

## Morphological Studies on Ovarian Mast Cells in the Cow

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**Abstract:** This study was conducted on cows in the estrual and luteal phases of the cycle to determine the staining properties, light and electron microscopic appearance and numerical distribution of mast cells from various areas of the ovary. The material for the study comprised ovarian specimen taken from 14 cows, 7 in the luteal phase and 7 in the estrual phase of the sexual cycle.

Specimens were taken from 3 different areas of the ovaries, including those surrounding the corpus luteum, the graafian follicle and the medulla. From all 3 areas, mast cells were demonstrated as metachromatic staining after treatment of paraffin-embedded sections with toluidine blue and as Ab(+)/SO(-) by the combined alcian blue/safranin O (Ab/SO) stain. In the electron microscopic studies, the mast cells were observed to have 2 types of granules, namely homogeneously dense granules and tiny particulate granules.

All 3 regions of the ovary were observed to have higher average mast cell counts per mm<sup>2</sup> during the estrual phase than during the luteal phase (P < 0.001). In the estrual phase, while mast cell counts demonstrated a marked increase in the periphery of the graafian follicle, a low number of mast cells was determined in the periphery of the corpus luteum. In comparison, the medulla was demonstrated to have a much higher concentration of mast cells in the medulla than the other 2 regions of the ovarium.

**Key Words:** Mast cell, ovarium, sexual cycle, cow

### İnekte Ovarial Mast Hücreleri Üzerine Morfolojik Çalışmalar

**Özet:** Bu araştırma, östrual ve luteal fazlarda inek ovaryumunun farklı bölgelerindeki mast hücrelerinin boyanma özelliklerini, ışık ve elektron mikroskopik görünümünü ve sayısal dağılımlarını belirlemek amacıyla yapıldı. Çalışmada 7'şer adet östrual ve luteal fazlardaki inek ovaryumu materyal olarak kullanıldı.

Ovaryumda korpus luteum çevresinden, graff follikül çevresinden ve medulladan olmak üzere üç bölgeden parça alındı. Her üç bölgeden alınan parafin kesitlerinde toluidin blue ile metakromazi gösteren, alcian blue / safranin O (Ab/SO) kombine boyamasında Ab(+) / SO(-) boyanma özelliği gösteren mast hücrelerine rastlandı. Elektron mikroskopik incelemelerde mast hücrelerinin homojen yoğun ve ince tanecikli olmak üzere iki tip granül içerdiği gözlemlendi.

Ovaryumun her üç bölgesinde de östrual fazda, luteal faza göre mm<sup>2</sup>'deki ortalama mast hücresi sayısının arttığı görüldü (P < 0,001). Östrual dönemde graaff follikülü çevresinde mast hücresi sayısında belirgin artış olurken, corpus luteum çevresinde az sayıda mast hücresine rastlandı. Ovaryumun üç bölgesi karşılaştırıldığında her iki fazda da mast hücre yoğunluğunun medullada olduğu gözlemlendi.

**Anahtar Sözcükler:** Mast hücresi, ovaryum, seksüel siklus, inek

### Introduction

Mast cells, basophils, platelets and endothelial cells are well-known sources of histamine in the ovary (1,2). Histamine has been reported to regulate blood flow and vascular permeability in ovarian tissue, with a role in follicular development and ovulation (1,3-5).

In rodents, mast cells are found only in the hilum of the ovary and not in the corpus luteum, the interstitium or follicles. In contrast, mast cells are found in all parts of the ovary in several other species, including humans, cows and monkeys (1). In rats, mast cells are absent from the theca externa of the graafian follicle and the corpus

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luteum, while the mast cell count in the medulla has been reported to change with the phase of the estrous cycle from a maximum during estrous, through moderate numbers in metestrous to a minimum in pro-estrous (1,6-8). Gaytan et al. (4) reported the presence of mast cells in the retracting corpus luteum in rats. In contrast to this finding in rats, in cows mast cells have been observed in the external capsule of the corpus luteum and the theca externa of the graafian follicle, as well as in the medulla (1,3,5).

In a study conducted on the ovary of cows (5), it was reported that throughout the estrous cycle the mast cell count in the medulla was higher than that in the cortex. The mast cells were reported to be even more concentrated in the theca externa of the dominant follicle in the same study. In the luteal phase, where the corpus luteum completes its development, a marked decrease in the number of mast cells was observed (5). In a study conducted on the human ovary during menopause and on polycystic ovaries, a remarkable decrease in mast cell numbers was reported (9).

Studies conducted on the oviducts of cows (10,11) showed that the mast cell count in the estrual and luteal phases was higher in the isthmus than in the ampulla region, while that in the luteal phase was higher than that in the estrual phase in both regions.

In another study carried out on the cow uterus (12), the mast cell count in the estrous phase was reported to be higher than that in the luteal phase. The same researchers drew attention to the increase in degranulation of the mast cell granules during the period of maximum estrogen levels.

This study was conducted with the objective of determining the appearance and numerical distribution of mast cells in the ovarian cortex and medulla of the cow during the estrual and luteal phases through their staining properties and appearance under light and electron microscopes.

## Materials and Methods

Ovarian material from 14 cows, 7 in the estrual phase and 7 in the luteal phase of the sexual cycle, was obtained from the Ankara abattoir. The period of the estrous cycle that each animal was in was determined by macroscopic examination and the serum progesterone levels of the

animals were determined by the RIA method (13). Material was taken from the areas surrounding the corpus luteum, the follicles and the medulla.

### Light Microscopic Examinations

A part of the tissue specimen obtained was fixed in 10% formol after washing, while the other part was fixed in isotonic formaldehyde acetic acid solution (IFAA, pH 2.9) for 12 h, and with 70% alcohol for another 12 h and then blocked in paraplasts after being passed through graded alcohols, methyl benzoate and benzol (14). Then serial 6  $\mu$ m sections were cut.

The sections of each of the blocks were either stained with 5% toluidine blue (prepared in a buffer of McIlvaine's citric acid disodium phosphate, pH 4), or they were stained with a combined alcian blue/safranin O (Ab/SO) method (15,16) as follows: from the above mentioned section series, 10 sections (each 6- $\mu$ m thick) were selected, i.e. sections 1 and 2 (next 5 sections omitted), sections 8 and 9 (next 5 sections omitted), sections 15 and 16 (next 5 sections omitted), sections 22 and 23 (next 5 sections omitted) sections 29 and 30. This yielded 5 pairs of sections, with a distance of 30  $\mu$ m between each of the pairs. One section each from the tissues fixed in 10% formol and IFAA were placed on the same slide and stained using an alcian blue/safranin O staining procedure as described below:

- Bring sections to water.
- Stain in 0.5% alcian blue 8GX in 3% acetic acid for 30 min.
- Wash in water for 5 min.
- Stain in 0.25% safranin in 0.125 N hydrochloric acid for 30 s.
- Dehydrate rapidly, clear and mount in synthetic resin.

Tissue samples from rat intestines were used as a control for the mucosal mast cells (MMCs), while tissue from the skin of rats was used as a control for the connective tissue mast cells (CTMCs).

### Electron Microscopic Examinations

The tissue samples obtained were kept for 24 h in glutaraldehyde-paraformaldehyde (pH 7.4) as described by Karnovsky (17). They were further washed in a cacodylate buffer for 3 h and fixed for a second time with 1.0% osmic acid. They were then left in 0.5% uranyl

acetate for 2 h and blocked in araldite M after passage through graded alcohols and propylene oxide. Sections 300-400 Å in thickness obtained from the blocks were contrasted according to the method given by Veneable and Coggeshall (18) and examined using a Carl Zeiss EM 9S-2 model transmission electron microscope.

#### Cell Counts and Statistical Analysis

To determine the numerical distribution of mast cells in the ovary in specimens stained with toluidine blue, cell counts obtained under a 100-square ocular micrometer (eye piece graticule) were used. For less than 40X magnification, the mast cells per unit area were counted. In each section, the numbers of cells from 10 different areas selected at random were counted. For the 40X magnification, the 100-square micrometer area was determined by means of a micrometer slide (19). Later, all the numerical data were converted to number of mast cells per unit area ( $\text{mm}^2$ ). Differences between the estrous and luteal phases examined were determined by Student's t-test (20). SPSS (5.0) was employed for this purpose.

#### Results

Mast cells demonstrating metachromatic staining properties with toluidine blue under light microscopic examination were found to be abundant, especially in the medulla and during estrous (Figure 1). In the Ab/SO combined staining, mast cells were seen as Ab(+), SO(-) (Figure 2). Serum progesterone levels of the animals are shown in Table 1.

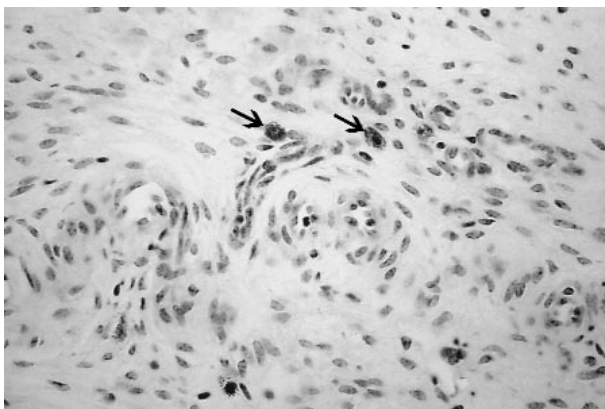


Figure 1. Mast cells (arrows) in medulla of the ovarium, estrous phase. Toluidine blue. x600.

Mast cells were seen to exhibit different forms and sizes. Mast cells in the luteal phase were noted to have a marked decrease in granule number. During this period, degranulated mast cells were also observed. In the estrual period, however, the mast cells were completely filled with granules (Figure 1). In the estrual phase, while mast cell counts demonstrated marked increases in the periphery of the graafian follicle (Figure 3), a low number of mast cells was determined in the periphery of the corpus luteum (Figure 4).

In the electron microscopic examinations, the mast cells were seen to contain 2 different types of granules, homogeneously dense granules and tiny particulate granules (Figure 5).

The average mast cell counts per  $\text{mm}^2$  in the ovarian cortex (surrounding the graafian follicle and corpus luteum) and medulla are shown in Table 2. The increase in the mast cell count during the estrual phase compared to the luteal phase was statistically significant ( $P < 0.001$ ). In both the estrual and luteal phases, mast cell counts in the periphery of blood vessels were greater than those in other areas.

#### Discussion

Mast cells have been identified according to their morphological, biochemical and physiological properties as MMCs and CTMCs (14,15,21). While MMCs have been demonstrated to be sensitive to formaldehyde fixation and Ab(+) staining granules with the combined Ab/SO stain, CTMCs on the other hand have been shown to

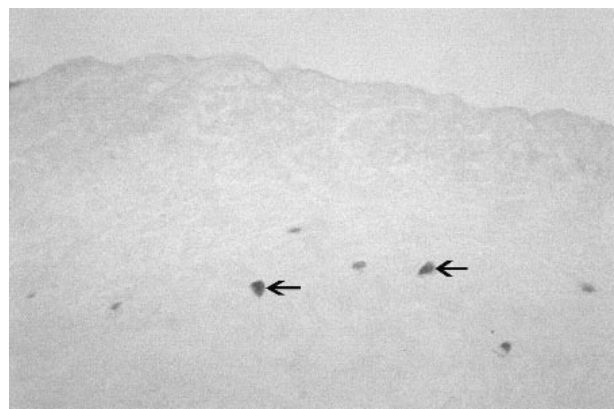


Figure 2. Ab(+) mast cells (arrows) in medulla in the luteal phase. Ab/SO. x600.

Table 1. Reproductive status and serum progesterone levels of the 14 animals examined.

Case no.	Reproductive status	Serum Progesterone Level (ng/ml)	Case no.	Reproductive status	Serum Progesterone Level (ng/ml)
1	Follicular phase	0.03	8	Follicular phase	0.15
2	Follicular phase	0.19	9	Follicular phase	0.11
3	Luteal phase	4.74	10	Luteal phase	4.71
4	Luteal phase	3.26	11	Luteal phase	2.43
5	Follicular phase	0.16	12	Luteal phase	3.19
6	Follicular phase	0.18	13	Luteal phase	2.25
7	Follicular phase	0.48	14	Luteal phase	5.12

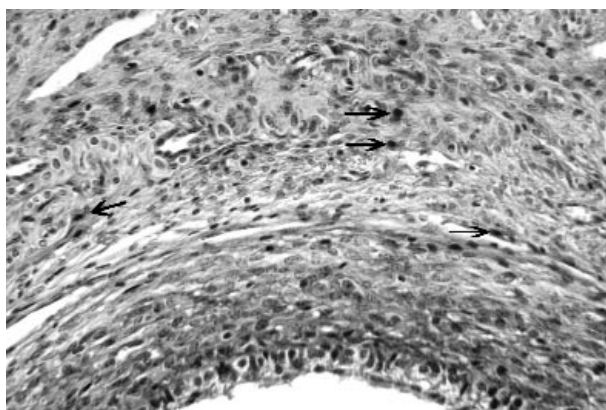


Figure 3. Mast cells in the periphery of the Graafian follicle (arrows). Toluidine blue. x500.

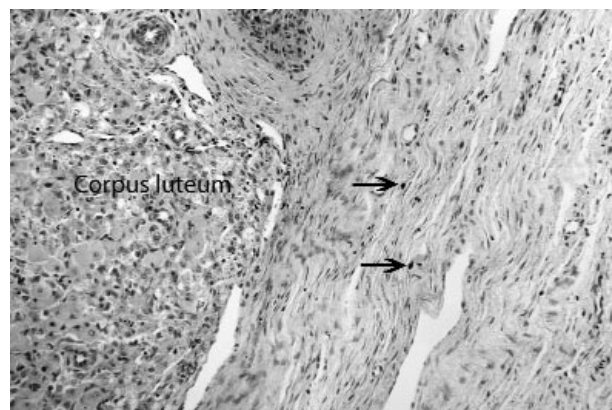


Figure 4. Mast cells in the periphery of the corpus luteum (arrows). Toluidine blue. x300.

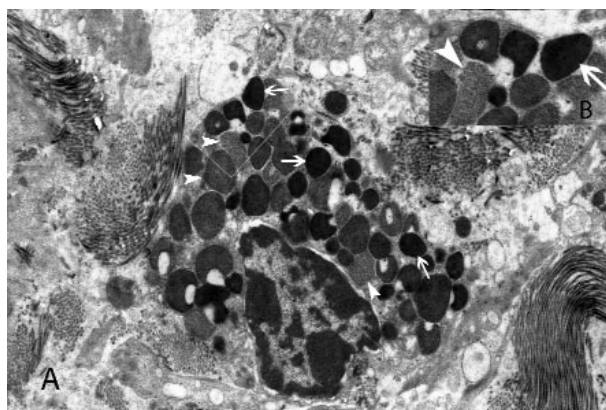


Figure 5. A. Mast cells in the medulla in the estrous phase. Arrows: Homogenously dense granules, arrow heads: Tiny particulate granules. x10,000. B. Arrow: Homogenously dense granule, arrow head: Tiny particulate granule. x15,000.

contain granules that are resistant to formaldehyde fixation but give SO(+) reactions (14,15,22). In the uterus (23) and oviducts of cows, mast cells exhibiting Ab(+)/SO(-) staining properties were reported in earlier studies (11). In the present study, mast cells showing metachromatic properties with toluidine blue were Ab(+)/SO(-) with the combined Ab/SO stain.

Electron microscopic examination of the uterus (23) and oviduct (11) has shown mast cells to contain 2 types of granules, i.e. homogeneously dense granules and tiny particulate granules. In the present study, similar types of granule were observed.

In a study conducted on cow ovaries (5) throughout the estrous cycle, more mast cells were encountered in the medulla than in the cortex, with most of the mast cells showing a wide distribution in the theca externa of the dominant follicle. However, in the luteal phase, in which development of the corpus luteum is completed, a

Table 2. Numerical distribution (mm<sup>2</sup>) of mast cells in the various regions of the ovary during the estrual and luteal phases.

Area	Group	n	X ± Sx	t
Surrounding the corpus luteum	Estrual	7	15.571 ± 0.480	4.608*
	Luteal	7	12.857 ± 0.340	
Surrounding the graafian follicle	Estrual	7	17.714 ± 0.474	4.899*
	Luteal	7	14.857 ± 0.340	
Medulla	Estrual	7	31.714 ± 0.918	8.583*
	Luteal	7	23.429 ± 0.297	

\*P &lt; 0.001

remarkable fall in the mast cell count was observed. In the present study, the finding of more mast cells in the medulla, increased numbers of mast cells in the periphery of the graafian follicle and a decrease in mast cell count all lend support to findings from previous investigations.

Reibiger and Spanel-Borowski (5) observed deposition of mast cells in the adventitia of thick-walled muscular arteries in the ovary of cattle, leading to suggestions of an effect on smooth muscle. In the present study, mast cells were found abundantly in the periphery of blood vessels from the ovarian medulla.

Eren et al. (23), in a study on the cow uterus, found more mast cells in the endometrium in diestrous than in estrous. Dubois et al. (10) and Özen et al. (11) in studies on cow oviducts reported marked increases in the mast cell count during the luteal phase. In another study on the cow uterus, the mast cell count was reported to be higher in the estrual phase than in the luteal phase (12). Results from the present study seem to agree with those published by Likar and Likar (12) on cow uterus, but conflict with findings by Eren et al. (23) on cow uterus, and by Dubois et al. (10) and Özen et al. (11) on cow oviducts.

Some researchers (1,3) have drawn attention to the fact that heparin and histamine increase capillary permeability and blood fluidity in the ovary, while heparin, by increasing mitotic activity and migration of

endothelial cells, might also contribute to the enlargement of the thecal and luteal vessels. The same researchers reported a remarkable increase in mast cell count in the dominant follicle on the 19<sup>th</sup> day of the cycle and a fall in the mast cell count in the periphery of the corpus luteum on the 4<sup>th</sup> day following ovulation. While the elevation in the mast cell count in the dominant follicle on the 19<sup>th</sup> day was attributed to its possible relation to follicular development, the fall in the mast cell count on the 4<sup>th</sup> day was suggested to be due to the degranulation process that takes place during this period. In the present study, the demonstration of increased mast cell counts in the estrual phase and decreased mast cell counts as well as degranulation in the luteal phase supports the findings reported by these researchers (1,3).

In conclusion, in both the estrual and luteal phases, mast cells found in the cortex and medulla of the ovary were determined to be metachromatic and Ab(+)/SO(-), with respect to histochemical staining properties. In electron microscopic examinations, mast cells were determined to contain 2 types of granules, namely homogeneously dense granules and tiny particulate granules. The increase in the number of mast cells in the periphery of the graafian follicle in the estrual phase suggests that these cells play a role in follicular development and ovulation.

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