

BLOOD LEUKOCYTES IN CARTHUSIAN MARES WITH ENDOMETRIOSIS OR ENDOMETRITIS IN COMPARISON WITH HEALTHY MARES

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Summary: Significant changes in total blood white blood cells (WBC) and populations are found in many generalized and localized pathological conditions in veterinary medicine. Endometritis is an inflammatory process that might lead to infertility in mares and it is associated with an influx of polymorphonuclear neutrophils (PMN) into the uterine lumen. Endometriosis is a chronic degenerative endometrial disease that causes severe alterations in the connective tissues of the uterus. We took blood samples from 6 Carthusian mares with endometritis (EM1) and 8 with endometriosis (EM2) in order to check whether these changes lead to changes in hematology. Further, 6 healthy mares of the same breed and similar age were also sampled (control group, CG). An analysis of variance was used to evaluate the differences between the three groups of mares. In comparison with CG, mares of group EM1 had significantly higher hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, segmented PMNs and neutrophils/lymphocytes ratio, and lower total white blood cells and lymphocytes. Mares of group EM2 presented lower red blood cell and white blood cell counts. No morphological changes were detected in the different types of WBC when examined in the blood smear. It is concluded that some hematological parameters differ in mares with endometritis and endometriosis in comparison to healthy mares. Even though the hematological study is not useful in the diagnosis of these diseases, the results of the present research are important in order to diagnose other clinical conditions that could coexist with these reproductive diseases.

Key words: endometritis; endometriosis; hematology; horse; mare

Introduction

Leukocytes (WBC) are vital for host defense, and for the initiation and control of inflammation and immunity. Although generally protective or supportive of the host tissue, WBCs are also involved in host-harmful allergic and immune-mediated diseases. Significant changes in blood WBC numbers and/or of their different populations, with or without morphological changes are observed in many diseases and they are of pivotal help in the diagnosis and prognosis in veterinary

medicine. The pattern of the WBC response depends on many factors, such as animal species, susceptibility of the patient, virulence of the pathogenic agent involved, immun status of the patient and extension of the inflammation and/or infection (localized vs. generalized) (1, 2). The WBC response to different reproductive diseases has been reported in small animals. Dogs with localized infections, such as pyometra exhibits leukocytosis with neutrophilia and sometimes, toxic changes in neutrophils and left shift, with increased band neutrophil numbers (3, 4, 5, 6).

Endometritis encompasses endometrial changes associated with acute or chronic inflammation and it is a major cause of mare infertility arising

ing from incapacity or failure to remove bacteria, spermatozoa and inflammatory exudates. Defects in genital anatomy, myometrial contractions, lymphatic drainage, mucociliary clearance, cervical function vascular degeneration and ageing underlie endometritis (7). It should be promptly diagnosed and resolved in order to avoid chronicity. Endometritis might be difficult to identify, because clinical signs, ultrasonographic and laboratory findings can vary according to the cause. Some cases are associated with an influx of inflammatory cells, mainly polymorphonuclear neutrophils (PMN) and fluid into the uterine lumen (8). Although affected mares do not show clinical signs associated with systemic disease, it is plausible to think that an acute inflammation might exist. Recently, it has been reported that mares with endometritis experimentally induced by intrauterine infusion of *E. coli* had increased serum concentrations of some acute-phase proteins (e.g. amyloid A and fibrinogen). These results indicated that endometritis in mares is linked to a systemic acute phase response (9).

Endometriosis is a chronic degenerative endometrial disease involving severe alterations in the uterine connective tissues. It is characterized by destruction of the uterine gland, including cystic dilation, hyperplasia and periglandular fibrosis, glandular epithelial hypertrophy, lymphatic lacunae or lymphangiectasia, angiosis (a vascular pathological condition with associated venous congestion) and, subsequently resulting in the development of a stromal fibrosis (10, 11). Briefly, the term 'endometritis' is used to describe inflammatory changes, whereas 'endometriosis' reflects chronic changes associated with age and parity (12, 13).

When an acute phase response appears, the number and percentage and/or the morphological

characteristics of the different blood WBC populations might change. The current study aims to compare the hematological profile in three groups of mares: healthy, mares with endometritis and with endometriosis. It is hypothesized that mares with endometritis will show modifications in the leukogram in response to the inflammation. Further, both of them could be chronic processes, and therefore, it is also plausible to evidence a leukogram of chronic disease.

Material and methods

This research was approved by the Ethic Committee for Animal Experimentation of the Cardinal Herrera-CEU University. Jugular venous blood samples were withdrawn from a total of 20 adult Carthusian mares, divided into three groups: healthy (n=6), considered as control group, CG, mares with endometritis (n=6), EM1 and with endometriosis (n=8), EM2. The 20 mares had previously foaled, although the rate of success was different. The reproductive history of the studied mares is presented in table 1. The mares were considered healthy (CG) when the echography of the reproductive system was unremarkable, physical examination did not provide any clinical indication of disease, all of them were pregnant without problems, have viable foals and had hematological parameters within the reference range established for this breed (14, 15). The diagnosis of mares EM1 was made with ultrasound (Pie Medical 480®), cytology and microbiological cultures. Mares of EM1 presented pus or fluid in the uterine lumen and endometrial edema and cytology showed predominant numbers of PMN. Diagnosis of mares of group EM2 was made by echography and endometrial biopsy. The echography showed increased echotexture in endometrial mucosa and endometrial

Table 1: Age and reproductive history of the 20 Carthusian mares included in this research (CG, control group; EM1, with endometritis; EM2, with endometriosis)

Mare groups	Age (mean±SD)	Number of previous successful foaling	Previous foal
CG (n=6)	8.345±2.45	3 foals (n=6)	Previous mating (n=6)
EM1 (n=6)	10.54±2.12	1 foal (n=3) 2 foals (n=2) 3 foals (n=1)	1 season without successful mating (n=4) 2 seasons without successful mating (n=2)
EM2 (n=8)	14.96±1.23	1 foal (n=4) 2 foals (n=4)	At least 3 seasons without successful mating

biopsy revealed a chronic endometriosis degree III (system of Kenney and Doig; 12, 13).

The mares of the three groups were sampled in the morning, before feeding. The samples were extracted in the follicular phase, 2-3 days before ovulation. Immediately after blood extraction, and directly from the syringe, without anticoagulant, a blood smear was carried out. The rest of the blood was poured into tubes with EDTA-3K for hematology and into glass tubes in order to obtain serum to measure total proteins. In all the cases, the samples remained refrigerated during the transportation to the laboratory and the analyses were performed within the first 12 hours after extraction.

The following hematological parameters were included in this research: red blood cells (RBC), hemoglobin concentration (HB), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) and platelets (PLT). These measurements were made using a semiautomatic counter (System F820®). The differential WBC count was performed in the blood smear, fixed with ethanol and stained with May-Grünwald-Giemsa technique. The absolute number of the WBC populations was quantified by microscope (Olympus CX 21®): lymphocytes (LYMP), polymorphonuclear

neutrophils (PMNs), segmented PMN (SPMN) and in band PMN (BPMN), eosinophils (EOS), monocytes (MON), basophils (BAS) and neutrophil/lymphocyte ratio (N/L). In the blood smear, we also assessed the presence of morphological changes in the PMNs, such as Döhle's bodies, basophilia, cytoplasmatic vacuolization, nuclear edema and/or hypersegmentation. They were subjectively scored over 5 points (0: absence of changes; 5: maximum morphological changes). Finally, the concentration of total serum proteins (TSP) was assessed by spectrophotometry with specific reagents (Metrolab, RAL®).

Data are presented as means±SD. Differences between the three groups were analyzed with an analysis of variance ANOVA. Statistical significance was set at $p < 0.05$. The statistical program Statistica v. 7.0 for windows was used (Statsoft®, Inc, 2001. Tulsa, Oklahoma, USA)

Results

The hematological parameters for the three groups of mares (CG, EM1 and EM2) are presented in table 2. Mares of group EM1 in comparison to CG had higher HB, HT, MCV, MCHM, SPMN and N/L ratio and lower WBC and LYMP. Mares of

Table 2: Mean±standard deviation of the hematological parameters in three groups of Carthusian mares (CG, control group; EM1, with endometritis; EM2, with endometriosis). Significant differences from the CG are indicated with an asterisk. Level of significance $p < 0.05$ (RBC, red blood cells; HB, hemoglobin concentration; HT, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; TSP, total serum proteins; WBC, white blood cells; SPMN, segmented polymorphonuclear neutrophils; BPMN, band neutrophils; LYMP, lymphocytes; EOS, eosinophils; MON, monocytes; N/L, neutrophil/lymphocyte ratio)

Parameter and unit	CG	EM1	EM2
RBC ($10^6/\mu\text{l}$)	9.43±1.58	9.49±2.29	8.90±2.34*
HB (g/dl)	12.60±1.40	13.50±2.06*	13.06±2.77
HT (%)	46.39±6.21	49.85±12.93*	47.51±13.76
MCV (fl)	50.39±2.33	52.43±2.86*	52.82±5.54
MCHC (g/dl)	26.86±5.23	27.82±3.84*	29.55±13.65
PLT ($10^3/\mu\text{l}$)	199.1±116	143.2±104	164.1±118
TSP (g/dl)	7.35±0.30	7.72±0.46	7.50±0.49
WBC ($10^3/\mu\text{l}$)	12.44±2.28	11.40±5.97*	10.46±8.01*
SPMN ($10^3/\mu\text{l}$)	4.92±0.15	5.45±1.46*	5.09±2.17
BPMN ($10^3/\mu\text{l}$)	0.21±0.01	0.24±0.12	0.24±0.28
LYMP ($10^3/\mu\text{l}$)	5.06±0.12	3.18±0.59*	4.55±2.30
EOS ($10^3/\mu\text{l}$)	0.67±0.04	0.69±0.48	0.65±0.12
MON ($10^3/\mu\text{l}$)	0.22±0.01	0.24±0.10	0.24±0.08
N/L	1.02±0.03	1.84±0.69*	1.21±0.64

group EM2 showed lower RBC and WBC (Table 2). No morphological changes in PMN neither in LYMP were found when assessing the blood smears.

Discussion

In the present research, we have found significant differences when comparing the hematological profile in mares with localized diseases (endometritis and endometriosis) with healthy mares. Both conditions are common causes of reproductive failure in mares and they are not accompanied by systemic clinical signs. However, they can cause changes in the uterine tissues that could lead to changes in the blood profile. Thus, endometritis is associated with an infiltration of inflammatory cells, mainly PMN in the stratum compactum (16, 17). Recently, it has been demonstrated that endometritis induces a systemic acute phase reaction, with increased serum amyloid (9). Traditionally, fever and changes in WBC numbers and/or morphology have been considered to be the hallmarks of inflammation and infection, even though many reports lately have confirmed that they are less sensitive than acute phase proteins (18, 19). Despite these ideas, it is plausible to think that endometritis could induce some variations in the hematological profile of the mare.

The higher HT of the EM1 mares seemed to result from the higher size of the erythrocytes (higher MCV), as RBC were similar in both groups. The reasons of these results are unknown. Firstly, this result was not associated with dehydration, as TSP did not exhibit significant differences between both groups. This was an unexpected finding, as chronic diseases lead to a normocytic normochromic anemia or do not exert any significant effect on the number of circulating RBCs (20, 21).

Increased PMN occurs during infectious and non-infectious inflammatory conditions (20, 21). Immature PMN, i.e. BPMN is the most sensitive differentiator of infectious inflammatory disease, but they are not seen as frequently in horses as in other animal species (21). In equids, BPMN are most commonly seen in severe acute bacterial infections or septicemic processes. This can be the explanation for the similar values for BPMN found in CG and EM1. The higher number of SPMN in EM1 is consistent with inflammation. It has been demonstrated that PMN increased in uterus exu-

dates at 30 min after experimental endometritis, induced by infusion of *Streptococcus zooepidemicus* (16). The lower LYMP in group EM1 could be the reflex of stress of the disease. Other causes of decreased LYMP, such as viral infections or glucocorticoid administration do not appear to be important in our mares. Probably both facts i.e. increased SPMN and decreased LYMP led to the higher N/L of the EM1 group in comparison with CG.

Endometriosis is a chronic degenerative endometrial disease associated with age and parity and with the presence of fibrosis (10, 11). The lower RBC in the mares of group EM2 in our study is consistent with the chronicity and degenerative condition of the process.

Surprisingly, the other types of WBC, mainly MON, were not different between groups. Increased MON are found during disease processes with increased tissue demand for phagocytosis of particles, such as tissue necrosis and infection (20, 21), as happen in endometritis. Finally, it is interesting to indicate that HT, MCV, TSP WBC, SPMN, BPMN, LYMP, EOS and N/L ratio had greater SD in both diseased groups (EM1 and EM2) than in CG. This result has two implications. Firstly, it could have limited the evidence of statistical significance when comparing the three groups and secondly, it might indicate the clinical differences between groups.

Our results indicate that some hematological parameters are different in Carthusian mares with endometritis and with endometriosis in comparison with a control group of mares of the same age and breed. Although the hematological profile is not used for the diagnosis of these diseases, it is important to establish whether the hematological parameters vary in mares with these reproductive problems in order to diagnose or to assess other clinical conditions that could co-exist with these conditions.

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LEVKOCITI V KRVI KARTUZIJSKIH KOBIL Z ENDOMETRIOZO ALI ENDOMETRITISOM V PRIMERJAVI Z ZDRAVIMI KOBILAMI

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Povzetek: Pri številnih splošnih in lokaliziranih patoloških stanjih ugotavljamo znatne spremembe vseh belih krvničk (WBC) kot tudi njihovih populacij. Endometritis je vnetni proces, ki lahko vodi do neplodnosti pri kobilah in je povezan z vdorom polimorfonuklearnih nevtrofilcev (PMN) v svetlino maternice. Endometrioza je kronična degenerativna endometrična bolezen, ki povzroča resne spremembe v vezivnem tkivu maternice. Odvzeli smo vzorce krvi šestih kartuzijskih kobil z endometritisom (EM1) in osmih z endometriozo (EM2), da bi preverili, ali te spremembe povzročijo spremembe v krvni sliki. Poleg tega smo odvzeli vzorce šestim zdravim kobilam iste pasme in podobne starosti (kontrolna skupina, CG). Z analizo variance smo ocenjevali razlike med tremi skupinami kobil. Kobile iz skupine EM1 so imele bistveno višjo vsebnost hemoglobina, višji hematokrit, višjo povprečno prostornino krvnih celic, višjo povprečno vsebnost hemoglobina v krvnih celicah, več polimorfonuklearnih celic in višje razmerje nevtrofilci/limfociti ter manjšo količino belih krvničk in limfocitov v primerjavi s CG. Kobile iz skupine EM2 so imele manj rdečih in belih krvničk v krvi. V krvnih brisih pri različnih vrstah WBC nismo zaznali morfoloških sprememb. Ugotovili smo, da se nekateri hematološki parametri razlikujejo pri kobilah z endometritisom in endometriozo v primerjavi z zdravimi kobilami. Čeprav krvna študija ni uporabna pri diagnosticiranju teh bolezni, so rezultati raziskave pomembni za diagnosticiranje drugih kliničnih stanj, ki so lahko tudi prisotna pri omenjenih reproduktivnih boleznih.

Ključne besede: endometritis; endometrioza; hematologija; konj; kobila