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RESEARCH ARTICLE

Investigation of Mast Cell Distribution in the Ovine Oviduct During Oestral and Luteal Phases of the Oestrous Cycles [1]

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Abstract

Mast cells are heterogeneous cell populations that play significant roles in many organs and systems and involve various physiological processes. We aimed to evaluate mast cells in the ovine oviduct mucosa by means of their staining and ultrastructural characteristics. The ovine oviduct samples of Akkaraman breed were collected from the slaughterhouse and they are categorized as luteal and oestral phases. They were fixed either with 10% formalin or IFAA and stained with Toluidine blue and Alcian blue and Safranin O (Ab/SO). Mast cells were located near blood vessels and basal membrane. Compared to 10% formalin fixed tissues, the number of mast cells were higher in IFAA fixed tissues (P=0.003). Importantly all mast cells Ab(+) and SO(-) so that they were categorized as mucosal type. The number of mast cells did not differ between luteal and oestral phases (P>0.05). However, there were significant differences among different regions of the oviduct with a less count in the isthmus regions (P=0.006). Transmission electron microscopy revealed that the oviduct mast cells contained two types of granules: an electron lucent, electron dense. Some electron lucent granules contained an eccentrically located crystal-like structure. The significance of less mast cell counts in the isthmus and the eccentrically located single crystal-like structure should be further investigated in future studies.

Keywords: *Mast cell, Oviduct, Ovine, Sexual cycle, Electron microscopy*

Östral ve Luteal Dönemlerdeki Koyunların Oviduktunda Mast Hücrelerinin İncelenmesi

Özet

Mast hücreleri birçok organ ve sistemdeki çeşitli fizyolojik süreçlerde önemli rolleri bulunan heterojen hücre topluluğudur. Bu çalışmada koyun ovidukt mukozasındaki mast hücrelerini boyanma özellikleri ve ince yapı düzeyinde incelemeyi amaçladık. Mezbahanede kesim sırasında östral ve luteal dönemleri Akkaraman koyunlarının oviduct örnekleri alındı. Alınan örnekler %10 formol ve IFAA ile tespit edilerek Toluidin blue ve Alcian blue-Safranin O (Ab/SO) ile boyandı. Mast hücrelerinin, kan damarlarının ve ovidukt epitelinin bazal membranına yakın olarak yerleştiği görüldü. IFAA ile tespit edilen dokulardaki mast hücre sayısının %10 formolle tespit edilen dokulara göre daha fazla olduğu belirlendi (P=0.003). Dikkat çekici olarak mast hücrelerinin tamamının Ab(+) ve SO(-) olduğu görüldü. Luteal ve östral dönemler arasında mast hücre sayısı yönünden fark bulunamadı (P>0.05). Fakat ovidukt bölgeleri arasında istatistiksel olarak önemli fark vardı ve mast hücre sayısı istmusta daha azdı (P=0.006). Elektron mikroskobik incelemelerde mast hücrelerinin elektron açık ve elektron koyu olmak üzere iki tip granül içerdiği ortaya konuldu. Bazı elektron açık granüllerde eksantrik yerleşimli elektron koyu kristal benzeri bir yapının varlığı gözlendi. İstmusta mast hücrelerinin daha az sayıda görülmesinin ve granüllerdeki eksantrik yerleşimli kristal benzeri yapının daha sonraki çalışmalarda araştırılması önemlidir.

Anahtar sözcükler: *Mast hücresi, Ovidukt, Koyun, Seksüel siklus, Elektron mikroskop*

INTRODUCTION

Mast cells are connective tissue cells that contain numerous basophilic granules in the cytoplasm and exhibit strong metachromasia due to heparin and highly sulfated proteoglycans present in their granules [1].

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Taking origin from the bone marrow, mast cells circulate in the blood stream without presence of granules and subsequently migrate to connective tissue sites and then differentiate to mature types that begin synthesis of specific granules [2]. Based on staining feature, size, and location, mast cells are classified, especially in rodents, into two groups: atypical mucosal mast cells (MMC) and typical connective tissue cells (CTMC) [3]. While MMCs are found especially in the lamina propria of the gastrointestinal (GI) tract and respiratory canal, CTMCs are found in the peritoneum, skin, and submucosa of the GI tract [4]. Mast cells can also be classified based on protease contents of the granules. The first type only contains tryptase such that MMCs are categorized in this category. The second type contains chymase carboxypeptidase and catepsin in addition to tryptase such that CTMCs are categorized in this category. The third type contains chymase and carboxy peptidase [5]. Such proteases directly affect tissues in which they are located [6]. Under influence of various mediators released into vicinity of connective tissue, mast cells can differentiate to each type, from MMC to CTMC and CTMC to MMC [4].

Mast cells are commonly found in connective tissues of the several organs and they play critical roles in hypersensitivity reactions and bacterial inflammations [4]. In addition, mast cells have critical roles in angiogenesis, inflammation and tissue regeneration [7]. Located near vasculature, numerous mast cells are found in skin and mucosa. In addition their critical roles in innate immunity, they also have roles in required immune response [8]. Among the mast cell granule contents are histamine and heparin, which are known to induce vascularization and endothelial cell proliferation [4].

In the reproductive tract, mast cells contribute to cellular immune response and to formation of the anti-bacterial barrier [7]. It is known that heparin is physiologically important for sperm capasitation [9]. It has been proclaimed that histamine inhibits cytotoxic lymphocyte activity [10]. In turn, reproductive hormones estrogen and progesterone can activate mast cells through receptors [11]. In response to estrodiol, mast degranulate and release a variety of bioactive substance. For instance, an *in vitro* study showed that estrodiol increases histamine release from rat mast cells [7]. Histamine increases the capillary permeability in the ovarium during ovulation. Increase in blood flow and vascular leakage result in edema in the oviduct [12].

Hormonal changes during sexual cycle may influence mast cell metabolism as they respond to sexual hormones. The mast cell involvement in physiology of the female reproductive tract and hormonal influence on mast cells as well as cascade of events following mast cell degranulation are still of a scientific interest [11]. The oviduct is an important region of the reproductive tract for being the fertilization sites. Mast cells are considered as important components of the reproductive physiology as bioactive molecules contained in granules directly involved in several physiological events such as vasodilatation and vasoconstrictions, which are very common during the sexual cycle. In support to this notions, histamine and 5-hydroxytriptamine (5-HT), commonly found in mast cells granules, are present in high concentration in the rat oviduct [12]. The sheep is an economically important domestic animal and, thus, we aimed to investigate mast cell distribution and ultrastructural characteristic of the ovine mast cells present in the oviduct.

MATERIAL and METHODS

The oviduct samples were collected from the Kazan Slaughterhouse. The ovine oviduct samples were collected from 14 sheep of Akkaraman breed, 7 samples representing oestral phase and 7 samples representing luteal phase of the sexual cycle. The phase of the sexual cycle was determined based on macroscopic evaluation of the ovarium [13] and RIA [14] test to determine progesterone concentration on blood samples collected at slaughter.

For light microscopic evaluation, the oviduct samples were further divided into three parts: fimbria, ampulla and isthmus. Such subregions were divided into two pieces and one piece was fixed in 10% formalin and the other was fixed in isotonic formaldehyte acetic acid (IFAA). Following routine histological procedure, all samples were embedded in paraffin blocks [3]. From the paraffin blocks, 5 µm thick two consecutive sections were cut for Toluidine blue and Safranin O staining. Such a double consecutive sectioning was repeated with a 30 µm interval for 10 times. One section was stained with 0.5% Toluidine blue (pH 4) prepared in Mc Ilvaine's citric acid disodium phosphate buffer and the other section was stained with Alcian blue/ Safranin O (Ab/SO) [15,16]. On a same slide, an IFAA fixed and a 10% formalin fixed sample were placed and stained. For staining positive control, the rat intestine and rat skin were used for MMC and CTMC, respectively.

For Transmission Electron Microscopy, a modified Karnovsky's method was followed [17,18]. Briefly, tissues were pre-fixed in gluteraldehyte-paraformaldehyte (pH 7.4) for 24 h, washed in cocdylate buffered for 3 h, and further fixed in 1% osmic acid for 2 h. Tissues were then kept in 0.5% uranyl acetate for 2 h, in graded alcohol, and propylene oxide and then embedded in Araldite M. The 300-400 Angstrom thick sections cut from these blocks were contrasted according to the Veneable and Coggeshall [19] methods and evaluated using the Carl Zeiss EM 9S-2 model transmission electron microscopy.

Mast cells in the lamina propria of the oviduct were counted in the Toluidine blue stained sections according to previously used methods [18,20]. Briefly, using an 100 -square ocular micrometer (eye piece graticule), mast cells were counted in per unit at the 40x objective. For each

section, 10 randomly selected areas were counted. Then all counted data of per unit area were converted into the number of mast cells in 1 mm^2 area.

Data processing was performed with the SPSS 15.0 (SPSS, Inc., Chicago, IL, USA). The normality of all data was assessed by Shapiro-Wilk Test. To compare the IFAA fixation with formol fixation, Mann Whitney U Test was used. The difference between oestral and luteal phase within the same oviduct region were assessed with either Mann Whitney U Test or Student T-test. The numerical distribution (mm^2) of mast cells with %10 formol fixation in the various regions of the oviduct during the luteal and oestral phases was evaluated by Kruskall-Wallis analysis of variance. *Post hoc* comparisons were performed using *Mann-Whitney U test with Bonferroni corrected*. Other parameters were analyzed by One-way analysis of variance (ANOVA). When the *F* values were significant, Duncan's Multiple Range Test was performed. *P* values less than 0.05 were considered as significant for all statistical calculations; however, in *Mann Whitney U test with Bonferroni corrected*, P value less than 0.016 was considered as significant.

RESULTS

Mast cells were generally localized to vessel surroundings and near the basal membrane *(Fig. 1A)*. Mast cells in Toluidine blue stained sections were observed oval in shape with metachromatically stained cytoplasmic granules and easily distinguishable nucleus *(Fig. 1B)*. Metachromasia was more prominent in the IFAA fixed tissues.

Mast cells were present in various tunics of the oviduct; however, we only counted those in the lamina propria. In general, IFAA fixed oviduct samples exhibited a higher number of mast cells compared to formalin

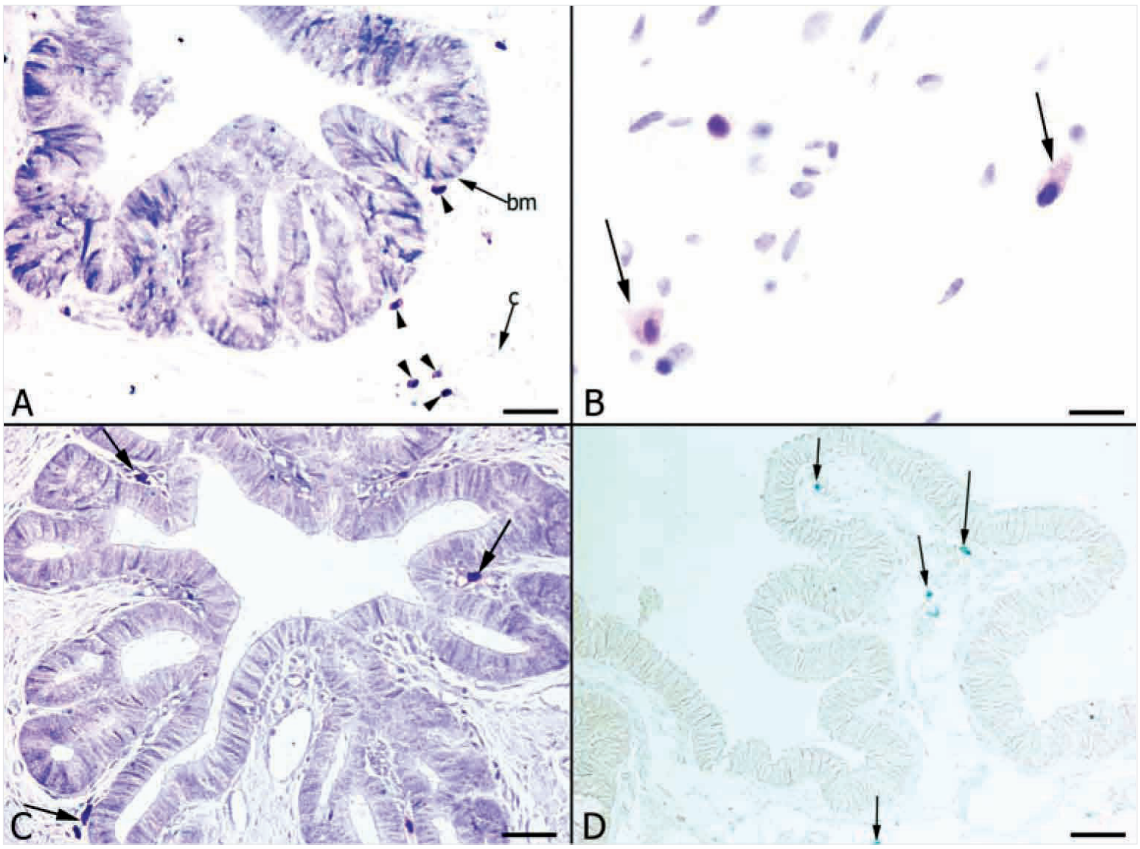

Fig 1. Light microscopic views of the ovine oviduct mast cells. **A-** Mast cells *(arrow heads)* are located around capillaries (c) and near the basal membrane (bm) as exemplifies in this ampulla region, **B-** Ovine oviduct mast cells *(arrows)* in higher magnification obtained in the fimbria, **C-** In the isthmus, the number of mast cells *(arrows)* is limited, but well illustrated in IFAA fixed samples, **D-** As exemplified this section of the fimbria, all oviduct mast cells in alcian blue/safranin O staining (Ab/SO) are Ab(+) and SO(-) A: oestral phase, IFAA fixation, B: luteal phase, 10% formalin, C: oestral phase, IFAA fixation, and D: oestral and 10% formalin fixation. Toluidine blue staining (A, B and C) and Alcian blue/Safranin O staining (D). Bar=120 µm in A and C, 40 µm in B and 160 µm in D

Şekil 1. Koyun ovidukt mast hücrelerinin ışık mikroskopik görüntüsü. **A-** Mast hücreleri *(ok başları)* kapillerlerin çevresinde (c) ve bazal membran (bm) yakınlarında görüldü, ampulla, **B-** Koyun ovidukt mast hücrelerinin *(oklar)* daha yüksek büyütmedeki görüntüsü, fimbriya, **C-** İstmusta mast hücre sayısı *(oklar)* sınırlıydı, ancak IFAA ile tespit edilen dokularda daha iyi belirlendiler, **D-** Resimdeki fimbriya bölgesinde görüldüğü gibi tüm ovidukt mast hücreleri Ab(+) ve SO (-) boyandı. A: östral dönem, IFAA tespiti, B: luteal dönem, %10 formol tespiti, C: östral dönem, IFAA tespiti, D: östral dönem, %10 formol tespiti. Toluidine blue boyaması (A, B ve C) and Alcian blue/Safranin O boyaması (D). Bar A ve C'de 120 µm, B'de 40µm ve D'de 160 µm

fixed oviduct samples, both in oestral and luteal phase (P=0.003) *(Table 1)*. No statistically significant difference was detected between oestral and luteal phase within the same oviduct regions and same fixative (P>0.05). The number of mast cells per counted area in different regions of the oviduct for luteal and oestral phases was presented in *Table 2* and *Table 3*.

In formalin fixed samples *(Table 2)*, there were significant differences among different regions of the oviduct both luteal and estral phases (P=0.006). The isthmus has significantly less mast cell counts compared to the other regions *(Fig. 1C)*. The ampulla tends to have a higher number of mast cells compared to the fimbria, but difference is not significant (P>0.05).

Table 1. *The numerical distribution (mm²) of mast cells in the ovine oviduct segments during sexual cycle (oestral and luteal phases): comparison of IFAA and 10% formalin fixation*

Tablo 1. *Seksüel siklusta (östral ve luteal dönem) ovidukt bölümlerindeki (mm²) mast hücrelerinin sayısal dağılımı: IFAA ve %10 formolde karşılaştırılması*

Parameter	IFAA Fixation	10% Formol Fixation	P value
Relative number of mast cells	88±9.11*	50.67±6.47	0.003

*The data were expressed as mean ± standart error. * There was a significantly difference between the groups within the same row*

Table 2. *The numerical distribution (mm²) of mast cells in the various regions of 10% formol fixed ovine oviduct samples collected during the luteal and oestral phases*

Tablo 2. *%10 formalinle tespit edilmiş östral ve luteal dönemlerdeki koyun ovidukt örneklerinin farklı bölgelerindeki (mm²) mast hücrelerinin sayısal dağılımı*

Phase of the Oestrous Cycle	n	Ampulla	Isthmus	Fimbria	P value
Luteal phase	7	82.29±11.80[a]	11.43±2.95[b]	61.71±15.78[a]	0.006
Oestral phase	7	82.29±15.39[a]	11.43±11.43[b]	54.86±9.14[a]	0.006

Data were analyzed with Kruskal Wallis test. Data were given as mean ± standard error. Mean values within the same row with different superscripts letters ([a, b]) are significantly different

Table 3. *The numerical distribution (mm²) of mast cells in the various regions of IFAA fixed ovine oviduct samples collected during the luteal and estral phases*

Tablo 3. *IFAA ile tespit edilmiş östral ve luteal dönemlerdeki koyun ovidukt örneklerinin farklı bölgelerindeki (mm²) mast hücresinin sayısal dağılımı*

Phase of the Oestrous Cycle	n	Ampulla	Isthmus	Fimbria	P value
Luteal phase	7	134.86±24.90[a]	29.71±6.47[b]	105.14±18.76[a]	0.002
Oestral phase	7	102.86±21.48	57.14±19.08	98.29±19.26	NS

The data were analyzed with One way ANOVA. Data were given as mean ±standart error. Mean values within the same row with different superscript letters ([a,b]) are significantly different, NS: Not significant

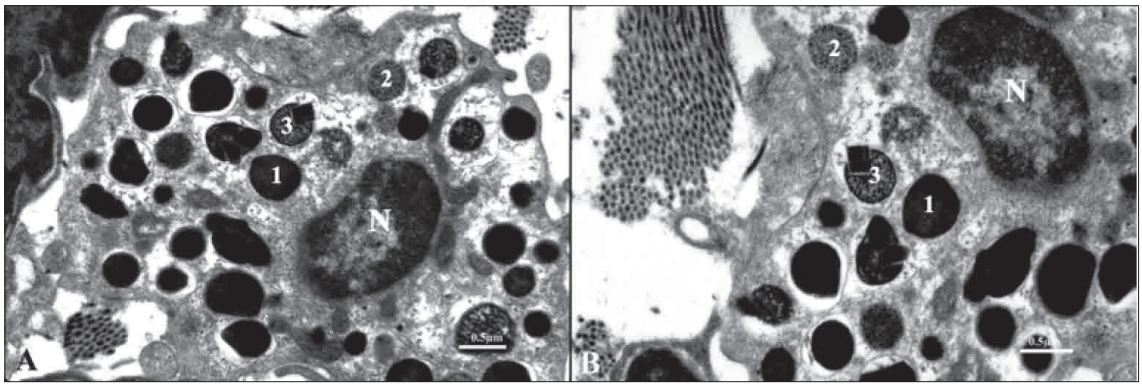

Fig 2. Transmission electron microscopic features of the ovine oviduct mast cells. **A-** Mast cells have an electron dense (1) and an electron lucent (2) granules. Some electron lucent granules have an eccentrically located crystal-like structure (3). **B-** The higher magnification of the mast cell granules. Luteal phase, isthmus (A,B)

Şekil 2. Koyun ovidukt mast hücrelerinin elektron mikroskobik özellikleri. **A-** Mast hücreleri elektron koyu (1) ve elektron açık (2) granüller içermektedir. Bazı elektron açık granüller eksantrik yerleşimli kristal benzeri yapı taşımaktadır (3). **B-** Mast hücre granüllerinin daha yüksek büyütmedeki görüntüsü. Luteal dönem, istmus (A,B)

In IFAA fixed samples *(Table 3)*, there were significant differences among different regions of the oviduct during the luteal phase (P=0.002). Similar to formalin fixed tissues of the luteal phase, the isthmus has significantly less mast cell counts compared to the other regions and the ampulla tends to have a higher number of mast cells compared to the fimbria, but difference is not significant (P>0.05). On the other hand, the regional differences during oestral phase was insignificant although the isthmus considerably less mast cell counts (P>0.05).

In Ab/SO stained tissues, mast cells Ab(+) and SO(-) in all regions of the oviduct in both oestral and luteal phase samples *(Fig. 1C)*.

In transmission electron microscopic evaluation, the oviduct mast cells exhibited two types of cytoplasmic membrane bound granules: electron dense, electron lucent granules *(Fig. 2A,2B)*. Some electron lucent granules had an eccentrically located crystal structure *(Fig. 2A,2B)*.

DISCUSSION

Mast cells execute numerous biological activities due to a wide range of bioactive molecules they contain in granules [4]. Mast cells with different types of granules may locate in different regions of the body. Based on their fixation and histochemical staining characteristics, mast cells were classified especially in rodents into two categories: MMC and CTMC [3]. The MMC granules contain chondroitin sulfate and little histamine contents; on the other hand, the CTMC granules contain heparin and higher amount of histamine content. MMCs are resistant to metachromatic staining when fixed in formalin based fixatives [4,21]. They can better preserve staining features in Carnoy's fixatives and fixatives containing acetic acid and low concentrated formalin and they are best stained with cationic dyes [6]. Due to formalin sensitivity of MMCs, Toluidine blue can stain well both MMCs and CTMCs in IFAA fixed tissues [3]. Mast cells studies conducted on the bovine uterus [22] and canine skin [23] indicated that the mast cell counts in IFAA fixed tissues were higher. Similarly, the results the present study indicated that the IFAA fixed ovine oviduct of Akkaraman breed had a higher number of mast cells per counter area in all regions. Thus, we also think that ovine mast cells exhibited some sensitivity to formalin. In the present study, we presumed that both types of mast cells (MMC and CTMC) were stained with toluidin blue in IFAA fixed ovine oviduct tissues. However, in AB/Safranin O staining, another staining technique used in differentiation between MMC and CTMC [16], we did not find any evidence regarding CTMC presence as all samples were Safranin O negative. It has been used to observe mast cell heterogeneity in various species. For instance, in bovine oviduct and ovarium [18,24] and uterus [22] mast cells in AB/Safranin O staining were AB(+) and SO(-). Similarly, the AB/SO combined technique to determine mast cell heterogeneity in the goat reproductive tract resulted in AB(+) and SO(-) [25]. Mast cells in the ovine respiratory system were AB(+) reactive [26]. In the present study, mast cells in the ovine oviduct were determined as AB(+) and SO(-). Such staining characteristics in the present study indicate that mast cells in the ovine oviduct are MMCs and CTMCs are either absent or limited in number. Furthermore, the ovine oviduct was similar to the bovine and caprine reproductive systems by means of mast cell staining feature. In the mean time, one should not forget that each mast cell type interchanges phenotypically to another type [4]. In our study, IFAA fixation did not affect AB/SO staining as all mast cells in formalin and IFAA fixed tissues were AB(+) and SO(-).

The oviduct is an import part of the genital tract as it is the place for fertilization and fosters embryo during embryogenesis [27]. As reported by two previous studies [10,24], the number of mast cells increases in the bovine oviduct mucosa especially in the isthmus during the luteal phase of the cycle. On the other hand, the number of mast cells increases in the bovine ovarium during the oestral phase compared to luteal phase [18]. Another study [22] reported that the number of mast cells in the cow endometrium increased during the luteal phase of the cycle. In the present study, the number mast cells in the lamina propria of the ovine oviduct did not change significantly between luteal and oestral phases of the cycle. However, there were significant differences among the oviduct regions. The isthmus had a less number of mast cell counts. As the isthmus serves as a sperm reservoir prior to fertilization, the significance of less mast cell counts in the isthmus should be investigated with respect to sperm deposition and microphysiology of the region.

Electron microscopic studies revealed that mast cells in the bovine endometrium [22], oviduct [24] and ovarium [18] contain two types of granules, one of which contains thin particular granules and the other one contains homogenous granules. Similar types of granules have been reported in mast cells found in the ovine respiratory system [26]. In addition to these two types, a third type has been defined, an intermediate type, between the former two types by means of electron density [26]. We classified the ovine oviduct mast cell granules into two categories: an electron dense and electron lucent. Notably, an eccentrically located electron dense crystal-like structure with well define edges was located in some of the electron lucent granules. It is known that the electron density and physical characteristics of the granule contents of mast cells granules is related to biochemical properties of mast cell granules, in which a number of bioactive molecules are entrapped [26]. Crystals with different shapes have been reported on human mast cells [28]. The significance of the eccentrically located crystal-like structure in the ovine mucosal mast cells should be further investigated.

In conclusion, mast cells in the lamina propria of the oviduct are classified as MMCs since they are AB(+) and SO(-). They are generally located near blood vessels. Mast cells are also found near the basal membrane. The number of mast cells in the lamina propria of the oviduct is not different between luteal and oestral phases. However, there are significant differences among different regions of the oviduct with a less count in the isthmus regions, significance of which should be investigated. The oviduct mast cells contain two types of granules: an electron lucent, electron dense. Some electron lucent granules contain an eccentrically located crystal-like structure. Such crystal-like structure should be further investigated for their content and biological significance.

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