiogram was obtained from all animals at defined time points. Heart rate after treatment was significantly higher in the ringer's with dobutamine (184.2 ± 14.75) than in ephedrine (99.6± 23.8) and dobutamine (108.8± 20/29). Heart rate in the ringers with ephedrine after treatment (110.5 ± 26.46) was significantly higher than the baseline. The changes in P wave, QRS, PR interval, QT, heart electrical axis, and ST segment shape were insignificant. It was concluded that the addition of ephedrine or dobutamine to conventional fluid therapy in normovolemic hypotensive dogs can be associated with higher heart rate values. According to the obtained results, dobutamine with ringer's solution increased the heart rate more than the other groups.

Key words: Hypotension, Dobutamine, Ephedrine, Electrocardiogram, Dog

Introduction

Hypotension occurs when the systolic and the mean arterial blood pressure become less than 80 and 60 mmHg, respectively. Early diagnosis of hypotension prevents the negative consequences, including insufficient tissue

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perfusion, renal, cerebral, and myocardial ischemia (Mazzaferro & Wagner, 2001). The most common cause of hypotension in animals is the loss of body fluids and a decrease in circulating blood volume (hypovolemia) (Haskins, 2012); however, other causes can lead to lower blood pressure as well (Klabunde, 2011).

Reduction of blood pressure without hypovolemia or normovolemic hypotension occurs in cases such as distributed shock (neurogenic, septic, anaphylactic), cardiogenic shock, spinal injuries, drug poisoning, electrolyte and acid-base disorders, and deep anesthesia (Secher and Van Lieshout, 2005; Guly et al, 2008; Summers et al, 2013; Silverstein and Hopper, 2014). The normovolemic hypotension can finally cause a substantial disturbance in oxygen supply to the tissues and even shock. If the animal does not receive emergency and effectual care, severe cellular hypoxia and organ damage will occur, and eventually, the animal may die (Laforcade and Silverstein, 2015).

Fluid therapy has considered the most effective way to improve the oxygen supply, maintain and restore blood pressure in hypotensive patients, and increase of cardiac output by improving preload (Davis, 2016). Various studies have shown that although fluid therapy is very effective in hypotensive patients, it is less effective or even ineffective in cases of normovolemic hypotension. In such cases, it is usually necessary to use certain drugs to manage blood pressure (Kudnig and Mama, 2002; Harold et al, 2013). Today, the intraosseous method is widely used in dogs for administration of drugs (Chitsaz et al, 2023; Gholami et al, 2025).

Ephedrine is a non-catecholamine sympathomimetic drug that directly or indirectly stimulates alpha and beta-adrenergic receptors (Hoffman, 2001; Plumb, 2018). It has positive inotrope effects, causes vasoconstriction, and increases the heart rate in bradycardia cases. It has also been used to treat anesthesia-induced hypotension (Ramsey, 2017).

Dobutamine is a potent alpha-1 adrenergic agonist with weak beta-1 and beta-2 adrenergic activities. Since most of the inotropic effects are related to increase cardiac alpha-1 activity, dobutamine, less than other adrenergic drugs, leads to tachycardia. Furthermore, it has no direct effect on vascular tone and resistance. Due to its inotropic effects, dobutamine primarily increases stroke volume and cardiac output in healthy animals. There is also a slight increase in heart rate, which has little contribution to the rise in cardiac output. An increase in cardiac output causes high blood pressure (Dubin et al, 2017).

According to the authors' knowledge, no study has investigated the simultaneous effects of fluid therapy and blood pressure-increasing drugs in hypotensive normovolemic dogs; therefore, this survey has aimed to compare ECG changes following either fluid therapy alone or combined with dobutamine and ephedrine in hypotensive normovolemic dogs.

Materials and methods

Before the study's begining, an ethical code (EE/1401.2.24.99424/scu.ac.ir) was obtained from the Research Ethical Committee of Shahid Chamran University of Ahvaz. Twenty-nine 1.5- to 2.5-year-old male and female dogs of Mixed breeds, weighing 20.1 ± 4.3 kg, were transferred to Veterinary Hospital and kept in separate cages for 2 weeks. Age detection was performed based on the dental formula and the amount of wear of the teeth. In the following, vaccines (hipradog + rabies) and anti-parasitic drugs (caniverm: 1 tablet per 10 kg) were given to them to eat. The studied dogs were collected from different parts of Ahvaz district through capture alive. Further, blood tests (CBC) and immunochromatography test were performed for *dirofilaria immitis*, and after ensuring the health of dogs, research was conducted on them. The dogs were

clinically healthy and in daily observations, none of them had any signs of liver, kidney, endocrine diseases. They were fed twice daily with free access to water. The animal health status was evaluated by performing a complete clinical examination and a CBC test. Their heart sounds, and blood pressure were normal before the experiment.

On the day of examination, the animals were transferred to the study place and kept for 30 minutes to adapt to the environment. Then the cephalic vein was catheterized with a suitable intravenous (IV) catheter. The dogs received 100% oxygen via a face mask for 5 minutes. Then, anesthesia was induced by administration of propofol (1%, Braun, Melsungen, Germany) titration. After intubation with an appropriate cuffed endotracheal tube, the dogs were placed in the right lateral recumbency and connected to a rebreathing anesthesia machine. Anesthesia was maintained by isoflurane (Forane, Abbott, Uk) in 100% oxygen. Mechanical ventilation was performed with a breathing rate of 8-10 times per minute and a vital volume of 10-15 ml/kg to maintain PaCO2 in the 35-45 mmHg range. Ringer's solution (Iranian Parenteral and Pharmaceutical Co., Tehran, Iran) was administered with a 3 ml/kg/h dose. Body temperature was attempted to maintain 37-38˚C using a blanket.

Under general anesthesia, the pedal artery was catheterized and connected to a direct blood pressure measuring device for continuous arterial blood pressure monitoring. The jugular vein was also catheterized with a central venous pressure measurement apparatus (Arrow International, PA, USA). An electrocardiogram (Digital ECG, Guangdong Biolight Meditech Co, BLT-1203 B, China) was taken continuously at a speed of 50 mm/second and a voltage of 10 mv.

The dogs were also connected to a multiparameter monitoring system (Burtons, PM-9000Vet, UK) to measure oxygen saturation of hemoglobin (SPO2), heart rate, non-invasive blood pressure (using a cuff at the metatarsal area), respiratory rate, rectal temperature, and end-tidal carbon dioxide (ETCO2).

After preparation, the dogs were maintained under general anesthesia with isoflurane 1.3% for 15 minutes. The data were recorded as time zero (Time 1). Then, the depth of anesthesia was increased using 3% isoflurane until the invasive arterial blood pressure reached below 60 mmHg and remained in this situation for 10 minutes (Chen et al, 2007). At this time, the data were measured and recorded (Time 2). The dogs were randomly divided into five groups and received one of five treatments: 1- Ringer's solution (1 ml/kg/min, R, n=5), 2- Ringer's solution (1 ml/kg/min) with intravenous administration of ephedrine (0.2 mg/kg; HBM Pharma s.r.o., Sklabinska martin, Slovak Republic) (RE, n=6), 3- Ringer's solution (1 ml/kg/min) with intravenous infusion of dobutamine (5 µg/kg/min; Hameln pharma gmbh inselstrabe, Hameln, Germany) (RD, n=6), 4- Intravenous administration of ephedrine (0.2 mg/kg, E, n=6) and 5- Intravenous infusion of dobutamine (5 µg/kg/min, D, n=6).

If, after treatment, the invasive blood pressure was reached over 60 mmHg and maintained for 15 minutes, the administration of drugs would discontinue, and the data would be recorded. Otherwise, the treatment would be repeated, and the data would be recorded 15 minutes later (Time 3). Then, within 15 minutes, the anesthesia was returned to isoflurane 1.3%, and the data would be recorded (Time 4). Afterward, the concentration of isoflurane was reduced to zero within 15 minutes, and the dogs recovered. In this investigation, ECG parameters were assessed including HR, heart rhythm, determination of the average of heart electrical axis, duration and amplitude of P and QRS waves, PR and QT intervals, and ST segment morphology.

Data were analyzed using GraphPad Prism 9 and Excel 2016 software. The

normal distribution of the data was checked and confirmed using the Fishers exact test. To compare the data of electrocardiogram changes, a Mix model (Repeated measure for ANOVA) and Bonferroni post hoc test were used to compare the data of electrocardiogram changes. Data are shown as mean ± standard deviation (SD). A level of $P< 0.05$ was considered significance.

Results

All dogs tolerated the anesthesia and hypotension processes well and were successfully recovered. No death or complications were seen related to the study's procedures until a follow-up of two weeks. Three dogs out of five in Ringer´s treatment, two dogs out of five in the dobutamine treatment, and one dog out of five in Ringer´s with Ephedrine, Ringer´s with dobutamine, and Ephedrine groups needed re-administration of therapy. One dog in Ringer´s, dobutamine, and dobutamine treatments did not show direct blood pressure above 60 mmHg after re-treatment. However, the blood pressure increased to 60 mmHg or more by reducing isoflurane concentration. In all dogs, after increasing the direct pressure to more than 60 mmHg, its decrease was not observed below 60 mmHg.

A comparison between treatments on heart rate showed that it was significantly higher in Ringer treatments with dobutamine and dobutamine alone ($p=0.0067$) than ephedrine alone ($p=0.0161$) at time 2. The within-group comparison showed that the heart rate in the Ringer´s with Ephedrine was significantly higher than the baseline at time 2 ($p=0.028$) (Table 1).

In most cases of electrocardiogram recording, the heart rhythm was sinus rhythm or sinus arrhythmia. The effects of time and group on the amplitude and duration of the P wave, QRS wave duration and ampitude, PR interval, QT interval, the heart electrical axis, and the shape of the ST segment were not significant ($P>0.05$). ST segment depression was not observed in any of the animals. ST elevation was seen in three dogs in Ringer´s with Ephedrine treatment at baseline, at times 1 and 2, and in four dogs at times 4 (Table 1). Wandering pacemaker and Sinus arrest were observed in the ringer´s and ephedrine treatments at time 4 in a dog (Figures 1 and 2).

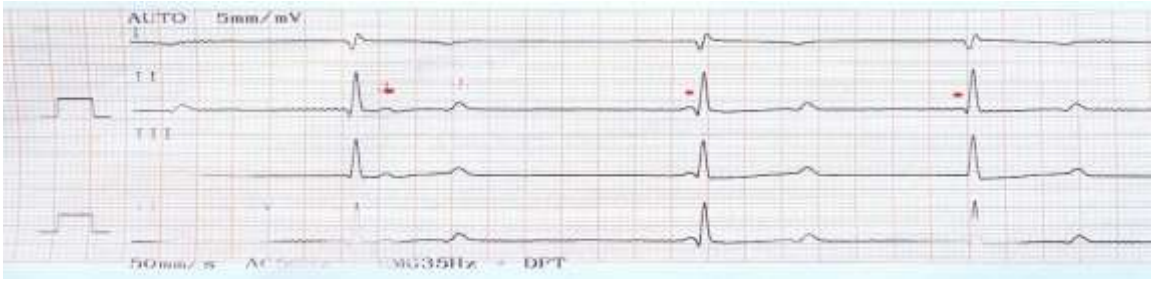

Figure 1: Wandering pacemaker in one of the dogs in group four that was received ephedrine alone

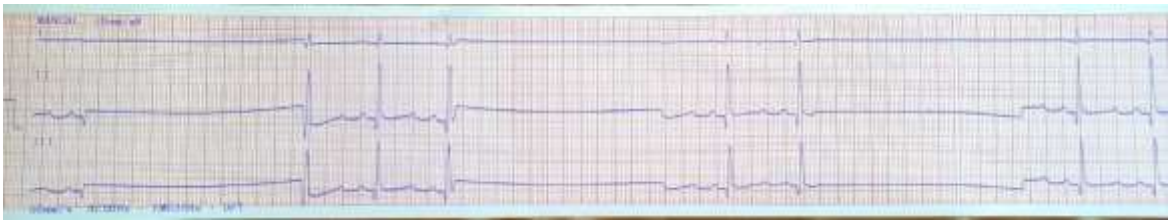

Figure 2: Sinus arrest in one of the dogs in group one that was received ringer solution alone

Table 1: Changes in electrocardiographic parameters in isoflurane-induced hypotensive dogs treated with 1- Ringer's solution (R), 2- Ringer's solution with intravenous administration (IV) of ephedrine (RE), 3- Ringer's solution with intravenous infusion of dobutamine (RD), 4- Intravenous administration of ephedrine (E) and 5- Intravenous infusion of dobutamine (D)

Parameters	Group	Baseline	Time 1 (hypotension induction)	Time 2 (treatment)	Time 4 (recovery)
Heart rate (beat/min)	R	23 ± 117	21 ± 101	20 [a] ±101	17 ± 105
	RE	13 ± 110	14 ± 88	26 [a] ±110	44 ± 126
	RD	28 ± 108	12 ± 92	15 [abA] ± 184	10 ± 119
	E	13 ± 113	18 ± 95	24 [ac] ± 100	38 ± 110
	D	32 ± 112	12 ± 107	20 [ac] ± 109	18 ± 114
P wave duration	R	0.03 ±0.01	0.04 ±0.01	0.04 ±0.00	0.04 ±0.01
	RE	0.04 ±0.00	0.04 ±0.00	0.04 ±0.00	0.04 ±0.00
	RD	0.04 ±0.00	0.04 ±0.00	0.04 ±0.00	0.04 ±0.00
	E	0.04 ±0.00	0.04 ±0.01	0.04 ±0.00	0.04 ±0.00
	D	0.03 ±0.01	0.04 ±0.01	0.04 ±0.00	0.04 ±0.01
P wave amplitude (mv)	R	0.18 ±0.06	0.23 ±0.07	0.21 ±0.06	0.23 ±0.04
	RE	0.20 ±0.09	0.16 ±0.08	0.20 ±0.07	0.25 ±0.07
	RD	0.21 ±0.05	0.19 ±0.07	0.21 ±0.07	0.21 ±0.02
	E	0.17 ±0.06	0.21 ±0.06	0.20 ±0.03	0.21 ±0.07
	D	0.16 ±0.05	0.16 ±0.06	0.20 ±0.02	0.18 ±0.04
QRS wave duration	R	0.04 ±0.00	0.04 ±0.01	0.05 ±0.00	0.04 ±0.01
	RE	0.04 ±0.00	0.04 ±0.00	0.05 ±0.00	0.05 ±0.00
	RD	0.05 ±0.00	0.05 ±0.00	0.05 ±0.00	0.05 ±0.00
	E	0.05 ±0.00	0.05 ±0.00	0.05 ±0.00	0.06 ±0.00
	D	0.04 ±0.01	0.05 ±0.00	0.05 ±0.00	0.05 ±0.01
QRS wave amplitude (mv)	R	1.45 ±0.75	1.70 ±0.53	1.42 ±0.99	1.47 ±0.43
	RE	1.14 ±0.26	0.78 ±0.39	1.06 ±0.16	0.90 ±0.48
	RD	1.16 ±0.65	1.20 ±0.48	1.52 ±0.42	1.24 ±0.47
	E	1.06 ±0.33	1.04 ±0.52	1.18 ±0.48	0.92 ±0.28
	D	0.97 ±0.50	0.78 ±0.58	0.85 ±0.65	0.88 ±0.70
PR duration	R	0.11 ±0.02	0.12 ±0.02	0.12 ±0.03	0.11 ±0.02
	RE	0.11 ±0.01	0.10 ±0.02	0.10 ±0.03	0.08 ±0.01
	RD	0.10 ±0.02	0.10 ±0.01	0.10 ±0.03	0.10 ±0.02
	E	0.09 ±0.02	0.10 ±0.00	0.10 ±0.01	0.06 ±0.03
	D	0.11 ±0.02	0.10 ±0.01	0.09 ±0.01	0.09 ±0.01
QT duration	R	0.24 ±0.04	0.24 ±0.02	0.25 ±0.03	0.24 ±0.05
	RE	0.27 ±0.03	0.26 ±0.03	0.26 ±0.04	0.23 ±0.05
	RD	0.25 ±0.04	0.25 ±0.01	0.25 ±0.04	0.24 ±0.01
	E	0.26 ±0.05	0.25 ±0.04	0.27 ±0.04	0.27 ±0.04
	D	0.23 ±0.03	0.23 ±0.02	0.24 ±0.04	0.24 ±0.02
Heart electrical axis (degree)	R	80.75 ±8.30	83.75 ±4.78	84.50 ±4.20	82.50 ±6.45
	RE	87.00 ±4.47	84.40 ±8.17	82.60 ± 7.50	79.40 ±10.81
	RD	79.00 ±12.45	76.40 ±18.01	81.40 ±10.24	76.80 ±17.80
	E	86.67 ±5.77	83.33 ±11.55	80.00 ±17.32	70.50 ±23.69
	D	68.83 ± 4.21	71.50 ±6.68	70.00 ±8.83	75.00 ±9.25

The different letters in each column indicate a significant difference ($P<0.05$).

Discussion

Various studies have shown that fluid therapy cannot alone restore normal pressure in cases of normovolemic hypotension in dogs. As a result, inotropes or vasoconstrictive have been tried to manage blood pressure (Kudnig and Mama, 2002; Harold et al, 2013). In cases of isoflurane-induced hypotension, as it decreases heart rate at high doses, myocardial contractile strength, peripheral vascular resistance, stroke volume, and cardiac output, so the reduction in isoflurane-induced compensatory responses has been proposed as the maintenance factor for the lack of response to the fluid infusion (Valverde et al, 2012; Yang et al, 2014). The current study evaluated the effect of ephedrine or dobutamine with fluid therapy on ECG parameters in managing the normovolemic hypotensive dogs induced by high doses of isoflurane.

In the present study, heart rate decreased slightly from the baseline compared with the time of hypotension induction in all groups, which was expected and directly related to the consequences of severe hypotension. After treatments, heart rate increased in ringer´s with dobutamine and ringer´s with ephedrine treatments; however, just heart rate in ringer´s with dobutamine was significantly higher than dobutamine and ephedrine alone treated dogs. Heart rate can be affected by the function of baroreceptors and respiratory cycle, as well as sympathetic and parasympathetic balance. Wagner et al, (1993) stated that ephedrine in both low and high doses increased blood pressure and decreased heart rate. Dubin et al, (2017) also reported that due to its inotropic effects, dobutamine primarily increases stroke volume and cardiac output and causes a slight heart rate rise. Goya et al, (2018), in a study on dogs, stated that the heart rate during anesthesia decreased with isoflurane and a low dose of dobutamine and increased with a high dose of dobutamine. Sousa et al, (2005) reported that heart rate increased in a dose-dependent manner with the increase in the dose of dobutamine, and at a dose of 10 μg/kg/min, it was not significantly different from the baseline. In the this study, it seems that the administration of dobutamine and ephedrine alone did not considerably change heart rate, which may be attributed to the ineffectiveness of used drugs to induce alterations in normovolemic hypotensive dogs. Interestingly, adding fluid therapy to ephedrine and dobutamine increased heart rate, highlighting the effect of increased preload in heart rate alterations; however, higher preload without supplemented drugs was ineffective in inducing heart rate changes, as we observed with ringer´s solution alone treatment.

Electrocardiogram measurements did not show significant differences among and within treatment's groups. The elevation of the ST segment observed in the ephedrine group can be caused by the increased catecholamines in the blood circulation and sympathetic stimulation by ephedrine (Adamson et al, 2004). The cause of deviations in the ST segment is caused by changes in the activity of the autonomic nerves of the coronary arteries or myocardium (Tilley and Smith, 2016). The most crucial reason for lowering this segment is myocardial ischemia and the rise of this segment in cases of myocardial hypoxia, in other words, lack of oxygen. This deviation was probably secondary to hypotension and did not indicate primary myocardial diseases.

In the current study, the changes in the P wave were insignificant between different times and treatments. As the amplitude and duration of P waves are considered normal up to 0.4 mv and 0.04 seconds in dogs, respectively (Nelson and Couto, 2019). The obtained results showed that the amplitude and duration of P waves were in this normal range. P wave indicates the depolarization of the right and left atria; therefore, it is an

indicator of the speed of transmission of electrical signals in the atria (Martin, 2015). Our results show that signal transmission speed does not seem to change significantly during hypotension in normovolemic dogs. Considering that P wave changes can occur in a rapid increase in heart rate, this finding can also indicate that the heart rate did not suddenly increase in this study.

The maximum duration of the QRS wave is 0.06 seconds, and the maximum amplitude of the QRS wave is three millivolts in large breed dogs (Nelson and Couto, 2019). Sousa et al, (2005) reported that P and QRS waves durations and amplitude did not change with increasing dosage f dobutamine. In all cases of electrocardiogram recording in this study, the duration and amplitude of the QRS waves were within their normal limits, so these changes were not significant at different times and among different treatment groups. The amplitude of the QRS fluctuated over time in all groups, but this difference was not statistically significant. Accordingly, hypotension and given treatments do not seem to cause a fundamental change in the speed of electrical signal transmission in the ventricles.

According to Tilley and Goodwin (2001), the shortening of the PR interval is caused by the conduction of the impulse through a secondary path to the AV node, and the lengthening of the PR interval is also a sign of first-degree AV block. In this study, in all cases of electrocardiogram recording, the PR interval was within the normal range of 0.06-0.13 seconds (Nelson and Couto, 2019). Tilley and Goodwin (2001) stated that variation in the size of the PR interval might occur with a change in the tonicity of the vagus nerve; the higher the HR, the shorter the PR interval and vice versa. The results of the present study are in line with the mentioned study, which can lead to the conclusion that electrical signal transmission in isoflurane-induced hypotension did not change significantly in the treated dogs.

Nelson and Couto (2019) reported that the normal QT interval was in the range of 0.15-0.25 and up to 0.27 seconds in dogs. The QT interval indicates the duration of depolarization and subsequent repolarization in the ventricles, and its changes are inversely related to heart rate. Autonomic nerve tone, drugs, and electrolyte disorders can cause changes in this interval. In all cases of electrocardiogram recording in this study, the QT intervals were within the normal range.

The mean of heart electrical axis indicates the general direction of the depolarizing electrical waves of the heart myocardium. In normal conditions and right lateral recumbency, where the left ventricle has an enormous volume of the myocardium, its expected value is between +40 and +100 degrees. Changes in the axis can be caused by how the animal is positioned, the difference in the chest structure, the presence of intraventricular conduction blocks, and the presence of structural heart diseases (Santilli et al, 2019). In the present study, the mean of heart electrical axis was within the normal range in all recorded cases. In conclusion, simultaneous use of fluid therapy and ephedrine or dobutamine can improve dogs' cardiovascular depressant effects of isoflurane-induced hypotension on heart rate. Dobutamine with ringer's solution increased the heart rate compared with ephedrine and other groups, due to positive inotropic effects.

Acknowledgments

The authors wish to express their gratitude to the Research Council of Shahid Chamran University of Ahvaz for financial support.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by a grant from the Shahid Chamran University of Ahvaz, Iran.

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Received: 2022.09.25
Accepted: 2022.11.20

Vol. 21, No. 3, Autumn, 2025, 98-102 *Case Report*
DOI: 10.22055/ivj.2025.490279.2751
Journal Homepage: http://www.ivj.ir/

Congenital vascular hamartoma of the gingiva in a calf

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Received: 24.11.2024 Accepted: 24.06.2025

Abstract

The aim of this study was to report the clinical and histopathological outcomes of a vascular hamartoma tumor of the gingiva in a calf and to evaluate the treatment results. A 3-month-old female Simmental calf was presented to the large animal surgery department of the Faculty of Veterinary Medicine, Shahid Chamran University, with a complaint of a mass in the region of the first lower incisor. The owner reported that the lesion had been present since birth and caused bleeding during feeding. Additionally, a small piece of the lesion had been torn off by the owner, resulting in severe bleeding. On clinical examination, an oval mass the size of a plum (30×20×5 mm) was observed on the surface of the lower incisors. The mass was attached to the gingiva and had pushed one of the incisors forward, displacing it out of alignment. After local anesthesia, the mass was completely removed using surgical excision. During the surgery, significant bleeding was observed, and it was found that there was a root of a deciduous tooth in the mass, which was extracted. The surgical area was closed with nylon sutures. Postoperatively, no feeding problems were observed in the calf. Based on histopathological examinations, the mass was identified as a vascular hamartoma. Gingival vascular hamartoma tumors are rare in cattle.

Key words: Congenital vascular hamartoma, Calf, Simmental breed, Pathology

Introduction

Vascular tumors in the oral and pharyngeal regions are rare, most of which originate from blood vessels (Head et al., 2002). Hemangiomas are uncommon in horses, pigs, and cattle and extremely rare in sheep and goats (Scott, 1988). The term "hamartoma" is derived from the Greek word "hamartion," meaning bodily defect (Sharma, 1998). Most vascular hamartomas are present at birth, shortly after birth, or during the neonatal period, considered as developmental anomalies (Tyler et al, 1995; Amniattalab et al, 2012; Gülbahar et al, 1999).

Bovine vascular tumors are generally benign angiomatous lesions that occur in various tissues and have been described as juvenile angiomas, hemangiomas, or hamartomas (Mohammadi et al, 2007). Hemangiomas are reported to be the most common tumors of infancy (Robbins and Cotran, 1984), but they are rare in animal

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species. Therefore, some researchers consider them as hamartomas or nevi (Mohammadi et al, 2007). The term hamartoma refers to a localized and excessive overgrowth of normal cells and tissues within an organ (Robbins and Cotran, 1984) which is a suitable term for a group of lesions that resemble tumors but are not true neoplastic tissues (Amniattalab et al, 2012; Robbins and Cotran, 1984). Hamartomas result from defective growth in an organ and consist of an abnormal mixture of similar cellular elements (Misdorp, 2002; Robbins and Cotran, 1984).

It has been reported that vascular tumors of the gingiva in the oral cavity are typically observed at birth, mostly originated from blood vessels (Nourani et al, 2007). The aim of the following case study is to describe the clinical and histopathological features of a vascular hamartoma of the gingiva in a calf and to evaluate the treatment outcomes.

Case History

The case was related to a 3-month-old female calf presented to the Large Animal Surgery Department at Shahid Chamran University of Ahvaz with a complaint of a mass on the surface of the lower incisor teeth. The owner reported that the lesion had been present since birth accompanied with bleeding during feeding. Additionally, a small portion of the lesion had been removed by the owner, resulting in severe bleeding. Clinical examination revealed an oval-shaped mass, approximately the size of a plum, located in the row of incisor teeth (Figure 1).

Figure 1: Observation of a mass, 30×20×5 mm in diameters, on the incisor region of the lower jaw in a Simmental calf

After proper immobilization, local anesthesia was administered by injecting 10 mL of 1% Lidocaine HCl around the base of the mass. The mass was excised using an elliptical incision at its base, ensuring that at least a 1 cm margin of healthy tissue was removed. Bleeding was controlled with topical epinephrine injection and electrocautery. The root of a deciduous tooth was extracted, and the gingival mucosa was closed using a simple continuous suture pattern with 1-0 nylon suture material (Figure 1). Additional bleeding was controlled by applying ice to the surgical site. The excised mass was sent to the pathology department for histopathological examination. It was noted that the mass was attached to the gingiva and had displaced one of the incisor teeth forward, causing it to misalign with the row.

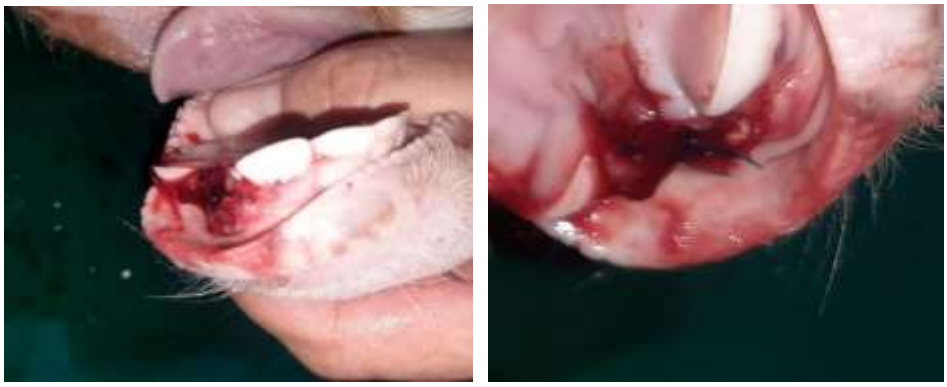

Figure 2: Postoperative view after excision of the mass and extraction of one deciduous tooth root

For postoperative care, the calf was treated with penicillin-streptomycin at a dose of 20,000 IU for five days, along with daily monitoring. It was observed that the calf experienced no complications during the postoperative period, and no recurrence of the lesion was noted after the surgery (Figure 2).

Pathology

Histopathological study revealed that the mass was composed of many small capillaries with different sizes. They were covered by endothelial cells which were bulged and irregular in size. Smooth muscles were seen in the wall of larger vessels. The surface of the mass was composed of stratified squamous epithelium. Although in some area necrosis and hemorrhage and inflammatory cells infiltration were observed. no mitotic figures were seen (Figure 3).

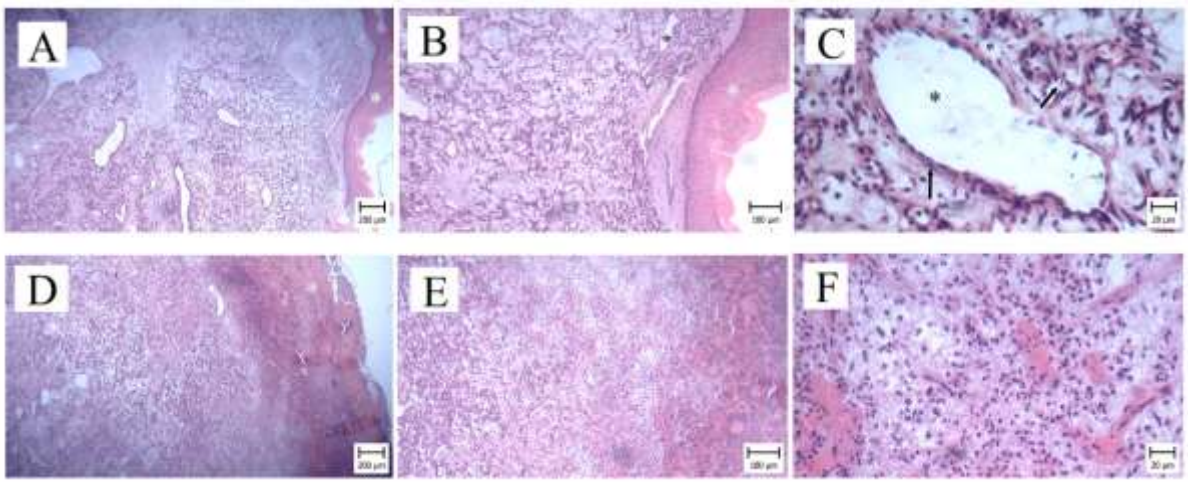

Figure 3: Microscopic figures of Hamartoma, Calf. A &D: The surface of mass is covered by ulcerated epithelium (white asterisks). B & E: Note to many small vascular structures (black asterisks) with different size. C & F: the vascular structures were covered by irregular endothelial cells (arrows). Also, inflammatory cells were infiltrated (Hematoxylin and Eosin staining. A & D: 200 µm; B & E: 100 µm; C & F: 20 µm).

Discussion

Contrary to the findings of Stanton et al, (1984), tooth extraction was not necessary, and neither electrocautery nor cryotherapy was employed in this case. The calf recovered without complications and gained weight normally in the subsequent months. No recurrence of the lesion was observed during the 12-month follow-up period (Yeruham et al, 2004).

The lesions were diagnosed as vascular hamartomas, characterized by the proliferation of numerous irregular capillary cells (not limited to endothelium) within a loose collagenous stroma. Blood vessels in hamartomas are structurally normal and retain all supportive elements, indicating that the proliferation is exclusively vascular tissue (Stanton et al, 1984; Wilson, 1990). Vascular hamartomas are developmental anomalies and are commonly described as congenital, non-neoplastic tumor-like lesions in various tissues (Gülbahar et al, 1999; Nourani et al, 2007; Benoit et al, 2005).

In this case, the anomaly was identified between the lower incisor teeth, presenting with surface ulcerations, three months after birth. As seen in this 90-day-old calf, vascular hamartomas are typically reported in the oral cavity of neonatal calves, appearing in varying shapes and sizes (Gülbahar et al, 1999; Robbins and Cotran, 1984). Similarly, it has been reported that half of all vascular hamartomas identified in young calves originate from gingival mucosa (Benoit et al, 2005).

Furthermore, such anomalies have been observed in various animal species of different ages and identified in other organs and tissues (Benoit et al, 2005; Bildfell et al, 2002; Nourani et al, 2007; Wilson, 1990). Vascular hamartomas are described as localized and excessive overgrowths of mature endothelial cells (Amniattalab et al, 2012).

In this case, the mass exhibited numerous thin-walled capillaries lined by endothelial cells. Additionally, it showed many thrombosed veins of varying diameters throughout the tissue, accompanied by hemorrhage and inflammatory cell infiltration dominated by neutrophils on the surface of the mass, likely resulting from secondary bacterial factors.

In line with our findings, many authors (Amniattalab et al, 2012; Gülbahar et al, 1999; Nourani et al, 2007) have reported that microscopically, vascular hamartomas are characterized by the proliferation of numerous blood vessels of varying sizes within loose connective tissue stroma, with necrosis and hemorrhage observed in the squamous epithelium and the superficial lamina propria. Moreover, vascular hamartomas are reported to show no mitotic activity, consistent with the findings in our case (Amniattalab et al, 2012).

It is emphasized that differentiating vascular hamartomas from hemangiomas is highly challenging. The only way to distinguish between the two lesions is through the classification of histopathological features (Amniattalab et al, 2012; Bildfell et al, 2002). While endothelial cells in vascular hamartomas are intact, well-differentiated, and irregular in size and proliferation, those in hemangiomas exhibit destructive growth, and smooth muscle cells are absent from the connective tissue of blood vessels. Therefore, distinguishing between the two lesions requires immunohistochemical confirmation (Amniattalab et al, 2012; Bildfell et al, 2002).

Additionally, this lesion must be distinguished from ameloblastoma, a benign tumor originating from odontogenic epithelium and pulpal mesenchyme. The definitive diagnosis of ameloblastoma is confirmed through histopathology (Imani et al, 2014).

Based on histopathological findings, this oral mass was identified as a congenital hamartoma. Surgical excision of the mass was performed, and the treatment was completed without recurrence.

Acknowledgments

The authors extend their gratitude to the Surgery and Pathobiology Departments of the Faculty of Veterinary Medicine.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This research was financially supported by the Vice Chancellor of Research of Shahid Chamran University of Ahvaz.

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Received: 24.11.2024
Accepted: 24.06.2025

DOI: 10.22055/ivj.2025.513762.2786

بررسی روند تداوم پادتن مادری ضد توکسین اپسیلون *کلستریدیوم پرفرنجنس* تیپ D در بزغاله‌ها

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تاریخ دریافت: ۱۴۰۴/۱/۴ تاریخ پذیرش: ۱۴۰۴/۳/۱۲

چکیده

انتروتوکسمی به صورت یک بیماری رایج و با مرگ و میر بالا در بزها رخ می‌دهد. این مطالعه با هدف بررسی پادتن‌های مادری ضد توکسین اپسیلون *کلستریدیوم پرفرنجنس* تیپ D انجام گرفت تا بتواند برنامه واکسیناسیون مقابله با انتروتوکسمی در بزها در شرایط ایران را بهبود بخشد. برای انجام این مطالعه نمونه خون از بزغاله‌های متولد شده از ۱۲ رأس بز که در ۶ و ۴ هفته قبل زایش با واکسن آنتروتوکسمی واکسینه شده بودند و همچنین نمونه خون از بزغاله‌های متولد شده از ۷ رأس بز غیرواکسینه، در زمان‌های ۰، ۳، ۱۴، ۲۸، ۴۲، ۵۶، ۷۰ و ۸۶ روز پس از تولد، گرفته شد. برای ارزیابی سرولوژیکی از الایزای مهاری استفاده شد. نتایج مطالعه نشان داد که درصد مهار در مادران واکسینه نسبت به مادران غیرواکسینه به صورت قابل توجهی بالاتر است. در بزغاله‌ها، درصد مهار در هر دو گروه تا سن ۴۲ روزگی تفاوت آماری نداشت. پس از این سن، درصد مهار در گروه کنترل به صورت قابل توجهی کاهش یافت و در سن ۵۶ و ۷۰ روزگی به صورت قابل توجهی نسبت به گروه تیمار پایین‌تر بود. در ادامه، از نظر آماری تفاوتی در درصد مهار در سن ۸۶ روزگی بین دو گروه مشاهده نشد. در گروه غیرواکسینه، درصد مهار در سن ۵۶ روزگی نسبت به درصد مهار در سن ۴۲ روزگی به صورت قابل توجهی پایین‌تر بود. در گروه واکسینه، درصد مهار در سن ۷۰ روزگی نسبت به درصد مهار در سن ۵۶ روزگی به صورت قابل توجهی پایین‌تر بود. در نتیجه، واکسیناسیون بزها در ماه آخر بارداری در افزایش تیتر پادتن‌های مادری ضد توکسین اپسیلون *کلستریدیوم پرفرنجنس* تیپ D در بزغاله‌ها مؤثر است. همچنین، سن ۵۶ تا ۷۰ روزگی در بزغاله‌های متولد شده از مادران واکسینه و سن ۴۲ تا ۵۶ روزگی در بزغاله‌های متولد شده از مادران غیرواکسینه به نظر می‌رسد زمان‌های مناسبی برای تجویز اولین دوز واکسن انتروتوکسمی باشد.

کلمات کلیدی: *کلستریدیوم پرفرنجنس*، انتروتوکسمی، توکسین اپسیلون، بزغاله، واکسن

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DOI: 10.22055/ivj.2025.512793.2783

حضور برخی ژن‌های بتالاکتاماز وسیع‌الطیف (ESBL) در سویه‌های مدفوعی *اشریشیا کلی* جدا شده از سگ‌ها و بررسی حساسیت آنتی‌بیوتیکی این جدایه‌ها

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تاریخ دریافت: ۱۴۰۳/۱۲/۲۶ تاریخ پذیرش: ۱۴۰۴/۴/۳

چکیده

اشرشیا کلی، یکی از اعضای خانواده *انتروباکتریاسه* و بخشی از فلور طبیعی روده در انسان و حیوانات خون‌گرم است. این باکتری می‌تواند طیف وسیعی از عفونت‌های گوارشی و خارج گوارشی را در انسان و حیوانات، از جمله سگ‌ها و گربه‌ها، ایجاد کند. سگ‌ها و گربه‌ها اغلب به عنوان مخازن بالقوه سویه‌های E. coli در نظر گرفته می‌شوند که قادرند عفونت‌های روده‌ای یا خارج‌روده‌ای را در انسان ایجاد کنند؛ از این رو، جنبه‌های زئونوزی انتقال این باکتری اهمیت بالایی دارد. استفاده از برخی آنتی‌بیوتیک‌ها و فشار انتخابی محیط، در انتخاب و گسترش ژن‌های مقاومت نسبت به آنتی‌بیوتیک‌های مشابه نقش داشته و درمان بسیاری از عفونت‌های باکتریایی را دشوار کرده است. هدف از این پژوهش، شناسایی برخی از ژن‌های بتالاکتاماز گسترده‌طیف (ESBL) در سویه‌های E. coli جدا شده از مدفوع سگ‌های سالم و مبتلا به اسهال و بررسی الگوی حساسیت آنتی‌بیوتیکی آن‌ها بود. در مجموع، ۱۰۰ جدایه E. coli به صورت فنوتیپی از نظر تولید آنزیم‌های ESBL با استفاده از دیسک‌های سفوتاکسیم و ترکیب سفوتاکسیم/کلاولانیک اسید بررسی شدند. حساسیت آنتی‌بیوتیکی سویه‌های تولیدکننده ESBL نسبت به ۱۲ آنتی‌بیوتیک از کلاس‌های مختلف ارزیابی گردید. همچنین، وجود ژن‌های blaTEM، blaSHV، blaCTX-M-1، blaCTX-M-9 و blaOXA-1 در جدایه‌های ESBL مثبت با استفاده از واکنش زنجیره‌ای پلیمراز چندگانه (Multiplex PCR) مورد بررسی قرار گرفت. نتایج نشان داد که ۳۱ مورد از ۱۰۰ جدایه E. coli از نظر فنوتیپی تولیدکننده ESBL بودند. ژن blaTEM به عنوان ژن غالب در ۲/۴۵ درصد از جدایه‌ها شناسایی شد، در حالی که ژن blaCTX-M-1 در ۸/۲۵ درصد وجود داشت. بیش‌ترین مقاومت آنتی‌بیوتیکی در برابر اریترومایسین و کم‌ترین مقاومت در برابر مروپنم مشاهده گردید. علاوه بر این، ۲۰ الگوی مقاومت آنتی‌بیوتیکی متفاوت در جدایه‌ها شناسایی شد. با توجه به جنبه‌های زئونوزی انتقال E. coli، انجام مطالعات اپیدمیولوژیک بیش‌تر و بررسی الگوی حساسیت آنتی‌بیوتیکی پیش از درمان، برای اطمینان از موفقیت درمان و جلوگیری از گسترش سویه‌های تولیدکننده ESBL توصیه می‌شود.

کلمات کلیدی: بتالاکتاماز گسترده طیف (ESBL)، *اشریشیا کلی*، حساسیت آنتی‌بیوتیکی، سگ‌ها

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DOI: 10.22055/ivj.2025.551126.2854

تشخیص مولکولی انگل های خونی منتقله از کنه (تیلریا، بابزیا و آناپلاسما) در سگ‌های بی صاحب با استفاده از Nested PCR

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تاریخ دریافت: ۱۴۰۴/۷/۱۲ تاریخ پذیرش: ۱۴۰۴/۹/۱۶

چکیده

عفونت‌های انگلی خونی اغلب در سگ‌های مناطق گرمسیری، از جمله عراق، مشاهده می‌شوند. سگ‌ها به چندین انگل خونی آلوده می‌شوند که منجر به بیماری‌های جدی در آن‌ها می‌شود. این تحقیق به منظور تشخیص میکروسکوپی و مولکولی انگل‌های خونی سگ در استان واسط عراق انجام شده است. این مطالعه مقطعی از ابتدای ماه مه ۲۰۲۴ تا پایان دسامبر ۲۰۲۴ انجام شد. در مجموع از ۲۸۰ سگ بی‌صاحب در مناطق مختلف استان واسط عراق نمونه‌برداری انجام شد. نمونه خون از ورید گردن سگ‌ها گرفته شد و برای آنالیز میکروسکوپی و مولکولی مورد استفاده قرار گرفت. گسترش‌های نازک خون تهیه شده و به منظور شناسایی آلودگی به بابزیا، تیلریا و آناپلاسما با رنگ گیمسا رنگ‌آمیزی شدند. در ادامه از نمونه‌های خون استخراج DNA انجام شد و آزمایش nested PCR با استفاده از ژن 16S rRNA برای گونه‌های آناپلاسما و ژن 18SrRNA برای تشخیص گونه‌های تیلریا و بابزیا استفاده شد. در نهایت محصولات PCR با الکتروفورز ژل آگارز تأیید شدند. توالی‌یابی نوکلئوتیدی، صحت ژن‌های تکثیر شده را تأیید کرد و توالی‌های آنها با توالی‌های مرجع ژن‌های 16S rRNA و 18S rRNA مقایسه شد و توالی‌های جدا شده در GenBank قرار گرفتند. در گسترش‌های خونی رنگ‌آمیزی شده گونه‌های بابزیا، تیلریا و آناپلاسما در سی و چهار سگ شناسایی شد. بررسی PCR گونه‌های آناپلاسما را در ۷۷ سگ (۲۷/۵٪)، گونه‌های بابزیا را در ۵۵ سگ (۱۹/۶٪) و گونه‌های تیلریا را در ۶۳ سگ (۲۲/۵٪) نشان داد. شباهت بین جدایه‌های این تحقیق و سویه‌های مرجع ۱۰۰ درصد یکسان بود. یافته‌های این مطالعه نشان می‌دهد که سگ‌های ولگرد مخازن گونه‌های آناپلاسما، بابزیا و تیلریا هستند که به طور بالقوه نقش مهمی در اپیدمیولوژی و انتشار انگل‌های خونی دارند و از این رو تهدیدی اساسی برای صنعت گاوداری محسوب می‌شوند.

کلمات کلیدی: عراق، انگل‌های منتقله از راه خون، سگ‌های بی صاحب، روش nested PCR

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DOI: 10.22055/ivj.2025.545068.2841

اثر حفاظتی الاژیک اسید بر تغییرات پروفایل اسپرم اپیدیدیمی و آپوپتوز بافت بیضه در موش‌های بزرگ آزمایشگاهی نر دریافت کننده نیکوتین

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تاریخ دریافت: ۱۴۰۴/۶/۱۳ تاریخ پذیرش: ۱۴۰۴/۹/۵

چکیده

نیکوتین اصلی‌ترین آلکالوئید موجود در تنباکواست و به طور قوی با سمیت تولید مثلی در مردان مرتبط می‌باشد که این ارتباط عمدتاً از طریق مکانیسم‌های مرتبط با استرس اکسیداتیو و آپوپتوز است. الاژیک اسید، یک پلی‌فنول طبیعی با خواص آنتی‌اکسیدانی و ضدالتهابی است که در مدل‌های مختلف تجربی برای مقابله با آسیب اکسیداتیو بافتی گزارش شده است. مطالعه حاضر، اثرات محافظتی الاژیک اسید را در برابر اختلال تولیدمثلی ناشی از نیکوتین در موش‌های بزرگ آزمایشگاهی نر بررسی کرد. بیست و چهار موش بزرگ آزمایشگاهی نر بالغ ویستار به طور تصادفی به چهار گروه (n=۶) تقسیم شدند که شامل گروه کنترل، الاژیک اسید (۶۰ میلی‌گرم/کیلوگرم، خوراکی)، نیکوتین (۱ میلی‌گرم/ کیلوگرم، داخل صفاقی) و نیکوتین همراه با الاژیک اسید بود. پس از ۳۰ روز تیمار، بافت‌های بیضه از نظر شاخص گنادوسوماتیک (GSI)، پارامترهای کیفیت اسپرم (تعداد، تحرک، زنده‌مانی و مورفولوژی) و سطوح بیان ژن‌های کلیدی آپوپتوز (Bcl-2، کاسپاز-۳، p53 و BAX) مورد بررسی قرار گرفت. تجویز نیکوتین به طور معنی‌داری شاخص گنادوسوماتیک، تعداد اسپرم، تحرک، زنده‌مانی و مورفولوژی طبیعی اسپرم را در مقایسه با گروه کنترل کاهش داد. همچنین نیکوتین به طور معنی‌داری بیان ژن‌های p53، کاسپاز-۳ و BAX را افزایش و بیان ژن Bcl-2 را کاهش داد. تیمار همراه با الاژیک اسید و نیکوتین به طور معنی‌داری این اثرات نامطلوب را کاهش داد و پارامترهای تولیدمثلی و بیان ژن‌های آپوپتوزی را بهبود داد. در نتیجه، این یافته‌ها نشان می‌دهند که الاژیک اسید با کاهش آپوپتوز، سمیت تولیدمثلی ناشی از نیکوتین را در موش‌ها کاهش می‌دهد که نشان‌دهنده پتانسیل آن به عنوان یک عامل درمانی برای اختلالات تولیدمثلی مرتبط با نیکوتین است.

کلمات کلیدی: نیکوتین، الاژیک اسید، سمیت تولیدمثلی، آپوپتوز، بیضه

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DOI: 10.22055/ivj.2025.535387.2818

تأثیر افزودن اسانس رزماری و سلنیوم به جیره غذایی بر بیان ژن‌های باروری در خروس مادر گوشتی

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تاریخ دریافت: ۱۴۰۴/۴/۲۷ تاریخ پذیرش: ۱۴۰۴/۷/۱۶

چکیده

در مرغ‌های گوشتی، عملکرد تولیدمثلی خروس‌ها با افزایش سن کاهش می‌یابد. آنتی‌اکسیدان‌های طبیعی مانند روغن رزماری و سلنیوم می‌توانند با کاهش استرس اکسیداتیو، عملکرد تولیدمثلی را بهبود بخشند. مطالعه حاضر تأثیر این آنتی‌اکسیدان‌ها را بر بیان ژن‌های باروری (StAR و PVRL3) در خروس‌های مسن بررسی کرده است. ۴۲ خروس در ۶ گروه و ۷ تکرار به صورت آزمایش فاکتوریل در قالب طرح کاملاً تصادفی مورد آزمایش قرار گرفتند. تیمارها شامل: ۱) جیره شاهد، ۲) جیره حاوی ۱۰۰ میلی‌گرم/کیلوگرم اسانس رزماری، ۳) جیره حاوی ۲۰۰میلی‌گرم/کیلوگرم اسانس رزماری، ۴) جیره حاوی ۰/۳ میلی‌گرم بر کیلوگرم مخمر غنی‌شده با سلنیوم، ۵) جیره حاوی ۱۰۰میلی‌گرم/کیلوگرم اسانس رزماری +۰/۳میلی‌گرم بر کیلوگرم مخمر غنی‌شده با سلنیوم و ۶) جیره حاوی ۲۰۰میلی-گرم/کیلوگرم اسانس رزماری +۰/۳میلی‌گرم بر کیلوگرم مخمر غنی‌شده با سلنیوم بودند. در آخر، بافت بیضه از سه حیوان هر تیمار جداسازی و در دمای ۸۰- نگهداری شدند. بیان ژن StAR و PVRL3 با واکنش Real-timeqPCR انجام و نرمال‌سازی نسبت به ژن مرجع β-actin سنجیده شد. نتایج نشان داد، افزودن اسانس رزماری به جیره خروس تأثیر معنی‌داری بر بیان ژن StAR نداشت، اما دوز بالا (۲۰۰میلی‌گرم/کیلوگرم) باعث کاهش معنی‌دار بیان ژن PVRL-3 شد، در حالی که دوز ۱۰۰ میلی‌گرم تأثیر معنی‌داری نداشت. مکمل سلنیوم (۰/۳ میلی‌گرم) بیان هر دو ژن StAR و PVRL-3 را به طور معنی‌داری افزایش داد. ترکیب سلنیوم با ۱۰۰ میلی‌گرم رزماری نیز بیان این ژن‌ها را افزایش داد، اما ترکیب با ۲۰۰میلی‌گرم رزماری اثر منفی دوز بالا را جبران نکرد. سلنیوم با کاهش استرس اکسیداتیو، سلامت تولیدمثلی و بیان ژن‌های مرتبط با تستوسترون را بهبود می‌بخشد، در حالی که رزماری در دوزهای پایین مفید است اما دوزهای بالا ممکن است باروری را کاهش دهد؛ بنابراین استفاده همزمان آن‌ها باید با احتیاط افزوده شود.

کلمات کلیدی: خروس مسن، PVRL3، رزماری، سلنیوم

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DOI: 10.22055/ivj.2025.535198.2817

ارزیابی مقایسه‌ای اثرات آرامبخشی، هماتولوژی و بیوشیمیایی تزریق وریدی زایلازین، دتومیدین، مدتومیدین و دکسمدتومیدین در اسبچه خزر

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تاریخ دریافت: ۱۴۰۴/۴/۲۹ تاریخ پذیرش: ۱۴۰۴/۷/۲۲

چکیده

پژوهش‌های داروشناسی در زمینه آگونیست‌های گیرنده آلفا-۲ آدرنرژیک، در اسبچه خزر بسیار محدود است. بنابراین، هدف مطالعه حاضر ارزیابی اثرات آرام‌بخشی، هماتولوژی و بیوشیمیایی تزریق وریدی زایلازین، دتومیدین، مدتومیدین و دکسمدتومیدین در اسبچه خزر بود. در این مطالعه، شش اسبچه خزر به صورت تصادفی به پنج گروه تقسیم شدند و هر گروه یکی از چهار آگونیست گیرنده آلفا-۲ یا سرم فیزیولوژیک را دریافت کردند. داروها شامل تزریق وریدی ۱ میلی‌گرم به ازای هر کیلوگرم زایلازین، ۲۰ میکروگرم به ازای هر کیلوگرم دتومیدین، ۱۰ میکروگرم به ازای هر کیلوگرم مدتومیدین، ۵ میکروگرم به ازای هر کیلوگرم دکسمدتومیدین یا ۵ میلی‌لیتر سرم فیزیولوژیک ۰/۹ درصد بود. سطح آرام‌بخشی و پاسخ‌های فیزیولوژیک، از جمله ضربان قلب، نرخ تنفس، حرکات دستگاه گوارش و دمای رکتال، بلافاصله قبل از تزریق دارو (زمان صفر) و سپس در فواصل ۵، ۱۰، ۱۵، ۳۰، ۴۵، ۶۰، ۷۵، ۹۰، ۱۰۵ و ۱۲۰ دقیقه پس از تزریق ارزیابی شد. نتایج نشان داد که تفاوت معنی‌داری در میانگین سطح آرام‌بخشی و عدم تعادل بین برخی گروه‌های مورد بررسی وجود نداشت. همچنین یافته‌ها بیان‌گر آن بود که در هیچ یک از زمان‌های اندازه‌گیری شده، تفاوت معنی‌داری در میانگین ضربان قلب بین گروه‌ها و گروه کنترل مشاهده نشد؛ اگر چه در نرخ تنفس، حرکات دستگاه گوارش، دمای رکتال و برخی پارامترهای هماتولوژی و بیوشیمیایی خون، در برخی از زمان‌های اندازه‌گیری شده پس از تزریق، اختلاف معنی‌داری دیده شد.

کلمات کلیدی: اسبچه خزر، گیرنده آلفا-۲ آدرنرژیک، آرامبخشی، هماتولوژی، بیوشیمیایی

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DOI: 10.22055/ivj.2025.534196.2837

ارزیابی هماتولوژی و برخی عوامل بیوشیمیایی سرم پس از آناستوموزتجربی یکطرفه حالب در سگ‌ها

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تاریخ دریافت: ۱۴۰۴/۵/۲۷ تاریخ پذیرش: ۱۴۰۴/۷/۶

چکیده

انسداد حالب یکی از مشکلات مهم بالینی در سگ‌ها است که اغلب نیاز به مداخله جراحی دارد. چنین اقداماتی می‌تواند منجر به تغییرات فیزیولوژیک قابل توجهی در شاخص‌های هماتولوژیک و بیوشیمیایی شود. این مطالعه با هدف ارزیابی تغییرات هماتولوژیک و برخی از عوامل بیوشیمیایی سرم پس از جراحی تجربی آناستوموز حالب در سگ‌ها انجام شد. پنج سگ سالم نژاد مخلوط (با وزن ۱۵ تا ۳۰ کیلوگرم و سن ۵/۱ تا ۵/۴ سال) تحت قطع حالب چپ و آناستوموز انتها به انتها با الگوی بخیه ساده تکی قرار گرفتند. بیهوشی پس از پیش‌بیهوشی با آسپرومازین، با تیئوپنتال سدیم القا و با هالوتان در سیستم بسته حفظ شد. نمونه‌های ادرار و خون وریدی قبل از عمل و در روزهای ۱، ۳، ۷، ۱۴، ۲۱، ۲۸، ۳۵، ۴۲ و ۹۰ پس از جراحی برای آنالیز ادرار، شمارش کامل خون، هماتوکریت و اندازه‌گیری نیتروژن اوره خون (BUN)، کراتینین، اسید اوریک، کلسیم، فسفر، کلرید، سدیم و پتاسیم جمع‌آوری شد. داده‌ها با استفاده از آزمون واریانس مکرر (ANOVA) و آزمون پس‌هنگام توکی تحلیل شدند. افزایش معنی‌داری در نیتروژن اوره خون، کراتینین و شمارش نوتروفیل‌ها در تمامی زمان‌های پس از جراحی مشاهده شد. سطوح نیتروژن اوره خون و کراتینین در روزهای ۱ و ۳ بالاتر از محدوده طبیعی بود. سطوح فسفر در روزهای ۱، ۳، ۷، ۱۴، ۲۱، ۲۸ و ۳۵ به طور معنی‌داری افزایش یافت، در حالی که سطوح پتاسیم در روزهای ۱، ۳، ۷ و ۱۴ افزایش معنی‌داری نشان داد. تغییرات اسید اوریک، کلسیم، کلرید و سدیم از نظر آماری معنی‌دار نبودند. با وجود مکانیزم‌های جبرانی کلیه و حالب سالم، بروز برخی تغییرات بیوشیمیایی پس از جراحی یک‌طرفه حالب اجتناب‌ناپذیر است و این امر بر ضرورت پایش دقیق پس از عمل برای پیشگیری از عوارض تأکید دارد.

کلمات کلیدی: آناستوموز، تغییرات بیوشیمیایی، سگ، حالب، آنالیز ادرار

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DOI: 10.22055/ivj.2025.542208.2838

مطالعه بافت‌زایی و تکوین لوزالمعده در جنین قرقاول

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تاریخ دریافت: ۱۴۰۴/۵/۲۸ تاریخ پذیرش: ۱۴۰۴/۷/۲۳

چکیده

هدف اصلی این مطالعه بررسی بافت‌شناسی و تکوین لوزالمعده قرقاول در مراحل جنینی بود. تعداد ۲۵ تخم قرقاول بارور شده در انکوباتور با دمای ۳۷/۵ درجه سانتی‌گراد و رطوبت ۵۸ تا ۶۲ درصد قرار داده شدند. سه رویان قرقاول در سنین ۱۳، ۱۵، ۱۷، ۱۹ و ۲۱ و همچنین سه جوجه یک روزه قرقاول جمع‌آوری شد. بعد از آماده‌سازی بافت شامل آب‌گیری، شفاف‌سازی و آغشته‌سازی با پارافین مذاب، نمونه‌ها برش داده شد (۵ میکرومتر). برش‌ها با رنگ‌های هماتوکسیلین و ائوزین، ماسون تری‌کروم و گوموری تری کروم رنگ‌آمیزی شدند. لوزالمعده قرقاول بین روزهای ۱۳ تا ۱۵ دوره جنینی شروع به تشکیل کرده بود. در جنین ۱۷ روزه قرقاول، علاوه بر لوب پشتی، تشکیل لوب شکمی نیز آغاز شده بود. مشابه جنین ۱۵ روزه قرقاول، لوزالمعده جنین ۱۷ روزه شامل سلول‌های اپیتلیال تمایز نیافته، بافت همبند و مجاری توسعه نیافته بود، اما تعداد سلول‌های آسینار افزایش یافته بود. در جنین‌های ۱۹ روزه، آسینوس تشکیل شده و عمدتاً سازماندهی شده بود. همچنین، جزایر لانگرهانس در این سن مشاهده شدند. در جنین ۲۱ روزه، بین لوبی و مجاری دفعی اصلی شناسایی شدند و تشکیل جزایر لانگرهانس افزایش یافته بود. در جوجه قرقاول ۱ روزه، قسمت برون‌ریز لوزالمعده، آسینوس، توسعه یافته‌تر بود. جزایر لانگرهانس نیز به وضوح قابل مشاهده بودند، زیرا این جزایر در لوب طحال بیش‌تر از سایر لوب‌ها بودند. نتیجه‌گیری می‌شود بافت‌زایی لوزالمعده قرقاول بین روزهای روز ۱۳ و ۱۵ اتفاق می‌افتد و تا پس از هچ ادامه می‌یابد. تکوین لوب پشتی زودتر آغاز شده بود. ساختار بافتی لوزالمعده از طریق تمایز پی در پی اجزای کلیدی شکل می‌گیرد. جزایر لانگرهانس در روز ۱۹ رویانی ظاهر شدند و به دنبال آن بافت آسینی برون ریز و سیستم مجاری تکوین پیدا کردند که نقطه اوج تشکیل اعضای جنینی را نشان می‌دهد.

کلمات کلیدی: جنین قرقاول، لوزالمعده، بافت‌زایی، تکوین

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DOI: 10.22055/ivj.2022.363499.2503

ارزیابی تغییرات الکتروکاردیوگرام در سگ‌های نرمولومیک دچار افت فشار خون، تحت درمان با مایع‌درمانی به تنهایی یا همراه با افدرین یا دوبوتامین

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تاریخ دریافت: ۱۴۰۱/۷/۳ تاریخ پذیرش: ۱۴۰۱/۸/۲۹

چکیده

افت فشار خون نورموولمیک، می‌تواند به دلیل اتساع عروق یا از دست دادن تون عروقی سیستم عصبی سمپاتیک رخ دهد. هدف از انجام مطالعه حاضر، بررسی تغییرات الکتروکاردیوگرافی و اختلالات ریتم قلب در سگ‌های دارای فشار خون نرمال، مبتلا به افت فشار خون ناشی از ایزوفلوران، و تحت درمان با افدرین، دوبوتامین و مایع درمانی بود. بیست و نه قلاده سگ نر و ماده بالغ، از نژاد مخلوط، با وزن ۴/۳ ± ۲۰/۱ کیلوگرم و در محدوده سنی ۱/۵ تا ۲/۵ سال انتخاب شدند. بیهوشی به ترتیب با پروپوفول و ایزوفلوران ۱/۵ درصد، در اکسیژن ۱۰۰ درصد القاء و حفظ شد. سپس با بیهوشی عمیق ایزوفلوران ۳ درصد، افت فشار خون القا گردید. به سگ‌های تحت مطالعه، یکی از پنج درمان ۱- محلول رینگر (۱ سی‌سی/کیلوگرم در دقیقه، تعداد: ۵ سگ)، ۲- محلول رینگر (۱ سی‌سی/کیلوگرم در دقیقه) همراه با تزریق داخل وریدی افدرین (RE، ۰/۲ میلی‌گرم/کیلوگرم، تعداد: ۶ سگ)، ۳- محلول رینگر (۱ سی‌سی/کیلوگرم در دقیقه) با انفوزیون داخل وریدی دوبوتامین (RD، ۵ میکروگرم/کیلوگرم در دقیقه، تعداد: ۶ سگ)، ۴- تزریق داخل وریدی افدرین (E، ۰/۲ میلی-گرم/کیلوگرم، تعداد: ۶ سگ) و ۵- انفوزیون داخل وریدی دوبوتامین (D، ۵ میکروگرم/کیلوگرم در دقیقه، تعداد: ۶ سگ) داده شد. هر زمان که فشار خون مستقیم پس از درمان، به بالای ۶۰ میلی‌متر جیوه می‌رسید، درمان قطع می‌شد و مقدار ایزوفلوران کاهش می‌یافت. در صورت عدم پاسخ، درمان یک بار دیگر تکرار می‌گردید. الکتروکاردیوگرام از تمام حیوانات در مقاطع زمانی مشخص گرفته شد. تعداد ضربان قلب پس از درمان در گروه رینگر با دوبوتامین (۱۴/۷۵ ± ۱۸۴/۲) نسبت به افدرین (۲۳/۸ ± ۹۹/۶) و دوبوتامین (۲۰/۲۹ ± ۱۰۸/۸) به طور معنی‌داری بیش‌تر بود. تعداد ضربان قلب در گروه رینگر با افدرین پس از درمان (۲۶/۴۶ ± ۱۱۰/۵) به طور معنی‌داری بیش‌تر از سطح پایه بود. تغییرات در دامنه و دوره موج P، دامنه و دوره QRS، فاصله PR، فاصله QT، محور الکتریکی قلب و شکل قطعه ST معنی‌دار نبود. نتیجه‌گیری شد که افزودن افدرین یا دوبوتامین به مایع درمانی معمولی، در سگ‌های با افت فشار خون نورموولمیک، می‌تواند با تعداد ضربان قلب بالاتر، همراه باشد. با توجه به نتایج به دست آمده، دوبوتامین همراه با محلول رینگر (گروه ۳)، به میزان بیش‌تری، باعث افزایش تعداد ضربان قلب نسبت به گروه‌های دیگر شد.

کلمات کلیدی: افت فشار خون، دوبوتامین، افدرین، الکتروکاردیوگرام، سگ

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DOI: 10.22055/ivj.2025.490279.2751

همارتوم عروقی مادرزادی لثه در یک گوساله

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تاریخ دریافت: ۱۴۰۳/۹/۴ تاریخ پذیرش: ۱۴۰۴/۴/۳

چکیده

هدف از این مطالعه، گزارش نتایج بالینی و آسیب‌شناسی یک تومور همارتوم عروقی لثه در یک گوساله و ارزیابی نتایج درمان بود. یک گوساله ماده ۳ ماهه از نژاد سیمنتال با شکایت وجود یک توده در ناحیه دندان پیشین نخستِ فک پایین به بخش جراحی دام‌های بزرگ دانشکده دامپزشکی دانشگاه شهید چمران ارجاع شد. صاحب گوساله گزارش کرد که ضایعه از هنگام تولد وجود داشته و در هنگام تغذیه موجب خونریزی می‌شده است. همچنین صاحب حیوان بخشی کوچک از ضایعه را جدا کرده بود که باعث خون‌ریزی شدید شده بود. در معاینه بالینی، یک توده بیضی‌شکل به اندازه یک آلو (۳۰×۲۰×۵ میلی‌متر) بر سطح دندان‌های پیشین فک پایین مشاهده شد. این توده به لثه متصل بود و یکی از دندان‌های پیشین را به جلو رانده و از محور طبیعی خود خارج کرده بود. پس از بی‌حسی موضعی، توده به طور کامل با عمل جراحی برداشت شد. در حین جراحی، خون‌ریزی قابل توجهی مشاهده گردید و مشخص شد که یک ریشه دندان شیری درون توده وجود دارد که خارج شد. ناحیه جراحی با نخ نایلون بخیه شد. پس از عمل، هیچ‌گونه مشکل تغذیه‌ای در گوساله مشاهده نشد. بر اساس بررسی‌های آسیب‌شناسی، توده به عنوان یک همارتوم عروقی تشخیص داده شد. تومورهای همارتوم عروقی لثه در گاو نادر هستند.

کلمات کلیدی: همارتوم عروقی مادرزادی، گوساله، نژاد سیمنتال، پاتولوژی

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Iranian Veterinary Journal

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This Journal has been granted the rating of **Scientific – Research** by the Commission for Evaluation of Iranian Scientific Journals, the Ministry of Science, Research and Technology, through the letter numbered 3.2910.545 dated 30.7.2006
This journal is indexed by Iran and Islamic World Scientific Citation Center (**ISC**)

Published by
Regional Information Center for Science and Technology and Islamic World Science Citation Center

IRANIAN VETERINARY JOURNAL

SCIENTIFIC – RESEARCH

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