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Contents

	Page
<u>Research Articles</u>	
1. Rats with Acute Gastritis Experimentally Induced with Ethanol Distribution of Mast Cells in the Liver, Lung and Kidneys Muhammed Kasım Aydın, Tuba Baksi, Zelal Karakoç, Nazan Baksi, Aydın Ketani	1-6
2. Regional Localization of Mast Cells and Eosinophils in the Small and Large Intestine of Bovine Fetuses during Pregnancy Eray Dişbudak, Fatma Çelenk, Uğur Topaloğlu, Asuman Arkaş Alklay, Hakan Sağsöz	7-14
3. Radiographic Evaluation of Thoracic Injuries in Traumatized Cats Hilmican ERGİN, Kurtuluş PARLAK	15-19
<u>Reviews</u>	
4. GöbekliTepe: A Window Into Early Human Rituals, Animal Symbolism, and Possible Pre-Veterinary Practices Bülent Başaran	20-22
<u>Case Reports</u>	
5. Abdominal Herniation of the Left Kidney in a Cat and Its Surgical Treatment: A Case Report Nahit Saylak, Berna Ersöz Kanay, Emine Çatalkaya, Laleş Taşdemir, Vedat Ozer, Sadık Yayla	23-26

Rats with Acute Gastritis Experimentally Induced with Ethanol: Distribution of Mast Cells and Eosinophils in the Liver, Lungs and Kidneys

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ABSTRACT

Mast cells are found in systems in contact with the external environment, such as blood vessels, nerves, respiration, and digestion. Mast cell proteases play important roles in regulating humoral and cellular events in the tissue. In rodents, mast cells are of two types: mucosal and connective tissue origin. This study aimed to examine mast cells histochemically and eosinophils in the possible damage that ethanol may cause in the liver, lung, and kidneys of rats with gastritis and also to determine whether pomegranate extract affects these cells. In this study, liver, lung, and kidney tissue samples taken from rats were stained with Alcian Blue/ Safranin O, Toluidine Blue to identify mast cells, and Congo Red to identify eosinophils. In the histological evaluation, it was determined that mast cells were located especially around blood vessels. It was determined that heparin-containing mast cells were dense in liver tissue, and histamine-containing mast cells were dense in lung tissue. It was determined that mast cells were less dense in the kidney than in the liver and lung tissues. When the locations and densities of mast cells in rats are considered, it can be said that they undertake similar tasks in functions such as vasopermeability and inflammatory cell response, as in other mammalian species.

Keywords: Heparin, histamine, mast cells, rat

INTRODUCTION

Mast cells and eosinophils are the main effector cells of innate immunity and play a fundamental role in defense mechanisms against bacterial, viral, and parasitic infections. These cells differ in their development, maturation, and location in tissues. Eosinophils mature completely in the bone marrow and enter the circulation as blood cells under physiological conditions. Mast cells leave the bone marrow as progenitor cells and mature in peripheral tissues. These cells are activated under the influence of chemical inflammatory stimuli and accumulate in inflamed tissues, participating in various pathological conditions together. Given their strategic location on major surfaces of the body, including the skin, kidneys, lungs, and the inner surface of the digestive system, mast cells are among the first cells to recognize danger signals from the external

stimulating other inflammatory cells. Eosinophils accumulate in organs and tissues following inflammatory and chemotactic stimuli produced as a result of inflammation. Mast cells and eosinophils are, therefore, simultaneously active in many diseases, including infections, allergic and autoimmune disorders, and cancer. Therefore, it is important to investigate the existence, role, and function of these two cells in various conditions (diseases, chemical stimuli, etc.) (1).

Mast cells are found in systems that interact with the external environment, such as blood vessels, nerves, respiratory and digestive systems. In humans and mammals, mast cell granules contain vital factors such as heparin, histamine, prostaglandin, neutral protease, β -glucuronidase, aryl sulfatase, tryptase, eosinophil chemotactic factor of anaphylaxis (ECF-A), and slow

reaction substance of anaphylaxis (SRS-A). These factors and mast cell proteases play important roles in regulating humoral and cellular events in the tissue (2). Mast cells in rodents also contain a substance called serotonin, which is not present in human mast cells and has an effect on the respiratory and digestive tracts (3,4).

In rodents, mast cells are classified as mucosal or connective tissue, depending on where they are located (5). Mucosal mast cells are located in the small intestinal mucosa, while connective tissue mast cells are located in the small intestinal submucosa, skin, skeletal muscle, and serosa (5). Eosinophils, sometimes called acidophils, are white blood cells. They are one of the components of the immune system responsible for combating multicellular parasites and certain infections in vertebrates. Together with mast cells and basophils, they also control mechanisms associated with allergy and asthma. Recent studies have provided important information on the selective infiltration of eosinophils into diseased tissues along with its molecular mechanism (6). It has been reported that eosinophil granule proteins stimulate various cells, including rat mast cells, neutrophils, respiratory goblet cells, basophils, and platelets (7).

Methyl alcohol and isopropyl alcohol are toxic and prohibited from being consumed. However, ethanol is an intoxicating substance and is found in beverages such as beer, wine, and raki (8). Excessive alcohol consumption can damage different organs, such as the brain, liver, heart, lungs, skeletal muscles, and bones. Alcohol taken orally is absorbed from all parts of the digestive tract, then quickly enters the bloodstream and spreads to all tissues (9). Studies have reported that even low doses of ethanol reduce several important functions of mast cells (10). It has been reported that ethanol inhibits histamine release in lung mast cells (10).

Alcohol consumption has harmful effects on the internal organs of the body, and rapid treatment is essential to prevent further deterioration of the situation in alcohol-induced gastritis. However, treatment methods have some negative effects. Therefore, it is important to determine the cellular and molecular pathogenic mechanisms that occur in various organs as a result of alcoholic gastritis. This study was planned to determine the regional localization, distribution, density, and quantitative distribution of mast cells and eosinophils in the liver, lungs, and kidneys damage that ethanol caused due to experimentally induced acute gastritis in rats. It was also planned to determine whether or not pomegranate extract given for prophylactic purposes affects the distribution and quantitative density of these cells.

MATERIAL AND METHODS

Tissue Procurement and Histochemical Procedure

In this study, liver, lung, and kidney tissues taken from the ethanol-induced gastritis in rats were used (2022/8). Twenty-four female Wistar albino rats (180-200 g) were used in the study. The animals were fed ad libitum with pellet feed and

water in a constant temperature (22 ± 3 °C) and humidity (50-55%) with 12 hours of light and 12 hours of darkness during the experiment. To create the experimental gastritis model with ethanol, the rats were fasted for 24 hours in advance but were only allowed access to water. Experimental groups include Control (1 mL saline oral gavage), Ethanol (2 mL ethanol oral gavage on the first day), and Ethanol+Pomegranate extract group (2 mL ethanol on the first day, then 0.5 mL (100 mg/kg) Pomegranate extract orally throughout the study (11). At the end of the study period, animals were sacrificed under xylazine-ketamine (10-90 mg/kg) anesthesia by taking blood from the heart, and tissue samples were taken. The tissue samples were fixed in 10% buffered formaldehyde. Tissue samples were embedded in paraffin blocks using the routine histological preparation method. Five μm thick sections were taken from each block and stained with Alcian Blue (AB, pH 0.3) / Safranin O (SO, pH 1.0), Toluidine Blue and Congo Red staining methods (12). Toluidine blue stained reveal mast cell granules, and Alcian Blue/Safranin O stained distinguish granules containing histamine and heparin. Preparations were examined and photographed under a research microscope (Nikon Eclipse E-400) with a digital camera (Nikon Coolpix4500) attachment.

Histochemical Evaluation

The numerical distribution of mast cells was evaluated by counting mast cells in 100 square unit areas with a magnification of 40 from each randomly selected area and converting them into the number of cells in 1 mm^2 unit area (2,13). Statistical analysis of the data was performed using the SPSS (IBM® Ver; 20.0 Windows, USA) package program. Parametric data were expressed as mean and standard deviation (M[SD]). Shapiro-Wilk test was used to check whether the data were normally distributed. ANOVA test was used to compare groups that did not show normal distribution. Kruskal Wallis H Test was used to compare groups that did not show normal distribution. The statistical significance level was defined as $P \leq 0.001$.

RESULTS

The numerical distribution of mast cells and eosinophils in rat tissues is given in Table 1.

Mast cells were found to be scattered, especially in the portal triad region in the liver, around the bronchi, bronchioles, and blood vessels in the lung, around the glomerulus in the kidney, and in the connective tissue between the tubules. Mast cells were observed to be round, spindle, or oval in shape in different sizes.

When the liver and kidneys were compared with the control group, it was determined that the number of mast cells was statistically significant in the groups given ethanol and pomegranate extract and in the lungs in the ethanol group (Figure 2-3, Table 1) ($P < 0.05$). It was determined that heparin-containing mast cells were common in the liver, and histamine-containing mast cells were common in the lung tissue. It was

Table 1. Numerical distributions of mast cells and eosinophils between groups in rat liver, lung, and kidneys (M/SD)

Groups		Mast cells	Eosinophils
Liver	Control	2,42±0,78 ^a	1,57±0,53
	Ethanol	2,71±0,75 ^b	1,71±0,48
	Ethanol+ <i>P.granatum</i>	2,85±0,90 ^b	1,85±0,37
Lung	Control	2,85±0,90 ^a	2,57±0,53
	Ethanol	2,42±0,78 ^b	2,57±0,53
	Ethanol+ <i>P.granatum</i>	2,85±0,90 ^a	2,57±0,53
Kidney	Control	1,42±0,53 ^a	1,28±0,48 ^c
	Ethanol	2,42±0,53 ^b	1,28±0,48 ^c
	Ethanol+ <i>P.granatum</i>	2,96±0,78 ^b	2,00±0,57 ^d

a, b, c, d: Difference between group means with different letters in the same column, $p < 0.05$

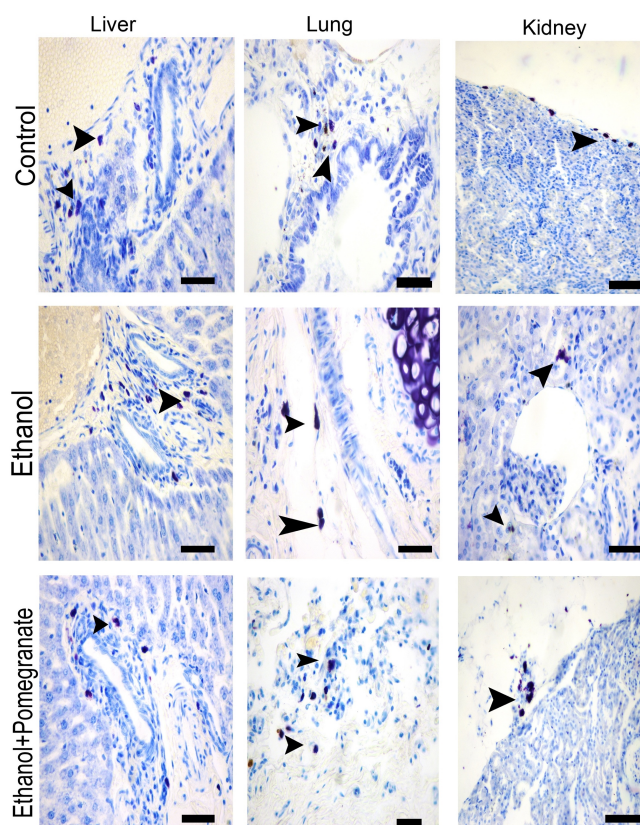


Figure 1: Distribution of mast cells in liver, lung and kidney in control, ethanol and pomegranate groups. Arrow head: mast cells containing heparin. Toluidine blue X40

determined that mast cells were less common in the kidney compared to the liver and lung tissues (Figure 2-3). In addition, histamine-containing mast cells were more common in the liver and lung groups compared to the control group.

It was observed that the localization of eosinophils in the liver, lung, and kidney was similar to mast cells. It was determined that eosinophils were widely distributed in the liver. The number of mast cells in the kidneys was found to be significant in the pomegranate extract group (Figure 3, Table 1) ($P < 0.05$).

DISCUSSION

Considering the frequency of gastric ulcers due to various chemicals and the side effects and costs of some existing synthetic drugs, the use of natural products is an important alternative for many people. In this sense, it has been stated that pomegranate extract is advantageous in the treatment of various disorders in laboratory animals and patients. In addition, it has been shown in short- and long-term studies that such plant-based extracts do not contain toxicity (14). As a

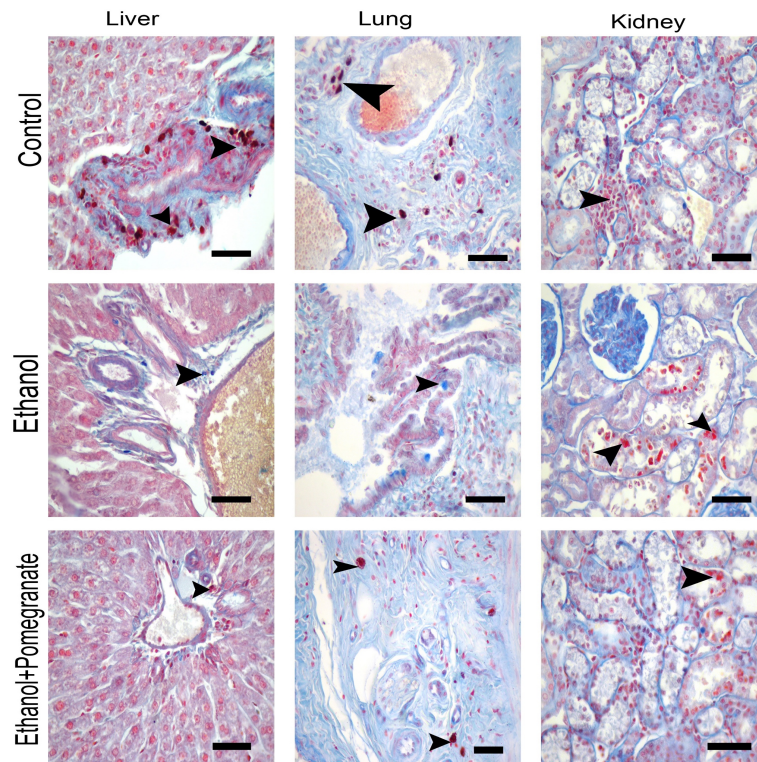


Figure 2: Distribution of mast cells in liver, lung and kidney in control, ethanol and pomegranate groups. Arrowhead: mast cells containing mixed pigment and histamine. Alcian blue/Safranin O X40

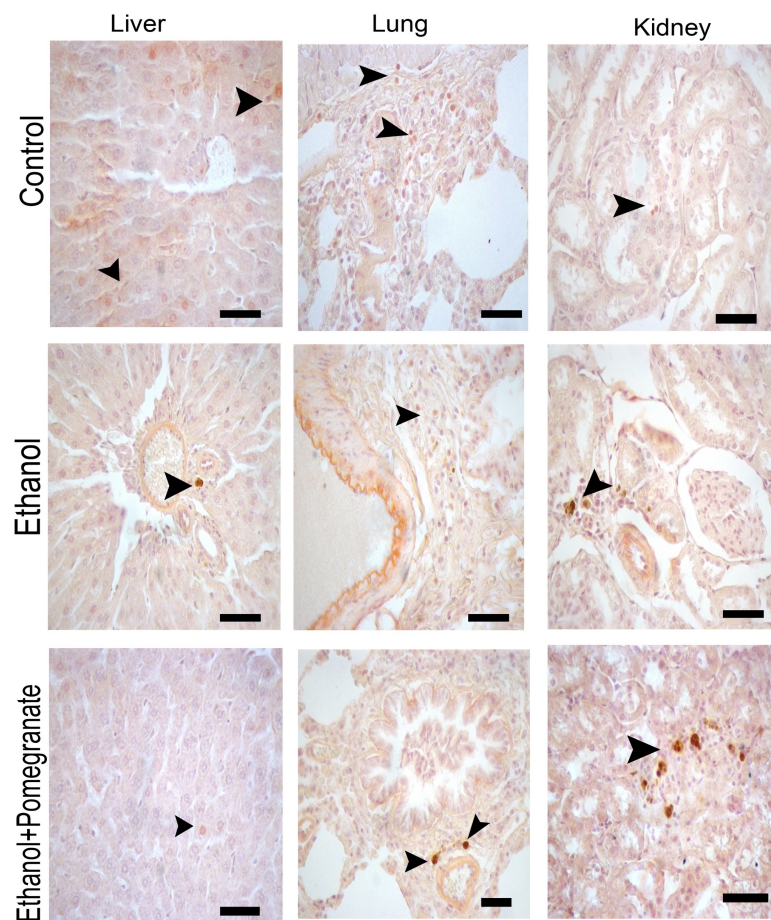


Figure 3: Distribution of eosinophils in liver, lung and kidney in control, ethanol and pomegranate groups. Arrowhead: eosinophils. Congo red X40

result of pomegranate extract given as a preservative revealed that the density of mast cells and eosinophils in the liver, lungs, and kidneys showed differences.

Although mast cells have been discovered for a long time, their functions have been associated with allergic diseases, and they were thought to have very few roles in other diseases and health. However, recent studies have reported that mast cells have very wide and diverse roles in both physiology and diseases (15). Mast cells act as a cellular interface between the external and internal environments and initiate and coordinate innate and adaptive immune responses by interacting with various cell types (16).

In normal rodent livers, there are few mast cells in the portal areas. After the liver injury, the number of hepatic mast cells increases, and they degranulate, releasing numerous growth mediators such as histamine, heparin, tryptase, TGF-1, TNF, ILs, cytokines, and basic fibroblast growth factor (bFGF) (16). Studies on mast cells in the liver have reported that ethanol-induced liver injury affects the function of mast cells (17). In the presented study, the finding of a significant difference in mast cells in the groups given ethanol and pomegranate extract compared to the control group supports the literature data.

In a study conducted on mice that were acutely poisoned with ethanol, it was reported that there was an increase in the number of mast cells due to damage in the lungs (18). In a study conducted on guinea pigs, it was reported that ethanol treatment of ethanol-induced gastritis in rats, it has been inhibited histamine release in lung mast cells (19). Studies have shown that ethanol has an effect on mast cells in the lungs (18,20). In the presented study, the significant number of mast cells in the ethanol group compared to the other groups supports the effect of ethanol on mast cells.

It is reported that mast cells are structurally found in small numbers in the kidneys, but their numbers increase in kidney diseases (21). Mast cells are thought to be related to the development of interstitial fibrosis in the kidneys (22). Ethanol has a direct effect on the kidneys, resulting in the diffusion of cell content into the intercellular space due to the increase in membrane fluidity (23). Studies have shown that the number of mast cells increases in the acute phase of renal diseases (21,22). In the presented study, the number of mast cells was found to be significant in the ethanol group compared to the control group. The number of mast cells was found to be significant in the pomegranate extract group compared to the ethanol group. It can be said that ethanol has a greater negative effect on the kidneys.

Recent studies have also provided important information about the selective infiltration of eosinophils into diseased tissues (24). It is known that mast cell-derived TNF- α and IL-1 α stimulate the production of eotaxin, a CC chemokine subfamily of eosinophil chemotactic proteins in epithelial and endothelial cells (24,25). In the presented study, it can be said that

eosinophils were not affected by the systemic effect of acute ethanol, but they affected the number of mast cells.

In conclusion, it has been determined that the locations and shapes of mast cells and eosinophils in rat lung, kidney, and liver tissues are similar to those in other mammalian species. It is thought that alcohol affects tissues systemically, that mast cells and eosinophils undergo changes in the affected tissues, and that pomegranate extract may be healing in tissues during acute alcohol consumption due to its antioxidant properties. More studies are needed to determine the effects of tissue damage on mast cells and eosinophils in alcohol consumption.

DECLARATIONS

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author (Z.K.).

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Regional Localization of Mast Cells and Eosinophils in the Small and Large Intestines of Bovine Fetuses during Pregnancy

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ABSTRACT

Mast cells and eosinophils are important immune system components and play pro-inflammatory and anti-inflammatory roles in complex processes associated with regulating the immune system. During the prenatal term, the development and maturity of the fetal digestive tract are affected by hormones (e.g., endogenous cortisol release), molecular factors, and various immune cell secretions (e.g., mast cells, eosinophils). We aimed to histochemically investigate the cellular localization and quantitative distribution of mast cells and eosinophils in the tissue layers of the small (duodenum, jejunum, and ileum) and large intestine (cecum, colon, rectum) of bovine fetuses during prenatal development. A total of 37 Holstein cow fetuses were used. Mast cells were observed in various sizes and appearances. The mast cell sizes were small, especially in the first period of pregnancy, and their sizes increased as the pregnancy progressed. Their shapes were oval-round or flat spindle-like. Eosinophils were localized in the lamina propria, submucosa layer and the tunica serosa layer and around the blood vessels of these layers. In conclusion, the presence of these cells and their biological roles in bovine fetal intestinal segments suggest that they are important for maintaining protective mucosal barrier integrity during pregnancy.

Keywords: Eosinophil, Fetal intestine, Histochemistry, Mast cell, Ruminant

INTRODUCTION

Mast cells and eosinophils are important components of the immune system and play pro-inflammatory and anti-inflammatory roles in complex processes associated with the regulation of the immune system. They are now recognized as motile cells that control or regulate multiple biological pathways and responses in health and disease. They store a large number of biologically active substances (proteins, cytokines, chemokines, enzymes) in specific granules ready for rapid release. Recent researches have revealed that these cells fulfill a multitude of previously unknown functions such as controlling inflammation, maintaining the epithelial barrier, contributing to tissue remodeling and bridging the gap between innate and adaptive immunity (1-3).

many tissues and organs in adults (2). So far, research on these cells has shown that they are produced in the bone marrow like most cells found in the blood. Mast cells produced during the embryonic period are called "primitive mast cells". These cells are produced on day 7 of embryonic development from the yolk sac, an extra-embryonic organ known to provide nutrients to the embryo and produce some blood cells. Mast cell progenitors enter the circulation and migrate to peripheral tissues where they complete their differentiation. Embryonic mast cell populations are gradually replaced by progenitor cells of definitive stem cell origin (4). In mice, these primitive mast cells are produced after day 8 of embryonic development. Given that an embryo and fetus are already protected by their mother's physical and immune barriers, researchers agree that

“primitive mast cells” serve no immune-related purpose (5). These mast cells have been reported to be present in the duodenum of human fetuses (6) and in the lamina propria, submucosa and tunica serosa of the small intestine of rat fetuses during gestation (7). During prenatal development, the development and maturation of the fetal digestive system is affected by luminal stimuli, hormonal factors, molecular factors and various immune cell secretions (e.g. mast cells, eosinophils). In many species, the maturation of organs is particularly rapid during prenatal development. The development of the digestive system is induced mainly by parenteral nutrition (via the placenta) before birth and only by the transition to enteral nutrition after birth. Therefore, in newborn pups, enteral food intake organizes structural and functional intestinal changes. However, responses vary according to species, food sources and specific digestive tract functions. The digestive system is one of the organs that is constantly exposed to pathogens from the internal (maternal transmission) or external world during pre- and postnatal development. Therefore, in mammals, offspring are protected by the maternal immune system and localized immune cells throughout intrauterine development (13, 14). Mast cells and eosinophils associated with the immune system are found in the stomach and intestines of some adult mammals including humans (1, 2, 9-11). Studies on the localization of mast cells and eosinophils in the small and large intestines during prenatal development are very limited (6, 7, 11). In the present study, we aimed to reveal the cellular localization and quantitative distribution of mast cells and eosinophils, which play important roles in the physiological and pathological responses of the immune system, in the small (duodenum, jejunum and ileum) and large intestine (cecum, colon, rectum) sections and tissue layers of bovine fetuses during prenatal development. Our study will provide histomorphological data on the functional importance of mast cells and eosinophils in the intestines of bovine fetuses during pregnancy.

MATERIAL AND METHODS

Collection of Tissue Samples

A total of 37 Holstein cow fetuses were used in this study. The fetuses were obtained from the Meat and Fish Institution and private slaughterhouses in Diyarbakır. In order to determine the gestational period of the obtained fetuses, the ages of the fetuses were calculated. For this purpose, after measuring the crown-rump length (CRL) of the fetuses, age determinations were made according to the method given by Harris et al. (1983), using the equation $y:54.6+2.46(x)$ obtained from the linear relationship between crown-rump length and fetal age (15). In this equation, “y” represents fetal age and “x” represents forehead-rump length. Based on this age determination, the fetuses were categorized into three groups as 1st (63-84 days), 2nd (94-170 days) and 3rd (187-271 days) stages of gestation.

In the material collection stage, an incision was made along the lateral edge to the horn of the uterus, where the pregnancy was

formed, wide enough for the pup to come out. After the membranes of the fetuses were cut and the fetal juices were drained, each fetus was collected in a separate place to measure the forehead-rump length. After the measurements, the abdominal region of the fetuses was opened and the stomachs and intestines were completely removed.

Tissue samples were obtained from the duodenum, jejunum, ileum and the cecum, colon and rectum and fixed in 10% formol alcohol solution for 18 hours. Following fixation, the tissues were dehydrated in graded alcohols (96% to absolute alcohol series), followed by clean in methyl benzoate and benzols and blocked in paraffin. Serial sections of 5 micrometer thickness were taken from the prepared paraffin blocks. To demonstrate the localization of mast cells and eosinophils during fetal development, 3 slides were prepared and each slide contained at least three sections for each organ at 50 μm intervals. Sections were mounted on slides coated with 3-ethoxysilane (APES) (Sigma-Aldrich Chemicals, St. Louis, MO, USA) and dried overnight at room temperature.

Histochemical Techniques

In the preparations, Toluidine blue (TB-pH: 0.5) and Combined Alcian Blue-Safranin O (AB/SO) staining methods were used to show mast cells, and Congo red method was used to show eosinophils. Mast cells showed AB/SO positive reaction were evaluated in 3 categories as AB (+) stained cells (only blue granules), SO (+) stained cells (only red granules) and mixed stained cells (containing both blue and red granules) according to their staining characteristics. Eosinophil granulocytes are stained in red/orange color with Congo red due to amyloid deposits in their granules (16).

Mast cells and eosinophils were examined and photographed on a Nikon Eclipse E400 (Nikon, Tokyo, Japan) microscope with a DS-R11 video camera (DS-U3, Nikon, Tokyo, Japan) attachment.

Counting Mast Cells

Mast cells were counted using three different sections. Two independent persons (E.D. and H.S.) counted the preparations using a consistent technique. Mast cells were counted around the lamina propria, submucosa and tunica serosa layers and blood vessels in small (duodenum, jejunum and ileum) and large (cecum, colon and rectum) intestinal sections (17). Counting of individual mast cells was performed at higher magnification (X40 objective magnification, 0.3 mm² field for all intestinal sections, 0.3 mm² area) using a light microscope (E-400; Nikon, Tokyo, Japan) equipped with a DS-R11 video camera (DS-U3, Nikon, Tokyo, Japan). Three different fields in each section were digitized by image analysis and computerized using NIS Elements D Imaging Software (Microvision, Evry, France) as described previously. Finally, all counts were converted to the number of mast cells per unit area (mm²).

Statistical Analysis

All data were entered into the computer and analyzed by means of the SPSS 15.0 system (SPSS 15.0, SPSS, Inc., Chicago, IL, USA). The non-parametric Kruskal-Wallis test was applied to determine whether there was any significant difference in staining throughout the gestation or between the different region of mast cells. The Mann-Whitney U test was used to determine in which particular different region of mast cells was significantly different from one another.

RESULTS

Localization of Cells during Pregnancy

Mast Cells; The lamina propria, submucosa and tunica serosa layers of the duodenum, jejunum and ileum of the small intestine and of the cecum, colon and rectum of the large intestine were examined during pregnancy. In TB and AB/SO stained sections, mast cells were observed in various sizes and

appearances; mast cell sizes were small especially in the first period of pregnancy and increased as the pregnancy progressed. Their shapes were oval-round or flat spindle-like. In general, intraepithelial mast cells were relatively rare in both small and large intestine during pregnancy. Mast cells were found in the tunica mucosa and tunica serosa between and within the crypts of the duodenum, jejunum, ileum, cecum, colon and rectum, around the villi, close to the blood vessels. In particular, fewer mast cells were found in the lamina propria and submucosa than in the tunica mucosa. The cells were mostly localized in the connective tissue in the tunica mucosa layer or around blood vessels. Regional localization of mast cells was similar in the three different gestational periods (Figs. 1 and 2).

The granules of TB (+) mast cells generally did not show metachromatic staining or showed a weak metachromasia, and

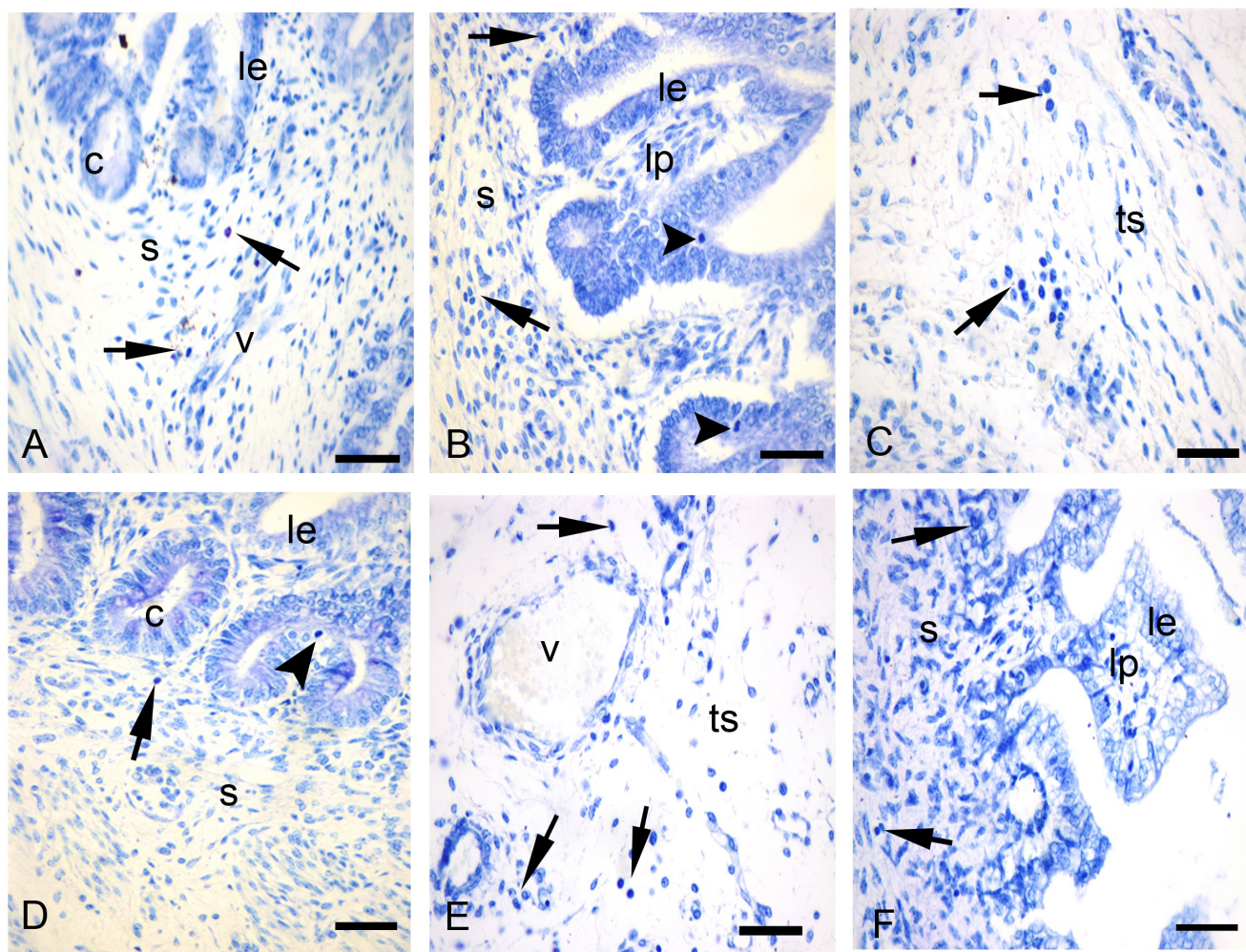


Figure 1. Localization of mast cells in the lamina propria, submucosa and tunica serosa layers of the small (A-C) and large intestine (D-F) sections (Scale Bar = 25 μ m). TB (+). Distribution of mast cells the lamina epithelialis (arrowhead), lamina propria (arrow), submucosa (arrow), tunica serosa (arrow) and around of blood vessels (arrow) of the duodenum (A), jejunum (B), ileum (C), cecum (D), colon (E) and rectum (F) during pregnancy. le; luminal epithelium, lp; lamina propria, c; crypt, s; submucosa, ts; tunica serosa, v; blood vessel. (A) 74 days of pregnancy, (B, D) 137 days of pregnancy, (C) 251 days of pregnancy, (E) 214 days of pregnancy and (F) 94 days of pregnancy.

the density of TB (+) cells was higher in all intestinal segments in the third stage than in the first and second stages of gestation. In addition, a high number of TB (+) mast cells were observed in the tunica serosa of all intestinal sections (Figure 1).

AB/SO staining revealed SO positive mast cells around the tunica mucosa and serosa layers and blood vessels in all intestinal sections during pregnancy. Mixed reacting mast cells were found predominantly in the tunica serosa in both small and large intestine sections during pregnancy. AB (+) mast cells were found sporadically in both intestinal sections and mostly in the tunica serosa layer during pregnancy. Degranulated mast cells were also sporadically observed in all intestinal sections (Figure 2).

Eosinophils; In both small and large intestine sections during pregnancy, eosinophils were localized in the lamina propria and submucosa layers of the tunica mucosa and the tunica serosa

layer and around the blood vessels in these layers. No intraepithelial eosinophils were found in the lamina epithelialis and crypts. Eosinophils were more abundant in both the small and large intestines during the third stage of gestation rather than in the first and second stages (Figure 3).

Variational distributions of mast cells

The total numbers of mast cells distributed in the lamina propria, submucosa and tunica mucosa of the small and large intestine sections of bovine fetuses during pregnancy are summarized in Table 1. During pregnancy, mast cell numbers were relatively similar from the small intestine to the large intestine in bovine fetuses. In addition, the number of mast cells in both the small and large intestine sections increased in the last stage compared to the first stage (Table 1, $p < 0.05$).

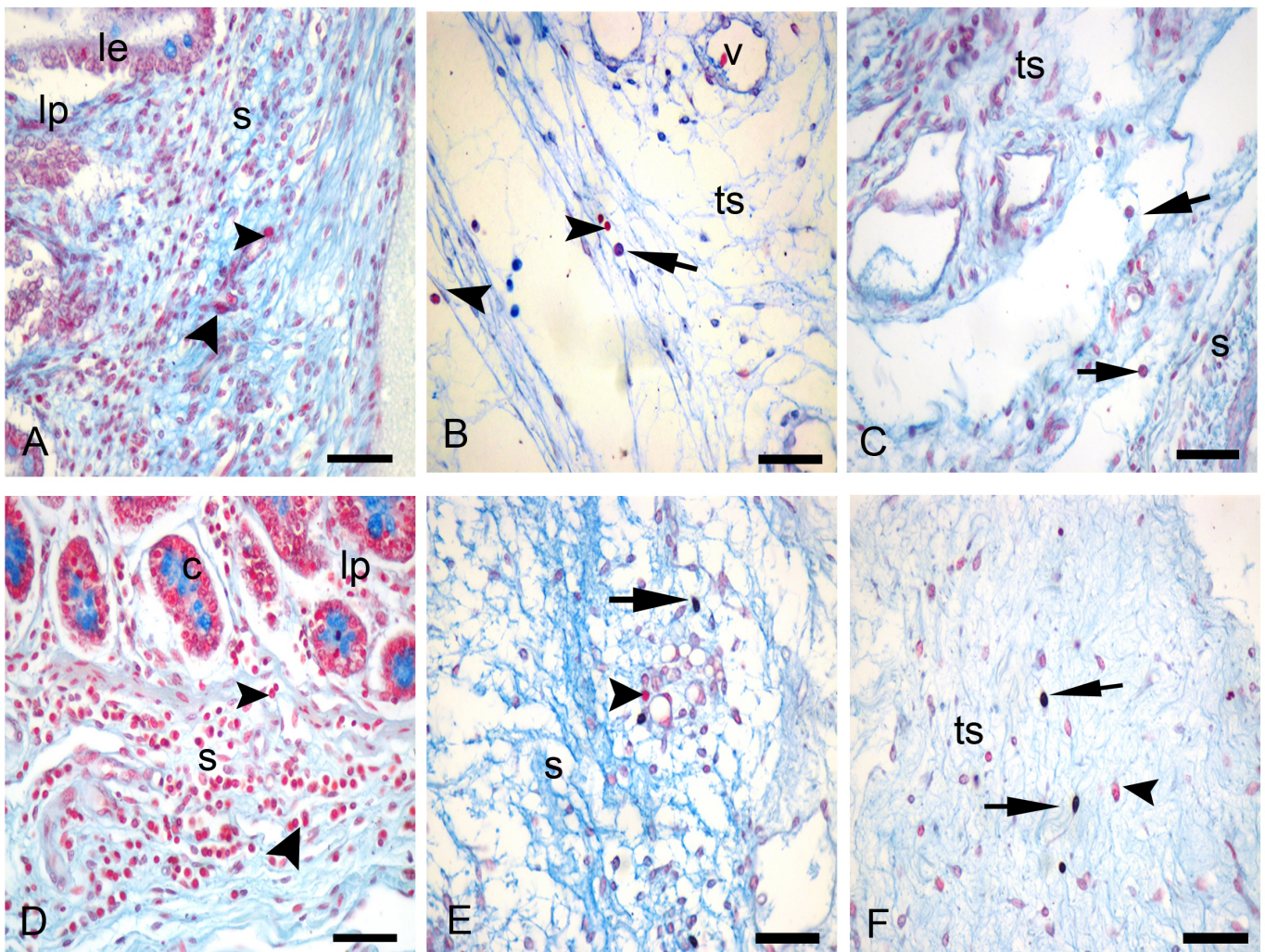


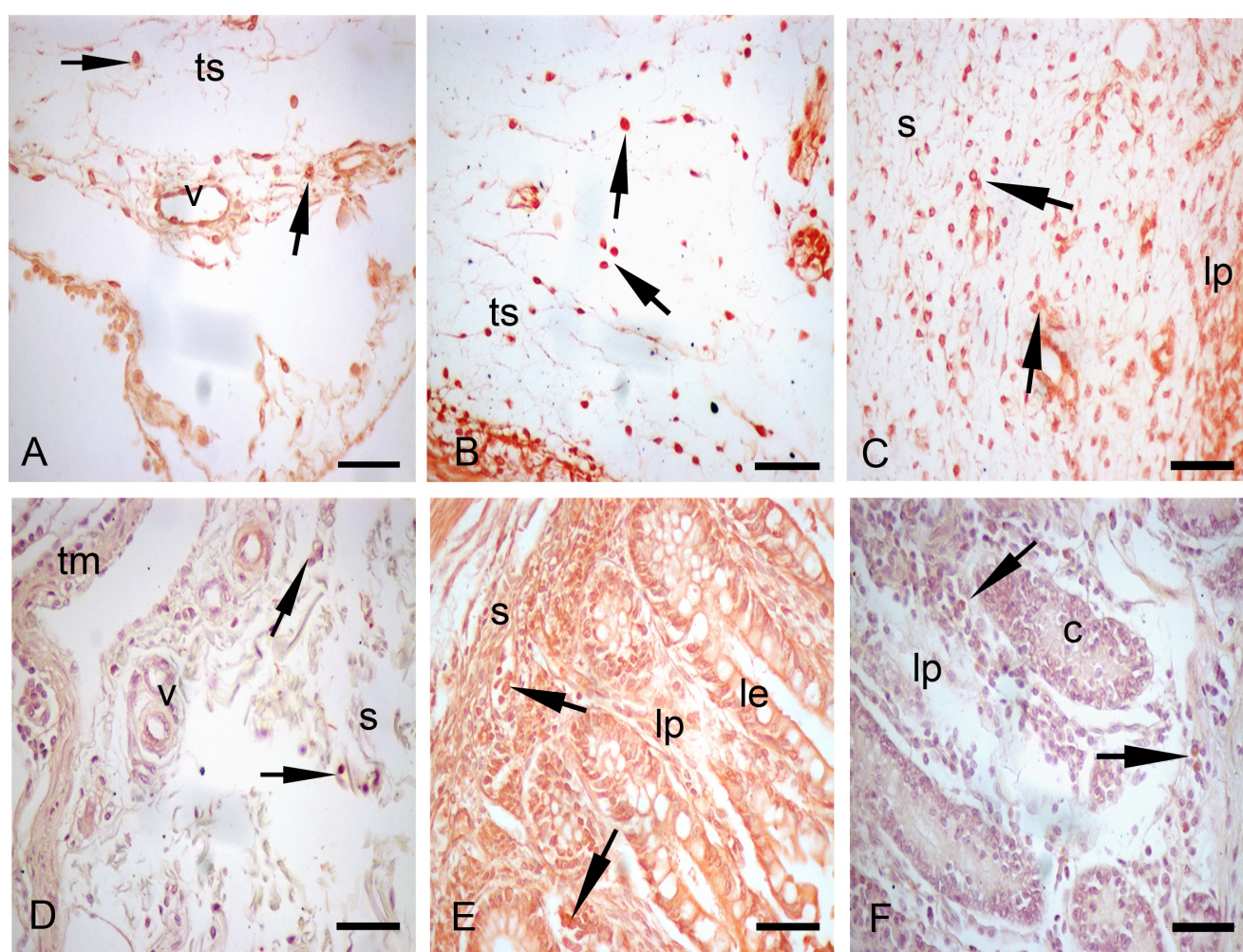
Figure 2. Localization of mast cells in the lamina propria, submucosa and tunica serosa layers of the small (A-C) and large intestine (D-F) sections (Scale Bar = 25 μ m). AB/SO (+). Distribution of mast cells the lamina epithelialis, lamina propria, submucosa, tunica serosa and around of blood vessels of the duodenum (A), jejunum (B), ileum (C), cecum (D), colon (E) and rectum (F) during pregnancy. le; lumianl epithelium, lp; lamina propria, c; crypt, s; submucosa, ts; tunica serosa, v; blood vessel, arrowhead; SO (+) mast cell, arrow; mixed reacting mast cells. (A) 214 days of pregnancy, (B) 98 days of pregnancy, (C) 137 days of pregnancy, (D) 251 days of pregnancy, (E) 133 days of pregnancy and (F) 192 days of pregnancy

DISCUSSION

In vitro studies have revealed possible soluble cross-talk pathways between mast cells and eosinophils. Data obtained over the years have shown that mast cells and eosinophils can affect the viability, functionality, trafficking, and activation of each other (1). Mast cells and eosinophils perform a fundamental defense and immunoregulatory function. The intestinal mucosa is the largest interface separating the internal and external environments that are constantly exposed to luminal contents. The ability to protect the body from harmful luminal contents and control mucosal permeability constitutes the intestinal barrier function. In this defense function, mast cells and eosinophils play a central role in both the adult and fetal intestinal tract. Specifically, mediators released by mast

intestines of bovine fetuses during pregnancy, and it was thought that these cells may perform the above-mentioned functions in the intestines of bovine fetuses as well.

It was reported that mast cells were distributed in the lamina propria, submucosa and tunica serosa layers of the duodenum, jejunum and ileum during pregnancy in rat fetuses, and mast cells changed numerically according to the layers and gestation periods. The number of mast cells increased in the 2nd and 3rd periods compared to the 1st period, but this increase was not statistically significant (7). In the duodenum of human fetuses, connective tissue mast cells were reported to be sporadic in the submucosa at 16th weeks old. However, mucosal mast cells were seen in the 18th week. In human fetuses, the granules in



Figs. 3. Localization of eosinophils in the lamina propria, submucosa and tunica serosa layers of the small (A-C) and large intestine (D-F) sections (Scale Bar = 25 μ m). Congo red. Distribution of eosinophils (arrow) the lamina epithelialis, lamina propria, submucosa, tunica serosa and around of blood vessels of the duodenum (A), jejunum (B), ileum (C), cecum (D), colon (E) and rectum (F) during pregnancy. le; luminal epithelium, lp; lamina propria, c; crypt, s; submucosa, ts; tunica serosa, tm; tunica muscularis, v; blood vessel. (A) 271 days of pregnancy, (B) 74 days of pregnancy, (C) 111 days of pregnancy, (D) 114 days of pregnancy, (E) 168 days of pregnancy and (F) 214 days of pregnancy.

cells and eosinophils in the intestinal mucosa affect epithelial integrity and viability, stimulate tissue remodeling, promote ion and water secretion (18). In this study, mast cells and eosinophils were found to be localized in the small and large

immature mast cells were stained in pale violet color, whereas they were stained in strong violet color in mature mast cells. Moreover, mast cells in the mucosa and submucosa layer were round or oval in shape and the cell bodies were large. In human

Table 1. Quantitative distribution of mast cells in both small and large intestinal sections of bovine fetuses. Results are expressed as means \pm standard deviations.

Intestinal Segments	Histological Layers	Sections	Fetal Gestation Periods		
			1 st period	2 nd period	3 rd period
Small Intestine	Lamina propria	Duodenum	0.50 \pm 0.58 ^a	1.0 \pm 0.0	1.33 \pm 0.52 ^b
		Jejunum	0.67 \pm 0.52	1.17 \pm 0.41	1.50 \pm 0.55
		Ileum	0.83 \pm 0.41 ^a	1.33 \pm 0.52	2.33 \pm 0.52 ^b
	Submucosa	Duodenum	0.67 \pm 0.52	0.67 \pm 0.52	1.46 \pm 0.52
		Jejunum	0.83 \pm 0.41	1.16 \pm 0.75	1.50 \pm 0.55
		Ileum	0.83 \pm 0.75 ^a	1.17 \pm 0.75	2.50 \pm 0.84 ^b
	Tunica mucosa	Duodenum	1.33 \pm 1.03 ^a	1.66 \pm 0.52	2.66 \pm 0.52 ^b
		Jejunum	1.33 \pm 0.89	1.33 \pm 0.52	2.23 \pm 0.82
		Ileum	1.17 \pm 0.75 ^a	1.67 \pm 0.52	2.67 \pm 0.82 ^b
	Around of blood vessels	Duodenum	0.93 \pm 0.75 ^a	2.0 \pm 0.63	2.50 \pm 0.84 ^b
		Jejunum	1.21 \pm 0.41	1.67 \pm 0.82	2.13 \pm 0.82
		Ileum	1.17 \pm 0.75 ^a	1.50 \pm 0.55	2.67 \pm 0.82 ^b
Cecum		0.50 \pm 0.55	0.67 \pm 0.52	1.0 \pm 0.63	
Colon		0.67 \pm 0.52	0.83 \pm 0.41	1.17 \pm 0.41	
Rectum		0.67 \pm 0.52	0.67 \pm 0.521	1.17 \pm 0.41	
Large Intestine	Lamina propria	Cecum	0.67 \pm 0.52	0.83 \pm 0.41	1.17 \pm 0.75
		Colon	0.83 \pm 0.41	1.0 \pm 0.0	1.33 \pm 0.52
		Rectum	0.67 \pm 0.52	1.0 \pm 0.0	1.33 \pm 0.52
	Submucosa	Cecum	0.83 \pm 0.41 ^a	1.0 \pm 0.63	2.17 \pm 0.75 ^b
		Colon	1.0 \pm 0.0 ^a	1.17 \pm 0.41	2.16 \pm 0.98 ^b
		Rectum	1.0 \pm 0.63 ^a	1.33 \pm 0.52	2.16 \pm 0.75 ^b
	Tunica mucosa	Cecum	1.0 \pm 0.0 ^a	1.17 \pm 0.75	2.17 \pm 0.98 ^b
		Colon	1.0 \pm 0.0	1.33 \pm 0.52	1.83 \pm 1.16
		Rectum	1.17 \pm 0.75	1.50 \pm 0.55	2.0 \pm 0.89

"a and b" indicate that the difference between pregnancy periods in the same line is significant ($p < 0,05$)

fetuses, the number of mast cells in the duodenal wall gradually increased during the gestation process. In the 22nd week of gestation, both types of mast cells were activated and distributed around the surrounding blood vessels and ganglia (6). In mice, mast cells contained characteristic granules were localized in the cornea on day 12.5 of embryonal development and in the skin on day 14.5 of embryonal development (5). Saad (12) reported that in the large intestines of boys and girls, mast cells were higher in the descending colon and lower in the rectosigmoid and mast cells were absent in the luminal epithelium of the colon and very rare in the crypt epithelium. He reported that mast cells were more numerous in the cecum than in the colon. Moreover, mast cells were more numerous in the cecum and descending colon in girls compared to boys (12). In the present study, mast cells were localized in the lamina propria, submucosa and tunica mucosa layers and around the blood vessels in intestines as reported in humans, rats and mice. In bovine fetuses, the size of the cells was found to be small in the early gestation period and gradually increased as the pregnancy progressed as reported in human fetuses. In contrast to immature mast cells in human fetuses, TB staining revealed that mast cells did not show metachromasia or a vague metachromasia in either the small intestine or large intestine of bovine fetuses during the entire gestation period. In addition, in AB/SO staining in the intestinal sections of bovine fetuses, mast cells showed predominantly SO (+) and

mixed reactions, while mast cells showing AB (+) reactions were in the minority. The mast cells with SO (+) reaction were predominantly localized in the lamina propria and submucosa layers, while mast cells with mixed reaction were localized in the tunica serosa layer in the present study. Contrary to Uslu and Tatar (7) reported in the small intestine of rat fetus, the number of mast cells gradually increased in all intestinal sections of bovine fetuses and the number of mast cells was significantly higher in the last period of pregnancy compared to the first period of pregnancy (Table 1).

Eosinophil migration and accumulation into tissues occurs largely in the active type-II immune response. In adults, under homeostatic conditions, eosinophils are concentrated in the mucosal tissues of various organs such as the thymus, intestine, uterus, lung and mammary gland. However, homeostatic eosinophil migration in the gastrointestinal tract (stomach, small and large intestine) and thymus occurs during fetal development (3,19). In particular, mice eosinophils were present in the fetal small intestine on day 19 of embryonic development at densities comparable to those of adult mice (3). Some researchers have reported that eosinophils were recruited to the intestinal lamina propria during fetal development and their numbers were generally maintained throughout the life cycle (19). In the postnatal period, the number of eosinophils in the lamina propria gradually

decreased from the cecum to the descending colon in boys and girls, but increased again in the recto-sigmoid. In addition, eosinophils in the surface and crypt epithelium were found more frequently in the cecum and recto-sigmoid (12). In our study, eosinophils were localized in the lamina propria and submucosa layers of the tunica mucosa, tunica serosa layer and around the blood vessels in both small and large intestine sections during pregnancy and the cell density was similar in both intestines. Intraepithelial eosinophils were absent in the lamina epithelialis and crypts of intestinal segments of bovine fetuses. In addition, eosinophils were relatively more abundant in both the small and large intestine during the last stage of gestation rather than in the early and middle stages.

Mast cells and eosinophils are powerful immune effectors with intracellular granules that can rapidly respond to harmful stimuli. Fetal mast cells can respond to environmental signals and stressors while the fetus is still in utero. Therefore, functional mast cells produced during the fetal stage help protect against immunologic threats and support the developmental processes of the fetus before the adaptive immune system is established (20). Eosinophils are a normal component of healthy intestines and have an important function in maintaining the homeostasis of the intestinal barrier (2). The largest eosinophil population is found in the gastrointestinal (GI) tract. They pass into the intestine independently of the microbiota in late pregnancy and early life (11). Eosinophils localized in the intestines are thought to participate in various physiological mechanisms such as providing immunity against pathogens in the intestinal lumen and establishing a link between innate and adaptive immunity (2, 3, 8). The presence of mast cells and eosinophils in the small and large intestines of bovine fetuses during pregnancy suggests that they are involved in the performance of the functions mentioned above.

In conclusion, mast cells were found to be higher in all intestinal sections in late pregnancy, whereas eosinophil counts were relatively similar throughout pregnancy. The presence of these cells in bovine fetal intestinal tracts during pregnancy and their biological roles suggest that they may function in a variety of homeostatic functions, including maintaining protective mucosal barrier integrity and contributing to gut-associated immunity. Therefore, further research is needed to elucidate the physiological importance of these cells in the intestinal tract of fetuses.

DECLARATIONS

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author (H.S.).

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Ethical Statement: The study did not require ethical approval with the decision of Dicle University Animal Experiments Local Ethics Committee dated 27.11.2024 and numbered 03/14.

Competing Interests: The authors declare that there is no competing of interest regarding the publication of this article.

Declaration of Generative Artificial Intelligence: The authors of the current study declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

Authors' Contributions: HS was responsible for the idea and concept of the paper. ED, FÇ, UT and AAA collected the placental tissue and contributed to the laboratory work. HS, ED and UT analyzed the results. HS wrote the manuscript. All the authors contributed to the manuscript editing and approved the final manuscript.

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Radiographic Evaluation of Thoracic Injuries in Traumatized Cats

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ABSTRACT

Trauma is a common health problem in cats that often leads to serious thoracic pathologies. Various imaging methods are used in the diagnosis of traumatic thoracic injuries. Thoracic radiography is the first preferred imaging method in post-traumatic emergencies due to its accessibility, speed, and cost-effectiveness. This study aims to retrospectively evaluate the pathologies observed in thoracic radiographs of trauma-affected cats. An assessment of 102 cats revealed that the leading causes of trauma were high-rise syndrome (83.3%) and motor vehicle accidents (6.86%). Prominent thoracic pathologies included pleural effusion, pulmonary edema, and pneumothorax. The findings underscore that radiography is a rapid and effective diagnostic tool for thoracic pathologies in trauma patients. The radiographic assessment facilitates the timely detection of critical conditions such as pneumothorax, hemothorax, and rib fractures, thereby expediting the initiation of appropriate treatment. Following trauma, 93% of the cats survived, while 6.8% succumbed. In conclusion, thoracic radiography should be the first-choice imaging modality for trauma-affected cats, with advanced imaging techniques serving as complementary tools when necessary.

Keywords: Cat, radiography, thorax, trauma

INTRODUCTION

Trauma is a common health problem in cats that often leads to serious thoracic pathologies. The main causes of trauma in cats are trauma mechanisms such as traffic accidents, falls from heights, and animal bites, and result in direct or indirect effects on the thoracic region. Depending on the type of trauma, lesions such as pneumothorax, hemothorax, costal fractures, lung contusions, and diaphragmatic hernia may occur. Early diagnosis of such pathologies is critical for determining appropriate treatment strategies (1,2,3).

Various imaging methods are used in the diagnosis of traumatic thoracic injuries. X-ray (radiography), computed tomography (CT), and magnetic resonance imaging (MRI) are the main methods used for this purpose. Thoracic radiography is the first preferred imaging method in post-traumatic emergencies due to its accessibility, speed, and cost-effectiveness (4,5). Radiography accelerates intervention by rapidly detecting

life-threatening conditions such as pneumothorax, hemothorax, and costal fractures. However, radiography can usually be performed with minimal sedation, while anesthesia is generally required in CT and MR imaging. This condition makes radiography a safer option for critically ill patients. Recently, with the increasing frequency of use, Thoracic Focused Assessment with Sonography for Trauma (T-FAST), when compared to thoracic radiography, has demonstrated superiority in dynamic and emergency assessments. However, it remains limited in evaluating detailed anatomical structures such as pulmonary parenchymal pathologies and bony structures, and its efficacy is highly dependent on the operator's experience (6,7,8).

This study aims to retrospectively evaluate the pathologies seen in thoracic radiographs in trauma-affected cats. The study analyzed the distribution of radiographic findings according to

the type of trauma, the incidence of these findings, and their relationship with clinical outcomes. The findings are expected to guide post-traumatic diagnosis and treatment processes in cats.

MATERIAL AND METHODS

The study material comprised 102 traumatized cats of different breeds, ages, and genders brought to the Surgery Clinic of the Faculty Animal Hospital between 2023-2024. Inclusion criteria included completeness of radiographic images and accessibility of clinical data. Patients with missing or insufficient radiographic data were excluded from the study. Cats brought to the clinic due to trauma were first triaged briefly, and their vital signs were quickly assessed. Simultaneously with the physical examination, intravenous access (V. cephalica antebrachia) was opened to the trauma-affected cats, blood samples were taken, and systemic blood gas (GEM Premier 3000, USA) and hemogram (MS4e, France) analyses were performed at the Central Laboratory of the animal hospital. Fluid electrolyte replacement was performed according to blood parameters, and intravenous medications were administered this way. Oxygen treatment was provided to traumatized patients in intensive care units specially designed for veterinary use.

After stabilization of trauma-affected cats, thoracic radiography (SIEMENS X-ray, Rayence Veterinary DR Device) was taken from each case and evaluated regarding lung pathologies in line with the clinical findings obtained. Thoracic pathologies such as pneumothorax, hemothorax, lung contusions, rib fractures, and diaphragmatic hernia were examined in radiographic analyses. The severity, distribution, and relationship with the type of trauma of the lesions were analyzed retrospectively.

Thoracocentesis was performed from the 8th intercostal space with a 21-gauge butterfly catheter in patients with thoracic pathologies such as pleural effusion and pneumothorax, and the radiographs of the patients were repeated at specific intervals (9). Laminectomy and vertebral stabilization were applied to cases with thoracic vertebrae fractures. After stabilization was achieved in flail chest cases, rib fractures were corrected with an operative procedure, and the thoracic wall was closed. In diaphragmatic hernia cases, the diaphragmatic defect was closed by laparotomy. In all cases, 0.1 mg/kg butorphanol hydrogen tartrate (Butomidor®-İnterhas- Turkey) was administered intramuscularly for analgesia.

Upon initial admission, data obtained from traumatized cats were recorded in patient follow-up formulas, and treatment protocols were arranged according to the symptoms. After the trauma, the patient owners were contacted, and information about the general condition of the patients was obtained and recorded.

RESULTS

The study evaluated 102 trauma-affected cats. The gender distribution was 49 male (48%) and 53 female (51%). The breed distribution was 48 Domestic shorthair (47%), 19 Scottish fold (18.6%), 26 British shorthair (25%), 1 Bombay (0.98%), 3 Siamese (2.9%), 2 Norwegian Forest Cat (1.96%), 1 Ankara Cat (0.98%), 1 Van Cat (0.98%) and 1 Persian Cat (0.98%). The trauma distribution of the cats in the study was 85 falls from heights (83.3%), seven traffic accidents (6.86%), four bite injuries (3.92%), one gunshot wound (0.98%), one blunt force trauma (0.98%) and four unknown causes of trauma (3.92%). Thoracic pathology was found in 67 of the patients evaluated (65.68%). Pleural effusion (46.7%), pulmonary edema (28.43%), pneumothorax (24%), lung contusion (12.7%), thoracic spine fracture (2.94%), subcutaneous emphysema, atelectasis (1.96%), rib fracture (0.98%), diaphragmatic hernia (0.98%) and sternum injury (0.98%) were observed (Figure 1). More than one thoracic pathology was observed in 36 cases (35%) included in the study. In the following process, the patients' owners were contacted about their general conditions, and it was learned that 95 patients were alive (93%) and 7 patients were dead (6.8%). In this study, rib fracture, pneumothorax, subcutaneous emphysema, pleural effusion, and flail chest were observed in 1 case due to a dog attack. A pre-anesthetic evaluation of the case was performed, and the intercostal mesh was applied to the patient deemed suitable for operative intervention. In the postoperative period, the patient was oxygenated in the intensive care unit. After the patient's vital signs became stable, the patient was discharged. In the clinical examination of the patient on the 7th postoperative day, it was observed that no complications developed due to operative intervention, and the patient's general condition was stable (Figure 2).

DISCUSSION

The findings indicate that trauma mechanisms in cats vary depending on their living environments and that high-rise syndrome is more common in areas with high-rise buildings (10,11,12). In this context, High-rise syndrome was determined to be the most common type of trauma (83.3%), and this rate was found to be consistent with the 66.6% rate in the study conducted by Pinar and Arican (13) in the same region. However, the data suggests that falls from heights have increased proportionally. This increase is associated with rapid urbanization and the growing number of domestic cats.

When the breeds exposed to trauma in cats were evaluated, it was determined that the Domestic Shorthair (47%), British Shorthair (25%), and Scottish Fold (18.6%) breeds were in the foreground. In this respect, this study parallels the study conducted by Lee et al. (14), who reported that 90% of cats exposed to trauma were Domestic Shorthairs. Similarly, a retrospective study conducted by Cojocar et al. (15) on 1306 trauma-affected cats stated that the European Shorthair (89.2%) was the most commonly traumatized breed. This

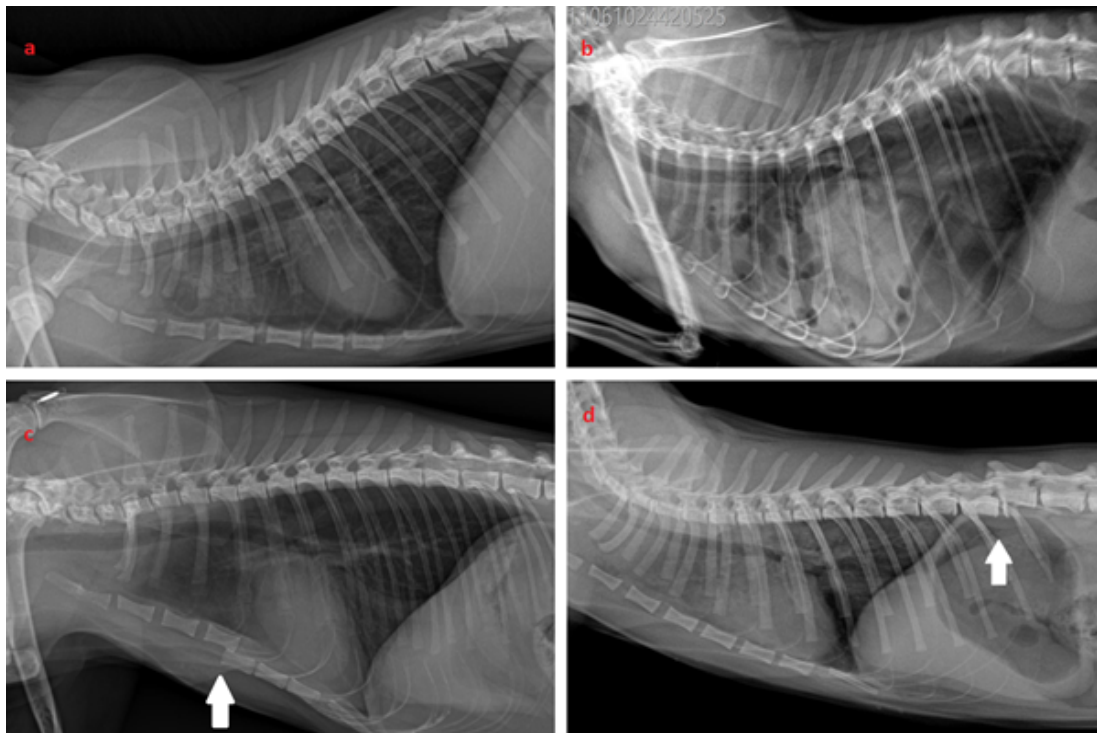


Figure 1: a) A case of pneumothorax and lung contusion in a cat with thoracic trauma due to falling from a height, b) A case of diaphragmatic hernia, lung contusion, and pneumothorax in a cat with thoracic trauma due to falling from a height, c) A case of sternum luxation and pleural effusion in a cat with thoracic trauma due to falling from a height, d) A case of T12-T13 fracture and pleural effusion in a cat with thoracic trauma due to falling from a height.



Figure 2: a) Latero-lateral thorax radiograph of a cat with thoracic trauma due to bite injury. b) Laceration image in the intercostal muscles. c) Mesh application d) Postoperative 7th day image of the patient.

suggests that the distribution between breeds may be related to regional population differences.

Life-threatening conditions such as pleural effusion, pulmonary edema, and pneumothorax are commonly observed in trauma related thoracic pathologies. Early diagnosis of such pathologies and determination of appropriate treatment protocols play a critical role in reducing mortality (16). In this study, pleural effusion (46.7%), pulmonary edema (28.43%), and pneumothorax (24%) were found to be the most common findings in the distribution of thoracic pathologies. In a retrospective study conducted by Vnuk et al. (17) on 119 cases of high-rise syndrome, pneumothorax (24%), lung contusion (13%), hemothorax (3.3%), and diaphragmatic hernia (1.6%) were reported as the most common findings. In contrast, in a study conducted by Frykfors and Halfacree (18) on 22 cats with thoracic trauma due to bite injuries, pneumothorax (61%), lung contusion (38%), and pleural effusion (33%) were the most common. These data show that the trauma mechanism has a significant effect on the distribution of thoracic pathologies.

Trauma-related mortality continues to be a significant problem in veterinary and human medicine. This study showed a 93% survival rate in traumatized cats, indicating that post-trauma management was implemented effectively. This finding is significantly higher than the 82% survival rate reported by Fisher et al. (19) on 251 patients with trauma and the 73% survival rate reported by Frykfors and Halfacree (18) in cats with bite injuries. Merbl et al. (20) reported an 83% survival rate in cats with falls from height, which is also lower than the results in this study. The success rate in this study can be attributed to the early diagnosis and optimization of treatment processes due to the rapid and effective use of thoracic radiography.

Various imaging methods, such as X-ray, CT, and MRI, are used in the diagnosis of traumatic thoracic injuries. However, radiography is often the first choice in post-traumatic emergencies due to its accessibility, speed, and cost-effectiveness (4,5). Radiography facilitates intervention by rapidly detecting life-threatening conditions such as pneumothorax, hemothorax, and costal fractures. In addition, the need for minimal sedation makes radiography a safer option, especially in critically ill patients (6,7,8). This study used radiography as a fast and secure method in all cases. It was an effective tool in detecting pathologies requiring urgent intervention, such as pleural effusion and pneumothorax.

In conclusion, this study revealed that thoracic radiography is an indispensable tool in early diagnosis and treatment management in trauma-affected cats. However, when necessary, the complementary use of advanced imaging methods may increase diagnostic accuracy in complex trauma cases. Future studies will further enhance knowledge in this area by examining the effects of different trauma mechanisms on radiographic and clinical outcomes in more detail.

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Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

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Ethical Statement: The present study is a retrospective study in which clinical data were evaluated and informed consent was obtained from the owners of the patients.

Competing Interests: The authors declare that there is no competing of interest regarding the publication of this article.

Declaration of Generative Artificial Intelligence: The authors of the current study declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.


Authors' Contributions: Motivation / Concept: KP, HE, Design: KP/HE, Control/Supervision: KP, Data Collection and / or Processing: HE, Analysis and / or Interpretation: KP, Literature Review: KP, Writing the Article: KP/HE, Critical Review: KP

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GöbekliTepe: A Window into Early Human Rituals, Animal Symbolism, and Possible Pre-Veterinary Practices

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ABSTRACT

This study explores GöbekliTepe (sometimes spelled “Göbekli Tepe”), a prehistoric site in southeastern Turkey, widely regarded as a transformative discovery in understanding early human civilization. Dated to approximately 12,000 years ago, GöbekliTepe challenges traditional views that monumental architecture and complex societies emerged only after the advent of agriculture. Its monumental T-shaped pillars and intricate carvings reflect advanced social organization and religious or ritualistic significance within a hunter-gatherer context. This study examines GöbekliTepe’s ritual architecture and symbolism, the cosmological and spiritual significance of animal figures, and early evidence of animal care, revealing the complex belief systems and large-scale cooperative capacities of hunter-gatherer societies. These findings highlight that early Neolithic communities had multifaceted relationships with animals, integrating practicality, spirituality, and social identity before the advent of settled agricultural life. Further interdisciplinary research is recommended to deepen our understanding of GöbekliTepe’s symbolic, social, and potential proto-veterinary practices, thereby continuing to redefine the trajectory of human history.

Keywords: Animals, Archaeo-zoological, GöbekliTepe, Veterinary Medicine.

INTRODUCTION

GöbekliTepe, located in southeastern Anatolia (modern-day Turkey), has been hailed as one of the most significant archaeological discoveries of the twentieth century. Dated to around 12,000 years ago—preceding the advent of agriculture—it upends conventional assumptions about the origins of civilization (1, 2, 3, 4). Formerly, it was believed that large-scale architectural projects and social complexity emerged only after established farming communities. However, GöbekliTepe’s massive T-shaped pillars, intricate animal carvings, and potential ritual significance highlight a sophisticated hunter-gatherer society (5, 6, 7).

Recent scholarship has also considered the possibility of early human-animal relationships at GöbekliTepe, including rudi-

mentary veterinary or animal-care practices (4, 8, 9). In this review, key themes and findings from the literature are compiled to illustrate the following:

- How GöbekliTepe’s architecture and art reflect ritualistic functions.
- The diversity of animal depictions and their potential symbolic connotations.
- Archaeo-faunal evidence and the suggestion of pre-veterinary or proto-veterinary practices.
- Broader implications for our understanding of early Neolithic societies.

1. Overview of GöbekliTepe and Its Significance

Discovery and Context

GöbekliTepe was first identified in the 1960s by American archaeologist Peter Benedict, though its importance was not fully realized until the 1990s excavations led by German archaeologist Klaus Schmidt (10). The site features multiple circular or oval enclosures constructed with towering T-shaped limestone pillars, some weighing several tons and quarried from nearby outcrops (3, 10, 11). The lack of domestic structures (e.g., storage rooms and clear residential units) has led many researchers to conclude that GöbekliTepe operated primarily as a ceremonial center rather than a permanent settlement (2, 3, 12).

GöbekliTepe as a Ritual Centre

Scholars emphasize that the absence of everyday artifacts or food-production installations suggests periodic gatherings for social or religious ceremonies (3, 10, 12). The enormous effort required to shape and transport the pillars indicates advanced social organization and a shared religious or cultural motivation (1, 3). Some interpret the T-shaped pillars as anthropomorphic, possibly representing deities, totemic beings, or ancestral spirits central to the beliefs of early Neolithic societies (2, 5, 13).

2. Animal Representations in GöbekliTepe's Art

Symbolism and Carving Styles

Animal motifs carved on GöbekliTepe's pillars are a prominent feature of the site, suggesting a complex symbolic repertoire. Scholars have discussed how these depictions likely encoded both cosmological beliefs and social identities (2, 5, 14). The animals most frequently identified in the carvings include:

- Snakes: Often shown in coiled or spiraling forms, frequently associated with chthonic (underworld) symbolism, regeneration, or fertility (2, 14).
- Foxes: Common in both carvings and faunal remains, pointing to possible totemic or mythic significance—perhaps as tricksters or intermediaries (5, 10).
- Wild Boar: Prominent in artwork but less abundant in bone assemblages, suggesting these animals had symbolic status beyond nutritional value (1).
- Aurochs (Wild Cattle): Notable in both art and faunal remains, likely revered for their strength and also valued as a substantial food resource (4, 9, 15).
- Cranes: Their depiction may reflect seasonal cycles or migratory patterns important to hunter-gatherers (3).
- Dodo: The idea that certain large, flightless bird carvings might represent a Dodo (*Raphus cucullatus*) has arisen in some commentary, though *Raphus cucullatus* was endemic to Mauritius and is widely believed extinct by the late 17th century

(18). Most specialists consider these carvings more plausibly local birds or vultures.

- Felids (Leopards/Lions): Emblems of power or protection, reflecting the predators' formidable presence (5, 10).
- Gazelles: Often associated with dry, open landscapes, possibly revered for speed and grace (2).
- Hemione (Asiatic Wild Ass): Indicative of open steppe environments and resource exploitation (14).
- Wild Sheep: Although not as common in the art, their occasional presence suggests a symbolic or ritual role (1).

3. Archaeofaunal Evidence: Insights from The Literature

Faunal Assemblages and Butchery

Archaeo-zoological studies of GöbekliTepe show a large number of animal bones with clear butchery marks, underscoring skilled meat processing and possibly organized feasting (10, 15, 16). Some species—like the aurochs—appear central to both subsistence and ceremonial activities, with carefully executed slaughter or cut marks that may signify sacrifice (9, 16). Fox remains in particularly high frequency could suggest their pelts or bones were utilized for tools, clothing, or ornaments (3, 5).

Pathological Findings and Possible Animal Care

Some faunal remains display signs of healed fractures or bone pathologies, indicating that animals survived injuries long enough for bones to heal—hinting at human intervention or care. Such observations have prompted suggestions of rudimentary “pre-veterinary” practices, wherein injured animals were protected to maintain their symbolic or utilitarian value (4, 8, 9). Snake venom or oils may have been used medicinally or ritually (14), and powerful animal bones (e.g., from bulls or sheep) could have been crafted into talismans or healing amulets (17).

4. Possible Pre-Veterinary Practices

Theoretical Context

While formal veterinary science would not emerge until much later in human history, evidence from GöbekliTepe alludes to early forms of animal management. This hypothesis aligns with anthropological theories on shamanic or healing roles within preliterate societies (5, 14). A caretaker or “proto-veterinarian” might have recognized the importance of tending to certain animals—be it for ritual, symbolic prestige, or practical resources such as milk, hides, or bone tools (1, 4).

Examples from the Literature

- Medical Use of Animals
 - o Snake Products: In many ancient cultures, snakes represent regeneration and healing; venom or snake oils

might have been applied in ceremonial or medicinal contexts (14).

o Bones/Skins: Strong animals (e.g., wild cattle, sheep) could be used as protective charms or therapeutic items (17).

• Animal Sacrifice in Rituals

o Selecting Healthy Specimens: Officials or spiritual leaders may have inspected animals for offerings to ensure their ritual purity (2, 16).

o Slaughter Techniques: Specific butchery methods—observed through cut marks—point to ritual protocols that also ensure the meat’s hygienic processing (1, 15).

5. Broader Impact On Prehistoric Studies

GöbekliTepe has reframed scholarly debates about the Neolithic Revolution, highlighting how monumental construction and intricate ritual frameworks could precede established agriculture (1, 6, 7, 9). The site’s emphasis on animal iconography and the possibility of early animal care challenges the traditional notion that humans viewed animals solely as sources of sustenance. Instead, it suggests a more complex and reverent relationship, merging practicality, spirituality, and communal identity (8, 9).

CONCLUSION

GöbekliTepe stands as a milestone in our understanding of early Neolithic societies, illustrating that hunter-gatherer groups could indeed undertake massive building projects and maintain intricate belief systems. The site’s extraordinary animal carvings—ranging from snakes and foxes to aurochs—reveal a vibrant symbolic world, where fauna was interwoven into the spiritual and communal fabric of daily life.

Moreover, the archaeo-faunal record indicates organized consumption, potential ritual sacrifice, and even traces of possible animal care or “pre-veterinary” intervention. While conclusive evidence of veterinary practices remains open to interpretation, the presence of healed animal bones and symbolic emphasis on certain species strongly points to early—and quite sophisticated—forms of human–animal interaction.

Future research might focus on comparing GöbekliTepe’s faunal assemblages and symbolic motifs with those of other contemporaneous sites, refining our grasp of the social, spiritual, and potentially medical significance of animals in the prehistoric Near East. By integrating archaeological, zoological, and anthropological scholarship, investigations at GöbekliTepe continue to challenge and enrich our understanding of the factors that shaped the trajectory of human civilization.

DECLARATIONS

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Abdominal Herniation of the Left Kidney in a Cat and Its Surgical Treatment: A Case Report

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ABSTRACT

A 4-year-old crossbred female cat, weighing 4.2 kg, was brought to the Animal Hospital of the Dicle University with a complaint of swelling in the left dorsolateral abdomen. Palpable, painless, and walnut-sized swelling in the left dorsolateral region of the abdomen had occurred after high-rise syndrome one year ago, guided by the anamnesis and old radiological images. As a result of the detailed clinical and radiographic examination, it was determined that osteosynthesis with cerclage wire was performed on the left corpus ilium in a private clinic, and there was an organ herniation as a swelling of the left dorsolateral region abdomen. It was decided that the case was stable according to physiological and laboratory examinations and would be removed operatively, and the surgery was planned. A skin incision was applied over the mass, the subcutaneous connective tissue was carefully cut under guidance, and the herniated kidney was examined macroscopically. There was no strangulation, and the herniated kidney was repositioned into the retroperitoneum. As a result, herniation of the kidney in abdominal dorsolateral hernias is a rare case. This case report will contribute to our colleagues in the clinical field to be more careful about abdominal hernias resulting from trauma.

Keywords: Cat, herniorrhaphy, kidney, traumatic abdominal hernia

INTRODUCTION

Traumatic abdominal wall hernias (TBWH) are defined as the protrusion of one or more abdominal organs through a defect in the abdominal wall due to trauma. The most common causes include blunt trauma, such as high-rise syndrome, traffic accidents, and ballistic or bite wounds. In cats, abdominal wall rupture is commonly known as prepubic, paracostal, inguinal, and dorsolateral (muscles torn from the transverse processes of the lumbar vertebrae) (1, 2). A comprehensive clinical examination and diagnostic imaging are essential criteria (3). Determining the etiology, abdominal wall rupture is often associated with concurrent injuries and complications (4). After initial stabilization and a thorough physical examination, laboratory tests (urinalysis, complete blood count, and measurements of total protein, albumin, and electrolytes) should be conducted to assess any potential organ dysfunction. Radiographs and ultrasound examinations can aid in evaluating

concurrent injuries, such as fractures, diaphragmatic rupture, ascites, and pulmonary contusions (5).

The most common clinical signs in abdominal wall tear cases include subcutaneous swelling, ecchymosis, or asymmetry in the abdominal contour. However, in some cases, swelling may not be noticeable until several days (up to 6 days) post-trauma when abdominal contents progress through the hernia (3, 6). Due to clinical symptoms, the veterinarian may overlook abdominal wall rupture and other injuries. In a study by Shaw et al. (2) involving 36 cats and dogs, five cases (4/26 dogs and 1/10 cats) had traumatic abdominal hernias that were not diagnosed until 24 hours after hospitalization, leading three cases to undergo orthopedic surgery without detecting abdominal defects. This highlights the difficulty of diagnosing traumatic abdominal defects in animals with multiple traumatic injuries (2).

This study aims to present the operative treatment and postoperative follow-up of a chronic case of a cat that underwent osteosynthesis for an ilium fracture in a private clinic after a fall from height but whose dorsolateral abdominal hernia remained undiagnosed.

CASE HISTORY

The material of this study was a 4.2 kg weight, 4-year-old crossbreed female cat brought to the Animal Hospital of the Faculty of Veterinary Medicine, Dicle University, with a complaint of swelling in the abdominal dorsolateral region. The patient's owner was informed about the case, and a consent form was obtained after being informed about the surgery. In the anamnesis, it was learned that she had fallen from the 5th floor 1 year ago and had an orthopedic surgery, as well as an OVH (ovariohysterectomy) 6 months ago. Since the detected swelling was in the dorsolateral part of the abdomen, it was learned that this swelling sometimes grew to the extent that it could be seen with the eye after falling from a high-rise syndrome. Still, it was usually felt by hand and rejected by palpation. While no negative findings were encountered regarding the patient in routine clinical examinations, it was determined that the swelling on the left side could be rejected by palpation. The radiographic ventro-dorsal (V/D) images showed that the kidney was herniated on the left side of the abdomen, and the osteosynthesis of the corpus ilium fracture was performed with a cerclage wire. As a result of clinical and radiographic examinations, it was observed that the fracture fragment healed despite the malunion, and there was a very slight lameness, while the hernia was seen in the radiograph (Figure 1).



Figure 1. The image taken from the patient owner from 1 year ago, the red arrow shows the herniation of the left kidney, and the blue arrow shows the osteosynthesis of the corpus ilium fracture

The case was stable according to physiological and laboratory examinations, and the chronic hernia of the preliminary diagnosis was operatively removed. In planning the surgery, the patient was subjected to 8-hour fasting and 2-hour water restriction. Before the surgery, Metacam (20 mg/ml Boehringer meloxicam) 0.5 mg/kg S/C was administered, and the surgical area was prepared aseptically. A prophylactic antibiotic, Cefazolin sodium 20-22 mg/kg I/V, was administered during the operation. Anesthesia protocol for the surgical procedure was established with medetomidine (40 mcg/kg) as a pre-anesthetic agent, propofol (3 mg/kg/IV) for induction, and isoflurane (2-2.5%) for inhalation anesthesia was completed with 100% oxygen. In addition, butorphanol tartrate, an opioid product used in routine cat and dog anesthesia, was applied at 0.2 mg/kg/IV for analgesia in pre-anesthetic anesthesia planning.

A skin incision was made on the mass, and the subcutaneous connective tissue was carefully cut under guidance. Afterward, it was realized that the herniated content was the kidney. The adhesions were carefully separated, and a detailed examination was performed to evaluate the kidney tissue macroscopically (Figure 2). We determined that there was no strangulation, and the kidney was placed in its position. The hernia hole was closed using the polydioxanone (USP: 2/0) inversion (Halstead) suture method (Figure 3). Subcutaneous



Figure 2. The incision line made on the mass and the image of the left kidney.



Figure 3. Halsted suture method used to close the area

connective tissue was closed using polyglycolic acid (USP: 2/0), and then the skin was closed with simple separate sutures using polyglycolic acid (USP: 2/0). After the surgery was performed and the hernia removed, a control X-ray was taken to check the condition of the hernia (Figure 4). For the postoperative period, amoxicillin/clavulanic acid (Synulox Pfizer) was applied at 20 mg/kg/IM for 5 days.



Figure 4. Control radiography image taken in the post-operative period

DISCUSSION

Traumatic body wall hernias (TBWH) are serious, life-threatening problems that occur after severe trauma in pets and can cause damage of varying severity in multiple areas in the same case. They usually occur as a result of blunt trauma or complications of surgical operations. Common locations of traumatic hernias include ventral hernias due to prepubic tendon rupture, dorsolateral hernias due to muscle rupture from the lateral processes of the lumbar vertebrae, and lateral hernias due to bite wounds or vehicle trauma (7-9). In the current study, the hernia is thought to occur due to muscle ruptures from the lateral part of the left lumbar vertebrae. Especially in 75% of traumatic cases after falling from high syndrome, there are other significant injuries (2, 10). In a study of 36 cases by Shaw et al. (2), abdominal hernias were diagnosed in five cases 24 hours after hospitalization. This report describes a rare case of traumatic chronic abdominal dorsolateral herniation of the kidney. In this study, clinical and radiographic evaluations and the anamnesis indicated that the hernia resulted from a fall from height over a year ago. The orthopedic surgery, conducted in a private clinic, involved osteosynthesis with cerclage wire for an ilium fracture, but the abdominal hernia remained undetected.

In a study of 10 cases of traumatic abdominal hernia in cats, 60% of the cats were male, 40% were due to bite injuries, 18% of the hernias were in the lateral paralumbal region, and the most frequently herniated organs were reported to be the omentum (50%), small intestines, and bladder (2). In the presented case, the cat was female and there was a dorsolateral hernia of the abdominal region accompanied by a corpus ilium fracture due to falling from a high-rise syndrome. It was determined that the herniated organ was the kidney, which is very rare, and there was no strangulation, incarceration, strangulation, adhesion, or loss of function due to the structure of the anatomical region of the chronic hernia.

When trauma occurs in animals, it is seen as the organ affected by the kidneys. A retrospective technique was performed by Shaw et al. (2) on traumatic wicks of the body wall in 26 dogs and 10 cats; only one case in the lines was affected in the examination, and everything could result in kidney rupture from the wounds due to deterioration. However, Andrade et al. (11) recently published a case study in which they interpreted the case kidney eventration as similar to our study and emphasized that it is very rare.

In reducible hernias, a local approach over the hernia ring is sufficient. A large skin area should be prepared aseptically because the hernia ring is usually much further away than can be estimated by palpation. Suture placement is more important than the type or pattern of suture for successful herniorrhaphy. Generous biting of healthy tissue is necessary (12). Slowly absorbable or non-absorbable mattress sutures that distribute tension are ideal (12, 13). Depending on the size and tension of the defect, muscle flaps or synthetic meshes may be used (14). In this case, a single incision over the mass was sufficient due to the anatomical location and size of the swelling, and no flap or synthetic mesh was required. Unlike most traumatic hernias, the herniated kidney was within the incision boundaries, eliminating the need for incision extension. Examination of the herniated kidney showed no lacerations, hematomas, torsion, or infarction, negating the need for nephrectomy.

As a result, it should not be forgotten that, especially in patients exposed to multiple trauma, in addition to orthopedic or neurological disorders, conditions such as abdominal wall hernia may also occur. In addition, in abdominal hernias, the herniated organ is often the omentum, intestines, urinary bladder, or uterus. At the same time, in rare cases, it has been observed that the kidney may also herniate. Therefore, it was thought that this case report of surgical treatment results would contribute to clinical practice.

DECLARATIONS

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author (NS).

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Ethical Statement: The necessary information about the operation was given to the patient's relative and a consent form was obtained.

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