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Morphometric Study of Red Blood Cells in Horses.

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ABSTRACT

This study was conducted on 88 horses belonging to Arabian thoroughbred and English thoroughbred breeds; the main aims is to reveal the influence of age and sex on the diameter, the circumference and the surface of erythrocytes; measurements were achieved while using the special software OPTIKA™ Vision Pro. Statistical analysis was undertaken using the T Student test. The results showed that generally the factors age and sex have no effect on the three morphometric parameters of red blood cells. This investigation allows us to propose new reference values for the circumference and the surface of red blood cells in horses; these two parameters appear most representatives as to mark changes in the morphometry of red blood cells.
Keywords: Morphometry, Red Blood Cell, Horses, Age, Sex.

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INTRODUCTION

As in all mammals, the mature red blood cells (RBCs) of horses are round in shape, elastic and biconcave with a central pallor; has a width of 5-6µm and have no nuclei and no organelles, with a lifespan of 140-155 days. Rouleaux formation is common in horses; and Howell-Jolly bodies can be seen occasionally in equine peripheral blood films in health [1, 2]. Polychromatophilic red blood cells are rarely detected in the peripheral blood of horses [3, 4], because equine reticulocytes mature in the bone marrow even when erythropoiesis is increased [5, 6].

The total number of horse erythrocytes is between 6×10^6/µl and 12×10^6/µl; many variations must be considered when establishing references values, the most important are due to the age, sex, breed, exercise and altitude [7, 8]. Young horses have appreciably greater red blood cells counts than adults; males have greater red blood cells values than those of females [9]; however, according to Gill and Rastawicka [10] and Piccione *et al.* [11] the Arabian thoroughbred stallion have consistently lower red blood cells values than Arabian mares. Animals raised at high altitudes have a higher number of erythrocytes (altitude polyglobulia) comparable to those of animals living at lower altitudes [12, 13].

While few references were found on the effect of these factors on red blood cells morphometry. This study was carried out on Arabian an English thoroughbred horses, it aims to investigate the influence the age and the sex on the diameter, the circumference and the surface of red blood cells.

MATERIALS AND METHODS

Region of the study and animals

This study was conducted in the region of Setif, located in the northeast of Algeria, 300Km from the capital city Algiers at an altitude of about 933m. For the realization of sampling, we selected 88 horses of two breeds, which are the Arabic thoroughbred and the English thoroughbred. 44 subjects were taken for each breed; clinically healthy animals were divided according to their age and sex in four subgroups as follows: 15 adult males, 15 non-pregnant adult females, 07 young males and 07 young females.

Blood samples and smears

Blood samples were taken from the jugular vein; smears were confectioned on microscope slides immediately after venipuncture without anticoagulants, which may interfere and induce some cytoplasmic and morphometric cell changes and on the extreme provoke degranulation of some blood cells [14, 15, 16]. Slides are precisely identified (order number, age, sex and breed) and classified in slides racks, which are equipped with an information sheet, including: age and sex of the animals following the order number mentioned on the slide; these informations are recorded immediately after each sampling and smear realization.

Blood smears staining

Staining of blood smears was carried out following the classical mixed Panoptic staining of Pappenheim, especially by the dye of May-Gründwald Giemsa (M.G.G, Romanowsky type) the best and most appropriate staining to mark the mammalians erythrocytes, respecting always the protocol cited by Bacha and Bacha [17] and Houwen [18].

Morphometric study of red blood cells

For several and even until the last years, morphometric studies of red blood cells are essentially based on linear measures of erythrocytes size. Using an ocular micrometer and a lens micrometer (micrometric slide or object rule), is the only valid and recognized method to measure the size of erythrocytes. The diameter of the red blood cells is measured and estimated roughly under an optical microscope at a magnification immersion (x100) [19, 20, 21]. Besides the tedious and monotonous use of the ocular micrometer, other difficulties in measuring non-linear structures make the use of the instrument in question disappearing especially with the exponential technological progress, the advent and the generalization of high performance microscopes.

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In the current study, we used a high-performance professional optical microscope of high quality: OPTIKA B-350 (Ver.4.0.0); it is a modern, ergonomic, binocular microscope equipped with a digital camera of high resolution OPTIKAM (Ver.4.1.0) enabling the display of the microscopic image of the smear placed in the microscope on a computer in real time. The morphometric study of red blood cells is performed with special OPTIKA™ Pro Vision software of the OPTIKA microscope; measurement operations of this software are the digital version of the more traditional techniques of morphometry with optical microscopes. This is an integral and ergonomic software, which in addition to the functions of image capture and camera control, it allows efficient processing and analysis of microscopic images in an easy manner and this for any type of application and research.

Before starting the actual morphometric study, it is necessary to scan the microscopic images of red blood cells. The taking of photos for all subjects of the two breeds, is an essential step, it allows fixing the microscopic images observed and to record so that you can manipulate them using the software. The information concerning morphometric data of red blood cells are accurate only if a scale has been correctly entered and well calibrated, this requires scanning the micrometer scale engraved on the micrometric slide at the immersion objective (x100).

To study the influence of the age and the sex on the morphometric parameters of red blood, we measured the diameter of red blood cells, which is still estimated in relation to the shape of the cell, and so we developed two new parameters that have not previously treated, which are the circumference and the surface of erythrocytes. The morphometric study of red blood cells was performed by always respecting the guidelines and instructions of the manufacturer of the software. For each horse, we measured the diameter, the circumference and the surface of 50 red blood cells, and then we determine the average of each parameter for all the animals of the two breeds.

Statistical study and analysis of results

To assess the significance of the results and the influence of age and sex on the morphometric parameters (diameter, circumference and surface) we have used the analytical Student T test of the MedCalc statistical software (version 12.7, Copyright © 1993-2013 MedClack software bvba). All values were expressed as mean ± standard error; and P value under 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The Table 1 shows the influence of the age on the diameter, the circumference and the surface of red blood cells. The results reveal a significant effect of age only in the Arabian thoroughbred horses, when erythrocyte circumference was greater ($P < 0.05$) in adult males (24.64 ± 1.55) than in young males (22.52 ± 1.50); however, in females, there is no significant differences between adults and youth. Regarding the diameter and the surface of red blood cells, it should be noted that the difference between adults and young horses, is never statistically significant.

Table 1: Influence of the age on the morphometric parameters of red blood cells.

Parameters	Groups	Arabian thoroughbred		English thoroughbred	
		Adult	Young	Adult	Young
Diameter (expressed in µm)	Global (n = 14)	5.56 ± 0.22	5.70 ± 0.31	5.78 ± 0.19	5.77 ± 0.35
	Males (n = 07)	5.59 ± 0.22	5.65 ± 0.34	5.75 ± 0.23	5.82 ± 0.41
	Females (n = 07)	5.54 ± 0.23	5.74 ± 0.29	5.80 ± 0.17	5.71 ± 0.30
Circumference (expressed in µm)	Global (n = 14)	23.50 ± 1.81	22.22 ± 0.69	22.22 ± 0.69	21.86 ± 0.79
	Males (n = 07)	24.64 ± 1.55*	22.52 ± 1.50	22.37 ± 0.76	21.86 ± 0.63
	Females (n = 07)	22.36 ± 1.28	22.49 ± 1.32	22.08 ± 0.63	21.87 ± 0.79
Surface (expressed in μm^2)	Global (n = 14)	29.20 ± 2.81	27.90 ± 3.26	27.68 ± 1.87	26.84 ± 2.20
	Males (n = 07)	30.06 ± 2.67	28.18 ± 3.63	28.01 ± 2.03	26.99 ± 1.32
	Females (n = 07)	28.34 ± 2.88	27.62 ± 3.11	27.35 ± 1.79	26.70 ± 2.95

* ($P < 0.05$)

Changes relative to the influence of the sex on the morphometric parameters of red blood cells are shown in Table 2; it seems that there is a significant effect of this factor ($P < 0.05$) just in English thoroughbred

horses, where the circumference of erythrocytes is greater in adult male (22.54 ± 0.72) than that of adult females (21.74 ± 0.78). In Arabian thoroughbred, as well as for young English thoroughbred, there is no significant difference in the circumference of erythrocytes between males and females. Concerning the influence of the sex on the diameter and the surface of red blood cells in horses, the statistical analysis of the achieved results, has not shown any significant effect of the sex on these two parameters in horses.

Table 2: Influence of the sex on the morphometric parameters of red blood cells.

Parameters	Groups	Arabian thoroughbred		English thoroughbred	
		Males	Females	Males	Females
Diameter (expressed in µm)	Global (n = 22)	5.56 ± 0.33	5.64 ± 0.24	5.78 ± 0.27	5.74 ± 0.22
	Adult (n = 15)	5.52 ± 0.32	5.59 ± 0.21	5.76 ± 0.19	5.75 ± 0.18
	Young (n = 07)	5.65 ± 0.34	5.74 ± 0.29	5.82 ± 0.41	5.71 ± 0.30
Circumference (expressed in µm)	Global (n = 22)	23.12 ± 1.85	22.24 ± 1.21	22.32 ± 0.75	21.78 ± 0.82
	Adult (n = 15)	23.41 ± 1.97	22.13 ± 1.19	22.54 ± 0.72*	21.74 ± 0.78
	Young (n = 07)	22.52 ± 1.50	22.49 ± 1.32	21.86 ± 0.63	21.87 ± 0.97
Surface (expressed in μm^2)	Global (n = 22)	28.33 ± 3.60	27.52 ± 2.79	27.88 ± 1.75	26.66 ± 2.27
	Adult (n = 15)	28.39 ± 3.71	27.47 ± 2.74	28.01 ± 2.03	26.99 ± 1.32
	Young (n = 07)	28.28 ± 3.63	27.62 ± 3.11	26.99 ± 1.32	26.70 ± 2.95

* ($P < 0.05$)

For the diameter of erythrocytes, it is clear that the values obtained are similar and still within the international reference values reported particularly by Kramer [1] and Grondin and Dewitt [7]. Concerning the circumference and the surface of red blood cells, as there was no studies and no published standards that provide detailed information on these two parameters, we tried to propose and advance reference values given by intervals in Table 3. Despite obtaining statistically interpretable results, it should be noted that to establish reference values, the ideal number of animals should be between 100 and 120 in order to be more representative and better characteristic.

Table 3: Overview of the morphometric parameters of red blood cells in the horse.

Parameters	Mean	Range
Diameter (in µm)	5.68	4.72-6.67
Circumference (in µm)	22.37	19.01-26.99
Surface (in μm^2)	27.60	19.38-34.32

From the observations made in the present investigation, we should note that the traditional linear measures of the diameter of red blood cells, with the ocular micrometer are quite difficult and imprecise. Measurements of the circumference and the surface with this type of software to perform comparative studies appears more representative, quite correct and form a significant assistance in limiting the human factor involved in the measurements realized with the ocular micrometer by choosing the best place. Additionally, we must emphasize that this new measurement method of morphometric parameters of red blood cells is very simple, direct, practical, easy to realize and very few expensive.

CONCLUSION

The morphometric study of red blood cells in horses performed on 88 blood smears in Arabian and English thoroughbred seems generally that the factors age and sex have no influence on the diameter, the circumference and the surface of erythrocytes in both equine breeds considered. Finally, and in light of the obtained results, it is more interesting to make new morphometric studies of red blood cell in all horse breeds, in order to see the exact effect of the age and sex on the morphometric parameters of red blood cells.

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