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Research Article

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-RI1 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-RI1 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 Kruskall-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

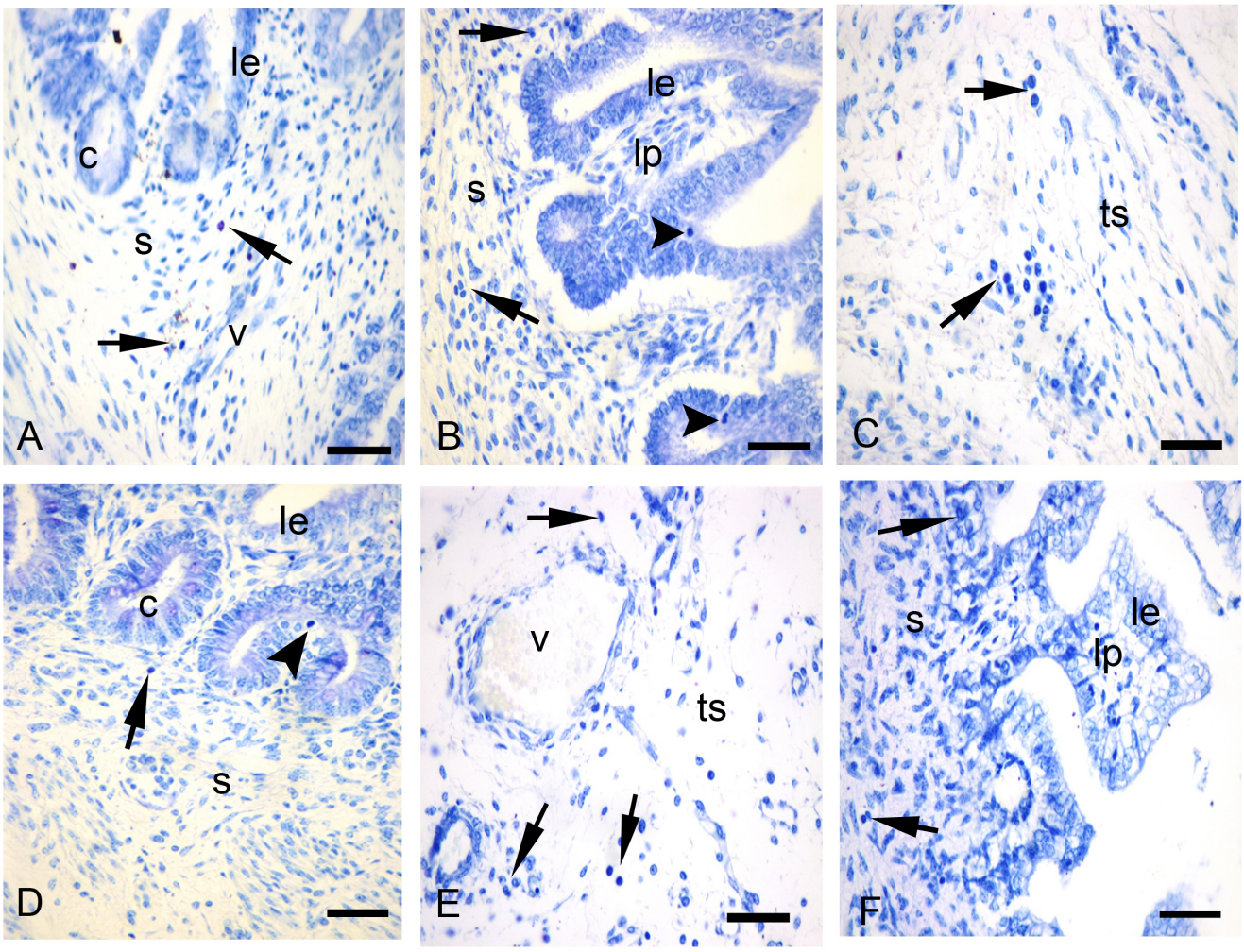


Figure 1. Localization of mast cells in the lamina propria, submucosa and tunica serosa layers of the small (A-C) and large intestine (D-F) sections (Scale Bar = 25 µm). TB (+). Distribution of mast cells the lamina epithelialis (arrowhead), lamina propria (arrow), submucosa (arrow), tunica serosa (arrow) and around of blood vessels (arrow) of the duodenum (A), jejunum (B), ileum (C), cecum (D), colon (E) and rectum (F) during pregnancy. le; lumianl 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, $p<0.05$).

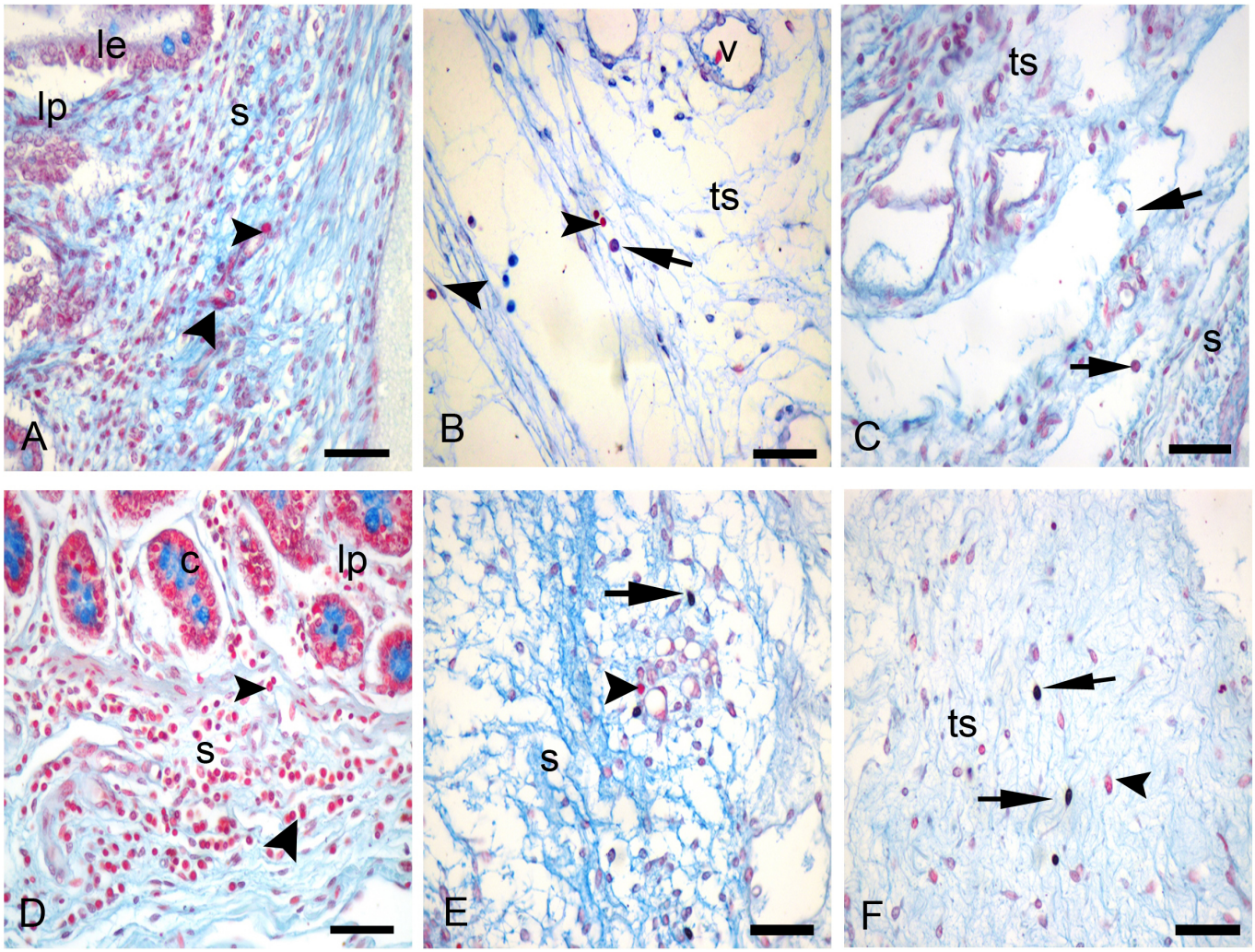
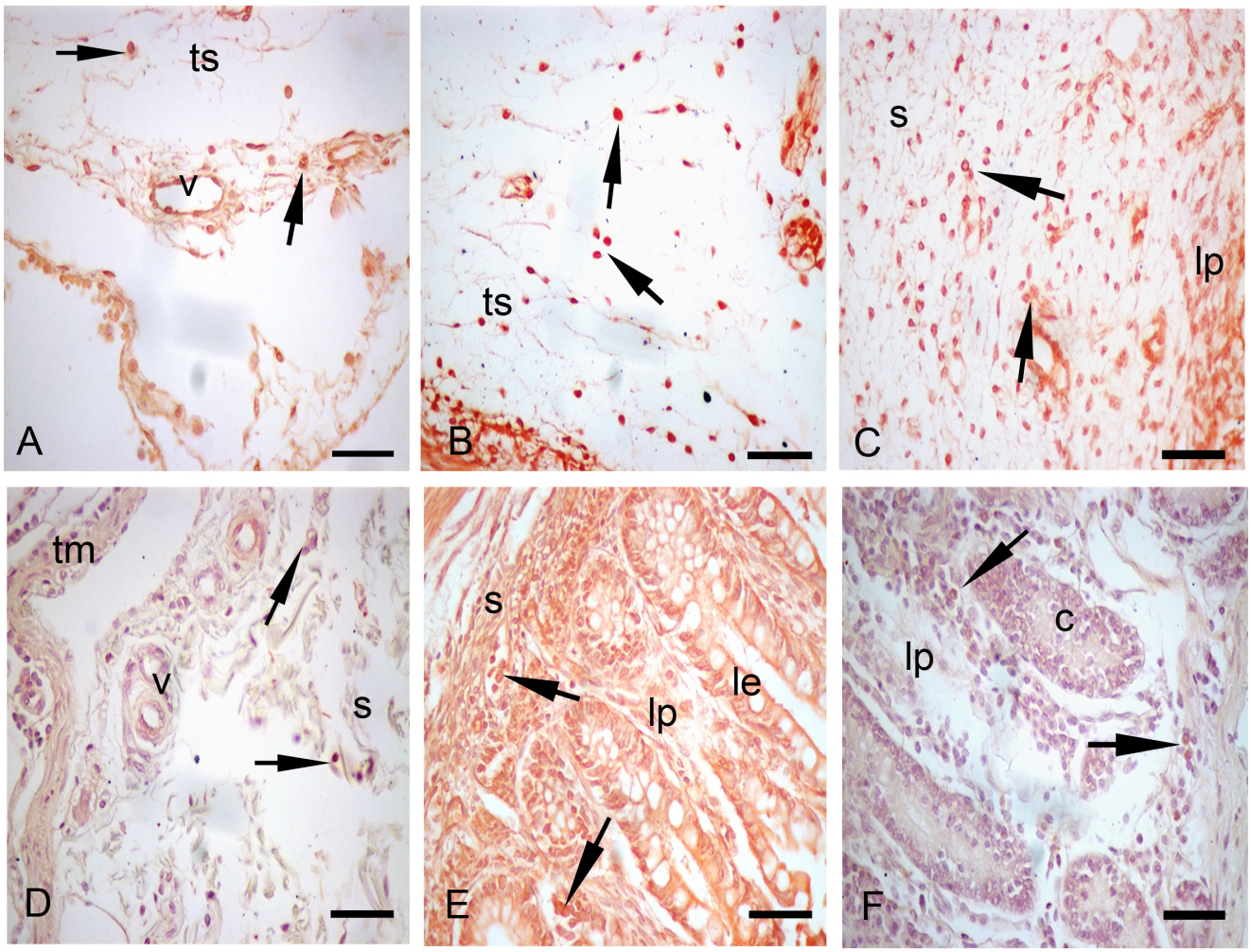


Figure 2. Localization of mast cells in the lamina propria, submucosa and tunica serosa layers of the small (A-C) and large intestine (D-F) sections (Scale Bar = 25 µm). AB/SO (+). Distribution of mast cells the lamina epithelialis, lamina propria, submucosa, tunica serosa and around of blood vessels 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 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 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 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



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 µm). 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.

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

Intestinal Segments	Histological Layers	Sections	Fetal Gestation Periods		
			1st period	2nd period	3rd period
Small Intestine	Lamina propria	Duodenum	0.50±0.58[a]	1.0±0.0	1.33±0.52[b]
		Jejunum	0.67±052	1.17±0.41	1.50±0.55
		İleum	0.83±0.41[a]	1.33±0.52	2.33±0.52[b]
	Submucosa	Duodenum	0.67±0.52	0.67±0.52	1.46±0.52
		Jejunum	0.83±0.41	1.16±0.75	1.50±0.55
		İleum	0.83±0.75[a]	1.17±0.75	2.50±0.84[b]
	Tunica mucosa	Duodenum	1.33±1.03[a]	1.66±0.52	2.66±0.52[b]
		Jejunum	1.33±0.89	1.33 ±0.52	2.23±0.82
		İleum	1.17±0.75[a]	1.67±0.52	2.67±0.82[b]
	Around of blood vessels	Duodenum	0.93±0.75[a]	2.0±0.63	2.50±0.84[b]
		Jejunum	1.21±0.41	1.67±0.82	2.13±0.82
		İleum	1.17±0.75[a]	1.50±0.55	2.67±0.82[b]
Large Intestine	Lamina propria	Cecum	0.50±0.55	0.67±0.52	1.0±0.63
		Colon	0.67±0.52	0.83±0.41	1.17±0.41
		Rectum	0.67±0.52	0.67±0.521	1.17±0.41
	Submucosa	Cecum	0.67±0.52	0.83±0.41	1.17±0.75
		Colon	0.83±0.41	1.0±0.0	1.33±0.52
		Rectum	0.67±0.52	1.0±0.0	1.33±0.52
	Tunica mucosa	Cecum	0.83±0.41[a]	1.0±0.63	2.17±0.75[b]
		Colon	1.0±0.0[a]	1.17±0.41	2.16±0.98[b]
		Rectum	1.0±0.63[a]	1.33±0.52	2.16±0.75[b]
	Around of blood vessels	Cecum	1.0±0.0[a]	1.17±0.75	2.17±0.98[b]
		Colon	1.0±0.0	1.33±0.52	1.83±1.16
		Rectum	1.17±0.75	1.50±0.55	2.0±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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