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Eosinophils and mast cells in the oviduct of heifers under natural and superovulated estrous cycles

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Abstract

Quantification of mucosal eosinophils and mast cells in the oviducts from 22 crossbred heifers was performed in both natural (estrus, metaestrus and diestrus) and superovulated (estrus and metaestrus) estrous cycles. The number of cells/square millimeter of mucosa was obtained through counts at different regions of the oviduct (infundibulum, ampulla, ampullary/isthmic transition and isthmus). Differences were not found ($P > 0.05$) among numbers of cells at the different phases of the natural estrous cycle nor between natural or superovulated cycles. When all animals were separated into two phases of the estrous cycle (estrus and metaestrus), the number of eosinophils at the ampullary/isthmic transition and isthmus was higher ($P < 0.01$) at the estrus cycle phase, and the number at the infundibulum was higher ($P < 0.001$) during the metaestrus phase than at estrus. The number of mast cells was highest at the isthmus regardless of the estrus phase, and highest at the infundibulum ($P < 0.001$) and ampulla ($P < 0.05$) during the metaestrus phase. Significant correlations were found between the number of mast cells and plasma progesterone levels at the infundibulum ($P < 0.001$; $r = 0.69$) and ampulla ($P < 0.03$; $r = 0.51$). No correlation was seen between numbers of eosinophils and mast cells, progesterone and 17β-estradiol concentrations. Therefore, the distribution of mucosal eosinophils in the oviduct of heifers is apparently not related to the circulating levels of 17β-estradiol and progesterone; however, the highest number of mast cells found at the metaestrus phase of the cycle, at least for the infundibulum and ampulla, appears to be related to the high circulating progesterone plasma levels in metaestrus phase compared to estrus phase. No differences were found between animals subjected or not to superovulation.

Keywords: bovine, eosinophils, estrous cycle, mast cells, oviduct.

Introduction

The presence of leukocytes in the mucosa and lumen of the female genital tract is well described in the literature, although its regulation and function are still controversial and subject of investigation. The eosinophil is a cell mainly involved with phagocytic, bactericidal and parasiticidal functions but also takes part in inflammatory and allergic processes in tissues (Young, 2000). It has receptors for histamine, glucocorticoids (Young, 2000) and estrogens (Tchernitchin *et al.*, 1974). Chemoattractant factors for eosinophils include the products of basophils, T lymphocytes and mast cells, as well as leukotrienes and histamine (Benitez-Briesca *et al*., 1989; Young, 2000).

Circulating estrogen evokes a migration of eosinophils to the uterus, thus causing an increase in vascular permeability and local edema through the release of histamine (Tchernitchin *et al*., 1974, 1998; Tchernitchin and Galand, 1983), possibly by the estrogenic stimulus for the production of endometrial eotaxin, a protein involved in the passage of eosinophils through the endometrial vessels in women (Zhang *et al*., 2000) and rat (Gouon-Evans and Pollard, 2001). A high number of eosinophils was seen in the outer layers of the rat uterus during the luteal phase, migrating to the endometrium at proestrus and degranulating at estrus (Lee, 1982), while Ramos *et al*. (2000) reported an inhibition of eosinophilia by progesterone at the rat cervix. Eosinophils have been reported in the oviducts of cows, especially at estrus (Matsuda *et al*., 1983).

Mast cells have been reported in the oviductal mucosa of cows (DuBois *et al*., 1980; Ozen *et al*., 2002) and are present in greater numbers during the progestagenic phase of the cycle, mainly at the isthmus (Ozen *et al*., 2002). These cells are known to produce histamine and heparin (Scott and Stockham, 2000). At this site histamine might play a role in the inhibition of cytotoxic activity of lymphocytes during estrus (DuBois *et al*., 1980) while heparin, which is found in large amounts at the time of ovulation (Parrish *et al*., 1989), would act in the local metabolism of lipids (DuBois *et al*., 1980) and sperm capacitation (Parrish *et al*., 1994).

Eosinophils stimulate degranulation of mast cells during inflammatory processes (Henderson *et al*., 1980) and mast cells stimulate production and activation of eosinophils (Abbas *et al*., 2000). Furthermore, enzymes released by mast cells, such as tryptase, and by

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eosinophils, such as eosinophilic peroxidase, take part in the processes of tissue remodeling (Abbas *et al*., 2000). However, Terada *et al*. (1985) did not find any effect of mast cells or histamine on uterine eosinophilia of healthy rats.

This study aimed to quantify these cells along the regions of the oviducts of heifers under natural or superovulated estrous cycles and to verify possible correlations between eosinophils and mast cells and circulating levels of 17β-estradiol (E2) and progesterone (P4).

Materials and Methods

Animals and treatments

The methodology employed in this study has been described in a previous paper (Valle *et al*., 2007). Briefly, crossbred heifers (*Bos taurus taurus* x *Bos taurus indicus*; n = 19) with ages ranging from 2 to 4 years and body weight from 350 to 430 kg were kept on pasture and checked for signs of behavioral estrus twice a day. As the animals displayed the first signs of estrus, they were randomly assigned to one of the following groups: a) slaughtered 17 hours after estrus initiation (Est group; n = 4); b) slaughtered 4 days after estrus initiation (Met group; n = 3); c) slaughtered 11 days after estrus initiation (Die group; n = 4); d) submitted to superovulatory treatment and slaughtered 17 hours after estrus initiation (Estsup group; n = 4); and e) submitted to superovulatory treatment and slaughtered 4 days after estrus initiation (Metsup group; n = 4). Superovulation was obtained by i.m. injections of follicle stimulating hormone (FSH) (Pluset; Calier, Brazil) in decreasing doses (total dosage of 350 iu) at intervals of 12 hours from the tenth day following estrus. At the twelfth day after estrus, 25 mg of dinoprost trometamina (Lutalyse; Rhodia, Brazil) was also i.m. injected.

Immediately before slaughter, 3 mL of venous blood were collected into assay tubes containing ethylenediaminetetraacetic acid (Vacutainer EDTA K3, B.D.; Brazil) and centrifuged for 10 minutes at 400 g. Blood plasma obtained was frozen at -20ºC until analysis by radioimmunoassay (Estradiol Maia Kit and Progesterone Maia Kit, BioChem ImmunoSystems; Italy). All samples were analyzed within the same assay. The intra-assay coefficients of variation for E2 and P4 were 7.0% and 8.1%, respectively. Minimum detection concentrations for P4 and E2 were 0.022 ng/mL and 1.0 pg/mL, respectively.

Tissue preparation

Tissue samples from the infundibulum, ampulla, ampullary/isthmic transition and isthmus of both oviducts were fixed in 10% neutral buffer formalin, embedded in paraffin wax and sectioned at 5μm. Cromotrope 2R (Lendrum, 1944) and Toluidine Blue (Luna, 1968) staining were employed for detecting the presence of eosinophils and mast cells, respectively.

Eosinophils and mast cells quantification

The number of eosinophils in the oviductal mucosa was estimated by counting these cells along the entire area of tissue present on the slide, at 1000X magnitude. Next, such area was determined with KS300 software using the image analysis equipment (Kontron Electronic/Carl Zeiss; Germany). Digitized images were obtained with the TK-1270/RGB microcamera (JVC; Japan), at 40X magnitude. Therefore, the concentration of eosinophils in the mucosa (eosinophils/mm^2) was obtained. The mast cell concentration in the mucosa (mast cells/mm^2) was determined by using the same procedure.

Statistical analyses

The Wilcoxon test was used for comparing right and left oviducts, as well as ipsilateral and contralateral oviduct in relation to the ovary containing either a corpus luteum in formation (Met group), a mature corpus luteum (Die group) or a preovulatory follicle (Est group), in those groups of heifers under natural estrous cycle. Comparisons among experimental groups, including data of hormonal concentrations, were performed by using Kruskal-Wallis and Mann-Whitney tests; Friedman method was employed for comparisons among different regions of the oviducts. The relationships among variables were identified by Spearman correlation coefficient. The level of significance considered for the statistical analyses was 5% ($P < 0.05$) and results were expressed as means ± standard deviation (age, body weight and hormonal concentrations) or medians (number of eosinophils and mast cells/mm^2).

Results

Age, body weight and circulating plasma E2 and P4 concentrations of the animals are shown in Table 1. Higher E2 levels ($P < 0.05$) were observed in Estsup group in relation to Metsup group, and lower P4 levels ($P < 0.005$) in Est group in relation to Metsup and Die groups.

Differences were not observed ($P > 0.05$) between right and left oviducts, as well as ipsilateral and contralateral oviducts in relation to the ovary containing a preovulatory follicle or a corpus luteum. Therefore, the mean values for the two oviducts were used for each animal.

Table 1. Mean ± SD for age, body weight and hormonal levels of heifers according to groups.

Group*	Age (years)	Body weight (kg)	Hormonal levels #	
			E2 (pg/mL)	P4 (ng/mL)
Est	3.0 ± 0.0	398.0 ± 30.2	10.27 ± 1.75^{AB}	0.18 ± 0.13^{A}
Estsup	3.3 ± 0.5	374.0 ± 17.9	27.40 ± 11.58^{B}	0.65 ± 0.42^{AC}
Met	2.7 ± 0.6	366.3 ± 18.6	12.23 ± 0.65^{AB}	0.73 ± 0.12^{AB}
Metsup	3.5 ± 0.6	397.5 ± 14.9	10.80 ± 1.24^{A}	21.58 ± 18.60^{BC}
Die	3.3 ± 0.5	368.3 ± 37.4	16.55 ± 10.55^{AB}	11.55 ± 7.07^{B}

*Est = estrus; Estsup = superovulated estrus; Met = metaestrus; Metsup = superovulated metaestrus; Die = diestrus.
#E2 = 17β-estradiol plasma concentration; P4 = progesterone plasma concentration.
^{A,B,C}within the same column indicate difference ($P < 0.05$).

Table 2 shows the results of eosinophilic counts at the different regions of the oviducts for each group. No differences ($P > 0.05$) were found among groups for each oviductal segment. When groups were combined according to estrus (Est + Estsup) and metaestrus (Met + Metsup) phases, highest numbers of eosinophils were found at the infundibulum ($P < 0.001$) during metaestrus phase; and during estrus phase, eosinophilic counts in the infundibulum were only higher ($P < 0.02$) than those found at the ampulla. At the ampullary/isthmic transition ($P < 0.01$) and isthmus ($P < 0.04$), these numbers were higher at estrus than at metaestrus phase. Despite these differences, no correlations ($P > 0.05$) were found between numbers of mucosal eosinophils and hormonal levels.

Table 2 depicts the results for the numbers of mucosal mast cells at the different oviductal regions. No differences ($P > 0.05$) among groups were found in numbers of mucosal mast cells at each oviductal segment. However, when groups were combined, at the infundibulum ($P < 0.01$) and ampulla ($P < 0.05$) the numbers were greater during metaestrus than during estrus phase. Nevertheless, at both estrus ($P < 0.001$) and metaestrus ($P < 0.02$) phases the highest number of mast cells were seen at the isthmus (Table 2).

Independently of the phase of the cycle, a positive correlation between mast cell number and P4 circulating levels was found only at the infundibulum ($P < 0.001$; $r = 0.69$) and ampulla ($P < 0.03$; $r = 0.51$). No correlation was found between mast cell number and E2 circulating levels ($P > 0.05$) and between eosinophil numbers and mast cell numbers ($P > 0.05$) at the oviductal mucosa.

Table 2. Median for mucosal eosinophils and mast cells (numbers/mm^2) at different regions of heifer oviduct, according to the phases of natural or superovulated estrous cycles.

Cell type	Group*	Region			
		Infundibulum	Ampulla	Ampulla/Isthmus	Isthmus
Eosinophil	Est	6.46	1.39	0.79	1.99
	Estsup	4.06	1.08	2.58	2.09
	Met	3.90	0.26	0.00	0.00
	Metsup	6.05	1.10	0.26	0.86
	Die	0.27	1.12	0.00	0.00
	Estrus phase	4.27^{b}	1.08^{a}	$2.05^{ab, A}$	$2.09^{ab, A}$
	Metaestrus phase	5.24^{b}	1.06^{a}	$0.00^{a, B}$	$0.00^{a, B}$
Mast cell	Est	6.50	3.44	10.38	35.90
	Estsup	8.37	7.17	1.85	25.27
	Met	6.80	8.64	18.86	28.85
	Metsup	14.04	12.92	0.00	38.70
	Die	9.43	8.69	37.72	42.35
	Estrus phase	$7.49^{b, A}$	$5.27^{ab, A}$	3.12^{a}	26.68^{c}
	Metaestrus phase	$12.72^{b, B}$	$9.80^{b, B}$	1.94^{a}	34.97^{c}

*Est = estrus; Estsup = superovulated estrus; Met = metaestrus; Metsup = superovulated metaestrus; Die = diestrus.
^{a,b,c}within the same line and ^{A,B} within the same column indicate difference ($P < 0.05$).

Discussion

A greater eosionophil concentration at the infundibulum in relation to the other regions of the oviduct, mainly during the estrus phase (Table 2), was observed in the present study. Matsuda *et al.* (1983) studied only the ampulla and isthmus and found no difference between these segments. This greater eosinophilic concentration at the infundibulum, mainly at estrus phase, could indicate that these cells play some role in the capture and initial transport of oocytes by the oviduct. Also, Lee (1982) found a greater concentration of eosinophils in the myometrium and perimetrium of rats during metaestrus and diestrus phases, and in the endometrium during proestrus and estrus phases. Since there is no clear mucosal, muscularis and serosal layers in the infundibulum (Lombard *et al*., 1950), the distribution of eosinophils in the individual layers cannot occur at the infundibulum.

The greatest concentration of eosinophils at the ampullary/isthmic transition and isthmus during the estrus phase of the cycle (Table 2) is in agreement with the findings of Brown and Nellor (1968), Tchernitchin *et al*. (1974), Matsuda *et al*. (1983) and Tchernitchin and Galand (1983). However, its functions under these circumstances are still unknown. Gouon-Evans and Pollard (2001) did not find any relation between endometrial eosinophils and fertility in rats; Grunert *et al*. (1984) have reported that the greater vascular permeability mediated by eosinophils increases the amount of luminal oviductal secretion, which could in some way influence fertility; and Wang *et al*. (2000) have stated that the presence of eosinophils at the mucosal female genital system is important for protection against invasion of infectious agents. This latter observation corroborates our finding of greatest numbers of eosinophils at the isthmus during the estrus phase of the cycle compared to metaestrus phase (Table 2), since the first oviductal region to be committed in an ascending infection during estrus would be the isthmus.

According to Lee (1982), Grunert *et al*. (1984) and Ramos *et al*. (2000), the uterine reduction in the number of eosinophils during the metaestrus phase of the cycle takes place through an unknown mechanism of P4 action, which would enhance degranulation of these cells. This could possibly explain the reduction of eosinophils in ampulla/isthmus and isthmus during the metaestrus phase in the present study (Table 2).

The use of superovulated animals in the present experiment allowed the establishment of circulating E2 and P4 levels greater than those normally found during the estrous cycle, thus increasing the chances of detecting correlations between circulating levels of these hormones and the concentration of the eosinophils and mast cells in the oviductal mucosa. However, no significant correlation was found between E2 and P4 circulating levels and eosinophils, not even at the ampullary/isthmic transition and at isthmus, where such difference was seen among estrus and metaestrus phases of the cycle (Table 2). This finding shows that, in the cow oviduct, the greatest incidence of eosinophils does not seem to be related to the increase of circulating E2, thus contrasting with the findings obtained in the endometrium of sows (Brown and Nellor, 1968) and women (Tchernitchim *et al.*, 1974; Tchernitchin and Galand, 1983). In rat cervix the reduction of eosinophils incidence is attributed to P4 (Ramos *et al*., 2000), but in this experiment the reduction of such cells should not be attributed to the effect of P4.

The greatest concentration of mast cells was seen at the isthmus (Table 2), which is similar to that observed by DuBois *et al*. (1980) and Ozen *et al*. (2002). Nevertheless, the concentration of mast cells was higher during the metaestrus phase than during estrus phase at the infundibulum and ampulla (Table 2) and was also accompanied by a positive correlation with the circulating levels of P4. The greatest concentration of these cells at the infundibulum and ampulla might be related to the tissue remodeling process, in which mast cells play a role (Abbas *et al*., 2000).

The greatest numbers of mast cells in the oviductal segments closer to the uterus could indicate, during estrus, a role of these cells in the mechanism of sperm capacitation since heparin, one of the components of oviductal secretions involved in that process (Parrish *et al.*, 1989), is also present in the secretion of mast cells (Scott and Stockham, 2000). Sperm capacitation begins with preovulatory P4 acting upon plasma and acrosomal membranes of the sperm at the isthmus, leading to intracellular changes that culminate with capacitation (Hunter, 1999), which in turn can be mediated by binding of heparin to receptors of the spermatozoon plasma membrane (Parrish *et al*., 1994; O'Flaherty *et al*., 1999). Conversely, during metaestrus, the isthmus is responsible for the maintenance of the embryo until its arrival in the uterus four days following ovulation (Harper, 1994). However, the greatest numbers of mast cells found at the isthmus (Table 2), regardless of the estrous cycle phase or of P4 circulating levels, does not mean that these cells are the source of heparin for the process of sperm capacitation or that they are related to the embryonic maintenance, thus making difficult to understand its role at this segment. In women, Weidinger *et al*. (2003) suggest the participation of mast cells in the inhibition of sperm motility, because these cells produce tryptase, an enzyme exerting this action upon spermatozoa.

No correlation was found between eosinophil and mast cell numbers, which is in agreement with previous findings of no effect of mast cells and/or histamine upon uterine eosinophilia in rats (Terada *et al.*, 1985).

The greatest number of eosinophils at the ampulla/isthmus and isthmus during estrus phase (Table 2) could not be linked to E2 and P4 circulating levels, or to the presence of mast cells or its products.

Thus, this increase of eosinophil population should be explained by other factors, like chemoattractant factors for eosinophils, such as eotaxin (Lee *et al*., 1989; Zhang *et al*., 2000; Gouon-Evans and Pollard, 2001) and leucotrienes (Benitez-Briesca *et al*., 1989). Conversely, the synergistic actions of eosinophils and mast cells in inflammatory processes (Henderson *et al*., 1980; Abbas *et al*., 2000; Young, 2000) and tissue remodeling (Abbas e*t al*., 2000) could not be detected in the oviducts.

Information about the mechanisms that regulate the presence of eosinophils and mast cells in the bovine oviducts is still lacking. The reason for the occurrence of greater numbers of eosinophils at the ampullary/isthmic transition and isthmus mucosa during the estrus phase of the cycle does not seem to be due to variations in E2 and P4 circulating levels, but the increase in mast cell numbers at the metaestrus phase is probably related to the greater P4 circulating levels. Furthermore, superovulation did not have an effect on the concentration of eosinophils and mast cells in the oviductal mucosa during estrus and metaesrus.

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References

Abbas AK, Lichtman AH, Pober JS. 2000. Immediate hypersensitivity. *In*: Abbas AK, Lichtman AH (Eds.). *Cellular and Molecular Immunology*. 4th ed. Philadelphia: W.B. Saunders. pp. 424-444.

Benitez-Briesca L, Villanueva C, Amezcua JL, Guevara R, Freyre R. 1989. Experimental uterine eosinophilia in the rat. The role of some inhibitors of arachidonic acid metabolism. *Archiv Invest Med*, 20:273-278.

Brown JE, Nellor JE. 1968. A relationship between ovarian and genital tract eosinophilic granulated cells. *Anat Rec*, 162:15-24.

DuBois JA, Wordinger RJ, Dickey JF. 1980. Tissue concentrations of mast cells and lymphocytes of the bovine uterine tube (oviduct) during the estrous cycle. *Am J Vet Res*, 41:806-808.

Gouon-Evans V, Pollard JW. 2001. Eotaxin is required for eosinophil homing into the stroma of the pubertal and cycling uterus. *Endocrinology*, 142:4515-4521.

Grunert G, Porcia M, Neumann G, Sepúlveda S, Tchernitchin AN. 1984. Progesterone interaction with eosinophils and with responses already induced by oestrogen in the uterus. *J Endocrinol*, 102:295-303.

Harper MJK. 1994. Gamete and zygote transport. *In*: Knobil, E and Neill, JD (Eds.). *The Physiology of Reproduction*. 2nd ed. New York: Raven Press. pp. 123-187.

Henderson WR, Chi EY, Klebanoff SJ. 1980. Eosinophil peroxidase-induced mast cell secretion. *J Exp* Med, 152:265-279.

Hunter RHF. 1999. Factors influencing sperm migration in the fallopian tubes of farm animals. *Reprod Domest Anim*, 34:227-235.

Lee SH. 1982. Uterine epithelial and eosinophil estrogen receptors in rats during the estrous cycle. *Histochemistry*, 74:443-452.

Lee YH, Howe RS, Sha S-J, Teuscher C, Sheehan DM, Lyttle CR. 1989. Estrogen regulation of an eosinophil chemotactic factor in the immature rat uterus. *Endocrinology*, 125:3022-3028.

Lendrum AC. 1944. A stain for eosinophil cells. *J Pathol Bacteriol*, 56:441-444.

Lombard L, Morgan BB, McNutt SH. 1950. The morphology of the oviduct of virgin heifers in relation to the estrous cycle. *J Morphol*, 86:1-23.

Luna LG. 1968. *Manual of Histologic Staining Methods of the Armed Forces, Institute of Pathology*. 3rd ed. New York: McGraw-Hill.

Matsuda H, Okuda K, Imori T. 1983. Tissue concentrations of eosinophils in the bovine oviduct and uterus at different stages of the oestrous cycle. *Res Vet Sci*, 34:369-370.

O'Flaherty CM, Beorlegui NB, Beconi MT. 1999. Reactive oxygen species requirements for bovine sperm capacitation and acrosome reaction. *Theriogenology*, 52:289-301.

Ozen A, Asti RN, Kurtdede N. 2002. Light and electron microscopic studies on mast cells of the bovine oviduct. *Dtsch Tierarztl Wochenschr*, 109:412-415.

Parrish JJ, Susko-Parrish JL, Handrow RR, **Sims MM, First NL**. 1989. Capacitation of bovine spermatozoa by oviduct fluid. *Biol Reprod*, 40:1020-1025.

Parrish JJ, Susko-Parrish JL, Uguz C, First NL. 1994. Differences in the role of cyclic adenosine 3', 5'-monophosphate during capacitation of bovine sperm by heparin or oviduct fluid. *Biol Reprod*, 51:1099-1108.

Ramos JG, Varayoud J, Kass L, **Rodriguez H, Muñoz de Toro M, Montes GS, Luque EH**. 2000. Estrogen and progesterone modulation of eosinophilic infiltration of the rat uterine cervix. *Steroids*, 65:409-414.

Scott MA, Stockham SL. 2000. Basophils and mast cells. *In*: Feldman BF, Zinkl JG, Jain NC (Eds). *Schalm's Veterinary Hematology*. 5th ed. Philadelphia, USA: Lippincott Williams & Wilkins. pp. 308-317.

Tchernitchin A, Roorijck J, Tchernitchin X, **Vandenhende J, Galand P**. 1974. Dramatic early increase in uterine eosinophils after oestrogen administration. *Nature*, 248:142-143.

Tchernitchin AN, Galand P. 1983. Oestrogen levels in the blood, not in the uterus, determine uterine eosinophilia and oedema. *J Endocrinol*, 99:123-130.

Tchernitchin NN, Tchernitchin AN, Mena MA, **Villarroel L, Guzmán C, Poloni P**. 1998. Effect of subacute exposure of lead on responses to estrogen in the immature rat uterus. *Bull Environ Contam Toxicol*, 60:759-765.

Terada N, Yamane T, Matsumoto K, **Asai H, Kitamura Y**. 1985. Estrogen-induced increase in eosinophil number and peroxidase activity in uterus of genetically mast cell-deficient W/WV mice. *Biol Reprod*, 33:899-901.

Valle GR, Cassali GD, Nogueira JC, **Castro AC**, **Reis AM**, **Cardoso FM**, **Figueiredo CB**, **Nascimento EF**. 2007. Nuclear estrogen and progesterone receptors in the oviduct of heifers under natural and superovulated estrous cycles. *Anim Reprod Sci*, 101:28-37.

Wang D, Ishimura R, Walia DS, **Müller H**, **Dai G**, **Hunt JS**, **Lee NA**, **Lee JJ**, **Soares MJ**. 2000. Eosinophils are cellular targets of the novel uteroplacental heparin-binding cytokine decidual/trophoblast prolactin-related protein. *J Endocrinol*, 167:15-28.

Weidinger S, Mayerhofer A, Frungieri MB, **Meineke V, Ring J, Kohn FM**. 2003. Mast cell-sperm interaction: evidence for tryptase and proteinase-activated receptors in the regulation of sperm motility. *Hum Reprod*, 18:2519-2524.

Young KM. 2000 Eosinophils. *In*: Feldman BF, Zinkl JG, Jain NC (Eds.). *Schalm's Veterinary Hematology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins. pp. 297-307.

Zhang J, Lathbury LJ, Salamonsen LA. 2000. Expression of the chemokine eotaxin and its receptor, CCR3, in human endometrium. *Biol Reprod*, 62:404-411.