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Evaluation of the hematological and biochemical changes of blood profile in Arabian horses under the effect of training and pregnancy

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تقييم التغيرات الدموية والكيميائية الحيوية لصورة الدم في الخيول العربية تحت تأثير التدريب والحمل

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Abstract:
Knowing more information about Arabian horses raised in Libya is considered valuable before enrolling in any research on this category of horse's breed. This study investigated the influence of racing and pregnancy on the hematological and biochemical parameters. The blood samples were collected from 65 healthy Arabian horses in Libya for hematology and biochemistry analyses. The (WBC), (HB) and (HCT) were higher in racing and pregnant compared with (non-racing and non-pregnant) horses. The (MCV), (RDW-CV) was higher in pregnant than (non-racing and non-pregnant) horses. However, (MCHC) was significantly decreased in racing and pregnant animals. On the contrary, (MCH), (MPV), (PLT), (PDW) and (PCT) did not change among the examined Arabian horses. Biochemical analysis revealed a significant decrease in (ALT), (ALP), (AST) and (LDH), in racing and pregnant compared to the (non-racing and non-pregnant) horses with no significant different in AST. Although the (TP), and (IBIL) was significantly increased in race and pregnant compared to (non-racing and non-pregnant) horses, no changes were reported in (Alb), (T BIL), (D BIL) and (Glu) levels. The serum (TC) and (HDL) significantly decreased in racing and pregnant compared to non-pregnant animals with no significant change of the (TG), (VLDL), (LDL), (Cr) and urea of all animals. Finally, serum (P) and (K) were significantly lower, but (Mg) was significantly increased in racehorses and pregnant than non-pregnant mares and no changes were found in serum (Ca), (Na) and (Cl). It is crucial to understand these physiological changes to provide mares with the finest care and medical attention available. These data will help clinicians assess and care for pregnant mares appropriately.

Keywords: Arabian Horse, Hematology, Biochemistry, Racing, Pregnancy.

الملخص
إن معرفة المزيد من المعلومات عن الخيول العربية التي تتم تربيتها في ليبيا يعتبر أمرًا ذا قيمة قبل الالتحاق بأي بحث ضمن هذه الفئة من سلالات الخيول. بحثت هذه الدراسة في تأثير السباق والحمل على المعايير الدموية والكيميائية الحيوية. تم جمع عينات الدم من 65 حصاناً عربياً سليماً في

ليبيا لتحليل معايير الدم والكيمياء الحيوية. كانت (WBC) و (HB) و (HCT) أعلى في خيول السباق والحوامل مقارنة بالخيول (غير المتسابقة وغير الحامل). وكان (MCV) و(RDW-CV) أعلى في الخيول الحامل منه عن (غير المتسابقة وغير الحامل) بينما انخفض مستوى (MCHC) بشكل ملحوظ في حيوانات السباق والحوامل. وعلى العكس من ذلك لم تتغير (MCH)، (MPV)، (PLT)، (PDW)، و (PCT) بين الخيول العربية المفحوصة. أظهر التحليل الكيموحيوي وجود انخفاض مهم في (ALT) و (ALP) و (AST) و (LDH) في الخيول المتسابقة والحوامل مقارنة بالخيول (غير المتسابقة وغير الحامل) مع عدم وجود اختلاف مهم في (AST). على الرغم من أن (TP)، و(IBIL) زادا قياسيا في السباق والحوامل مقارنة بالخيول (غير المتسابقة وغير الحامل)، إلا أنه لم يتم الإبلاغ عن أي تغييرات في (Alb)، (T BIL)، (D BIL) و(Glu). انخفض قياسيا في مصل الدم (TC) و (HDL) في السباقات والحوامل مقارنة بالحيوانات غير الحامل مع عدم وجود تغير معنوي في (TG)، (VLDL)، (LDL)، (Cr) واليوريا لجميع الحيوانات. وأخيرا، كان مصل الدم (P) و (K) أقل قياسيا، ولكن (Mg) ارتفع قياسيا في خيول السباق والحوامل مقارنة بالأفراس غير الحوامل ولم يتم العثور على تغييرات في مصل الدم (Ca) و (Na) و (Cl). من الضروري فهم هذه التغيرات الفسيولوجية لتزويد الأفراس بأفضل رعاية وعناية طبية متاحة. ستساعد هذه البيانات الأطباء على تقييم ورعاية الأفراس الحوامل بشكل مناسب.

الكلمات المفتاحية: الحصان العربي، أمراض الدم، الكيمياء الحيوية، السباق، الحمل.

Introduction

Pregnancy has been demonstrated to cause hematological and biochemical abnormalities that may resemble illness patterns seen in people who are not pregnant. Hormonal milieu alterations are the primary cause of physiological changes during pregnancy [1, 2]. Pregnancy in many mammals, including mares, is associated with physiological changes that are reflected in hematological and biochemical profiles [2]. Although hormonal changes around the parturition have been well studied in the field of endocrinology [3], hematological and biochemical changes have been studied little [4].

Arabian horses are bred in stud farms where they live together with their conspecifics until they are 3 years old, and then they are taken to hippodromes where they are socially isolated from their conspecifics for flat races [5]. One of the most important roles of research in equine physiology is to obtain new useful information on characteristics that make the horse such a super athlete [6, 7]. In equine medicine, biochemistry and hematology are essential diagnostic techniques. It is crucial to distinguish these physiological changes from pathological ones that could endanger the health of the mare and/or fetus and necessitate rapid medical attention. These alterations have been connected to fetal development and are ascribed to the mares' health [8]. The hemogram and biochemistry are particularly important when working with sport horses subjected to heavy workloads. It allows tracking of the function of blood components, workability and performance of horses [9, 10]. It was reported that long-duration activities, such as endurance races, cause significant changes in hematological and biochemical components as a result of central and peripheral exhaustion [11]. A significant increase of 36% for the leukocytes (WBC, $x10^3/\mu L$) was described in endurance horses immediately after exercise during the training season but no significant changes were noted for the erythrocytes (RBC, $x10^6/\mu L$), hemoglobin (HB, g/dl) and hematocrit (HCT, %) [12]. After activity, deviations from the normal range in one or more blood components either above or below the range's boundaries might indicate a response to exhaustion by a working horse [11]. Moreover, an increase of RBC (19.1%), Hb (18.6%) and HCT (19.4%) were reported in race horses with increased Alanine aminotransferase (ALT, U/L) and total protein (TP, g/dl) significantly immediately after effort in endurance and race horses [12].

Previous studies have examined hematological and biochemical parameters in equine. these studies evaluated the blood values to provide normal reference intervals. To date, there is little information available about normal blood values during various phases of mare pregnancy or racing [13-15]. Several studies have reported hematology and biochemistry profiles in pregnant mares of different breeds including Spanish purebred [16], Carthusian [13], Holstein [17], and others [14, 18]. Thus, this study aimed to compare the hematological and biochemical values among three different horse physiological status (non-racing and non-pregnant, racing and pregnant mares).

Material and Methods

Animal welfare and ethical statements

All the experimental procedures and management were carried out in accordance with the Committee guidelines in the faculty of veterinary medicine, university of Tripoli, Tripoli, Libya. Regarding the protection of animals for experimental purpose and experimental conditions were accepted by institutional ethical committee.

Experimental animals

A total of sixty-five clinically healthy Arabian horses were used in this study at the winter season. The animals were grouped into (non-racing and non-pregnant) horses (n=34), racehorses (n=15) and pregnant mares (n=16) from one geographical area.

The animals were kept and fed by the owners and there were no changes in routine nutrition or husbandry. Prior to blood sampling, attending veterinarians ensured good health in all Arabian horses including assessment of medical history, physical examination, and routine anthelmintic and vaccine administration. If an animal showed any sign of disease, blood was not collected. Pregnancy dates were calculated based on the conception date.

Blood sampling

The blood samples were drawn from the left jugular vein using an 18-gauge needle and a 10-mL syringe then transferred into K_3EDTA. The samples from pregnant mares were collected during the last trimester of pregnancy (6 -10 months).

All specimens were properly marked with the subject identification number to avoid sample mix-ups. Blood serum was centrifuged at 3000 rpm for 20 min and subsequently stored at −20°C until the realization of biochemical analysis.

Hematological and biochemical analysis

All hematology and biochemistry analyses were performed by a reference laboratory (Esraa's and Al-shefaa's clinical laboratories, Tripoli, Libya). The hematological indicators included in the study were as follow: leukocytes (WBC, $x10^3/\mu L$), erythrocytes (RBC, $x10^6/\mu L$), hemoglobin (Hb, g/dl), hematocrit (HCT, %), mean cell volume (MCV, fl), mean cell hemoglobin (MCH, pg), mean cell hemoglobin concentration (MCHC, %), red cell width distribution-coefficient of variation (RDW-CV, %), red cell distribution width-standard deviation (RDW-SD, fl), platelet distribution width (PDW, fl), and plateletcrit (PCT, %). The described indicators were examined with an automatic hematological analyzer (Celltac α, nihon kohden, tokyo, japan). platelets (PLT, $x10^3/\mu L$), mean platelet volume (MPV, fl), platelet distribution

The serum biochemical indicators included in the study were as follow: alanine aminotransferase (ALT, U/L), alkaline phosphatase (ALP, U/L), aspartate aminotransferase (AST, U/L) and lactate dehydrogenase (LDH, U/L), glucose (Glu, mg/dl), total protein (TP, g/dl), albumin (Alb, g/dl), total and direct bilirubin (T BIL, D BIL , mg/dl),total cholesterol (TC, mg/dl), triglycerides (TG, mg/dl), very low density lipoprotein (VLDL, mg/dl), low density lipoprotein (LDL, mg/dl), high density lipoprotein (HDL, mg/dl), urea (mg/dl), creatinine (Cr, mg/dl), calcium (Ca, mg/dl), phosphorus (P, mg/dl), and magnesium (Mg, mg/dl) were measured using an automated blood chemistry analyzer (pz Cormay ACCENT M320) as well as serum sodium (Na, mg/dl), potassium (K, mg/dl), and chloride (Cl) were determined using easylyte plus automatic analyzer using ion-selective electrode. Indirect bilirubin (I BIL) was estimated as the arithmetical difference between serum total bilirubin and direct bilirubin values.

Statistical analysis

The data was submitted to statistical analysis using the computational program SAS version 25. Values were compared using ordinary one-way ANOVA method and the level of significance was set to: $p< 0.5$, $p < 0.01$, $p < 0{,}001$, $p < 0.0001$ and column statistics were calculated. For the non-normally distributed data, a Kruskal-Wallis analysis was used and when data were statistically significant.

Results and discussion

Table 1&2 summarizes the hematological indices of (non-racing and non-pregnant) horses, racehorses and pregnant mares. The WBC, Hb and RBC showed an increase in racing and pregnant mares but this did not significantly different in comparison with the (non-racing non-pregnant horses). However, the HCT values was significantly ($p< 0.005$) higher in racing and pregnant mares compared with (non-racing and non-pregnant) horses. Similar findings obtained by [19] who reported that WBC, HCT, HGB, PLT, RDW-SD, and RDW-CV were not significantly modulated ($p > 0.05$) in Thoroughbred racehorses. Also, [2, 20] stated that total WBC counts and differentials were not statistically significant between pregnant and non- pregnant mares. Physiologically, higher RBC, HCT and HGB levels can play a role in better physical condition, such as in sports activities [21]. Previous investigations displayed significantly lower values of RBC, HGB, and HCT in pregnant mares compared with non-pregnant mares [2, 20]. According to fetal growth that occurs in that period of pregnancy produces a greater oxygen demand [22]. This greater need for oxygen is compensated by the endocrine system that stimulates the release of erythropoietin by the renal tissue [23]. The secretion of this circulating glycoprotein stimulates increased production of erythrocytes in the bone marrow [24]. This phenomenon, which has been described in pregnant rabbits [25] and pregnant women [22], has also been observed in Arabian pregnant mares [20, 21]. Additionally, studies on rabbits stated an increase in RBC, HCT, and HGB during the 2nd trimester followed by a reduction in the 3rd trimester [26, 27]. A previous study on 16 draft mares reported no significant changes in RBC, HCT, and HGB before and

after delivery [4]. Moreover, there was no significant difference in the erythrocyte variables between pregnant and non-pregnant Campeiro mares [28] or pregnant and non-pregnant Warmblood mares [29] or between pregnant and non-pregnant Barb mares [30]

The MCV (p=0.01), RDW-CV (p<0.001) and RDW-CV (p<0.001) showed a significant higher value (p<0.01) in pregnant mares than non-racing non-pregnant horses. The same findings obtained by previous report [31] who found significantly higher values for the MCV in pregnant Crioula mares. The results found for MCV and RDW suggest an increase in the number of immature erythrocytes, which would be in accordance with findings in pregnant rats, rabbits and women reported by [20, 24, 25]. However, MCHC was significantly (p-<0.001) decreased in racing and pregnant animals. Decreased circulating lifespan of erythrocytes and hemodilution have been proposed as mechanisms for decreased RBC and HB concentrations during pregnancy in humans and rabbits. The MCH was non-statistically different among all animals. Our results partially agree with [2] who reported that MCV, MCH, and MCHC did not significantly change between pregnant and non-pregnant mares. Previous studies on pregnant mares reported no significant changes in MCV, and MCHC [4, 14, 32]. Similarly, no significant differences were established in MCV, MCH and MCHC in endurance horse's pre and post 120 km race [9]. However, [33] observed an increase in RBC count, Hb and HTC in endurance horses competing at a distance from 30 km to 100 km.

Table 1. Hematological values in non-racing non-pregnant horses (n=34), racehorses (n=15) and pregnant mares (n=16) (Mean ±SD).

Items	Phys.	Mean		Std. Deviation	Kruskal-Wallis test	
					test statistics	*p*-value
WBC (x10^3/µL)	no Race, no pregnant	6.82	±	1.92	0.657	0.720
	Race	6.77	±	1.31		
	pregnant	7.07	±	1.24		
(g/dl)	no Race, no pregnant	12.06	±	2.13	1.455	0.483
	Race	13.05	±	2.57		
	pregnant	12.39	±	1.65		
RBC (x10^6/µL)	no Race, no pregnant	6.94	±	1.03	5.647	0.059
	Race	7.70	±	1.14		
	pregnant	7.44	±	0.98		
HCT (%)	no Race, no pregnant * (p-value < 0.005)	30.91	±	5.74	12.414	0.002
	Race *	36.05	±	5.35		
	Pregnant *	35.81	±	4.99		
MCV (fl)	no Race, no pregnant * (p-value =0.010)	44.48	±	3.82	8.225	0.016
	Race	46.99	±	3.43		
	Pregnant *	48.46	±	4.61		
MCH (pg)	no Race, no pregnant	17.07	±	1.23	1.543	0.462
	Race	16.38	±	1.79		
	Pregnant	16.36	±	1.84		
MCHC (%)	no Race, no pregnant * (p-value <0.004)	38.67	±	3.84	16.912	0.000
	Race*	34.88	±	2.65		
	Pregnant*	33.91	±	1.27		
RDW-CV (fl)	no Race, no pregnant * (p-value <0.003)	18.41	±	2.89	15.891	0.000
	Race *	21.52	±	2.71		
	Pregnant *	21.60	±	2.61		
RDW-SD (%)	no Race, no pregnant * (p-value <0.002)	28.42	±	9.26	16.523	0.000
	Race*	37.81	±	6.76		
	Pregnant*	38.51	±	6.88		

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

Table 2. Platelet count and platelet indices in non-racing non-pregnant horses (n=34), racehorses (n =15) and pregnant mares (n=16) (Mean ±SD).

Items	Phys.	Mean		Std. Deviation	Kruskal-Wallis test	
					test statistics	*p*-value
PLT ($x10^3\mu L$)	no Race, no pregnant	81.96	±	49.08	1.328	0.515
	Race	82.01	±	50.44		
	pregnant	73.72	±	60.20		
MPV (fl)	no Race, no pregnant * (p-value =0.02)	7.15	±	0.57	6.297	0.043*
	Race	7.40	±	0.43		
	Pregnant *	7.57	±	0.57		
PDW (fl)	no Race, no pregnant	15.16	±	1.38	5.566	0.062
	Race	15.25	±	2.86		
	pregnant	15.97	±	2.14		
PCT (%)	no Race, no pregnant	0.07	±	0.09	0.617	0.735
	Race	0.06	±	0.03		
	pregnant	0.10	±	0.16		

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

Concerning platelets evaluations (Table 3), with the exception of mean platelet volume (MPV), PLT count, platelet distribution width (PDW) and plateletcrit (PCT) values reported no significant changes among the examined Arabian horses (Table.2). This agree with [4, 14, 32]. The MPV, PDW and PCT parameters are indicators of PLT, they indicate changes in the form and activation of platelets, and of them PDW is the most specific marker [34]. According to [35], the analysis of MPV changes may be a potential marker in pregnant cattle. As described in other studies [36], no statistically significant differences were found in the MCH, mean platelet volume (MPV), thrombocrit or red blood cell distribution width (RDW) in the pregnant Polish Holstein-Friesian black-and-white cattle. Also our results were parallel to those obtained by [37] who reported that pregnant women showed a statistically significant increase in mean platelet volume (MPV) and the other platelet parameters showed differences, but were not statistically significant.

With reference to the biochemical results in this research, a significant decrease in serum ALT, ALP, and LDH activities were recorded in racing (6.53±5.36, 104.83±32.50, 324.22±78.99 U/L) and pregnant animals (5.11±3.06, 140.34±50.48, 305.76±109.15 U/L) compared to the non- racing non-pregnant horses (8.48±3.90, 182.09±89.74, 382.72±96.25 U/L). Moreover, there is lower activity of AST but did not significantly different as shown in Table 3.

On contrary to our results, the greatest increase in serum enzyme activity post-race was for those indicators of muscular injury, that is, CK, ALT, and AST. These enzymes were all within a normal reference range pre-race and changed dramatically, beyond the normal reference range, during the race [38]. According to a study by [39] the magnitude of increase of both CK and AST is not related to fatigue or performance (e.g., speed). In horses, the most accepted blood indicators of muscle damage are LDH and AST activities [12]. Other authors stated that activity of hepatic parameters is a health state monitoring factor in all organisms. Increased activity of AST is usually a result of muscle or liver diseases [40]. The AST activity decreased in racing or pregnant horses thus may be connected with the highest muscle fatigue during these conditions. Besides, the CPK and AST values are related to muscle mass and composition [41]. Furthermore, plasma CK, AST, and LDH activities of the horses that were not assessed to be poor performers were within normal RIs [15].

Table 3. Serum enzymes in non-racing non-pregnant horses (n=34), racehorses (n=15) and pregnant mares (n=16) (Mean ±SD).

Items	Phys.	Mean	Std. Deviation	Kruskal -Wallis test	
				test statistics	*p*-value
ALT (U/L)	no Race, no pregnant	8.48 ±	3.90	9.893	0.007*
	Race	6.53 ±	5.36		
	pregnant	5.11 ±	3.06		
AST (U/L)	no Race, no pregnant	263.65 ±	74.06	5.489	0.064
	Race	254.09 ±	101.04		
	pregnant	231.71 ±	102.47		
ALP (U/L)	no Race, no pregnant	182.09 ±	89.74	11.986	0.002*
	Race	104.83 ±	32.50		
	pregnant	140.34 ±	50.48		
LDH (U/L)	no Race, no pregnant	382.72 ±	96.25	10.282	0.006*
	Race	324.22 ±	78.99		
	pregnant	305.75 ±	109.15		

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

As illustrated in Table 4, serum total protein (TP) and indirect bilirubin (I BIL) was significantly (p<0.05) increased in race (7.19 g/dl, 0.42 mg/dl) and pregnant horses (7.41 g/dl, 0.30 mg/dl) in relation to non-racing non-pregnant horses (6.80 g/dl, 0.64 mg/dl) but there is no statistical changes in serum albumin (Alb), total and direct bilirubin (T BIL, D BIL) and glucose (Glu) levels in between the studied experimental animals.

The increase in TP in racing and pregnant mares was in agreement with [28] who recorded significantly higher (P<0.05) values of TP in pregnant mares than non-pregnant mares. According to [42], during pregnancy, there is usually a decrease in serum albumin concentrations and an increase in globulin concentrations, followed by a decrease during the postpartum period due to the production of colostrum. Increase in globulin concentration due to pregnancy may have contributed to the increase in total protein concentration in pregnant mares.

About serum Alb results, analogous study reported no gestational changes in serum albumin levels in purebred Spanish and Holstein mares [16-18] .On the other hand, other investigators specified that Alb significantly decreased during the 2nd trimester of pregnancy [2]. Also, plasma concentrations of total protein, and albumin were reduced (p<0.001) from mid-gestation [43]. A similar pattern has been reported in women during the 3rd trimester. It has been stated that the reduction in Alb levels causes a decrease in oncotic pressure, leading to hemodilution in pregnant women, thus predisposing them to physiological edematous states and pre-eclampsia [44]. Additionally, plasma Alb concentration decreases during pregnancy and hemodilution is the more likely explanation may be related to reduced synthesis or increased catabolism of Alb [45]. There are some disagreements in previous reports regarding bilirubin levels. Former results show lower levels of T BIL (p< 0.001), D BIL (p< 0.001), and I BIL (p< 0.001) in pregnant compared with non-pregnant mares [2]. However, [18] reported higher T BIL concentrations in mid and late gestation in mares while [32] found that T BIL and D BIL were significantly higher in prepartum mares than in non-pregnant mares. Lower levels of T BIL, I BIL, and D BIL have been observed in humans as well, likely due to hemodilution [46]. Presumably, alterations in albumin levels and hemodilution are linked to alterations in bilirubin levels. Similarly, research on pregnant monkeys revealed a drop in liver functioning indicators, such as decreased levels of total bilirubin [47]. The serum glucose concentration decreased significantly during the race, while the triglyceride concentration increased substantially [38], findings that are consistent with those of earlier studies [48, 49]. However, the glucose level of thoroughbred race horses showed significant increase (p <0.05) [6] and this increases may be attributed to hyper activity of sympathetic system and adrenaline release which activate hepatic glycogenolysis [50].

Table 4. Serum biochemical profile in non-racing non-pregnant horses (n=34), racehorses (n=15) and pregnant mares (n=16) (Mean ±SD).

Items	Phys.	Mean	Std. Deviation	Kruskal -Wallis test	
				test statistics	*p*-value
TP (g/dl)	no Race, no pregnant	6.80 ±	0.98	9.752	0.008*
	Race	7.10 ±	0.52		
	pregnant	7.41 ±	0.38		
ALB (g/dl)	no Race, no pregnant	3.69 ±	0.51	2.421	0.298
	Race	3.74 ±	0.33		
	pregnant	3.60 ±	0.30		
T BIL (mg/dl)	no Race, no pregnant	0.55 ±	0.41	5.185	0.075
	Race	0.93 ±	0.67		
	pregnant	1.12 ±	1.02		
D BIL (mg/dl)	no Race, no pregnant	0.25 ±	0.19	5.424	0.066
	Race	0.39 ±	0.27		
	pregnant	0.42 ±	0.28		
I Bill (mg/dl)	no Race, no pregnant	0.64 ±	0.64	8.307	0.016*
	Race	0.42 ±	0.31		
	pregnant	0.30 ±	0.33		
Gluc (mg/dl)	no Race, no pregnant	64.90 ±	14.22	0.652	0.722
	Race	65.21 ±	16.31		
	pregnant	65.99 ±	11.33		

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

Serum lipid profile and renal biomarkers (Cr and urea) in the present study showed a significant (p<0.05) decrease in serum TC and HDL in racing (88.55 mg/dl, 46.01 mg/dl) and pregnant mares (92.71 mg/dl, 51.14 mg/dl) compared with non-pregnant animals (103.00 mg/dl, 55.82 mg/dl), while the other determined parameters included TG, VLDL, LDL, Cr and urea were insignificantly changed among all animals as demonstrated in Table 5. However, in our study TG increased in pregnant mares but not statistically different. Similar results were obtained by [51] who showed a significant increase in TC , and TG in late pregnant thoroughbred Arabian race mares. The authors attribute the increase in TG to high very low-density lipoprotein (VLDL) production in liver increase due to negative energy balance in pregnancy and hyperlipemia may occur. Additionally, pregnancy in the Spanish broodmare induced hyperlipidemia with hypertriglyceridemia and increased plasma concentrations of total, direct and indirect bilirubin ($p<0.05$) [52].

On contrary to our findings, [6] stated that the urea and creatinine of thoroughbred race horses showed significant increase (p<0.05). Other investigation stated that blood ALT, AST, ALP, albumin, TB, and TC, TG, Cr, Na, and inorganic phosphate increased significantly with endurance racing, whilst chloride, glucose, iron, and potassium decreased significantly compared to pre-race levels [38].

Table 5. Serum lipid profile and renal biomarkers in non-racing non-pregnant horses (n=34), racehorses (n=15) and pregnant mares (n=16) (Mean ±SD).

Items	Phys.	Mean		Std. Deviation	Kruskal -Wallis test	
					test statistics	p-value
TC (mg/dl)	no Race, no pregnant	103.00	±	22.40	6.750	0.034*
	Race	88.55	±	15.82		
	pregnant	92.71	±	15.71		
TG (mg/dl)	no Race, no pregnant	35.15	±	13.15	1.624	0.444
	Race	30.53	±	14.69		
	pregnant	40.75	±	29.22		
HDL D (mg/dl)	no Race, no pregnant	55.82	±	11.32	11.207	0.004*
	Race	46.01	±	6.05		
	pregnant	51.14	±	7.41		
VLDL (mg/dl)	no Race, no pregnant	14.97	±	4.74	3.860	0.145
	Race	13.13	±	3.18		
	pregnant	13.19	±	3.21		
LDL (mg/dl)	no Race, no pregnant	33.10	±	10.81	4.143	0.126
	Race	28.91	±	9.66		
	pregnant	27.24	±	9.97		
CREA (mg/dl)	no Race, no pregnant	1.29	±	0.20	2.602	0.272
	Race	1.37	±	0.22		
	pregnant	1.35	±	0.24		
UREA (mg/dl)	no Race, no pregnant	36.09	±	6.89	1.324	0.516
	Race	33.36	±	5.26		
	pregnant	35.16	±	5.95		

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

Concerning the mineral analysis in the experimental animals, serum P (p= 0.001) and K (p= 0.001) were significantly lower, meanwhile serum Mg was significantly increased in racehorses and pregnant mares than in non-pregnant mares. In addition, serum Ca, Na and Cl showed non-significant changes as illustrated in Table 6. The lower phosphorus and potassium levels in pregnant mares compared to non-pregnant mares may be due to fetal mineral consumption [53].

Maintaining optimal levels of Ca, P, Na, K and Cl is of particular importance for the water-electrolyte balance of the cell, which favors the transmission of nerve impulses and muscle contractions [54]. Prolonged exertion during endurance races or pregnancy is invariably accompanied by loss of electrolytes through sweat, urine, and to some extent with faeces. The loss of electrolytes through sweat leads to fatigue and weakness in horses [9]. Similar results were reported by [30] who stated that compared to non-pregnant Barb mares, P levels significantly decreased during the first, second, and third trimesters of pregnancy ($p<0.014$), ($p<0.002$) and ($p<0.0001$), respectively. P and Ca are essential for several body functions, including bone and energy metabolism. Most fetal Ca and P are deposited in the last 2 months of pregnancy, implying that most skeletal growth occurs at that time [55].

Serum K was significantly decreased in racing and pregnant horses and also sodium and chloride decreased in racehorses comparing with non-racing non-pregnant animals but did not statistically different. As described by other studies, [56] and [57] found a significant decrease in sodium as well as potassium and chloride during an endurance race in Australia and USA, respectively. Horses are known to secrete hypertonic sweat and lose sodium, chloride, and potassium through perspiration [54]. Also, the low concentration of potassium in the present study is most likely due to a combination of sweat loss, renal loss, water intake related dilution, and expected alkalosis as reported by [38]. Other study revealed that plasma concentrations of Na, K, urea, Cr, TG and TC were significantly reduced ($p<$

0.001) during pregnancy [43]. Decreased K concentration after a ride is a common finding [57-59], associated with loss in the sweat and increased activity of the Na-K-ATPase pumps during recovery.

Table 6. Serum mineral profile in (non-racing and) non-pregnant horses (n=34), racehorses (n=15) and pregnant mares (n=16) (Mean ±SD).

Items	Phys.	Mean	Std. Deviation	Kruskal -Wallis test	
				test statistics	*p*-value
CA (mg/dl)	no Race, no pregnant	11.76	1.31	0.792	0.673
	Race	12.01	0.50		
	pregnant	11.78	0.69		
P (mg/dl)	no Race, no pregnant	4.82	1.23	14.084	0.001*
	Race	4.14	0.96		
	pregnant	3.44	1.13		
Mg (mg/dl)	no Race, no pregnant	1.78	0.30	9.309	0.010*
	Race	1.93	0.20		
	pregnant	2.03	0.23		
Na (mEq/L)	no Race, no pregnant	133.04	4.93	2.595	0.273
	Race	134.08	3.44		
	pregnant	135.26	4.31		
K (mEq/L)	no Race, no pregnant	4.86	0.53	13.965	0.001*
	Race	4.56	0.65		
	pregnant	4.20	0.57		
Cl (mEq/L)	no Race, no pregnant	102.24	4.43	1.563	0.458
	Race	103.69	3.44		
	pregnant	104.62	4.33		

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

Conclusion

Understanding such physiological changes is imperative to providing the best care and medical treatment in mares. These data will assist clinicians in better evaluating and treating any problems in the animals either race horses or pregnant mares.

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