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RESEARCH ARTICLE

Distribution and heterogeneity of mast cells in different regions of the genital canal in female quails during the prepubertal and postpubertal periods

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Puberte öncesi ve sonrası dönemlerde dişi bıldırcınların genital kanalının farklı bölümlerindeki mast hücrelerinin dağılımı ve heterojenitesi

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Öz

Amaç: Bu çalışmada dişi bıldırcınlarda *(Coturnix coturnix Japonica)* puberte öncesi ve sonrası dönemlerde genital kanalın farklı bölgelerinde yer alan mast hücrelerinin heterojenitesi ile dağılımının farklı tespit solüsyonları ve boyama yöntemleri ile ortaya konulması amaçlanmıştır.

Gereç ve Yöntem: Yapılan çalışmada 10 adet puberte öncesi ve 10 adet puberte sonrası dönemde sağlıklı dişi bıldırcınlara ait genital kanaldan (infundibulum, magnum, istmus, uterus, vajina) alınan doku örnekleri materyal olarak kullanıldı. Her iki gruptan alınan doku örneklerinin bir kısmı %10'luk nötral formaldehit, bir kısmı ise izotonik formaldehit asetik asit tespit solüsyonunda tespit edildi. Dokulardan alınan kesitlere Toluidin mavisi ve alcian blue/safranin O boyama yöntemleri uygulandı.

Bulgular: Hazırlanan preparatlar ışık mikroskopta incelendiğinde her iki tespit solüsyonunda da yuvarlak, oval ya da mekik şekilli mast hücreleri özellikle kan damarları çevresinde yerleşmiş olarak genital kanalın tunika mukoza, tunika muskularis ve tunika seroza katmanlarında gözlendi. Mast hücrelerinin hem puberte öncesi hem de puberte sonrası dönemde en çok uterusta yerleştiği; ardından sırasıyla istmus, magnum, vagina ve infundibulumda gittikçe azalan yoğunluk gösterdiği gözlendi. Mast hücrelerinin infundibulumda puberte sonrası dönemde puberte öncesi döneme göre önemli oranda ($p<0,05$) arttığı belirlendi. İFAA ile tespit edilen tüm dokularda mast hücrelerinin daha belirgin boyandığı ve sayısal olarak değerlendirmede de %10'luk nötral formaldehit tespitine göre daha fazla olduğu gözlendi.

Öneri: Sonuç olarak özellikle puberte sonrası dönemde infundibulumda mast hücre sayısının arttığı; bu artışın yumurta oluşumu ve yumurtlamaya yönelik etkisinin olabileceği kanaatine varıldı.

Anahtar kelimeler: Bıldırcın, genital kanal, mast hücre dağılımı.

Abstract

Aim: This study aimed to determine the distribution and heterogeneity of mast cells located in different parts of the genital tract of female quails (*Coturnix coturnix japonica*) during the prepubertal and postpubertal periods using different fixation solutions and staining methods.

Materials and Methods: For this purpose, tissue samples were taken from the genital tract (infundibulum, magnum, isthmus, uterus, vagina) of healthy female quails, including 10 prepubertal and 10 postpubertal animals. The tissue samples of both groups were fixed in either 10% neutral formaldehyde or isotonic formaldehyde acetic acid solution. The tissue sections were stained with the toluidine blue and combined alcian blue/safranin O staining methods.

Results: The light microscopic examination of the tissue sections treated with both fixatives demonstrated that mast cells were round to oval or spindle-like in form and localized especially around blood vessels in the tunica mucosa, tunica muscularis and tunica serosa layers. Mast cells were found to be distributed primarily in the uterus during the prepubertal and postpubertal periods; followed in descending order by the isthmus, magnum, vagina and infundibulum. It was determined that mast cell counts significantly increased in the infundibulum in the postpubertal period ($p<0.05$) compared to the prepubertal period. Fixation in IFAA was associated with a more prominent staining and higher count of mast cells in all tissues, compared to fixation in formaldehyde.

Conclusion: The number of mast cells in the infundibulum was determined to have increased in the postpubertal period, and this increase was considered to may affect egg formation and egg-laying.

Keywords: Genital canal, mast cell distribution, quail.

Introduction

Mast cells are resident immune cells of the connective tissue, which show critical effects against allergic diseases and IgE-dependent allergic factors (Skaper et al 2001). They are involved in hypersensitivity reactions, wound healing and various conditions including fibrosis. These cells are large in size with a diameter of 20-30 micrometers (µm), have an oval nucleus and contain heavily stained basophilic granules. Electron microscopic observations have shown that there are many microvilli and folds on the surface of mast cells. A small amount of granular endoplasmic reticulum, mitochondria and advanced Golgi devices are noted in the cytoplasm. Mast cells show strong metachromasia due to the heparin and sulfate proteoglycans contained in their granules (Ergün 2016). Mast cells are observed in the connective tissue of various structures and organs, especially in the mucous membranes of the skin, gastrointestinal tract and respiratory system, all which are associated with the external environment, as well as on the brain side of the blood-brain barrier and in the leptomeninges (Erpek 2004, Silver and Curley 2013, St John 2013, Da Silva et al 2014, Espinosa and Valitutti 2017). Due to their strategic localization to the periphery of blood vessels and nerve endings, mast cells can quickly interact with other hematopoietic effector cells (Krystel-Whittemore et al 2016). Although mature mast cells are normally not present in the bloodstream, they migrate to tissues as mast cell progenitors under the influence of the stem cell factor (SCF) and other cytokines. These cells, which complete their development under the influence of microenvironmental tissue factors, need the SCF, which binds to the C-kit receptor, in order to survive and develop (Molderings 2010).

Mast cell research (Özkorkmaz 2008, Uslu and Yörük 2013, Yıldız et al 2016) has revealed that these cells are either formaldehyde-sensitive or formaldehyde-resistant. As formaldehyde-sensitive mast cells are mostly located in the mucous membranes, they are referred to as mucosal mast cells (MMCs), whilst formaldehyde-resistant mast cells are located in connective tissue and are referred to as connective tissue mast cells (CTMCs) (Özkorkmaz 2008). To distinguish the heterogeneity of these two subtypes of mast cells, the terminology proposed by Enerback for the rat intestine is used. Accordingly, mucosal mast cells are distributed particularly in the lamina propria of the gastrointestinal tract and respiratory mucosa, whilst connective tissue mast cells are localized to the submucosa of the peritoneum, skin and digestive tract (Welle 1997). Mucosal mast cells have a smaller diameter (5-10 µm) and contain fewer granules than mast cells located in other organs. Connective tissue mast cells are larger (10-20 µm) and found in the skin, the periphery of blood and lymph vessels, and the serosa of visceral organs (Karaca and Yörük 2005).

While many detailed studies are available on the number and distribution of mast cells in the mammalian uterus, very little work has been done on the presence and heterogeneity of mast cells in the genital canal of poultry species. In this study, it was aimed to investigate the heterogeneity and distribution of mast cells in the different regions of the genital canal of female quails during the prepubertal and postpubertal periods.

Material and Methods

Material

The study material comprised of tissue samples taken from different regions of the genital canal (infundibulum, magnum, isthmus, uterus, vagina) of healthy prepubertal and postpubertal female Japanese quails (*Coturnix coturnix japonica*) raised at a private farm in Konya.

Method

Tissue samples were taken after the quails were sacrificed by decapitation. Part of the tissue samples were fixed in 10% neutral formaldehyde, while the remaining were fixed in isotonic formaldehyde acetic acid (IFAA, (40 ml formaldehyde, 100 ml distilled water, 0.5 ml glacial acetic acid); pH 2.9). After 12 h of incubation, the samples were kept in 70% alcohol for 12 h without being washed, and were subsequently submitted to routine histological processing (Enerback 1966a). Five-micrometer (µm) serial sections were cut from the paraffin blocks. The sections, prepared for determining the distribution and number of mast cells in the genital canal, were stained for 30 minutes (Harem et al 2011) in a 0.5% toluidine blue solution (pH 0.5). The sections used for determining the heterogeneity of mast cells and the distribution of mast cell subtypes in tissues were cut from blocks of tissue fixed in either 10% neutral formaldehyde or IFAA. All sections were stained with the combined Alcian blue/Safranin O (AB/SO) staining method (Enerback 1966b).

Cell quantification

The distribution of mast cells during the prepubertal and postpubertal periods was determined by counting cells in randomly selected 10 different areas in the tunica mucosa, tunica muscularis and tunica serosa of toluidine blue-stained tissue samples from different regions of the genital canal (infundibulum, magnum, isthmus, uterus, vagina). Cell counts were performed in an area covering 100 square ocular micrometers. The arithmetic mean of the mast cell counts of the different serial sections was calculated. Thereby, the mean number of mast cells in an area covered by 100 square

ocular micrometers was determined. All data calculated for an area of 100 square ocular micrometers under 40x magnification using a micrometric slide were converted into mast cells in a unit area of 1 mm2 (Uslu and Yörük 2013). Before moving onto statistical analysis, data were tested for parametric test assumptions.

Statistical analyses

The Kolmogorov-Smirnov test was used to assess the normality of data distribution. The statistical significance of the differences determined for the number of mast cells in the tissues fixed in IFAA and 10% formaldehyde was determined by the Wilcoxon signed test. Differences between the prepubertal and postpubertal quails for the mast cell counts of the tissue sections from the infundibulum, magnum, isthmus, uterus and vagina were determined by the t-test, taking into account the fixatives used. All statistical analyses were evaluated with a minimum margin of error of 5%. The SPSS v22 statistical software package was used for statistical analyses (SPSS 2013).

Results

In the tissue sections prepared from the different regions of the genital canal of prepubertal and postpubertal female quails, mast cells were observed in oval, round and polygonal shapes of different sizes, which varied with their localization (Figure 1). The nuclei of the mast cells were mostly located in the center of the cell, often masked by the granules. In the tissue samples, which were fixed in IFAA, a more prominent metachromatic staining and greater number of mast cells were observed, compared to those fixed in 10% formaldehyde (Table 1). Mast cells were observed in the tunica mucosa, tunica muscularis and tunica serosa of the sampled genital canal regions, mostly in the connective tissue. While mast cells showed perivascular localization in the submucosa and tunica serosa (Figure 2, Figure 4 and Figure 6), they were observed as individual cells in the lamina propria (Figure 3) and as groups of cells in the subepithelial region.

In the preparations examined, it was determined that the oviduct of the female quail consisted of 5 parts: the

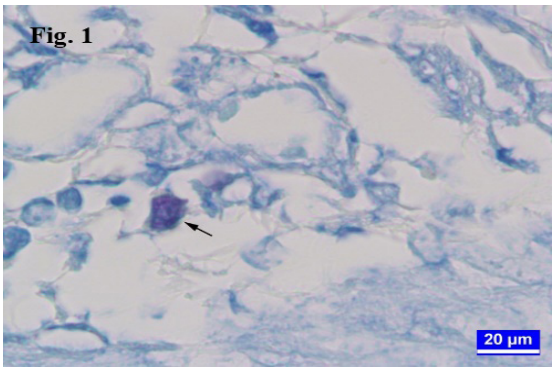

Figure 1. A section of the postpubertal isthmus tissue fixed in IFAA. Arrow: mast cell in the lamina propria. Toluidine blue staining

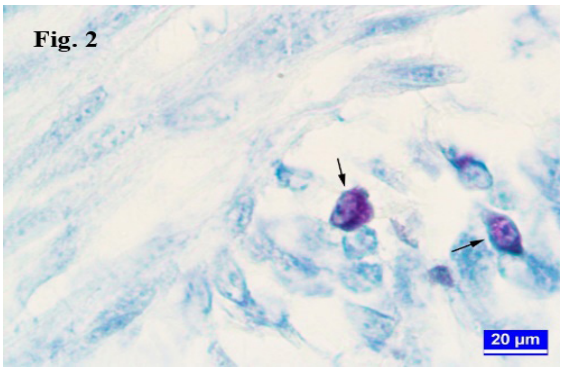

Figure 2. A section of the postpubertal uterine tissue fixed in IFAA. Arrows: mast cells in the tunica muscularis. Toluidine blue staining

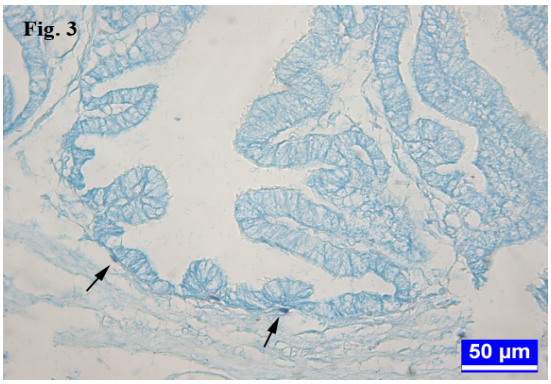

Figure 3. A section of the postpubertal infundibulum tissue fixed in 10% neutral formaldehyde. Arrows: mast cells in the lamina propria. Toluidine blue staining

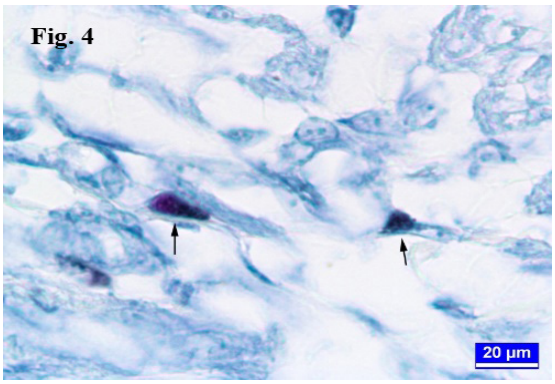

Figure 4. A section of the postpubertal vaginal tissue fixed in IFAA. Arrows: mast cells in the submucosa. Toluidine blue staining

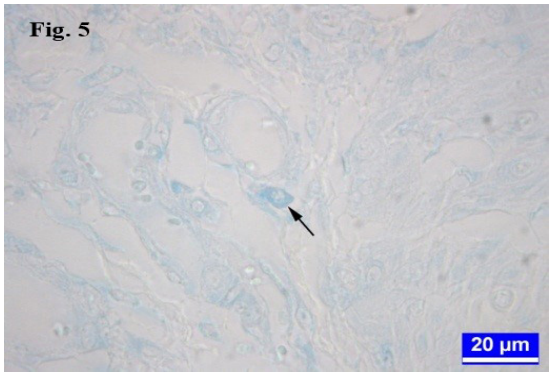

Figure 5. A section of the postpubertal infundibulum tissue fixed in IFAA. Arrow: AB (+) mast cell in tunica muscularis. Alcian blue/Safranin O staining

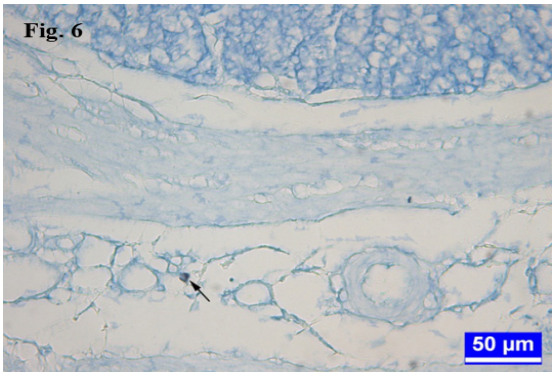

Figure 6. A section of the postpubertal magnum tissue fixed in IFAA. Arrow: mast cells in the submucosa. Toluidine blue staining.

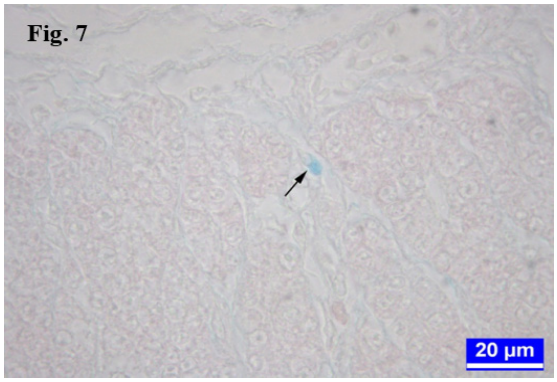

Figure 7. A section of the postpubertal magnum tissue fixed in IFAA. Arrow: mast cell in the lamina propria. Alcian blue/Safranin O staining

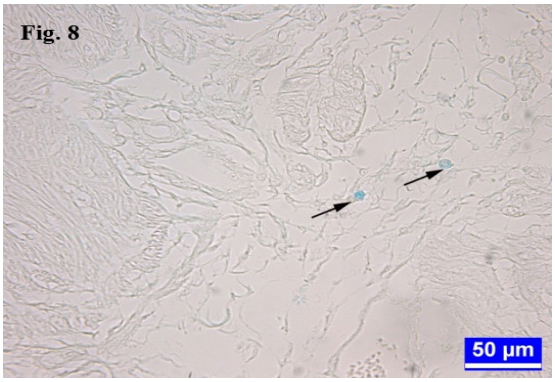

Figure 8. A section of the postpubertal uterine tissue fixed in IFAA. Arrows: AB (+) mast cells in the tunica muscularis. Alcian blue/Safranin O staining

Table 1. Mast cell counts in genital tissue sections fixed in IFAA and 10% neutral formaldehyde during the prepubertal and postpubertal periods

	IFAA		10% Formaldehyde	
	Before Puberty	After Puberty	Before Puberty	After Puberty
Infundibulum	3,60±2,22^{cB}	8,00±3,09^{cA}	0,50±0,70^{cC}	0,20±0,63^{cC}
Magnum	12,1±1,37^{bA}	12,0±4,02^{bcA}	1,10±0,31^{cB}	1,2±1.03^{bB}
Isthmus	12,9±4,04^{bA}	14,60±6,38^{bA}	2,70±1,49^{aB}	1,10±1,37^{bB}
Uterus	20,3±3,80^{aA}	19,00±7,93^{aA}	2,20±1,03^{bB}	2,10±1,59^{aB}
Vagina	10,0±3,16^{bA}	12,70±2,35^{bA}	1,90±0,56^{bB}	0,90±0,87^{cB}

Groups with different superscripts in the same column$^{(a,b,c)}$, and in the same row$^{(A,B,C)}$ differ significantly ($p<0,05$)

infundibulum, magnum, isthmus, uterus and vagina. Combined AB/SO staining was performed to determine the heterogeneity of the mast cells in the different regions of the genital canal. AB (+) mast cells were localized to the periphery of the blood vessels in the lamina propria, and to the subepithelial region, and were greater in number than the SO (+) (Figure 5 Figure 7 Figure 8).

Data pertaining to mast cell counts in the IFAA- and formaldehyde-fixed tissues from the different regions of the prepubertal and postpubertal genital canal are presented in Table 1. Accordingly, mast cell numbers in the infundibulum ($p<0.05$), isthmus and vagina were higher during the postpubertal period, compared to the prepubertal period. Mast cell counts were low in the magnum and uterus during both periods.

Discussion

While mast cells have traditionally been known as the leading cells for type 1 allergic reaction (Harvima and Nilson 2011); today, many researchers believe that mast cells are part of the external and acquired immune system. These cells are reported to be found in all tissues, blood vessels, mucous membranes of the skin, and the respiratory, digestive and urogenital systems, which are associated with the external environment (Weller et al 2011, Espinosa and Valitutti 2017, Ertuğrul 2022). Proteins, epithelial barriers, dendritic cells, macrophages, natural killer cells and mast cells constitute the main components of natural immunity (Rehman et al 2017). According to some researchers, mast cells, which are considered leukocytes, originate from hemopoietic progenitor cells in the bone marrow (Mirjam et al 2012, Wernersson and Pejler 2014, Siebenhaar et al 2018). Immature mast cells, which pass into the blood circulation, migrate to tissues transendothelially, under the influence of microenvironmental factors in the tissue to which they are localized (Gurish and Austen 2012, Dahlin and Hallgren 2015, Siebenhaar et al 2018).

To date, studies have mostly focussed on the number and distribution of mast cells in the mammalian genital canal (Karaca et al 2007, Kurum et al 2014, Özen et al 2014). To our knowledge, the presence and heterogeneity of mast cells has not been investigated before in the genital system of poultry. Thus, the data obtained in the present study was discussed in comparison to studies on mast cell distribution in the genital system and various organs of animal species other than poultry.

Mast cells are oval, have a centrally located round to oval nucleus, and contain metachromatic granules in their cytoplasm. The mast cell nucleus is usually covered by metachromatic granules found in their cytoplasm (Shao-Heng 2004, Molderings 2010). In the present study, it was observed that the morphological structure of the mast cells in all regions of the genital canal was consistent with literature reports, and these cells were distributed in the tunica mucosa, tunica muscularis and tunica serosa. Furthermore, it was observed that mast cells in the tissues fixed in IFAA displayed a more prominent metachromatic staining, such that those in tissues fixed in 10% neutral formaldehyde were slightly paler. Studies in chickens (Ertuğrul 2012) and cattle, sheep and goats (Ertuğrul and Kurtdede 2017) have demonstrated that mast cells show strong metachromatic staining with the use of both IFAA and neutral formaldehyde for tissue fixation. In previous studies on the digestive and respiratory systems and various tissues, including the skin, of quails (Chen et al 1990, Karaoglu et al 2010, Harem et al 2011), it was observed that mast cells produced metachromasia with the use of toluidine blue. Mast cell granules are metachromatically stained with toluidine blue and azure A methylene blue, owing to their sulfate proteoglycan content (Enerback 1966b, Eşrefoglu 2009). In our study, mast cells showed metachromasia in all regions of the genital canal during both the prepubertal and postpubertal periods.

In our study, tissue samples from the different regions of the genital canal of prepubertal and postpubertal female quails were fixed in either 10% neutral formaldehyde or IFAA. The histological examination of these tissue sections revealed that, in both groups, mast cells were localized mainly to the lamina propria and tunica serosa, in the periphery of blood vessels and nerves. Mast cells localized to these regions were either oval or round. Some researchers (Eşrefoğlu 2009, Karaca and Yörük 2005, Krystel-Whittemore et al 2016) have reported mast cell localization to areas with dense connective tissue, including the tunica mucosa and tunica serosa.

The present study demonstrated that the number of mast cells was higher during the postpubertal period. Tissue fixation with IFAA was found to be more effective in determining the increased population of mast cells (Table 1). Our findings also showed that the mean number of mast cells per unit area of the tissues was significantly higher in tissue specimens fixed in IFAA, compared to those fixed in 10% neutral formaldehyde (Table 1). Similarly, combined staining with AB/SO to determine mast cell heterogeneity in the genital tract of the female quail produced a higher number of AB (+)/SO (+) cells in the tissue samples fixed in IFAA. In tissue samples fixed in neutral formaldehyde (10%), mast cell granules displayed a paler staining with AB/SO. Moreover, we determined that mast cells were greater in number during the postpubertal period in tissue samples from all regions of the genital tract fixed in both IFAA and 10% neutral formaldehyde (Table 1) Traditionally, mast cells are classified according to their tissue localization and granular content. In humans, mast cells are classified as MC(T) and MC(TC) according to their tryptase and chymase content. In rodents, the classification of mast cells is based on the heparin content of granules. While mast cells containing heparin in their granules are classified as connective tissue or serosal mast cells (CTMCs), those with less or no heparin in their granules are classified as mucosal mast cells (MMCs) (Frossi et al 2018). Several studies have reported that the heterogeneity of mast cells can be determined according to their response to different fixatives (Chen et al 1990, Karaca et al 2007, Özen et al 2014). The staining characteristics of mast cell granules vary with the fixation solutions used. Special fixation solutions such as IFAA, Bouin's solution and Carnoy's solution are needed to demonstrate mucosal mast cells and connective tissue mast cells that are resistant to fixation with neutral formaldehyde (10%) (Enerback 1966a). In the present study, the combined AB/SO staining method was used to determine mast cell heterogeneity in the

genital tract of the female quail. In result, AB (+)/SO (+) mast cells were observed in the infundibulum, magnum, isthmus, uterus and vagina. These cells were mostly distributed in the lamina propria, in the periphery of blood vessels, and in the subepithelial region. In their study on the lung tissue of pubertal quails, Harem et al (2011) reported the presence of AB (+) cells throughout the lung tissue and SO (+) mast cells around large blood vessels in the connective tissue. Another study on the porcine oviduct (Özen et al 2014) reported the presence of AB (+)/SO (-) mast cells throughout the oviduct. Studies on the bovine uterus (Eren et al 1999) and oviduct (Özen 2002) demonstrated AB (+)/SO (-) mast cells in these organs. In their study on the ovine oviduct, Kurum et al (2014) reported the presence of AB (+)/SO (-) mast cells. In the present study, AB (+)/SO (+) mast cells were distributed in all regions of the quail oviduct. Accordingly, AB (+) MMCs were present in all 5 parts of the quail genital tract, as reported by Enerback (1966a, 1966b). The results of the present study were found to be consistent with the findings of Özen et al (2014) and Eren et al (1999).

In the genital canal sections fixed in IFAA, the highest number of mast cells was determined in the uterus during the prepubertal period, followed in descending order by the magnum, vagina and infundibulum (Table 1). In the tissue sections fixed in neutral formaldehyde (10%), the same descending order was determined (Table 1). In postpubertal tissue sections fixed in IFAA and 10% neutral formaldehyde, the highest number of mast cells was determined in the uterus, followed in a descending order by the isthmus, magnum, vagina and infundibulum (Table 1). In a study on the distribution of mast cells and histamine in the chicken oviduct, mast cells and histamine were most numerous in the infundibulum (Hrabia et al 2001). The primary location of mast cells in the duck and chicken oviduct was reported to be the infundibulum by Valsala et al (1986) and Wight and Mackenzie (1970), respectively. These mast cells were reported to show a subepithelial localization between the mucosal folds. The findings of the present study are consistent with literature data, excluding those determined for the infundibulum.

The mean number of mast cells in the infundibulum was 3 during the prepubertal period and 8 during the postpubertal period ($p<0.05$) (Table 1) Studies in chickens (Wight and Mackenzie 1970, Hrabia et al 2001) and ducks (Valsala et al 1986) showed that mast cells were most numerous in the infundibulum section of the oviduct. The significant increase detected in the number of mast cells in the infundibulum during the postpubertal period may be an indication that the infundibulum significantly contributes to egg formation.

It was ascertained that the number of mast cells was highest in the uterus and differed significantly from other tissue sections both during the prepubertal and postpubertal periods. In a study conducted by Aydin et al (1998) in rats, the number of mast cells was determined to be higher in the ovaries and uterine endometrium, particularly during the estrus phase of the sexual cycle. Karaca et al (2007) reported that the number of mast cells was higher in the uterus during the estrus and metestrus stages in rats. Valle et al (2009) showed that, in heifers, the number of mast cells was significantly higher during metestrus. Considering that ovulation occurs during the metestrus phase in cows and rats, it can be said that the number of mast cells increases during the period of ovulation in both species, and the oviduct shows significant changes during the passage of the egg cell. Similarly, the increase detected in the number of mast cells during the postpubertal period and at the stage of ovulation in the present study may indicate that mast cells have a significant role in the function of the oviduct during the formation and passage of the egg.

Conclusion

It is considered that our findings on the distribution and heterogeneity of mast cells in the female quail genital tract during the prepubertal and postpubertal periods will provide input for future research on mast cells in poultry.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Ethical Approval

The Ethics Committee approved the study procedure of Kyrgyzstan-Türkiye Manas University, Animal Experimentation Ethics Committee (Date: 15/09/2015 and Decision no: 2015/4).

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