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Physiological Factors in the Interpretation of Equine Hematological Profile

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1. Introduction

Cellular components of the blood reflect specific changes in an organ or body system or more often, a general response of the individual to some physiological or pathological conditions. In this chapter, we will review the main physiological factors that should be considered when interpreting equine hematological profiles. The interpretation of the hematological profile in conjunction with history and physical findings directs the clinician in the selection of other diagnostic, imaging and sampling techniques. Additionally, a hematological profile provides invaluable information concerning the severity of the disease and the response to a treatment, and it helps in establishing a prognosis. Further, horses can have different hematological disorders, making hematology important in equine medicine. A special consideration should be made for equine athletes, since the assessment of a hematological profile is pivotal in the diagnosis of a reduced performance.

2. Erythron

2.1 Introduction and specific characteristics of the equine erythrocytes

The term erythron refers to red cell precursors, the tissues in which production takes place and mature erythrocytes themselves and its functional unit, the red blood cell (RBC). The erythron is assessed from peripheral blood samples, by calculating the number of circulating RBC, hemoglobin concentration (HB), packed cell volume (PCV), volumetric indices, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), a morphological examination at microscopic level (Messer, 1995; Kramer, 2000) and sometimes, examination of the bone marrow (Lording, 2008). When interpreting an equine hematological profile, some specific characteristics of this species should be kept in mind.

PCV unstable. The horse is somewhat unique compared to most other mammalians in that the spleen is a very capacious organ, storing between 6 and 12 L of red-cell-rich blood at rest (Persson, 1967; McKeever et al., 1993). Large numbers of RBCs temporally sequestered in the spleen can be rapidly transferred into the circulation in response to excitement (handling,

venipuncture, loss blood, twitching, and pain) and intense exercise (Persson, 1967; 1983). This response is induced by the release of catecholamines and therefore, the resting PCV in horses should be carefully assessed under different excitation levels (Persson, 1967; Schalm and Carlson, 1982). By contrast, tranquilizers and anesthetics decreased circulating RBCs, because of splenic sequestration (Jain, 1986). Comparable changes are not found in splenectomized horses following excitement and strenuous exercise or tranquilization (Kunugiyama et al., 1997).

The intensity of changes in circulating RBC in relation to spleen activity depends on individual variations, age, breed and fitness level and in the case of exercise, duration and intensity. The time required for RBCs to return to resting values is dependent on the degree of the excitement, and it can vary from 40 to 60 minutes to up to several hours (Jain, 1986).

Rouleaux formation and erythrocyte sedimentation rate. Rouleaux formation is the result of the aggregation of RBCs in linear stacks and depends on the number of RBCs and their tendency to aggregate. Rouleaux formation is a characteristic finding in healthy horses, as a result of weak surface changes on RBC membranes (Brockus et al., 2003). There is a positive correlation between the rate of rouleaux formation and the rate of setting of RBCs in anticoagulated blood (erythrocyte sedimentation rate). Rouleaux formation is accentuated in some diseases associated with hyperproteinemia, because high concentrations of plasma proteins, particularly fibrinogen and immunoglobulins, have an insulating effect that reduces the RBC surface membrane charge, promoting RBC aggregation (Schalm and Carlson, 1982; Brockus et al., 2003).

Autoagglutination can be seen in some horses without hemolysis as a result of cold antibodies, with a maximal activity at 4-20°C or as a result of unfractioned heparin treatment (Monreal et al., 1995). Macroscopically, agglutination has a granular appearance and microscopically, appears as grape-like clusters of RBCs. It should be differentiate from rouleaux by using the saline dilution test. Typically a 1:2 dilution will disperse rouleaux but not the autoagluttinated RBCs. Infrequently, a higher dilution (up to 1:10) may be needed to disperse rouleaux. Agglutination causes erroneous MCV values and RBC numbers determined by impedance, because the aggregates may interfere with the electronic or optical evaluation of the erythrocytes. Pre-treating cell suspensions from agglutinated heparin-treated horses with trypsin might reverse the agglutination, improving the accuracy of cell counts (Grondin and Dewitt, 2010).

Absence of peripheral signs of regeneration. Life span of equine RBC in the circulation is approximately 140 to 150 days (Schalm and Carlson, 1982). RBCs are released from bone marrow as mature cells and the horse is unique in failing to release reticulocytes into peripheral blood when there is a regenerative response to hemorrhage or hemolysis. Therefore, morphological features indicative of regeneration, such as reticulocytes and polychromasia are rare in equine blood films. Reticulocyte counts may be performed on marrow aspirates in anemic horses. Values greater than 5% are consistent with accelerated erythropoiesis. Further, in those cases where anemia derives from decreased erythropoiesis, bone marrow examination may identify the cause and enable to carry out a definitive diagnosis (Schalm and Carlson, 1982).

The only indication of maximally stimulated erythropoiesis in routine hematology data is anisocytosis and increased MCV (by up to 10 to 15 fl above baseline levels for an individual

horse) (Jain, 1986). Other way to evaluate regeneration is to interpret RBC distribution width or RDW. It is a coefficient of the degree of anisocytosis of circulating RBCs. This parameter will be increased in anemias with significant macrocytosis and/or microcytosis. The RDW is wider in healthy horses than in other species, and normal values range between 14 and 25% (Kramer, 2000). Similarly, assays of RBC creatine concentrations enable a more accurate evaluation of the erythropoietic response in horses. Mean RBC creatine concentration is significantly higher in young RBC populations (Wu et al., 1983), it shows a positive correlation with the reticulocyte count in bone marrow aspirates and a negative correlation with myeloid-erythroid ratio (Lording, 2008). However, it is not a common clinical procedure.

The response to hemolysis is greater than following hemorrhage, although the regenerative capability of horses is relatively poor compared with other species (Brockus et al. 2003). Complete recovery from very severe hemolytic or hemorrhagic anemia may take approximately 1 to 2 months and 2 to 3 months respectively (Lumsden et al., 1975a,b).

Howell-Jolly bodies. They are nuclear remnants of DNA that occasionally are seen in healthy equine peripheral blood films. They are small, round, purple inclusions. Increased number can be seen with enhanced erythropoiesis and with decreased or compromised splenic function (Schalm and Carlson, 1982; Kramer, 2000; Grondin and Dewitt, 2010).

RBC morphology. Equine RBC is relatively small compared to other animal species, with a mean diameter of 5-6 μm and a MCV of approximately 40 to 52 fl (Lassen and Swardson, 1995; Kramer, 2000; Grondin and Dewitt, 2010). They exhibit a mild degree of anisocytosis and RBC size may differ between horse breeds. Breeds dedicated to sport, such as Thoroughbred racehorses or Standardbred horses have an MCV lower than other breeds. Because HB is spread over a larger number of cells, the total surface of the red cell mass is increased. This adaptation of sport equine breeds appears to be a mean to achieve an easier gas exchange during exercise (Allen and Powell, 1983; Kramer, 2000).

2.2 Physiological factors influencing erythron in horses

2.2.1 Sampling handling

In relation to the anticoagulant used, blood parameters in horses are not altered after anticoagulation in heparin-lithium or EDTA. On the contrary, when using sodium citrate, most hematological parameters significantly decrease, compared with the other anticoagulants (Sharif et al., 2010).

Once the blood sample has been taken, another parameter to consider is the time to conduct the blood test. This should be done as soon as possible, preferably within the first 6 hours after collection, in order to avoid the damage caused by storage. The most common change associated with storage is an increase in the RBC size, a fact that artefactually leads to increased MCV and PCV (Allen et al., 1988). However, within limitations in some hematological parameters, equine blood samples stored in EDTA at 4ºC for a maximum of 72 hrs may be adequate for blood tests (Sharif et al., 2010).

Exposure of the sample to high temperatures or direct sunlight can cause hemolysis, resulting in altered RBC values (Rose and Hodgson, 1994).

2.2.2 Accuracy of measurements

The accuracy of the determinations results from the characteristics of the equipment of analysis. Therefore, it is very important to know the sources of errors according to the type of equipment. It has been recommended to conduct repeated measurements, allowing a more reliable interpretation of the results (Persson, 1975; Jain, 1993; Rose and Hodgson, 1994). In fact, Persson (1975) described a variation of up to 30% in baseline HB in three Standardbred horses in which blood samples were obtained in 7 consecutive days.

2.2.3 Attitude and degree of excitement of the horse

Another aspect that might influence the interpretation of RBC parameters is the attitude and the degree of excitement the horse has before and during blood withdrawal (Rose and Hodgson, 1994). Excitement leads to a rise in circulating RBC, HB and PCV. This is the result of the splenic contraction produced by the release of adrenaline and noradrenaline (Kurosawa et al., 1998). The main limiting factor is the time to collect the blood sample. Venipuncture for longer than 30 sec significantly alters the hemograma, as it involves splenic mobilization, resulting from the actions of the sympathetic-adrenal and hypothalamic-pituitary axis (Persson, 1967; Kurosawa et al., 1998).

There are many physiological factors that cause stress in the horse, such as exercise (whether in training or competition), adverse environmental conditions, particularly high heat and humidity, but also dust and very cold or windy weather, long-distance transport, insufficient rest between athletic events, lack of sleep at shows (e.g. late night events or activity in the boarding barn, stall too small for the horse to lie down and rest comfortably), new experiences during training or competition, confinement, removal from familiar environment and social group, changes in daily routine when traveling and at shows, strange environments (e.g. boarding at shows), presence and activity of strange horses and people at shows and increased stress levels in the handlers and rider and weaning, among others (Friend, 2001).

2.2.4 Method of venipuncture

An early study showed that the use of vacuum tubes for blood collection can cause cell damage (Archer, 1977). The use of higher gauge needles leads to satisfactory results according to most of the authors (Jeffcott, 1977; Messer, 1995). In our experience, additional care should be having in blood samples taken in maximally exercised horses in order to avoid hemolysis. Recently, it has been demonstrated that there are not significant differences when comparing hematological parameters obtained using two different methods: venipuncture and intravenous catheter (May et al., 2010).

2.2.5 Feeding

Another factor to take into account when interpreting equine erythrogram is the food and the time of blood sampling in relation to time of feeding. Significant increases in PCV and total plasma proteins (TPP) are found in animals after be fed. This fact has been associated with loss of fluids through the saliva and other gastrointestinal fluids, as well as fluid shifts from the circulation to the gastrointestinal system (Kerr and Snow, 1982). Similarly, there are variations in RBC parameters in horses subjected to different nutritional regimes (Greppi et

al., 1996), as well as in animals which common salt is added to the food, supplied 8 hrs before blood collection. In main lines, it is recommended to avoid collecting blood samples within 3 hrs of feeding a large concentrate meal or hay ration or at least ensure that samples are collected at the same time each day.

2.2.6 Circadian biological rhythms

RBC parameters exhibit diurnal infradian, circadian and ultradian rhythms, both in athletic and sedentary horses (Gill and Rastawicka, 1986). Gill and Rastawicka (1986) described elevations in PCV and HB overnight in comparison to light time. Hauss (1994) relates this variation to the influence exerted by alternation between periods of light and darkness on erythropoiesis. After that, Greppi et al. (1996) corroborated these results, and they also found a significant effect of biological rhythms in TPP.

2.2.7 Gender

Hematological differences linked to gender seem to have limited importance in horses. Indeed, minor differences between adult females and males have been reported. However, the results of research in this field are subjected to controversy. Males have slightly higher RBC, HB and PCV, while females have higher MCH and MCHC (Jain, 1986; Hernández et al., 2008; Satué et al., 2009). By contrast, Gill and Rastawicka (1986) observed in Thoroughbred racehorses and Quarter Horses that RBC, PCV and HB were higher in mares than in males. Persson and Ullberg (1974) had reported that baseline hematologic values were higher in stallions than in mares and geldings, probably because of the effect of androgens on erythropoiesis. However, this feature was not seen during exercise in this paper. The authors explained these results indicating that mares and geldings established a hypokinetic circulation with increased oxygen uptake by active muscle during exercise (Persson and Ullberg, 1974).

2.2.8 Season

Season is an exogenous factor that modulates the dynamic of blood components in horses, both in cycling and pregnant mares (Gill and Wanska, 1978; Gill and Kownacka, 1979; Satué, 2004; Satué et al., 2011). Indeed, the patterns of seasonal changes on RBC, HB and PCV in Thoroughbreds and Arabian horses have showed decreased values in winter (Gill and Wanska, 1978; Gill et al., 1979). In Carthusian mares, Satué et al. (2011) confirmed these results. RBC, PCV and MCV in summer were significantly higher than in spring, autumn and winter. However, HB in spring was significantly higher and MCV and MCH in spring and summer were significantly lower than in other seasons, without modifications in MCHC (Satué et al., 2011). These variations could be related to the effect of some factors, such as the breeding season (Satué et al., 2011). Furthermore, these patterns could be subjected to a different degree of tolerance to the cold, and dissimilar changes in ambient temperatures in different locations (Ruiz et al., 2004). It has been suggested that intense cold decreases RBC due to the reduction in the half-life (Ruiz et al., 2004).

2.2.9 Altitude

Horses subjected to high altitude have significantly higher RBC, HB and PCV values, compared to animals that live at less altitude. It is considered a compensatory mechanism

for the lower content of oxygen in the atmospheric air, which is proportionally reduced to the altitude (Wickler and Anderson, 2000).

2.2.10 Age

The influence of age on hematological parameters have been evaluated in different horse breeds (Ralston et al., 1988; McFarlane et al., 1998; Cebulj-Kadunc et al., 2002; 2003; Satué, 2004; Hernández et al., 2008; Satué et al., 2009). Most of the studies on age with hematology have focused on foals from birth to 4 years of age (Harvey et al., 1984; Jain, 1993), even though geriatric horses have received much attention recently, probably because of the increase of the age population (McFarlane et al., 1998).

Newborn foals have RBCs of fetal origin, large size and high RBC, HB and PCV. These parameters are reduced sharply within 12-24 hrs of life, then decline more gradually over the subsequent 2 weeks, and after that, they remain in the lower limit of the adult reference internal during the first year of life (Jain, 1986; Harvey, 1990; Grondin and Dewitt, 2010). The initial hematological changes in foals at birth are thought to be due to the increase in fetal RBC destruction, inadequate iron supplementation, needed for HB synthesis, catecholamine release and expansion of plasma volume as adjustments of fluid balance as a result of the osmotic effect of colostral immunoglobulins. Declines in these values are attributed to decreased RBC survival time, decreased iron delivery to the bone marrow, decreased stimulus for erythropoietin production as a result of higher HB saturation, increased blood oxygen content, and enhanced delivery of oxygen to the tissues due to lower 2,-3 diphosphoglycerate concentrations (Harvey, 1990). Normal adult hematological values are attained at 1-2 years of age. MCV are high at birth and then decrease, reaching its lowest values at 3-5 months of age (Jain, 1986; Harvey, 1990). They do not increase to adult values until approximately 1 year of age (Harvey, 1990). Microcytosis in foals has been attributed to a decrease in serum iron as a result of increased demand for growth. These RBCs may be too small to be recognized as erythrocytes by some impedance counts, hence generating erroneous MCV, RBC and PCV values. Mild anisocytosis is also a typical finding in young foals (Harvey, 1990). MCHC remains constant after birth and is similar to adult values (Harvey, 1990).

Stewart et al. (1970) found that PCV and HB were lower in foals younger than 2 years. Between 3 and 4 years of age, there was a gradual increase in MCV and MCH and since HB and PCV remained unchanged during this period, the increase in MCV was accompanied by a slight reduction in RBC. In Carthusian pregnant mares and in Spanish Purebred horses, Hernández et al. (2008) and Satué et al. (2009) found a reduction of RBC with a compensatory increase in MCV and MCH associated with aging. These results agree with those presented for other equine breeds, such as Standardbreds (Jain, 1986; Ralston et al., 1988), Lipizziano (Cebulj-Kadunc et al., 2002) and wild horses (Plotka et al., 1988). However, McFarlane et al. (1998) found a decreasing trend in geriatric horses, without achieving statistical significance. This fact was linked to a reduced regenerative capacity of the bone marrow (McFarlane et al., 1998).

As indicated before, increased MCV appears to be a common finding associated with aging in the horse (Ralston et al., 1988; McFarlane et al., 1998; Satué, 2004) and it has been explained as the result of changes in the dynamics of maturation of the RBCs (McFarlane et al., 1998).

2.2.11 Breed

Breed in horses exerts a significant effect on the erythron. Light horse breeds or 'hot-blooded breeds' have higher RBC, HB and PCV and blood volume compared to draft horses or 'cold-blooded breeds' (Jain, 1993; Kramer, 2000; Grondin and Dewitt, 2010). Thus, PCV as low as 24% can be found in healthy draft horses and pony breeds. Further, Thoroughbreds have smaller MCV than draft horses. Breeds ancestrally closer have minor differences in HB, MCH and MCHC (Jain, 1986). American miniature horses have lower RBC, HB and PCV but higher MCV, MCH and MCHC than other breeds (Harvey et al., 1984). Donkeys have similar RBC, HB and PCV than ponies, but much higher MCV (Jeffcott, 1977).

2.2.12 Exercise

Exercise has variable effects on the erythrogram, depending on exercise duration and intensity (short-term high intensity or maximal exercise and long-term low intensity or submaximal prolonged exercise), fitness and training levels and environmental conditions. In main lines, exercise results in increased RBC, HB and PCV. At the onset of the exercise, this rise derives from the mobilization of splenic RBC under the influence of the catecholamines. The direct effect of this increased RBC is a greater oxygen transport capacity and therefore, aerobic performance. Both the intensity and the duration of the exercise determine the magnitude of the catecholamine response (Kurosawa et al., 1998). The extent of the increase in PCV is a function of the exercise intensity in maximal exercises and in increasing-intensity exercises and a linear relationship between PCV and speed has been described (Persson, 1983; Muñoz et al., 1998; 1999). This relationship is maintained until the maximum PCV is achieved (60-65%) (Persson, 1983).

Even though the majority of the increase in PCV in high-intensity exercises is due to the splenic contraction, exercise-induced fluid shifts also have a role. A decrease of 5-10% in plasma volume is expected in short-term and in incremental-intensity exercises (McKeever et al., 1993; Muñoz et al., 1998). This reduction is attributed to the loss of sweat in order to dissipate heat produced by muscle contraction and to the exchange of fluids between the different body compartments, because of changes in blood pressure (McKeever et al., 1993; Muñoz et al., 1998; 1999).

The rise in PCV during exercise is linked to higher HB and RBC. If we consider the importance of increased HB in the oxygen transport capacity, it is plausible to think that increased PCV, HB and RBC leads to higher aerobic capacity and therefore, exercise performance (Muñoz et al., 1997). Indeed, several studies carried out in splenectomized horses have demonstrated a marked reduction in exercise performance (McKeever et al., 1993; Kunugiyama et al., 1997). On the other hand, there is a close relationship between increased PCV and blood viscosity. As a consequence, there should be a limit in the elevation of PCV that offsets improved oxygen-carrying capacity (Muñoz et al., 1997). This fact has been implied in the loss of performance of horses with red cell hypervolemia (Funkquist et al., 2000).

Other changes associated with brief maximal exercises are small increases in MCV and decreases in MCH and MCHC. Additionally, RBC in blood samples obtained after this type of exercise seems to be more resistant to osmotic stress (Smith et al., 1989), although a later study found a reduced RBC deformability (Geor et al., 1992).

On the other hand, prolonged submaximal exercise or endurance exercise leads to moderate increase in PCV, associated with loss of fluids because of the quantitative importance of sweating. In this case, the increased PCV, HB and RBC are good indices of dehydration. We have found that endurance horses with PCV higher than 50% are disqualified from competition and some of them require intensive intravenous fluid therapy (Muñoz et al., 2010; Trigo et al., 2010). The increase in PCV should be equal to the increase in TPP. In these cases where PCV increases and TPP remains unchanged, other reasons different from dehydration should be considered in order to explain these results. In fact, we found that the most rapid endurance horses in competitions can have higher PCV than the slower horses. Other reason that can lead to increased PCV in endurance horses is pain. We studied 13 endurance horses that had increased PCV (PCV> 52%) with non-increased TPP (TPP<7.2 g/dl) during a competition. One of them had laminitis, 2 had heart arrhythmias, 2 had colic, 3 were retired from competition by the owners and the remaining 5 were able to finish the competition (Trigo et al., 2010).

2.2.13 Training

Although training has limited effects on RBC parameters at rest, some differences are found between horses undergoing high-intensity and endurance training. Speed-trained horses have higher RBC, HB and PCV, which is considered an adaptation for a greater demand for oxygen uptake, stimulating RBC production. However, it is very difficult to obtain a 'true' basal blood sample in these horses, because of their demeanor and nervous temperament. The increased excitability of a horse as it gets fitter could result in elevations of RBC, HB and PCV values (McKeever et al., 1993). On the other hand, regular monitoring of the hemogram during training has little value for assessing the fitness of the horse, but it is very helpful in order to detect subclinical problems that can significantly reduce exercise performance. Decreases in PCV have been reported as a consistent finding in horses with viral respiratory tract disease and in gastric ulcers (McGowan, 2008; Nieto et al., 2009).

In Standardbred trotters, prolonged and/or intensive training can result in an excessive increase in red cell mass, phenomenon known as red cell hypervolemia, which results in a significant reduction of racing performance. Some authors have related the hypervolemia with overtraining (Golland et al., 2003). It has been hypothesized that increased blood viscosity leads to reduced capillary perfusion and inadequate utilization of oxygen by contracting muscles.

By contrast, endurance-trained horses have lower resting RBC, PCV and HB than sprint-trained horses (Muñoz et al., 2010; Robert et al., 2010; Trigo et al., 2010). In fact, in our experience, is very common to find PCV as low as 30-32% in endurance healthy horses with good performance (Muñoz et al., 2010). There are two main reasons to explain these results. Firstly, it has been indicated that feeding fibrous diets might increase water consumption and then plasma volume (Robert et al., 2010). The second reason is the effect of a greater release of aldosterone, which promotes water and electrolyte-conserving mechanisms in the kidneys and gastrointestinal tract (McKeever et al., 2002). These changes appear at the beginning of the training program, with retention of water and electrolytes after only 3 days of endurance training (McKeever et al., 2002). The advantage of plasma expansion in endurance horses is to provide extra total body water for the maintenance of cardiovascular and thermoregulatory stability during prolonged exercise in order to compensate the significant losses associated with sweating (McKeever et al., 2002; Robert et al., 2010). Despite these results, it is clear that hematological measurements are of little value in assessing the fitness or progress of endurance horses in training.

2.2.14 Reproductive status

The researchers that have evaluated the hematological changes resulting from pregnancy in the mare have provided controversial results. Studies in Thoroughbred, Arabian, Carthusian, Brazilian and Breton pregnant mares have described a significant increase in RBC parameters during pregnancy (Berlink et al., 2000; Satué, 2004; Satué et al., 2008). There is not a reasonable explanation to justify these results, although it has been hypothesized that the increased fetal metabolic requirements might condition this response (Satué, 2004). In an early study, a mild anemia appeared at the end of pregnancy (Trum, 1952). This result agrees with studies carried out in pregnant women (Bailit et al., 2007) and other animal species (Steinhardt et al., 1994; Zvorc et al., 2006). The decrease of RBC parameters in pregnancy has been associated with an absolute gain of plasma, RBC and HB. As the increase in RBC and HB is slower than the rise in plasma, a relative oligocythemia is found, despite the increased erythropoietin concentration probably derived from placental prolactin. The hypervolemia of pregnancy has been associated with water and sodium retention after an activation of the renin-angiotensin-aldosterone axis, stimulated by estrogens (Satué and Domingo, 2008). The hypervolemia of pregnancy is necessary in order to meet the demands of the gravid uterus, to protect the mother and the fetus from the harmful effects of decreased venous return and to prevent the mother from suffering the adverse effects of blood loss during delivery (McMullin et al., 2003).

It has been suggested that iron deficiency anemia is common in the pregnant mare. Detlef (1985) studied the effect of iron supplementation in pregnant mares compared with untreated control mares. In the treated mares, RBC and HB were not changed during pregnancy, whereas a decline in RBC parameters was found in the control group. Additionally, the foals born from supplemented mothers had higher HB and RBC than the foals born from the untreated mares (Detlef, 1985).

Near the parturition, RBC parameters do not change (Taylor-Macallister et al., 1997). After delivery, RBC parameters increase slightly, until the total blood volume is restored by releasing the attachments and fetal fluids. On the other hand, lactation induces a reduction in RBC, HB and PCV (Harvey et al., 1994; 2005).

2.2.15 Administration of sedatives-tranquilizers

The administration of tranquilizing compounds, such as phenothiazine derivatives (acepromazina, chlorpromazine…) and adrenergic α-2 agonists (xylazine, romifidine, detomidine…) significantly affect the RBC parameters. These drugs lead to a relaxation of the smooth muscle in the splenic capsule, promoting the storage of RBC (Jeffcott, 1977). Further, these drugs, after an initial short phase of hypertension, have a prolonged hypotensive effect. Hypotension leads to increased plasma volume, with the subsequent hemodilution and reduced RBC parameters at rest (Jain, 1986).

3. Leukon

The term leukon refers to the set of data derived from total and differential count of white blood cells (WBC) and the analysis of WBC morphology (Grondin and Dewitt, 2010). Circulating WBC represents the outcome of the dynamic production of the bone marrow, the release of the cells to the peripheral blood and, the storage in different organs or pools.

Cells can coexist in different stages of maturation, being fully mature cells (neutrophils, NEU, eosinophils, EOS, monocytes, MON, lymphocytes, LYM and basophils, BAS) and immature (band neutrophils, metamyelocytes, myelocytes and progranulocytes) (Messer, 1995; Welles, 2000; Grondin and Dewitt, 2010).

3.1 Neutrophils

The release of NEU from the bone marrow into the circulated depends on the tissue demands and the production of different humoral substances. After passing into the blood, NEU can be in the circulating pool or stored in the marginal pool (in the endothelium of several organs, such as lungs or intestine). Three main locations of the NEU can be described: 1) Bone marrow. There is a proliferating population of NEU, including promyelocytes, myelocytes, metamyelocytes, and mature ENU, prepared for release into peripheral blood; 2) Blood. In the blood compartment, mature NEU appear as round cells, 10-15 μm of diameter, with clear cytoplasm, neutral or granules stain pink and with the nucleus polymorphic and segmented and the chromatin arranged in the form of knots; 3) Tissues. In inflammatory processes, there is a release of chemotactic substances that promotes NEU migration from the vascular bed into the tissues. Marginal pool of NEU adheres to the vascular endothelium, mainly in the small vessels. This fact facilitates the migration to the tissues, while serving as a reserve, so there is a continuous exchange between circulating and marginal pools (Lassen and Swardson, 1995; Grondin and Dewitt, 2010; Smith, 2000; Welles, 2000).

Barr bodies (sex chromatin lobe/ drumstick) can be recognized in females and resemble a small purple body attached to the nucleus by a thin chromatin strand. Further, in peripheral blood, both mature and immature or band NEU can be found. Equine band NEUs are less frequently seen because horses do not exhibit marked left shifts during inflammatory insults compared to dogs and cats. In cases of bacterial infection, the band NEUs might represent between 1 and 10% of the total WBC differential count (Welles, 2000). Band NEUs have a polymorphic nucleus, without constrains, with a less condensed chromatic pattern than the segmented NEUs. The cytoplasm is similar to this of the mature NEU (Jain, 1993; Welles, 2000). Hypersegmented NEUs are rarely seen in healthy and they have five or more lobes separated by filaments. Prolonged storage of blood may lead to the artefactual development of hypersegmented NEUs. Idiopathic hypersegmentation of NEUs have been described in Quarter Horses that lacked evidences of clinical disease. Hyposegmented NEUs have also been reported in apparently healthy Arabian horses, which were diagnosed as Pelger-Huët anomaly (Grondin et al., 2007).

Circulating NEUs have a half-life of 10.5 hours, renewing approximately 1.5 times per day (Lassen and Swardson, 1995; Welles, 2000). Then, they leave the bloodstream and migrate into the tissues. It is a unidirectional movement, because they do not return to the peripheral circulation. In the tissues, NEUs are functional for 1 to 2 days, and then, they are fagocyted by the monocyte-macrophage system or by the mucosal surfaces (Welles, 2000).

3.2 Lymphocytes

LYM are the second largest population of circulating WBCs, after NEUs and the main components of the immune system. They are smaller than NEUs and the other granulocytes,

with a dark-staining nuclei, coarse chromatin pattern and scant amount of blue cytoplasm. The mature cell has a diameter of 7-12 μm, an eccentric, round nucleus with a notch on some occasions (Latimer and Rackich, 1992). LYMs are made up to 38-66% T cells, 17-38% B cells with the remaining being null cells (Tizard, 2009). Occasionally, larger LYMs are present, and they have smooth chromatin patterns and large amounts of pale blue cytoplasm (Jain, 1986). Reactive LYMs or immunocytes are rarely seen in health. They are slightly larger than small LYMs, with scalloped nuclear margins, moderately aggregated chromatin, scant to moderate amounts of intensely basophilic cytoplasm and sometimes, they have a pale-staining Golgi zone (Latimer, 1999; Grondin and Dewitt, 2010).

The half-life of LYMs varies between 20 and 200 days (Schalm and Carlson, 1982), with a mean duration of transit through the blood of 30 hrs. Blood LYMs have the ability to recirculate in the blood, lymphatic channels, lymphoid and peripheral tissues and they are able to have mitosis, allowing amplification of the immune response (Jain, 1986; Latimer, 1999; Welles, 2000). Most of the LYMs are originated in the peripheral lymphoid tissues, and only a small percentage comes from central lymphoid tissues, i.e. bone marrow and thymus. The circulation time depends on the LYM subtype and the tissue of origin. T cells circulate more rapidly than B cells and migration through the splenic parenchyma is faster than through the lymph nodes (Hopkins and McConnell, 1984).

3.3 Eosinophils

EOS are cells slightly larger than neutrophils that contain large, reddish-orange granules in the cytoplasm, often obscuring the nuclei and giving a raspberry-like appearance, with a pale blue cytoplasm (Kramer, 2000; Smith, 2000). The lobulated nucleus seldom shows fine filamentation. Degranulated EOSs are vacuolated and are rarely seen in health (Latimer, 1999; Grondin and Dewitt, 2010). The amount of EOS in peripheral blood is low, because most of these cells migrate into tissues, such as the bronchial mucosa, gastrointestinal tract…The half-life of circulating EOS is about 2 to 12 hrs (Latimer, 1999; Young, 2000).

3.4 Monocytes

MON are the largest WBC in circulation, with a large, broad, variable in shape nuclei (oval, bilobed, horseshoe) with lacy chromatin and gray-blue cytoplasm with small azurophilic granules. The cytoplasm can also have a few clear vacuoles of variable size, located in the cell periphery and with a foamy appearance (Jain, 1993; Bienzle, 2000). After their production in the bone marrow, MON are released into the bloodstream. In circulation, they distributed between the circulating and marginal pools, with a ratio of 1/3.5 between them. This ratio remains constant in different physiological states and in response to disease. The mean circulating MON life is about 8.4 hrs, and there is not exchange at the tissue level and blood. In the tissues, the MONs mature into macrophages, a transformation that is accompanied by changes in ultrastructure, in the appearance of cellular receptors or by metabolic changes. The half-life of macrophages ranges from several days to months (Bienzle, 2000; Welles, 2000).

3.5 Basophils

BAS are cells slightly larger than the NEUs, with a lobulated nucleus, although to a lesser extent than NEUs, a cytoplasm from blue to gray, with large amounts of granules

distributed irregularly, and with an intense purple stain that vary in size and shape and can mask the nucleus (Jain, 1993; Kramer, 2000).

3.6 Physiological factors influencing leukogram in horses

There are two main WBC responses, physiological leukocytosis and stress leukocytosis. Physiological leukocytosis refers to changes in circulating WBC associated with the intervention of the sympathetic-adrenal axis resulting from splenic contraction in cases of fear, excitement, or high intensity exercise. There is a mobilization of the marginal pool of NEUs and/or LYM, because of a reduction in NEU adherence capacity, increased blood flow through the microvasculature and splenic contraction (Latimer, 1999). These events result in leukocytosis with mature neutrophilia and/or lymphocytosis. In some cases, eosinophilia and monocytosis are also found (Snow et al., 1983; Welles, 2000). These changes are transient and the marginal pool of NEUs is restored again in 20-30 min after the onset of the response and the LYM counts returned to baseline after 1 hr (Rossdale et al., 1982).

Stress leukocytosis is associated with cortisol release under certain stressful situations. This hormone induces neutrophilia without left shift, lymphopenia and eosinopenia. Neutrophilia derives from the mobilization from the marginal pool, the reduced ability to migrate from the blood to the peripheral tissues and the increased mobilization of the population of bone marrow reserve. Lymphopenia is the result of LYM sequestration from lymphoid tissues and the eosinophenia derives from the marginalization of EOS in the blood vessels and the decreased release from the bone marrow (Caracostas et al., 1981; Welles, 2000). This response appears between 2 and 4 hours after the elevation of the endogenous cortisol concentrations or after exogenous administration of corticoids (Rossdale et al., 1982; Burguez et al., 1983). Normal values are recovered in 24 hrs. This response has been also found after an endurance exercise and in response to a great variety of pathological processes (Welles, 2000).

3.6.1 Breed

Minor differences have been found among equine breeds in relation to WBC, with the hot-blooded horses having higher WBC compared to cold-blooded horses (Jain, 1986; Harvey et al., 1984). Thoroughbreds and Arabian have a mean NEU/LYM ratio of 1.0, whereas cold-blooded horses and miniature horses have ratios of 1.7 and 0.67, respectively (Jain, 1986).

3.6.2 Time of the day

In Thoroughbred racing horses, Allen and Powell (1983) described that LYM count has higher in the evenings and lower in the mornings. These findings have been attributed to the circadian variations in the release of endogenous corticoids. It is well known that maximum cortisol concentrations appear in the morning (McKeever, 2011).

3.6.3 Gender

WBC and granulocytes are higher in females than in stallions, as recently found in Spanish Purebred horses (Hernández et al., 2008; Satué et al., 2009). On the contrary, previous researchers performed in warm-blooded horse breeds reported higher values in males and

in females (Lassen and Swardson, 1995; Cebulj-Kadunc et al., 2002). Other study failed to find significant differences between sexes (Lacerda et al., 2006).

3.6.4 Age

NEU number is low in the fetus (<1,500/µl, before 300 days of gestation), increases after birth in response to cortisol (8,000/µl) and then decrease to mean adult values (4000/µl) at about 4-6 months of age. Band NEU do not exceed 150/µl in healthy foals (Harvey, 1990; Allen et al., 1998). Foals born at term have higher NEU count than foals born prematurely. LYM numbers in foals are low at birth (average 1,400/µl), increase to 5,000/µl at 3 months of age, and reach adult values at 1 year of age (Jain, 1986; Harvey, 1990). LYMs further decline during adulthood while NEU count remains the same, resulting in a higher NEU/LYM ratio in aged horses compared to foals (Jain, 1986). The ratio NEU/LYM reaches values of 2/1 in geriatric horses (Jain, 1993; Lassen and Swardson, 1995; Hernández et al., 2008). The progressive trend towards lymphopenia in geriatric horses is characterized by a reducing in B cells CD4+ and CD8+, in relation to immune senescence (Smith et al., 2002; Hernández et al., 2008; Satué et al., 2010).

EOSs are not routinely detected in the fetus and in foals at birth, achieving a mean of 400/µl by 4 months of age (Harvey, 1990). EOSs count increases with aging due to prolonged exposure to allergens through life (Jain, 1993; Satué et al., 2009). However, McFarlane et al. (1998), Cebulj-Kadunc et al. (2003) and Hernández et al. (2008) did not found differences in EOS count between young and adult horses. Band NEU, MON and BAS do not seem to change with aging in horses (Harvey, 1990; Jain, 1993; Lassen and Swardson, 1995; Cebulj-Kadunc et al., 2003; Satué, 2004; Hernández et al., 2008).

3.6.5 Exercise

WBC show different responses according to the type of exercise. Sprint exercise is associated with leukocytosis because of neutrophilia but mainly because of lymphocytosis, with a decrease in NEU/LYM ratio. These changes are likely secondary to catecholamine release and splenic contraction. At 3 hr after exercise, there is an increase in NEU/LYM ration, because the increase of NEU and decrease in LYM associated with cortisol concentrations. The NEU/LYM ratio returns to baseline values by 6 hrs after exercise (Snow et al., 1983).

Endurance exercise is associated with leukocytosis, resulting from a neutrophilia and lymphopenia (Snow et al., 1982). Probably this is combined effect of increased circulating corticosteroids and splenic contraction. Horses that completed an endurance event at a faster speed have higher NEU/LYM ration than slower horses (Trigo et al., 2010). Additionally, it has been demonstrated that exhausted endurance horses had left shift in the NEUs and significantly lymphopenia (Trigo et al., 2010).

3.6.6 Training

Total WBC is unchanged during training for racing and for endurance events. In addition, there are no alterations in the proportions of the different WBC populations. Some overtrained horses develop eosinophenia together with clinical signs of disease, and this result has led to the hypothesis that EOSs may be a more sensitive indicator of training stress than other types

of WBCs (Tyler-McGowan et al., 1999). It is important to take into account that decreased NEU count and later, increased LYM count is consistent with systemic or respiratory disease, that are common causes of loss of performance in trained horses (McGowan, 2008).

3.6.7 Reproductive status

Although in main lines, estrous cycle and pregnancy do not change substantially the leukogram (Berlink et al., 2000; Da Costa et al., 2003), some studies have found a reduction in WBC, NEU and EOS counts during pregnancy (Satué, 2004; Satué et al., 2010). The reduction in WBC in Thoroughbred mares appears during the first 4 months of pregnancy, with a trend toward increase from half of pregnancy and it is maintained until the time of delivery (Gill et al., 1994). During delivery, leukocytosis with neutrophilia, lymphopenia and eosinopenia appear, in association with the hypothalamic-pituitary-adrenal stimulation and glucocorticoid release (Silver et al., 1984; Harvey et al., 1994). However, this idea has not been confirmed by all the authors (Taylor-Macallister et l., 1997). Finally, lactation induces leucopenia with an intensity proportional to the degree of stress during the period of maximum milk production (Harvey et al., 1994).

4. Platelets

Platelets or thrombocytes are cytoplasmic fragments of megakariocytes. Equine platelets stain very lightly with Wright-Giemsa stain and sometimes can be difficult to discern on blood films. They are round, oval or elongate, measuring 2.5-3.5 µm in diameter, with light blue cytoplasm with fine azurophilic granules (Kramer, 2000). The survival time of equine platelets in circulating blood is 4-7 days (Jain, 1993). Equine platelet concentrations are some of the lowest reported for mammals. Finding 6-10 platelets/field of high resolution in a peripheral blood film indicates an adequate platelet concentration. Mean platelet volume (MCV) and mean platelet mass have been reported in horses: 4.3-5.6 fl and 0.47-0.96 10^6/fl respectively (Boudreaux and Ebbe, 1998).

Morphologically, giant platelets, greater than the diameter of a RBC, are associated with accelerated thrombocytopoiesis. Platelet clumping indicates platelet activation and aggregation during blood collection, and might lead to erroneously low platelet concentrations. EDTA-dependent pseudo thrombocytopenia has been reported in a Thoroughbred gelding (Hinchcliff et al., 1993).

4.1 Physiological factors influencing platelets in horses

4.1.1 Anticoagulant

The use of EDTA as anticoagulant, although it can produce aggregation in normal situations, it is more common in patients with severe gastrointestinal disease due to platelet activation by circulating endotoxins and formation of aggregates of platelets and leukocytes (Hinchliff et al., 1993; Saigo et al., 2005).

4.1.2 Blood sample collection and analytical time

Repeated venipuncture, alterations in blood flow or delay in carrying out the analysis alter platelet count. It is advisable to perform the analysis within the first 2 hrs after collection, as MPV can be altered if the EDTA-sample is kept refrigerated. On the other hand, it is

interesting to use sodium citrate as an anticoagulant in order to measure platelet size (Sellon, 1998; Seghatchian, 2006).

4.1.3 Breed

In Quarter Horses, Jeffcott (1977) found that the number of platelets in this breed was higher than in other equine breeds. A clear explanation for this result is lacking, although factors others than the breed should be taken into consideration.

4.1.4 Age

Platelet numbers in foals do not change during the first year of life. In adult horses, age determines a progressive decrease in platelet count (Ralston et al., 1988; Jain, 1993; Satué, 2004; Satué et al., 2009), as well as in other species (Zinkl et al., 1990). By contrast, other studies in horses did not agree with these results (McFarlane et al., 1998).

4.1.5 Exercise and training

The effect of exercise on platelet parameters seems to be intensity dependent. Short brief or maximal exercise results in significant increases in platelet numbers, whereas moderate exercise does not appear to alter platelet numbers (Bayly et al., 1983). Further, some studies reported reduced platelet aggregability in response to high-intensity exercise (Bayly et al., 1983), but other authors described increased aggregability and activation of platelets (Kingston et al., 2002). One possible explanation for the diverse results is the modifications in blood pH and hemoconcentration, with changes in ionized calcium concentrations and platelet activity. In addition, and given many of the methodological and technical problems when working with equine platelets, it is unknown if training alters platelet function.

4.1.6 Reproductive status

In human beings, laboratory animals and female elephants, a marked activation of the megakaryopoiesis has been found at the end of pregnancy. This fact continues during the initial weeks after delivery, possibly in association with high concentrations of estrogen, progesterone and other steroid hormones (Jackson et al., 1992). In the mare, most of the studies concluded that pregnancy does not exert significant effects on circulating platelets (Harvey et al., 1994; Berlink et al., 2000). However, Satué (2004) and Satué et al. (2010) described a progressive decline in platelet numbers during pregnancy in Carthusian broodmares. Hormonal dynamic during pregnancy, coupled with increased levels of thromboxane B2 produced by the placenta, chorion and amnion.

While in other species delivery leads to thrombocytosis (Suárez et al., 1988; Jackson et al., 1992), mares during delivery do not develop significant changes in platelet numbers (Harvey et al., 1994). This response is attributed to the combined effect of stress and increased release of estrogen, progesterone and other steroid hormones. Finally, lactation does not exert evident influence of circulation platelet numbers in mares (Harvey et al., 1994; Satué, 2004).

5. Conclusion

Hematological profile is frequently used in horses as an aid for the diagnosis and/or consequences of systemic, infectious and some parasitic diseases. It can also provide

significant information about the response to treatment, the severity of the process and the metabolic state of an animal. Despite the wide use of hematology, interpretation is challenging because many exogenous and endogenous factors significantly modify blood parameters. The present chapter reviews the current knowledge of the influence of physiological factors on erythrocytes, leukocytes and platelets in horses.

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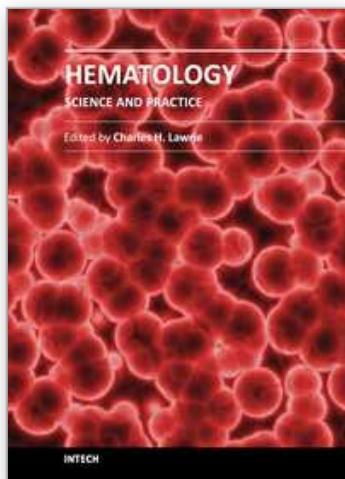
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Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

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