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Original article

The Effect of Sex Variation on Hemato-Biochemical Profiles of Arabian Horses Raised in Libya

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ABSTRACT

As essential components of a minimal database, the hematological and biochemical blood profile are potent and significant diagnostic tools. Thus, the current study aimed to ascertain the serum biochemical and hematological characteristics, as well as any potential sex-related variations, of apparently healthy Arabian horses raised in Libya. The blood samples were collected from 65 clinically healthy horses of both sexes; males (n=26) and females (n=39) at winter season 2022 and included to analyze blood constituents and biochemical profile. The results of this study revealed that differences among Arabian horses according to gender factor not significant (p> 0.1) of all the parameters including the total white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), mean cellular volume (MCV), mean cellular hemoglobin (MCH), mean cellular hemoglobin concentration (MCHC), red cell width distribution-coefficient of variation (RDW-CV), red cell distribution width-standard deviation (RDW-SD), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT). Additionally, biochemical analysis showed no significant differences (p> 0.05) between stallions and mares of tested breed with exception of serum bilirubin, creatinine (Cr), urea, potassium (K) and chloride (Cl). This study shows no significant sex differences between male and female Arabian horses raised in Libya, whereas gender had only slight effect on the hematological and biochemical parameters.

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INTRODUCTION

The horse (*Equus caballus*) is a long domesticated mammal belonging to the Equidae family, comprising of asses, horses and zebras [1]. Equine are raised for different purposes in Libya which surged recently. For instance, breeding, track racing, and participation in traditional equestrian events [2]. There are no official statistics for the horse population in

Libya yet. Nevertheless, it is believed that the most common breed in Western Libya is the Thoroughbred. This breed was coming by crosses between stallions of Arabian origin and a poorly defined group of mares imported mainly from the UK [3,4]. In addition to the physical examination, the hematobiochemical investigations in equine medicine provides significant information that indicates the health condition. It aids in diagnosis and evaluates the performance and response to the therapy of horses [5,6]. Moreover, this profile provides useful information about the disease severity and prognosis [5,7].

Breed-specific variations in the hematobiochemical data are typically affected by age, gender, management and environmental factors [4,6–9]. To date, numerous published studies defined the standard of hematobiochemical profile for equids and showed the impact of particular characteristics on the performance of horses in various parts of the world especially horses and donkeys (*Equus asinus*) in the UK [10–13]. As a result, accurate measurement of the reference values for each parameter is essential and may affect the context's readability and dependability [4,7,14–16].

The effects of age and gender on some basic hematological parameters in Bosnian mountain horses are available in literature; however, no data of the effect of age and gender on serum biochemical parameters have been published [17,18]. However, it is known that the blood profiles of horses vary with sex, age, and season [19,20]. Some studies showed that male horses had higher levels of hemoglobin, PCV, RBC, TWBC, MCH, MCV and MCHC than female horses, although these differences were however, not statistically significant (p>0.05) [21]. Others stated that female horses had higher levels of AST, ALP and total protein than the males [15].

The objective of this study was to examine the values of hematological and some biochemical parameters in clinically healthy adult Arabian horses in Libya, revealing gender-related differences and to compare the obtained results with previously reported one.

MATERIAL AND METHODS

Animals

Horses were randomly chosen from pure Arabian breed. Different ages and both sexes with a total of sixty-five apparently healthy horses were involved in this study. According to sex, the horses were divided into two experimental groups: males (n= 6) and females (n=39). The horses involved in this study were from a farm located in Qasr Ben Geisher; the suburban area of Tripoli.

Blood collection

Blood samples were collected in the winter time of the year. at the morning. Thirteen milliliters of blood were collected from the jugular vein of each horse by disposable plastic syringe and a 19G needle. Three milliliters of blood were transferred to EDTA anti-coagulant containing tubes for hematological analysis while the remained ten milliliters of blood were distributed into clean dry plain tubes for serum analysis. Subsequently, all blood samples were transferred to the laboratory. The blood allowed to clot and after centrifugation at 3000rpm for 10 min, the serum samples were aliquoted in dry clean Eppendorf capped tubes and stored at -80º C for later analysis. The samples were sent to the Esraa's and Al-shefaa's clinical laboratories, Tripoli, Libya for hematological and biochemical analysis, respectively.

Hematological analysis

The blood samples were analyzed within 45mins from sampling. The EDTA- anti coagulated blood was used to determine total white blood cells (WBC) count, red blood cells (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell width distribution- coefficient of variation (RDW-CV), red cell distribution width - standard deviation (RDW-SD), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) using Automated hematologic analyzer (Celltac α, Nihon Kohden, Tokyo, Japan).

Biochemical analysis

The blood samples collected without EDTA were centrifuged at 3000rpm for 10 min to obtain serum. The activities of [alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)] and the concentrations of [serum glucose (Glu), total protein (TP), albumin (Alb), total and direct bilirubin (TB & DB), total cholesterol (CHOL), triglycerides (TG), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL, urea, creatinine (Cr), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K)] were determined, and were measured using an automated blood

chemistry analyzer (pz Cormay ACCENT M320) and Easylyte plus for electrolytes using Labtest Diagnóstica® kits. Moreover, the iIndirect bilirubin (IB) was estimated as the arithmetical difference between serum total bilirubin and direct bilirubin values.

Statistical analysis

Descriptive statistic was used to established frequency and percentage. The computer software, SPSS version 25 was used for analysis. A non-parametric test was used, because the data collected did not follow a normal distribution (Kolmogorov- Smirnov Test). The data obtained were analyzed using independent two samples (Mann-Whitney test) and one-way analysis of variances (Kruskal-Wallis test). The mean values were considered significant at p <0.05.

RESULTS

Hematological indices

Descriptive statistics for hematological parameters in the present work was demonstrated in table 1. The maximum and minimum of WBC, RBC and PLT counts were 3.80-13.33 with mean 6.86±1.62, 5.26-9.68 with mean7.23±1.07, 2.70-228.00 with mean79.94±51.58 respectively.

Table 1. Descriptive Statistics of hematological parameters

CBC	Minimum	Maximum	Mean	±	Std. Deviation
WBC (x10^{3}µL)	3.80	13.33	6.86	±	1.62
RBC (x10^{6}µL)	5.26	9.68	7.23	±	1.07
Hb (g/dl)	8.60	17.61	12.37	±	2.14
HCT (%)	21.50	49.40	33.21	±	5.96
MCV (fl)	40.87	51.03	45.98	±	4.24
MCH (pg)	16.34	18.19	16.75	±	1.54
MCHC (%)	40.00	35.65	36.70	±	3.80
RDW-CV (%)	14.70	25.90	19.85	±	3.16
RDW-SD (fl)	19.50	47.20	32.88	±	9.48
PLT (x10^{3}µL)	2.70	228.00	79.94	±	51.58
MPV (fl)	5.90	8.60	7.31	±	0.56
PDW (fl)	9.00	19.10	15.37	±	1.98
PCT (%)	0.01	0.67	0.08	±	0.10

Results of the analyzed blood parameters in Arabian horses of different gender are shown in tables 2-4. Comparative study revealed that all parameters; total WBC, RBC, Hb, erythrocyte indices (MCV, MCH &MCHC), RDW, PLT, MPV, PDW and PCT were not significantly different (p> 0.1) according to sex variation.

Table 2. Hematological parameters in the male (n=26) and females (n=39) Arabian horses (Mean ±SD).

CBC	Gender	Mean	±	Std. Deviation	Mann-Whitney test	
					test statistics	*p*-value
WBC (x10^{3}µL)	Male	7.01	±	2.07	382.00	0.884
	Female	6.76	±	1.25		
RBC (x10^{6}µL)	Male	7.30	±	1.13	387	0.675
	Female	7.18	±	1.05		
Hb (g/dl)	Male	12.83	±	2.33	400.50	0.154
	Female	12.07	±	1.98		
HCT (%)	Male	33.75	±	6.78	391.50	0.727
	Female	32.86	±	5.44		

Table 3. Erythrocyte indices in the male (n=26) and females (n=39) Arabian horses (Mean ±SD).

CBC	Gender	Mean	±	Std. Deviation	Mann-Whitney test	
					test statistics	*p*-value
MCV (fl)	Male	46.11	±	4.27	397.00	0.792
	Female	45.90	±	4.28		
MCH (pg)	Male	17.18	±	1.42	314.50	0.122
	Female	16.48	±	1.57		
MCHC (%)	Male	37.58	±	4.23	329.50	0.189
	Female	36.14	±	3.45		
RDW-CV (fl)	Male	19.07	±	2.92	323.00	0.157
	Female	20.36	±	3.25		
RDW-SD (%)	Male	31.65	±	9.58	376.50	0.560
	Female	33.67	±	9.47		

Table 4. Platelets count and their indices in the male (n=26) and females (n=39) Arabian horses (Mean ±SD).

CBC	Gender	Mean	±	Std. Deviation	Mann-Whitney test	
					test statistics	*p*-value
PLT (x103µL)	Male	88.72	±	52.83	403.50	0.166
	Female	74.09	±	50.57		
MPV (fl)	Male	7.25	±	0.50	411.00	0.459
	Female	7.34	±	0.61		
PDW (fl)	Male	14.90	±	2.17	372.00	0.194
	Female	15.69	±	1.79		
PCT (%l)	Male	0.07	±	0.04	387.50	0.281
	Female	0.08	±	0.13		

Results of serum biochemical parameters are presented in tables 5-7. A total of twenty- three biochemical parameters were determined. Serum ALT, AST, ALP and LDH enzymes activity, as well as serum levels of TP, Alb, TC, TG and lipoproteins exhibited non-statistically differences between stallions and mares of Arabian horses as shown in Tables 5&6. On the other hand, gender significantly affected serum total bilirubin (TB) and direct bilirubin (DB). The values of serum TB and DB were significantly (p <0.05) higher in males (0.92±0.57, 0.41±0.24) than females (0.69±0.77, 0.27±0.23), respectively (tables 5).

Table 5. Serum enzymes, total protein and albumin levels in the male (n=26) and females (n=39) Arabian horses (Mean ±SD).

Chemistry	Gender	Mean	±	Std. Deviation	Mann-Whitney test	
					Test statistics	*p*- value
ALT (U/L)	Male	7.71	±	4.05	437.500	0.352
	Female	6.86	±	4.46		
AST (U/L)	Male	234.04	±	52.98	430.000	0.302
	Female	266.61	±	103.34		
ALP (U/L)	Male	158.54	±	101.46	457.000	0.503
	Female	150.95	±	57.72		
LDH (U/L)	Male	364.42	±	92.81	430.000	0.303
	Female	340.85	±	105.81		
TP (g/dl)	Male	7.01	±	0.58	482.500	0.743
	Female	7.02	±	0.94		
Alb (g/dl)	Male	3.77	±	0.35	384.000	0.099
	Female	3.62	±	0.47		

Table 6. Serum bilirubin, glucose and lipid profile in the male (n=26) and females (n=39) Arabian horses (Mean ±SD).

Chemistry	Gender	Mean	±	Std. Deviation	Mann-Whitney test	
					test statistics	*p*- value
TB (mg/dl)	Male	0.92	±	0.57	348.500	0.034
	Female	0.69	±	0.77		
DB (mg/dl)	Male	0.41	±	0.24	334.000	0.020
	Female	0.27	±	0.23		
IB (mg/dl)	Male	0.47	±	0.32	482.500	0.743
	Female	0.53	±	0.64		
Gluc (mg/dl)	Male	66.66	±	13.24	497.500	0.899
	Female	65.24	±	14.47		
TC (mg/dl)	Male	99.03	±	20.13	467.500	0.597
	Female	95.86	±	20.56		
TG (mg/dl)	Male	33.37	±	13.35	484.000	0.758
	Female	36.86	±	21.59		
VLDL (mg/dl)	Male	14.73	±	3.53	416.500	0.223
	Female	13.69	±	4.48		
LDL (mg/dl)	Male	33.45	±	10.03	393.000	0.127
	Female	28.85	±	10.57		
HDL D (mg/dl)	Male	51.72	±	10.40	455.000	0.486
	Female	52.86	±	10.10		

** Indicate significant difference (p<0.05%) among the different sex.*

As showed in table 7, serum Cr was significantly *(p* <0.05) higher in males (1.40 mg/dl) than females (1.28 mg/dl), while serum urea was significantly *(p* <0.05) higher in females (11.84 mg/dl) compared to males (11.78 mg/dl). All serum minerals analysis in the current investigation showed non-significant differences except serum K and CL (Table 7). The serum value of K was significantly *(p* <0.0001) higher in males (4.94 mEq/L) than females (4.41 mEq/L), while serum Cl was significantly *(p* <0.05) higher in females (103.93 mEq/L) compared with the values in males (102.01 mEq/L) as demonstrated in Tables 7. The only parameters affected by gender was bilirubin, Cr, urea, K and Cl.

Table 7. Serum Cr, urea, and mineral levels in the male (n=26) and females (n=39) Arabian horses (Mean ±SD).

Chemistry	Gender	Mean	±	Std. Deviation	Mann-Whitney test	
					test statistics	*p*- value
Cr (mg/dl)	Male	1.40	±	0.23	348.000	0.033*
	Female	1.28	±	0.18		
Urea mg/dl)	Male	32.98	±	6.42	342.000	0.027*
	Female	36.73	±	5.88		
Ca (mg/dl)	Male	11.78	±	0.68	417.500	0.231
	Female	11.84	±	1.22		
P (mg/dl)	Male	4.66	±	1.14	382.000	0.094
	Female	4.09	±	1.31		
Mg (mg/dl)	Male	1.85	±	0.22	439.500	0.366
	Female	1.89	±	0.32		
Na (mEq/L)	Male	133.39	±	1.77	421.500	0.252
	Female	134.12	±	5.65		
K (mEq/L)	Male	4.94	±	0.34	217.000	0.000*
	Female	4.41	±	0.68		
Cl (mEq/L)	Male	102.01	±	1.96	337.000	0.023*
	Female	103.93	±	5.16		

** Indicate significant difference (p<0.05%) among the different sex.*

DISCUSSION

Arabian horse is considered as one of the most famous top ten popular horse breeds around the world and their land of origin is the Middle East [22]. However, there is a lack of published hematological and biochemical reference values for Arabian horses raised in Libya which is make it inevitable to create a blood profile reference of normal apparently healthy pure Arabian horses for both clinician and researchers in Libya. The current study, attempted to determine the normal values of some hematological and biochemical blood constituents in Libyan healthy horses of Arabian breed and compare its finding with the other studies conducted previously elsewhere. Blood collection, storage and analysis were performed with the same technical procedures to minimize the variation that could affect the accuracy of the obtained data.

In the present investigation, male horses had higher levels of WBC, RBC, Hb, HCT, MCH, MCV and MCHC than female horses though these differences were not statistically significant (p >0.05). This finding is in agreement with the previous study conducted on Miranda donkey reported that differences between males and females in the hematological parameters were not significant [23].

Studies performed in Catalan donkeys and in mixed-breed donkeys has shown no significant differences between different sexes [24,25]. This insignificant variations in the hematology of both sexes could be attributed to the fact that both athletic stallions and mares are used for the same purposes from time to time throughout the year. In contrast, other researches stated that males had a noticeably larger erythrocyte count and immature neutrophilic granulocytes compared to female Przewalski horses [26]. The later authors stated that stallions were also observed to have a relatively higher Hb concentration and leucocyte and monocyte counts than mares but was not statistically significant. Earlier, some colleagues from France, Serbia and Bósnia has performed a study on donkeys demonstrated a significant elevations for several hematological parameters in males than females [18,21,27-29]. Their findings were in the same line with our results. Either, this is in accordance with previous study reported that stallions have slight elevation in RBC counts, Hb levels and PCV than the females though was not significantly different [15]. In parallel, other studies reported that no significant differences in hematological values among stallion and mares [30]. Total WBC count, MCV, and neutrophil counts varied significantly between the sexes in a study done to establish baseline values for hematology in working donkeys [31].

Previously conducted studies showed that male horses have slightly higher PCV, RBC and Hb than female horses [15,32]. The authors in the latter attributed the slight increase in erythrocytic parameters in males to the effect of androgens which may stimulate erythropoiesis [33]. Notably, a study has find female donkeys had higher values of MCHC, leukocyte count and neutrophil counts than adult males [34]. Interestingly, female Zematukai horses have higher Hb level, PCV, RBC count and MCHC than male horses [9]. This might indicate that the gender had no drastic effect on the hematological readings except for HCT, MCHC, and RDW, with significantly higher values in females than males [2]. In contrast, total and differential leukocyte counts did not vary significantly with gender.

The RDW is an important marker of erythrocyte diseases. However, it is not fundamentally affected by gender as reported previously [35]. In respect to the platelets count and their indices, the males had higher PLT count and lower MPV, PDW and PCT but was not significantly different. The foregoing study reported that the normal value of platelets in horses varies between 100-350,000/µl and that stallion had higher platelet values than the mares [36]. In recent report, the gender of horses had a significant influence on RDW and it might be a reflection of RBC counts and Hb levels alterations [2].

With regard to the biochemical analysis in this study, the statistical differences were not significant considering the serum enzymes activity of (ALT, AST, ALP and LDH) under the effect of gender in Arabian horses. This finding was in line with previous results recorded that sex factor among horses had no considerable effect on the activity of most of the analyzed enzymes indicative of liver function (AST, ALT and LDH) [32]. The blood enzymes like AST observed within the range compared to Arabian horses, Lahor horses and Sell Italian horses [14,37,38]. The ALT ranges were higher in Kathiawari breeds [39]. However, ALT is not a useful indicator of liver disease in large animals as this enzyme activity is low in liver tissue of these species [40]. The ALT, ALP and GGT serum activities did not significantly differ between the males and females in the current work. This finding support the previous results which described no sex-related differences ($p > 0.05$) in equine for the values of ALT, IB, albumin and triglycerides [27,41–44] Neither the type of use nor the gender significantly influenced lipid parameters in the horses which is in agreement with studies by other authors [32,45,46].

This study, showed that most of variables such as ALT, ALP, LDH, Alb, Glu, TC and lipoproteins (VLDL, LDL and HDL) were higher in males than females but were not statistically different. Supplementary studies showed That AST, ALP, and TP serum activities were clearly higher in female horses than the males [15,41,42,47].. The high AST recorded in the horses might be due to their use as sport horses because AST is known to be associated with the increase in

muscular activity and the skeletal myocytes are a major source of AST [48]. Increased ALP levels may be attributed to bone changes during osteoclastic activity, while higher AST levels reflect more muscular activities in the animals. Some studies reported that males showed greater means of ALP ($p < 0.01$), serum albumin and glucose ($p < 0.01$) and TC ($p < 0.01$) [1,42,47,49].

The male's horses have significantly higher serum creatinine and lower urea values compared with the female's values. Similarly, the males showed greater means of creatinine ($p < 0.01$) and female Breton horses had higher serum urea than males [27,31,50]. Our findings are in partial correlation with other investigations that observed higher levels of blood urea and creatinine in female Kathiawari horses as compared to male groups [39]. Nevertheless, there were no sex-related differences ($p > 0.05$) for serum urea and Cr [41,50]. In another report, serum urea was significantly ($p = 0.018$) increased in males (8.89 mg/dl) matched with female values (7.34 mg/dl), but serum Cr level did not significantly differ regarding gender [18]. Previous study, did not specify breed, sex, or age of horses reported a considerably lower urea values (10-24 mg/dl) than the current work (20.90- 49.40 mg/dl)[51]. However, the Cr values (1.2 to 1.9 mg/dl) were the closest to the study (0.90-1.92mg/dl) [51]. This variation may have arisen from breed, environmental conditions, muscle mass, nutrition, as well as timing of blood sampling [12].

Concerning the values of electrolytes in the serum of both horse sexes in the current study were within the normal ranges. Na, K, Ph, Ca, Mg and Cl. Ca, Ph, Mg, and Na revealed non-significant alterations among horses' gender while serum K was significantly ($p < 0.0001$) higher in males than females (4.41mEq/L). Meanwhile, the serum Cl was significantly ($p < 0.05$) higher in females compared with the male's values. Similar findings were previously found any significant changes in Ca between the sexes [52,53]. The serum Ca level was higher in mares than stallions but the differences between both sexes not significant. In contrast, other researchers have found sex impact on the Ca level and stated that the serum total calcium was higher ($p < 0.05$) in female Brazilian donkeys [47]. On the contrary, there was no significant variations in serum Ca between males and females, but serum Ph showed a higher ($p = 0028$) increase in females compared with male Bosnian mountain horses [18]. The results of no sex-related differences ($p < 0.05$) in serum Mg, and Na in this study were in agreement with other finding [41]. However, Ph and Mg levels were higher ($P < 0.01$) for Pêga males than females [43].

Generally, as stated by another reporters the variations in blood profile and biochemical parameter values that have been observed may result from variations in the horses' breed, geographical, physiological, seasonal, climatic variables, diet, care and sample size. These variations may also result from using various reagents, techniques and applicable instruments.

CONCLUSION

The present study has proved no significant influence of gender of Arabian horses on blood hematological and biochemical parameters with exception of serum bilirubin, Cr, urea, K and Cl. Our results showed that variations associated with the sex occur regardless of the physiological state of the animals. The blood values determined in this investigation may serve as reference values for male and female Arabian horses raised in Libya and could be used in clinical disease diagnosis, prognosis as well as in preventive programs conducted in Libya.

Conflicts of interest

The authors declare that they have no competing interest.

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دراسة تأثير اختلاف الجنس على الصفات الدموية والكيموحيوية في الخيول العربية في ليبيا

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المستخلص

تعتبر الكيمياء الحيوية للدم وتعداد الدم الكامل أدوات تشخيصية قوية وهامة في مجال البحوث الطبية لما لها من قدرة على إعطاء صورة حقيقية (كمرآة) عن الوضع الفسيولوجي والأيضي للعينة المختبرة وأيضا باعتبارها مكونات أساسية لقاعدة بيانات بسيطة. لذلك كان الهدف من هذه الدراسة هو البحث في الخصائص البيوكيميائية والدموية في الدم ومدى تأثرها بعامل الجنس في الخيول العربية الأصيلة التي ولدت وترعرعت في ليبيا. تم جمع عينات الدم من 65 حصاناً سليماً سريرياً من كلا الجنسين؛ الذكور (العدد = 26) والإناث (العدد = 39) في فصل شتاء 2022 وضمنت للتحليل الدموي والكيميائي الحيوي. أظهرت نتائج هذه الدراسة أنه بين الخيول الذكور والإناث لا توجد فروق معنوية ($P> 0.1$) في جميع العوامل بما في ذلك إجمالي خلايا الدم البيضاء، خلايا الدم الحمراء، الهيموجلوبين، متوسط حجم الكريات، متوسط الهيموجلوبين الجسيمي، متوسط تركيز الهيموجلوبين داخل الكرية، معامل التباين لتوزيع عرض الخلية الحمراء، الانحراف المعياري لعرض توزيع الخلايا الحمراء، عدد الصفائح الدموية، متوسط حجم الصفائح الدموية، عرض توزيع الصفائح الدموية, والصفائح الدمويةالمضغوطة داخل حجم من البلازما. على مستوى الخصائص الكيموحيوية، أظهرت التحاليل عدم وجود فروق معنوية ($P> 0.05$) بين الذكور والإناث باستثناء مستويات البيليروبين في الدم والكرياتينين واليوريا والبوتاسيوم والكلوريد. أظهرت نتائج هذه الدراسة أن الجنس كان له تأثير طفيف على المعايير التي تم بحثها في الخيول العربية البالغة السليمة سريريا والتي نشأت في ليبيا مما قد يكون له عظيم الفائدة عند البحث في هذه السلالة.

الكلمات الدالة. الخيول العربية، التنوع الجنسي، علم الدم، الكيمياء الحيوية