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RESEARCH ARTICLE

Distribution and density of mast cells in the bovine reproductive tract during the follicular and luteal phases

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Özet

Saruhan BG, Sağsöz H, Akbalık ME. Östrus siklusunun folliküler ve luteal fazlarında inek genital sistemindeki mast hücrelerinin dağılımı ve yoğunluğunun belirlenmesi.

Abstract

Saruhan BG, Sagsoz H, Akbalik ME. Distribution and density of mast cells in the bovine reproductive tract during the follicular and luteal phases.

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Amaç: Dişi cinsiyet hormonlarının mast hücre davranışları üzerine etkilerinin olduğu bilinmektedir. Bu düşünceden yola çıkılarak, ineklerde genital kanalda östrus siklusu boyunca histokimyasal teknikler kullanılarak mast hücrelerinin dağılımları incelenmiştir.

Aim: Female sex hormones have long been suspected to have an effect on mast cell (MC) behavior. Based on this idea, we determined MC content in reproductive tract throughout the estrus cycle by using histochemical techniques.

Gereç ve Yöntem: Sunulan çalışmada, yerel bir kesimhaneden elde edilen 23 adet sağlıklı hayvan kullanıldı. Buna göre hayvanlar foliküler (n:13) ve luteal (n:10) faz olmak üzere 2 gruba ayrıldı. Doku örnekleri FA solüsyonunda tespit edildi. Daha sonrasında alınan 5-6 µm kalınlığında kesitlere mast hücrelerini belirlemek için Toluidin Blue (TB) ve Alcian Blue/Safranin O (AB/SO) boyama metotları uygulandı.

Materials and Methods: Genital tracts of 23 healthy cows were collected from a local slaughterhouse. The animals were classified into two groups as follicular phase (n=13) and luteal phase (n=10). The tissue samples were taken and fixed in formaldehyde-alcohol solution (FA). Then, histological sections of 5-6 µm thickness were prepared and stained by toluidin blue and alcian blue/safranin O methods.

Bulgular: TB ile boyanan kesitlerde, mast hücrelerinin metakromazi gösterdiği izlendi. Mast hücreleri bütün genital organların yüzeyel epitelinde, ovaryumun korpus luteum ve teka internasında yerleşmemişti. Genel olarak, tüm gruplarda mast hücrelerinin kan damarlarına yakın yerleştiği saptandı. AB/SO kombine boyama metodu uygulandığında mavi [AB(+)], kırmızı [SO(+)] ve karışık renkli [AB/SO(+)] olmak üzere üç tip mast hücre popülasyonunun olduğu gözlendi. Östrus siklusunun foliküler ve luteal fazlarında mast hücre sayılarının değişken olduğu belirlendi (P<0.05).

Results: Mast cells (MCs) demonstrated metachromatic staining properties with toluidine blue. MCs were not observed in the follicle of theca interna, and corpus luteum of the ovary and, surface epithelium of the reproductive organs. MCs generally associated with blood vessels in all samples. Three types of cells, including AB() cells with blue cytoplasm, pink-red coloured SO (+) cells and blue-pink coloured AB/SO(+) cells were indicated by AB/SO staining in the reproductive organs. There was a difference in the number of MCs between the follicular and luteal phases of the estrus cycle (P<0.05).

Öneri: Sonuç olarak çalışmamızda inek üreme sisteminde mast hücrelerinin östrus siklusunun foliküler ve luteal fazlarında histokimyasal ve morfolojik farklılıklar gösterdiği ortaya konuldu. Fertiliteyle ilgili fonksiyon bozukluk mekanizmalarının ortaya konulabilmesi için, genital sistemin tüm bölümlerinde özellikle mast hücrelerinin içerik ve yapısına ilişkin daha ileri çalışmaların sürdürülmesi tavsiye edilmektedir.

Conclusions: This study showed histomorphometrical changes of mast cells in the bovine reproductive tract in both follicular and luteal phases of estrus cycle. We suggest that further studies related to structures and granular contents of MCs should be sustained in all parts of genital systems in order to understand possible roles of MCs in mechanisms of unknown causes of infertility.

Anahtar kelimeler: İnek, mast hücresi, genital organlar, histokimya

Keywords: Cow, mast cell, genital organs, histochemistry

Introduction

Mast cells are granular cells that reside in the connective tissue. They originate from the CD34+ multipotent progenitor stem cells in the bone marrow and circulate in the blood as precursor cells (Benoist et al 2002). Mast cells then located within the skin and the mucosal membranes lining the digestive, respiratory, urinary and genitals systems in the males and females. The mast cells particularly the ones residing in close to the blood vessels and lymphatic vessels perform certain physiological functions (Karaca et al 2007). Mast cells can be activated via various pathways and their activation induces the release of a wide spectrum of mediators such as histamine, heparin, proteases, enzymes, cytokines, chemokines, growth factors, arachidonic acid metabolites, reactive oxygen and nitrogen species (Moon et al 2010). The release of these substances stored in the granules of the mast cells triggers vascular dilatation, chemotaxis of eosinophils and basophils, increases vascular permeability, and impairs integrity of vascular wall (Dong et al 2012, Zierau et al 2012).

The histamine released by the mast cells residing in the female genital tract is shown to play a critical role in follicular development and ovulation through regulating blood flow in the ovary (Aydın et al 1998, Özen et al 2007). Heparin is reported to contribute to thecal and luteal vascularization (Nakamura et al 1987). In addition, the mast cells are disclosed to be part of the cellular immune response of the genital tract and they are also stated to play a potential role in remodeling of the cervix with the effect of ovarian steroids at birth (Karaca et al 2008).

There are studies published concerning histochemical features and quantitative distributions of the mast cells residing in the female genital tract during the estrus cycle in the rat (Karaca et al 2007), mice (Padilla et al 1990), hamsters (Brandon et al 1983), opossums (Mahoney et al 2002) and cows (Likar 1964, Özen et al 2002, Valle et al 2009). The quantitative distributions of the mast cells are shown to alter particularly with respect to the estrus cycle at the different parts of the female genital tract and even among the different layers of the genital organs (Reibiger and Spanel-Borowski, 2000, Karaca et al 2007, Özen et al 2007). Nevertheless, recent studies indicate that mast cell activities are regulated by sex hormones and chiefly the estrogen is revealed to stimulate increased histamine release in the uterine tissue by affecting the mast cells (Welle et al 1997, Aydın et al 1998).

While the roles of the mediators released by the granules of the mast cells are well known in allergic and inflammatory reactions, their physiological roles are not well established in the female genital tract (Moon et al 2010). In addition, there are conflicting reports regarding the heterogenity of the mast cells and their quantitative distributions in the female genital tract during the estrus cycle. Therefore, in the present study we aimed to evaluate quantitative distributions, morphological and histochemical features of the mast cells in addition to their physiological roles in the female genital tract during the estrus cycle.

Materials and Methods

Animals and samples

This study, we benefited from the tissue blocks in our archive and in our previous studies used (Project number: 06-VF-13, 2007). We obtained 23 reproductive tracts from Holstein cows that were kept in a local slaughterhouse where the tissues of the animals were removed immediately after their sacrifice. All the animals were free of genital diseases. Blood samples were also collected from the animals before their sacrifice to measure estrogen and progesterone levels. The blood serum concentrations of estradiol- 17β (DRG Intl. Co., Inc. DRG Aurica Elisa Estradiol Kit, cat. no. EIA-2693, Marburg/Germany) and progesterone (DRG Intl. Co., Inc. DRG Aurica Elisa Progesterone Kit, cat. no. EIA-1561) were measured using commercially available measurement kits. The stage of the sexual cycles in the animals was determined through morphologic evaluations of the ovaries, histological appearance of uterine epithelium and glands along with monitoring the blood serum hormone levels. Accordingly, the animals were classified into two groups as follicular phase (n=13) and luteal (n=10) phase. Female reproductive tract (ovary, oviduct, uterus, cervix and vagina) samples were fixed in FA for 18h at room temperature, and then they were dehydrated, cleared and embedded in Paraplast for the histochemical determination of mast cells. Two slides were prepared from each staining of each animal. The slides were previously screened and standardized and each slide contained a minimum of 5µm thick 4 serial sections, taken at least 50µm apart.

Histochemical techniques

Toluidine blue staining protocol

In this protocol, 0.5 g Toluidine blue powder (TB-pH: 0.5) was dissolved in 100 mL of 0.5 N HCl (pH: 0.5). The sections were stained in the TB solution for 30 min, washed in distilled water for 2 min, dehydrated in graded alcohol, cleared in xylene, and mounted by Entellan, a water-free mounting medium (Enerback 1966). Binding of Toluidine Blue to sulphated mucopolysaccharides via ionic linkages results in the metachromatic staining of mast cell granules (Bancroft et al 1990).

Combined alcian blue-safranin O (AB/SO) protocol

In this protocol; 0.9 g Alcian blue powder, 0.045 g safranin O and 1.2 g ferric ammonium sulphate were dissolved in 250

mL of acetate buffer (pH: 1.4). The sections were stained in the AB/SO solution for 15 min, washed in distilled water for 2 min, dehydrated in tertiary butyl alcohol, cleared in xylene, and mounted by Entellan (Enerback 1966). Alcian blue-tetrakis (methylpyridinium) chloride (Sigma, Cat. No. A4045, 90% dye content) was used. In the combined AB/SO staining procedure, Alcian blue, a copper phthalocyanine dye, reacts with "carboxylated" and "O-sulphated" GAGs, while safranin reacts with "N-sulphated" GAGs (Combs 1965). In the current study, the mast cells were classified into three groups according to their staining features with AB/SO as follows: AB (+) mast cells that showed only blue granules, SO (+) mast cells that showed only red granules, and mixed staining-AB/SO (+) mast cells that showed both blue and red granules.

Counting of mast cells

The number of the mast cells was determined using four different sections prepared. Two independent researches (B.G.S and H.S.) counted the mast cells on the sections. The count of the mast cells residing around ovarian cortex, medulla, and vessels, oviduct (infundibulum, ampulla, and isthmus), uterus, and cervix in addition to vaginal stroma, muscles layers, and vessels was completed. The count of individual mast cells was performed at higher magnification (X40 objective). The counting of individual mast cells was performed at higher magnification (X40 objective, ovaries, uterus, cervix and vagina 0.3 mm2 per field, oviduct 0.2 mm2 per field), 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 the NIS Elements D Imaging Software (Microvision, Evry, France) (Pansrikaew et al 2010). Each slide was examined at least twice by the same researcher after an interval of 2 weeks. Finally, all the counts were converted to number of mast cells per unit area (mm2).

Statistical analysis

The data obtained were analyzed using 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 follicular and luteal phase or between the different regions of mast cells. The Mann–Whitney U test was used to determine if particular different regions of mast cells were significantly different from one another. Correlation analyses were performed using Pearson analysis. Differences were interpreted as significant for $P < 0.01$ and $P < 0.05$.

Results

General characteristics of mast cells

Mast cells of various sizes and appearances were observed in the sections stained with TB and AB/SO. Their shapes ranged from oval-round to flat spindle-like. In general, no intraepithelial mast cells were encountered throughout the female genital tract (Figures 1A-E). Similarly, we noted no TB (+) or AB/SO (+) mast cells in the theca interna of the ovary and corpus luteum during the follicular and luteal phases (Figures 2A-B). The granules of the TB (+) mast cells showed metachromatic staining and the number of the TB (+) cells was higher in the follicular phase than the luteal phase throughout the genital tract except for the ovary (Table 1). By contrast, the number of the TB (+) mast cells was greater in the luteal phase than the follicular phase in the ovary (Figures 2C-D). Besides, rather numerous TB (+) mast cells were observed within the longitudinal muscle layer of the myometrium (Figure 3A). We determined that while the number of the AB (+) mast cells was higher during the follicular phase than the luteal phase, the number of the SO (+) and mix (+) mast cells was more plentiful during the luteal phase (Table 1). Particularly, AB (+), SO (+), AB/SO (+) mast cells were condensed in the stroma of the cells and around the vessels (Figures 3B-C). However, no AB (+) mast cells were documented in the muscular layer of the cervix during the luteal phase (Figure 3D). Besides, while no SO (+) mast cells were encountered in the stroma of the cells and around the vessels of the ampulla (Figure 4A), rather extensive number of mast cells was noted in all three layers of the vagina during the luteal phase (Figures 4B-C). Degranulated mast cells were also observed sporadically in entire of the female genital tract (Figure 4D).

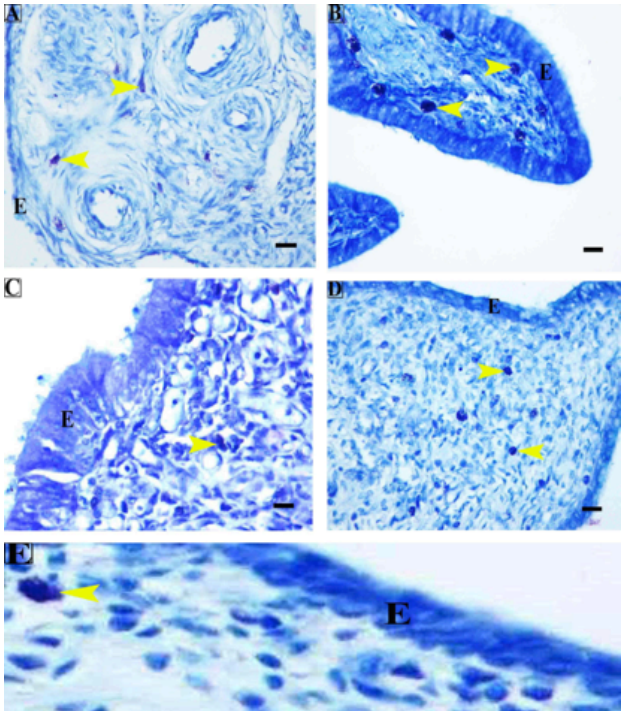

Figure 1. There were no mast cells in the luminal epithelium of the reproductive organs. (A) Ovary, (B) Tuba uterina, (C) Uterus, (D) Cervix, (E) Vagina. (E) luminal epithelium, TB (+) mast cells (arrowheads). TB staining, Bar: 12.5 µm.

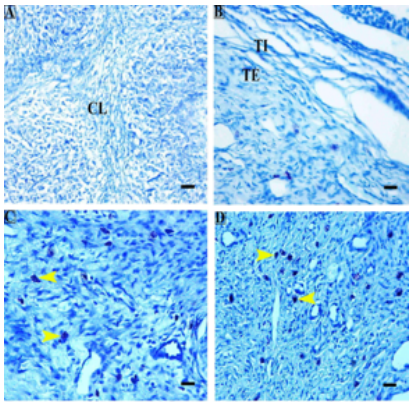

Figure 2. Mast cells never found in the theca folliculi interna and corpus luteum; however, they were observed in the theca externa of follicles. (A) Corpus luteum (CL), (B) Theca folliculi interna (TI), theca externa (TE). Mast cells in the ovarian cortex, (C) follicular phase, (D) luteal phase, TB (+) mast cells (arrowheads). TB staining, Bar: 12.5 µm.

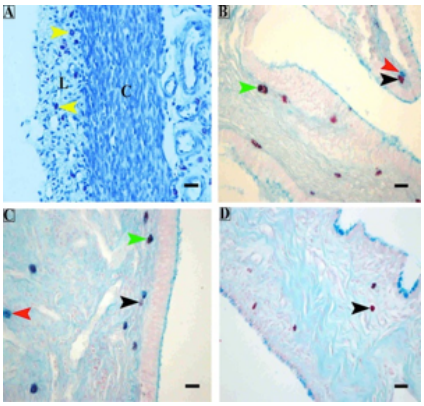

Figure 3. Mast cells (yellow arrowheads) in the myometrium. (A) Circular layer (C), longutudinal layer (L), TB staining. The appearance of mast cells by staining with AB/SO. (B) Tuba uterine, (C) Uterus, (D) Cervix, black arrowhead: SO (+), red arrowhead: AB (+), green arrowhead: mix reaction. Bar: 12.5 µm

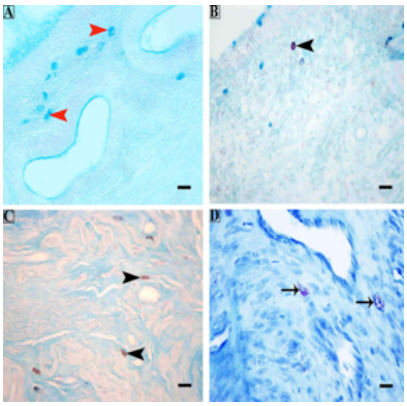

Figure 4. (A) AB (+) mast cells (red arrowhead) in the ampulla of the luteal phase. SO (+) mast cells (black arrowhead) in stroma (B) and surrounding of blood vessels (C) in the vagina. (D) The appearance of the degranulated mast cells (arrows) in bovine reproductive organs. Bar: 12.5 µm

Variational distributions of mast cells

The total numbers of the mast cells observed in the bovine reproductive tract throughout the estrus cycles are summarized in Table 1. Statistical differences for all stainings were determined with regard to the stages of the estrus cycle. On the whole, the number of the mast cells stained with TB was comparatively higher in the follicular phase than the luteal phase in the female genital tract. Moreover, at the AB/SO staining while the number of the AB (+) mast cells was more numerous in the follicular phase, the number of the SO (+) mast cells and AB/SO (+) (mixed) mast cells was more abundant in the luteal phase. Even though the number of the TB (+) mast cells was higher in the luteal phase than the follicular phase of the ovary, the difference was not statistically significant (P>0.05). When a comparison was made for the presence of the mast cells among the parts of the oviduct during the follicular phase, the isthmus was the area containing the highest number of the mast cells. In addition, the number of the SO (+) mast cells in the ampulla of the oviduct

Table 1. Mast cell distribution in the bovine reproductive tract throuhout the estrus cycles.

			Histochemical Stains							
			AB/SO							
			TB		AB (+)		SO (+)		Mix	
		Layers	FP	LP	FP	LP	FP	LP	FP	LP
Ovaries		Cortex	4.90±0.659*	5.74±0.913*	3.09±0.383*	2.83±0.254*	0.48±0436*	0.74±0.568*	0.26±0.362a*	2.65±0.469b*
		T.interna	0.0±0#	0.0±0#	0.0±0#	0.0±0#	0.0±0#	0.0±0#	0.0±0#	0.0±0#
		T.externa	5.51±0.664a*	6.84±0.834b*	2.73±0.669&	1.84±0.354&	1.13±0.712*	0.89±0.788*	0.83±0.301a&	1.71±0.538b&
		Medulla	6.49±0.992*&	6.59±0.840*	2.83±0.341&	2.05±0.141&	2.0±0.831&	1.90±0.812&	1.48±0.708a&	2.35±0.652b*
		Vessels	5.54±0.641*	6.15±1.063*	2.51±0.471&	1.98±0.577&	1.85±0.789&	1.90±0.911&	1.40±0.656a&	2.19±0.579b*
Tuba uterina	Infundibulum	Stroma	3.56±0.597a*	2.98±0.365b*	4.54±0.512a*	2.35±0.374b	0.49±0.356a	1.85±0.160b*	2.65±0.495a	2.01±0.485b
		Muscle	2.90±0.568#	2.45±0.346#	2.54±0.256#	2.18±0.324	0.50±0.297a	0.98±0.468b#	2.13±0.253	2.01±0.188
		Vessels	3.96±0.665a*	2.50±0.297b#	2.58±0.301#	2.18±0.324	0.45±0.307a	1.93±0.138b*	2.40±0.316	2.18±0.324
	Ampulla	Stroma	3.83±0.562a	2.94±0.238b	0.56±0.520a	3.31±0.657b*	2.31±0.348a	0.0±0b*	2.58±0.341a	0.38±0.265b
		Muscle	4.04±0.821a	2.83±0.301b	0.33±0.296a	2.70±0.256b#	2.44±0.396a	0.26±0.362b#	2.48±0.575a	0.55±0.160b
		Vessels	3.65±0.667a	2.81±0.223b	0.54±0.587a	2.95±0.307b*#	2.10±0.185a	0.0±0b*	2.24±0.272a	0.65±0.325b
	Isthmus	Stroma	4.65±0.728a*	2.75±0.407b	2.66±0.486a	3.54±0.342b*	1.23±0.649a	0.59±0.155b	3.41±0.695a*	2.33±0.296b
		Muscle	4.63±0.645a*	2.88±0.361b	2.31±0.383	2.61±0.335#	0.76±0.829	0.45±0.307	2.49±0.356#	2.28±0.430
		Vessels	3.56±0.408a#	2.74±0.192b	2.49±0.356a	3.64±0.311b*	1.20±0.526a	0.63±0.212b	2.76±0.342*#	2.48±0.436
Uterus		Stroma	5.86±0.555a*	4.35±0.465b*	3.29±0.422a*	1.04±0.495b	0.96±0.410a*	3.01±0.210b*	2.45±0.282a*	4.20±0.504b*
		C. muscle	2.49±0.356#	2.41±0.290#	2.65±0.325a#	0.95±0.462b	1.35±0.437a#	2.65±0.282b#	2.39±0.188a*	3.11±0.522b#
		L. muscle	7.33±1.015&	7.09±1.124&	3.48±0.806a*	0.95±0.539b	2.05±0.752a#	3.71±0.264b&	2.98±0.238a#	4.69±0.482b*
		Vessels	3.38±0.361aα	4.40±0.509b*	2.63±0.399a#	0.86±0.388b	1.16±0.272*	1.64±0.447α	2.48±0.138a*	2.89±0.405b#
Cervix		Stroma	3.51±0.339a*	2.83±0.353b*	0.69±0.864	0.91±0.707*	1,11±1,099	0.75±0.746	1.26±0.745a	2.09±0.589b
		Muscle	2.0±0.0#	2.09±0.247#	0.31±0.511a	0.0±0b#	1.19±1.227	0.26±0.520	1.64±1.207	2.03±0.395
		Vessel	2.63±0.138&	2.65±0.325*	0.39±0.566	0.39±0.454*	1.06±1.146	0.44±0.396	1.69±0.968	1.51±0.470
Vagina		Stroma	5.24±0.528a*	3.40±0.866b*	3.46±0.531a*	1.85±0.277b	0.70±0.160a	3.25±0.462b	2.40±0.346a*	1.23±0.501b
		Muscle	2.26±0.362#	2.05±0.141#	1.88±0.353#	2.18±0.324	0.78±0.138a	3.10±0.453b	1.85±0.226#	1.64±0.534
		Vessel	2.53±0.395#	2.36±0.324#	1.90±0.738#	2.25±0.462	0.86±0.302a	2.95±0.381b	1.78±0.520#	1.68±0.465

Table 2. Correlations between serum hormonal levels and mast cells in the bovine reproductive tract.

Reproductive Tract Section		Estrus cycles			
		Follicular phase		Luteal phase	
		Estradiol-17β	Progesteron	Estradiol-17β	Progesteron
		r	r	r	r
Ovaries		0.36	0.21	0.26	0.27
Tuba uterina	Infundibulum	0.07	-0.22	-0.71*	-0.66*
	Ampulla	-0.03	0.57	0.58	0.57
	Isthmus	0.04	0.35	-0.55	-0.19
Uterus		0.53	-0.23	0.36	0.15
Cervix		-0.33	0.23	-0.11	-0.06
Vagina		-0.61	-0.42	-0.44	-0.11

was markedly higher in the follicular phase than the luteal phase (P<0.05) (Table 1).

Moreover, we noted no TB (+) and AB/SO (+) mast cells in the theca interna of the ovary, and no SO (+) mast cells in the stroma of the cells and around the vessels of the ampulla (P<0.05). More TB (+) and AB/SO (+) mast cells were found in the myometrium of the uterus, particularly in the longitudinal smooth muscle layer, with respect to the rest of the other parts of the uterus. The number of TB (+) and AB/SO (+) mast cells in the stroma of the cells and around the vessels of the cervix and vagina was more dominant (P<0.05) (Table 1).

Correlations between steroid levels and mast cells

Tables 2 summarize the correlations between serum hormonal levels and mast cells in the according to the bovine reproductive tract regions. In cycling bovines, positive correlations were found between mast cells and serum levels of estradiol-17β in the ovaries, tuba uterina and uterus, whereas negative correlations were found between mast cells and serum levels of estradiol-17β in the cervix and vagina.

Discussion

Distribution sites of the mast cells throughout the female genital tract and different staining features of their granules indicate the presence of heterogeneity among the mast cells (Özen et al 2007, Karaca et al 2007, Karaca et al 2008). Although there are several studies available on the heterogeneity and histochemical features of the mast cells, in the present study we investigated the effects of sex hormones on the number of mast cells and their physiological functions in the genital tract of the cows during the estrus cycle.

While Nakamura et al (1987) noted presence of the mast cells both in the cortex and medulla of the bovine ovary, Krishna et al (1989) noticed existence of the mast cells only in the ovarian medulla of rodents such as rat and hamsters. Özen et al (2007) noted the presence of increased number of mast cells at the periphery of Graafian follicles of the cow at TB staining during the follicular phase and they proposed that these mast cells may play a critical role in follicular development and ovulation. Besides, Reibiger and Spanel-Borowski (2000) observed no mast cells within the corpus luteum and the follicles at TB staining during the estrus cycle; however, intense number of mast cells located within the interstitial cortical stroma and ovarian medullary area and therefore they considered that mast cell distribution might be unstable through the ovary in the cow. On the other hand, Nakamura et al (1987) were able to count mast cells in the capsule of the corpus luteum and in the layers of theca externa of the dominant follicles (tertiary or Graafian follicle), but they did not detect the presence of the mast cells within the interstitial cortical stroma. Some researchers (Nakamura et al 1987, Reibiger and Spanel-Borowski 2000, Özen et al 2007) interpreted this observation such that theca externa of the dominant follicles was formed by a couple of cell layers and there was no prominent border between itself and the stroma. Furthermore, in the ovary of the cow, Reibiger and Spanel-Borowski (2000) observed mast cells stored in the tunica adventitia of the thick-walled muscular arteries at TB staining and they proclaimed that these mast cells were effective on the smooth muscles. The studies carried out in the rats depicted that while there were increased mast cells around the blood vessels at TB staining, there were no mast cells nearby theca externa of the Graafian follicle and the corpus luteum.; in addition, the number of the mast cells was at maximum during the estrus, moderate during the metestrus, and minimum during the proestrus (Najafpour et al 2011). Additionally, mast cells located around the ovarian medulla, particularly nearby the blood vessels in the hamster at TB staining and asserted that these mast cells contribute to the gonadotropins that induce preovulatory events (Krishna et al 1989). In the present study at TB staining, even though the number of the mast cells was higher during the follicular phase, their number was further increased during the luteal phase, suggesting that the progesterone can synergistically work with the estrogen to stimulate mast cell activity.

In the golden hamster, while Shinohara et al (1987) found no AB (+) mast cells on the wall of the ovarian bursa during the estrus cycle at AB/SO staining; all the mast cells they observed were stained with safranin reddish-orange color (Shinohara et al 1987). Özen et al (2007) reported the presence of AB (+) and SO (–) mast cells in the ovarian medulla of the cow during the luteal phase at AB/SO staining. In the rat, Aydın et al. (1998) did not observe mast cells in the cortex at AB/SO staining, but noted AB (+) and AB/SO (+) mast cells in the medulla during the proestrus. Furthermore, they observed SO (+) mast cells in the cortex, SO (+) and AB/SO (+) mast cells in the medulla during the estrus; AB (+) and AB/SO (+) mast cells in the cortex and medulla during the metestrus; SO (+) and AB/SO (+) mast cells in the cortex and medulla during the diestrus. The presence of more AB (+) mast cells in the ovarian cortex during the follicular phase at AB/SO staining suggests that the histamine released by the mast cells might involve in follicular development and deactivation of the cytotoxic effects of the lymphocytes during the preparation to ovulation. The appearance of the increased SO (+) mast cells in the ovarian medulla and around the ovarian blood vessels implies that the heparin released by these mast cells might increase capillary permeability and blood flow in the ovary, contribute to the dilatation of thecal and luteal vessels via amplifying mitotic activity of the endothelial cells and cell migration; these are important for preserving follicular development and protecting egg cell.

The mast cells along with basophils and endothelial cells play a crucial role in managing of vascular permeability, blood flow regulation and follicular development in the ovary and female genital organs. Earlier studies demonstrate a connection between degranulation-activation of the mast cells and angiogenesis-neovascularization (Najafpour et al 2011). Mast cells in several organs are the main source of the secretory vasoconstrictor factor mediated via histamine and serotonin. The number of the mast cells is shown to be increased in cases of reduced blood flow to the ovary to physiologically return ischemic situations to their normal states (Yildirim 2003). Several studies report the presence of copious mast cells around the small and moderate size of the blood vessels in the ovarian medulla and in the layers of other genital organs, an observation comparable to the present findings showing numerous AB (+) mast cells around the vessels, and in the ovarian cortex and medulla and further supports the reported effects of the histamine in the ovary (Reibiger et al 2000, Najafpour et al 2011).

The reason for the existence mechanism of the mast cells in the oviduct of the cow is still not well known. Nonetheless, other studies indicate that the presence of greater amount of heparin during ovulation might be momentous for local metabolism of sperm capacitation and biochemistry of sperm maturation (Parrish et al 1994, Scott et al 2000). Our observation showing an increase in the number of the mast cells at TB staining during the follicular phase with respect the luteal phase of the estrus cycle further supports the concepts of these studies. Du Bois et al (1980) and Ozen et al (2002) reported the presence of the highest number of the mast cells residing in the isthmus, like our findings. The increased number of the mast cells in the isthmus, closer part of the oviduct to the uterus, can provide more heparin and histamine while the former might play a vital role in the production of oviductal secretion and regulation of sperm capacitation mechanisms, the latter can control tonus and motility of the smooth muscles in the area. Our findings revealing the existence of the more copious mixed mast cells in the isthmus as illustrated in Table 1 promotes the these explanations.

The endometrium lining the uterine cavity undergoes cyclic changes in response to sex hormones. The number of the mast cells residing within the endometrium and myometrium of the uterus is shown to be regulated by sex hormones (Çerçi et al 1998). There are several studies demonstrating that estrogens induce mast cells to release histamine; indeed, these studies also indicate that most of the histamine is generated by the mast cells residing within the reproductive system including the uterus and vagina (Levier et al 1966, Liu et al 2004). Moreover, remodeling, vasodilatation, edema of the uterus during the estrus are shown to be initiated by histamine released from the granules of the estrogen-induced uterine mast cells (Pedilla et al 1990). Histamine generated by the uterine mast cells is indicated to be critical for the regulation of zygote implantation owing to its ability to induce permeability of the uterine vessels and stromal decidualization (Johnson et al 1980). At their study on the mouse uterus, Padilla et al (1990) reported that histamine concentration was higher under the effect of the progesterone than that of the estrogen. Likewise, similar other studies, which further support our present observations, reported that the concentration of the histamine released by the mast cells in the uterus of the mice in estrus or received estrogen injections was less than the mice in diestrus or received progesterone injections (Drudy et al 1990). In the endometrium of the rat, Aydin et al. (1998) revealed that the number of the mast cells at TB staining was high at estrus, moderate at metestrus, and quite low at diestrus and proestrus. The same researches also reported that at AB/SO staining the mast cell profiles were AB (+) / mixed (+) at proestrus, safranin (+) / mixed (+) at estrus, AB(+) / mix (+) at metestrus, and safranin (+) / mix (+) at diestrus (Aydin et al 1998). In contrast to these studies, in the present study as summarized in Table 1, the numbers of the mast cells were found to be higher at all layers of the uterus during the follicular phase at TB staining as reported that Eren et al (1999); at AB/SO staining while the number of the SO (+) mast cells was higher during the luteal phase, that of AB (+) mast cells was at large during the follicular phase of the estrus cycle. Likewise, a previous study (Likar et al 1964) performed on the uterus of the cow, whose results are comparable with the present results, showed that

the number of the mast cells was higher during the estrus than the luteal phase. They also noted that degranulation of the mast cell granules was augmented at the maximum levels of the estrogen. Similar to the present study, several other studies (Shimizu et al 1987, Hore et al 1988, Pedilla et al 1990, Eren et al 1999) demonstrated that the number of the mast cells was more numerous in the myometrium than the endometrium of the uterus. The presence of more abundant mast cells in the myometrium may suggest that high concentration of the histamine released by the mast cells is a critical factor among the other factors to initiate uterus contractions for the beginning of parturition.

Our present results are comparable with a previous study carried out in mare using the TB staining showed that there were no mast cells residing in epithelium (Wehrend et al 2005) and the number of the mast cells locating within the cervical connective tissue was increased during the follicular phase (Walter et al 2012). While local immune response can protect female genital tract from invasion of the potential environmental infectious agents, it also contributes to remodeling of the cervix during estrus cycle and pregnancy. In mammals, the cervix undergoes major structural changes during the estrus cycle. While staying tightly closed during diestrus, the cervix becomes opened during estrus; consequently, it becomes vulnerable to increased risk of infections after mating or insemination. The local immune response also serves as a barrier against the spread of infections; thereby, protects the sperms against the adverse effect pathogens. The regulation mechanisms of mast cells in this protection, although not fully understood, might contribute to structural changes in the uterus during the estrus cycle (Wehrend et al 2005, Walter et al 2012). Our current results showed that the number of the mixed mast cells (secreting both heparin and histamine) in the cervix was more abundant than that of the mast cells releasing only histamine or heparin. This observation suggest that the mixed mast cells might involve in increased vascular permeability in the cervix and regulation of cervical smooth muscle tonus through histamine release in addition to both controlling formation of fibrous connective tissue by inducing production of collagen fibers and facilitating the slide of collagen fibers over one another via lubricating effect of heparin secretion.

Studies completed in different species on the localization and distribution of the mast cells within vaginal tissue indicates that these cells show anisotropic features (Mahoney et al 2002, Mahoney et al 2003). On the vagina of the dog, the number of the mast cells visualized with TB staining was more at diestrus than proestrus and estrus (Goericke et al 2010). In the present study as summarized in Table 1, the number of the mast cells in the vaginal connective tissue was higher during the follicular phase. The mast cells in species such as Brushtail Possum are shown to locate in close proximity to the border of the epithelium and epithelial connective tissue (Mahoney et al 2002, Mahoney et al 2003). Similarly, in the present study we did not find the mast cells residing in the organ epithelium of the the vagina.

The studies (Eren et al 2010, Walter et al 2012) imply that while the histamine released by the vaginal mast cells induces vasodilatation and smooth muscle contraction, and regulates vaginal mucous secretion, heparin works synergistically with estrogen to regulate cell mechanisms such as cell proliferation and growth. The localization of the vaginal mast cells is shown to be critical in the control of pathogenic invasions (Mahoney et al 2002) and the clustering of the mast cells in the female genital tract might be in response to inflammatory and immunological reactions in addition to the chemotactic signals. Hughes and Rodger (1971) proposed that vaginal mast cells, spermatozoal interference, mucus-sperm compliance, and sperm capacitation. Likewise, our observations showing prominent increase in the number of the mast cells secreting heparin in all vaginal layers further supports earlier studies (Mahoney et al 2002, Eren et al 2010, Walter et al 2012).

Conclusions

Our results indicate that mast cells possess a dynamic structure; various types of mast cells reside in different tissues at diverse numbers. In addition, the function and phenotype of the mast cells can alter with reference to their content of proteoglycan. The estrogen and progesterone hormones released during estrus cycle can prepare mast cells for acquiring appropriate function with respect to the environmental ambiance through affecting granules of the mast cells. Besides, the present results indicate that the estrogen and progesterone hormones might work synergistically to positively increase the number of the mast cells. In brief, environmental ambient continuously interact with mast cells and this mutual interaction can define the mast cells response and behavior.

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