



Research article

Comparison of some blood and synovial fluid biomarkers of Local Arabian horses and donkeys

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Abstract:

The study was achieved on 16 local Arabian horses and 16 local donkeys. The aim of the study was to compare of some blood and synovial fluid biomarkers of local Arabian horses and donkeys. Blood analyses were done to measure PCV, Hb, WBC counts, and RBC counts. Synovial fluid analysis was done to estimate some physical and biochemical markers, total protein, glucose, alkaline phosphatase, urea, creatinine, creatinine kinase, cholesterol, uric acid. The differences of the mean \pm SE of PCV and Hb values were non-significant at $P \leq 0.05$. Whereas the variances of the mean \pm SE of WBC counts and RBC counts were significant. The physical markers of the synovial fluid samples were clear for both horses and donkeys nevertheless the volume was greater and more viscous for horses with straw color though it was pale amber for donkeys. The inconsistencies of the mean \pm SE of total protein, and uric acid were not significant at $P \leq 0.05$. But the variances of the mean \pm SE of glucose, alkaline phosphatase, urea, creatinine, creatinine kinase, and cholesterol were significant.

Keywords: Blood, Synovial fluid, Arabian horses, Donkeys.

Introduction:

Earlier many decades, a lot of investigators studied many features of equine anatomy, physiology, pathology, biomechanics of equine limbs...etc(1). Equids have spent long time being used by man, even though little attempts have been made to study their different aspects. (2). They are the most important draught animals played important roles in the agriculture zone in Iraq. Nowadays, many countries have documented the normal reference levels of clinical, hematological and serum biochemical parameters for their local animals species (3). The horse is unique in comparison to most other mammalian species in that the spleen is a large capacity organ, storing up to one third of the red blood cells, and this reserve can be mobilized by stress, excitement, or exercise (4). Arabian breed horses are considered hot-blooded(5). These animals are characterized by their higher metabolic supplies, and subsequently

have higher levels of packed cell volume (PCV), red blood cells counts (RBC), and hemoglobin (Hb), associated to horses of the other breeds, according to discoveries informed by(6,7,8). Physiologically, Packed cell volume, higher RBC, and hemoglobin levels can play an important role in well physical state, for instance in sports deeds(9). For the valuation of horse reply to the action and assessment of the applied training approaches, Consistent blood testing achieved at rest permits, although a single investigation of resting blood guides is applied chiefly for comparing the levels with physiological norms (10). Normal cell physiological processes, water and food consumption, body structure, organ function, and diseases are subjected to periodic variations related to biological rhythms in a number of species, containing human beings and horses(11). Hematological evaluations are frequently accomplished in equine



medicine for variant reasons, as screening tests on healthy animals, to observe aged patients, to identify a disease, to recognize conditions that might make an animal a deprived candidate for anesthesia or surgery, to determine its severity, to express a prognosis, to display the reaction to remedy and in sport medicine(12). Clinical pathology parameters, and predominantly hematological variables, are exposed to biological rhythms. Consequently, the acquaintance of the rhythmic deviations of the most used laboratory checks has imperative diagnostic, therapeutic, exploration and epidemiological consequences that assurance a profound research(13). As any other organic fluids, the valuation of synovial fluid may be distributed to three biomarkers : physical (color, volume & turbidity) , chemical (concentration of total proteins and formation of mucin precipitate) and cytological (nucleated cell count and swab analysis)(14). Normal synovial fluid of

domestic animals has many enzymes. Alkaline phosphatase (ALP), and lactic dehydrogenase(LDG), acid phosphatase are existing in measurable amounts. The proportions of enzyme level in the synovial fluid to their levels in the serum in same animal differ conferring to the incidence and severity of a certain pathological circumstance(15,16). It's worthwhile to indicate that, biomarkers can be used in altered means: to distinguish between affected and non- affected joints/ animals (as diagnostic test), to recognize joints/ animals probable to display fast progression or to guess reply to treatment(as a prognostic test) & to evaluate the severity, observed alteration in disease grade or monitored response to therapy (as an evaluative test) (17). The goal of this work is to document the comparison of some blood and synovial fluid components between Iraqi Arabian horses and local donkeys.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study.

The study was achieved in Al-Dewania province on 16 Iraqi Arabian horses (group1) and 16 local donkeys (group2). Their ages ranged 2-9 years and included both sex. Both groups had the identical food, alfalfa, hay, grains and free drinking water. These trial animals were examined clinically for one week to check for any unhealthy observations. The horses were kept in stables while donkeys were lived in open farmhouses, but well care for.

Blood collection & analyses:-

Blood sample 10 ml. was drawn aseptically from the jugular vein of each animal in the mornings. 5 ml. out of the 10 ml. of blood which collected was permitted into a tubes with EDTA for hematologic assessment while the remaining 5 ml. was

transported into another tubes without EDTA for serologic exam. The blood without EDTA was centrifuged instantaneously at 3000 rpm for 5 minutes, to isolated the serum. The latter were stored at - 20°C till used. PCV, Hb, WBC counts, RBC counts values were evaluated using the typical method (17).

Biochemical analyses:-

The serum samples were examined by Reflotron® plus (Roche Diagnostics GmbH Mannheim Germany) by using equine commercial reagent kits (Roche Diagnostic GmbH Sandhofer Strasse 116 D-68305 Mannheim, Germany) to determine total protein (mg/dl), glucose (mg/dl), alkaline phosphatase (μ/L), urea (mg/dl), creatinine (mg/dl), creatinine kinase (u/L), cholesterol (mg/dl) and uric acid (mg/dl).

Synovial fluid collection & analyses:-

All the animals submitted to local anesthesia, lidocaine 2% 5ml., had arthrocentesis of their right carpal joints aseptically according to the routine surgical



method. Synovial fluid samples 2-3 ml. were collected from the mid- carpal joint via using an 18- gauge sterile needles and syringes. Subsequent aspiration of the samples, they were transported to vaccutainer tubes and referred in ice box to laboratory analysis by Reflotron® plus as well. Physical markers were documented, clarity, color, viscosity by dropping the synovial fluid from the end of the needle and record the stringing out as much as 5 cm. before separating and mucin clot development by adding 0.5 ml. of

synovial fluid sample to 2 ml. of 2% acetic acid and mixed it quickly with glass rod. The biochemical markers of synovial fluids were documented, total protein (mg/dl), glucose (mg/dl), alkaline phosphatase (u/L), urea (mg/dl), creatinine (mg/dl), creatinine kinase (u/L), cholesterol (mg/dl) and uric acid (mg/dl).

Statistical analysis: The differences of the results were analyzed by SPSS program version 32 at level $P < 0.05$.

Results:

The differences of the mean \pm SE of PCV and Hb values were not significant at $P \leq 0.05$. PCV rate of horses was 35.438 ± 1.595 and of donkeys was 32.221 ± 1.104 . Hb value of horses was 15.317 ± 0.529 and of donkeys was 14.043 ± 0.862 . Whereas the variances of

the mean \pm SE of WBC counts and RBC counts were significant at $P \leq 0.05$. WBC counts value of horses 12.45 ± 0.935 and of donkeys was 17.54 ± 0.735 . RBC count of horses was 7.991 ± 0.986 and of donkeys was 5.26 ± 0.355 as in table-1.

Table(1): The mean \pm SE of specific blood parameters in Arabian horses and donkeys.

Animals	PCV %	Hb (g/L)	WBC (cells $\times 10^3$)	RBC (cells $\times 10^6$)
Horse (n= 16)	35.438 a ± 1.595	15.317 a ± 0.529	12.45 a ± 0.935	7.991 a ± 0.986
Donkey (n=16)	32.221 a ± 1.104	14.043 a ± 0.862	17.54 b ± 0.735	5.26 b ± 0.355

*Different letters mean the variances were significant.

The variances between physical markers of the synovial fluid of horses and donkeys are presented in table-2. The appearance is clear of both, the volume is alternated (2-3 ml.) from the mid- carpal joint of the horses while it is (1-2 ml.) from the mid- carpal joint of the donkeys. The viscosity is greater

in the synovial fluid of the horses than that of the donkeys, similarly the degree of the clot formation is higher in the synovial fluid of the horses than that of the donkeys. The horses has straw color synovial fluid, however the donkeys has pale amber color synovial fluid.

Table (2): Differences of physical markers of the synovial fluid in horses and donkeys.

Experimental animals	Appearance	Volume	Viscosity	Clot formation	Color
Horses	clear	2-3 ml.	+++	+++	straw
Donkeys	clear	1-2 ml.	++	+	Pale amber

Mild: + Moderate: ++ Severe: +++

The differences of the mean \pm SE of total protein, and uric acid were not significant at $P \leq 0.05$. The mean \pm SE values of total protein of horses was 7.481 ± 0.393 and of donkeys

was 7.143 ± 0.587 . The mean \pm SE of uric acid of horses was 3.931 ± 0.112 and of donkeys was 4.100 ± 0.146 . Though the variances of the mean \pm SE of glucose,



alkaline phosphatase, urea creatinine, creatinine kinase, and cholesterol were significant at $P \leq 0.05$. The mean \pm SE values of glucose of horses was 70.986 ± 2.086 and of donkeys got 68.316 ± 6.087 . The mean \pm SE values of alkaline phosphatase of horses was 6.418 ± 0.359 and of donkeys recorded 7.756 ± 0.382 . The mean \pm SE values of urea of horses reached 69.125 ± 2.350 and of

donkeys was 58.315 ± 3.460 . The mean \pm SE values of creatinine of horses showed 1.991 ± 0.280 and of donkeys was 1.032 ± 0.131 . The mean \pm SE values of creatinine kinase of horses listed 78.637 ± 2.705 and of donkeys was 125.517 ± 7.915 . The mean \pm SE values of cholesterol of horses became 90.031 ± 1.281 and of donkeys registered 102.225 ± 1.145 as exhibited (table-3)

Table(3): Biochemical parameters of synovial fluid of local Arabian horses and donkeys.

Animals	Total protein (mg/dl)	Glucose (mg/dl)	Alkaline phosphatase (u/L)	Urea (mg/dl)	Creatinine (mg/dl)	Creatinine Kinase (u/L)	Cholesterol (mg/dl)	Uric acid (mg/dl)
Horses	7.481a ± 0.393	70.986a ± 2.086	6.418b ± 0.359	69.125a ± 2.350	1.991a ± 0.280	78.637b ± 2.705	90.031b ± 1.281	3.931a ± 0.112
Donkeys	7.143a ± 0.587	68.316b ± 6.087	7.756a ± 0.382	58.315b ± 3.460	1.032b ± 0.131	125.517a ± 1.915	102.225a ± 1.145	4.100a ± 0.146

*Different letters mean the variances were significant.

Discussion:

The results of the physical and biochemical analysis of the synovial fluid components displays many alterations between horses and donkeys joint markers. The results of table(1) demonstrate no significant variances of PCV and Hb between horses and donkeys might because of the fact that both are belonged to the similar family genetically. Whereas there are differences of WBC and RBC counts values, these results may be interpreted the hard work of horses for instance sports. Table(2) indicates that the synovial fluid of all trial animals are clear that point to all the samples are taken from well healthy animals under restrict aseptic conditions and the needles are introduced properly in the joint space. (9) documents that the synovial fluid of the horse is straw color but (2) mentions that the pale amber color (xantho chromic) is due to former hemorrhage and is most frequently related with chronic traumatic arthritis. The extreme synovial fluid volume that can be attained from normal joint is just about 3.5 ml. (between 0.1 and 3.5 ml.) (11). The results express that the volume of synovial fluid of some trial horses are more greater quantity in the carpal joint due to its great joint space than those of donkeys. The synovial fluid is

more viscous which play very important role in joint lubrication of horses because of its importance in the dynamic movements of the joint throughout exercising. Normal synovial fluid does not clot. This property is attributed to absence of fibrinogen as well as other clotting factors (comprising prothrombin, factor V, factor VII and tissue thromboplastin) (14). The results of the table-3 demonstrates that the mean values of glucose, alkaline phosphatase, creatinine kinase and cholesterol of the synovial fluid of donkeys are greater than that of the horses. These consequences may be due to daily exercise of the experimental horses (much more movements of the joints) which lead to decrease the stages of these important constituents while donkeys may become less movements. (19) measures in donkeys, the normal level of glucose is (55.4 mg/dl) and alkaline phosphatase (13.0 U/L) respectively in Egypt, although in this study, they are (116 mg/dl) for glucose level and (6.3 U/L) for alkaline phosphatase. These results may be due to the variances between the local breeds of donkeys. Whereas total protein, creatinine and uric acid are nearly equal, possibly due to all experimental animals acquire the same feeding alfalfa, hay, grains.



Only the urea constituent of the horses are greater than that of the donkeys, this result may be due to the higher permeability of the synovial membranes of horses which clarify the higher sedimentation of urea in the horses joints. The results of this work illustrate

different values of many biomarkers between horses and donkeys even they belong to the same family (Equidae). A lot of researches may require to explore how we can use synovial fluid as a biological material for preparation of biological cures.

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