

Blood Constituents, Antioxidant Activities and Hormonal Profile in She-camels (*Camelus dromedarius*) during different Physiological Statuses in the North Western Coast of Egypt

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ABSTRACT

To monitor blood constituents and hormonal profiles in dromedary she-camels at different physiological statuses in the Northwestern Coast of Egypt, twenty-four she-camels, aged 7-10 years were used from December 2016 to March 2019. Animals were divided according to estrous behavior and ultrasound scans of ovarian structures into estrus and non-estrus groups before natural mating. Pregnancy was diagnosed 30 days after mating by ultrasonography. Blood samples were collected from all animals during estrus and non-estrus and monthly during gestation and lactation periods. During the follicular phase GLC and P₄ levels decreased ($P < 0.05$). Levels of E₂ and PRL increased ($P < 0.05$). Follicles number and diameter in both ovaries increased ($P < 0.05$) in estrus group. Cyclic status correlated negatively with GLC, P₄, total number follicles of left ovary (TLO) and diameter follicles of left ovary (DLO) and positively with AST, E₂ and DLO. In 1st trimester of pregnancy, GLC levels decreased. Higher ($P < 0.05$) activity of ALT (2nd and 3rd trimesters) and ALP (1st and 2nd trimesters) were recorded. SOD and HP changed ($P < 0.05$). Pregnancy status correlated negatively with GLC, ALP, HP and SOD and positively with TG and ALT. During lactation, ALB, CHOL, TG, ALT, ALP, P₄ and PRL were affected ($P < 0.05$). SOD and HP were higher ($P < 0.05$) in 2nd and 1st trimester, respectively. Lactation status correlated negatively with P₄ and PRL and positively with CHOL, ALB, ALP, ALT and TAC. In conclusion, the knowledge the values of hormonal, blood biochemical, antioxidant activities are necessary for diagnostic interpretation of pathological and adjusting the different functions throughout different physiological statuses under arid conditions.

Key words: Ovarian activities; Blood constituents; Antioxidants biomarkers; She Camel; Physiological statuses.

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INTRODUCTION

In Egypt, the one-humped camel (*Camelus dromedarius*) is one of the important recourses for meat and milk in the economy and social life of a large sector of pastoralists in arid, semi-arid, regions in several localities especially in North Cost of Egypt and their total population are 120,000 heads (Abd El-Hamid et al. 2015). Meat of camels contributes about 6% of total red meat and enter many food industries such as meat burgers and cheese (Ibrahim and Nour 2010; Heydari et al. 2015). Milk is a good source of proteins, fat, vitamin C and energy. All essential nutrients found in bovine milk also exist in the camel milk and can be used to combat the hunger during drought season in arid and semi-arid-zones (Gassem et al. 2015; (Chemweno et al. 2019; Mahran et al. 202). Reproductive inefficiency in the one-humped camel is a major problem in most of herds due

to late first service and induced ovulatory (non-spontaneous) nature with ovulation induced mainly by coitus (Anouassi and Tibary 2013). Additionally, short breeding period, long calving interval, long gestation period, limited breeding opportunities, late post-partum estrus and a relatively high embryonic loss reaching 35%, might be the main factors attributing in low fertility in camels (Pratap et al. 2012). Blood hormones and biochemical concentrations in one-humped camel might be a potential aid in characterizing the different physiological problems and are frequently used because of their significant correlation with the nutritional status (Claypool et al. 1975). Therefore, studies about the biochemical constituents, hormonal concentrations and antioxidant enzymes activities are very important for clinicians in the field during different physiological phases including estrus cycle, pregnancy and lactation (Omidi et

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al. 2015). However, fewer reports have been published concerning the influence of different physiological statuses on hormonal profiles, serum biochemical parameters and antioxidant status in one-humped camel under arid zones (Abd-El-Rahman et al. 2017). Generally, oxidative stress is associated with the generation of reactive oxygen species (ROS) Herrera et al. (2012) and can be produced by many factors, such as prenatal hypoxia, excessive glucocorticoid exposure, hyperketonemia, ketosis and negative energy balance (Al-Qudah 2011) and it is considered a very important tool in evaluation of fertility and development of embryos (Symonds et al. 2007; Mohebbi-Fani et al. 2012). The increasing it leads to weakness of biological and enzymatic activities, decrease secretion of steroids hormones and its receptors during estrus and pregnancy periods, thus reducing fertility in animal herds (Egbuna and Ifemeje 2017). As a consequence, the present study aimed to study the pattern of some blood biochemical constituents, hormonal profiles and oxidative stress status included total antioxidant capacity (TCA), malondialdehyde (MDA), superoxide dismutase (SOD), hydrogen peroxide (HP) and nitric oxide (NO) in one-humped camel with various physiological statuses and to set reference values to help in early prediction and diagnosis of metabolic diseases related to disturbance in oxidative stress as well as ensuring proper health in very challenging physiological state in one-humped camel.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

All ethical protocols have been followed in the conduct and preparation of this manuscript in conformity with the EU Directive for protection of experimental animals (2010/63/EU).

Animals Management

This study was carried out at Mariout Research Station (Latitude 31° 00' N; Longitude 29° 47' E) belonging to Desert Research Center, Egypt. Twenty-four mature one-humped non-lactating she-camels (*Camelus dromedarius*) aged 7-10 years with average body weights of 450±20 kg, good body condition score (2.5±0.4) arbitrary units according to (Faye et al. 2001) were used in this study from December 2016 to March 2019. The animals were housed in an open paddock throughout the experimental period. The camels were allowed to graze daily from 9:00 AM to 3:00 PM. Each animal received 4kg/head/day of pelleted concentrate mixture that contained 65% total digestible nutrients and 14% crude protein in addition to Egyptian clover (*Trifolium alexandrinum*) hay as roughage *ad libitum*. Fresh water was presented twice daily. Animals were examined clinically and were found to be free of any disease and physical abnormalities or reproductive disorders.

Ultrasound Examination and Ovarian Activity

Ovarian follicular dynamics in all she-camels were monitored using a Dynamic Imaging Concept MLV scanner ultrasound device (Eickemeyer Magic 5000 Digital), integrated with a dual frequency (5.0–7.5 MHz) linear probe. The examined animals were restrained in a crush in a standing position during the sessions. The

number and size of different types of follicles were recorded according to their ovarian activity into the following different ovarian phases: growing phase (0.5cm< follicle <1.1cm), mature phase (1.1cm< follicle <1.9cm) (Swelum and Alowaimir 2015). The females were divided according to estrous behavior and ultrasound scan into two statuses: 1. Estrus and 2. Non-estrus: before natural mating.

Early Pregnancy Diagnosis

After 30 days from natural mating, all animals were examined by ultrasonography to detect anechoic uterus, allantoic fluid and embryo proper or fetus which considered positive signs of pregnancy (Fig. 1).

Reproductive Performance

Reproductive performance including conception rate (number of conceived she-camels at day 30 evaluated by ultrasonography/ total number of she-camels×100), pregnancy rate (number of conceived she-camels/total number of she-camels×100), twinning rate (number of she-camels twinning lambd/number of she-camels lamed×100). Survival, motility rate and sex ratio of calve camels were recorded.

Blood Samples

Blood samples were collected from jugular vein of all animals in plasma vacutainer tubes (K₃ EDTA) in early morning (07:00 AM) throughout estrus (n=18) and non-estrus (n=8) stages. Blood sampling started after 30 day of natural mating and continued monthly during pregnancy and lactation trimesters (first, second and third). Plasma was harvested after centrifugation at 1500g for 10min, and then stored at -20°C for later analysis.

Blood Biochemical Analysis

Commercial kits (Stanbio Laboratory, USA) were used to analyze plasma total proteins (TP, Cat. # 0345), Albumin (ALB, Cat. # 0285), Glucose (GLC, Cat. # 1070), cholesterol (CHOL, Cat. # 1010), triglycerides (TG, Cat. # 2200) according to (Weichsbaum 1964; Scheletter and Nussel 1975; Trinder 1969; Finley et al. 1978; Young et al. 1972), respectively. Alanine aminotransferases (ALT, Cat. # 113100), aspartate aminotransferase (AST, Cat. # 043360) and alkaline phosphatase (ALP Cat. # 151050) were determined using colorimetric kits (Biomed, Germany), according to (Henry 1964; Tietz 1976; Belfield and Goldberg 1971), respectively.

Biomarkers of Antioxidant Enzymes Activities

Antioxidants enzymes activities of total antioxidant capacity (TCA, Cat. # TA2513), malondialdehyde (MDA, Cat. # MD2529), superoxide dismutase (SOD, Cat. # SD2521), hydrogen peroxide (HP, Cat. # HP25) and nitric oxide (NO, Cat. # NO2532) were calorimetrically assayed using commercial kits (Biodiagnostic Research, Egypt) according to previous reports (Koracevic et al. 2001; Satoh, 1978; Nishikimi et al. 1972; Aebi, 1984; Montgomery and Dymock, 1961) in order.

Hormonal Assay

Estradiol 17-β (E₂, Cat. # MO4925-300) and progesterone (P₄, Cat. # MO4825-300) hormones were analyzed using ELISA kits (Monobind, USA) according to

Abraham (1974). The intra -and inter-assay CV's are 9.3 and 9.7%, respectively. Triiodothyronine (T_3 , Cat. # MBS9914463) and prolactin (PRL, Cat. # MBS089793) hormones were analyzed using specific camels ELISA kit (MyBioscience, USA). The intra-and inter-assay CV's are less than 15%.

Statistical Analysis

All results were found as mean \pm SEM. The changes in ovarian activities, biochemical blood parameters, biomarkers antioxidant activities and hormonal profiles throughout experimental periods were statistically analyzed by one-way analysis of variance (ANOVA) to test the significance among means. Chi square of independence was used to analyze fertility-related parameters post parturition. The differences between means were detected by Duncan's post-hoc test. The correlations between all parameters and status were obtained by Pearson correlation coefficient. Significance was set at 5% and data were analyzed by IBM-SPSS statistics program (IBM-SPSS 2013).

RESULTS

Changes in Blood Biochemical Constituents and Hormonal Profiles During Cycle Status

Overall mean of plasma GLC was affected ($P<0.05$) during the follicular phase of the cycle status, where lower concentration was recorded during estrus status than in non-estrus. On the other hand, plasma concentrations of TP, ALB, CHOL, TG, ALT, AST and ALP in addition to antioxidant enzymes activities including TCA, MDA, SOD, HP and NO did not differ significantly (Table 1).

Concerning sexual hormones, the present findings demonstrated that overall means of E_2 levels were higher ($P<0.05$) in estrus than non-estrus during follicular phase of the cycle status. Moreover, plasma PRL concentrations increased ($P<0.05$) in estrus compared with non-estrus during follicular phase of the cycle status. On contrarily, plasma P_4 level decreased ($P<0.05$) in estrus compared with non-estrus during the follicular phase of the cycle status. On the other hand, overall mean of plasma T_3 concentration were not significantly affected throughout follicular phase of the cycle status (Fig. 2).

Changes in Ovarian Follicular Activities

The results showed that overall means of total number follicles in both ovaries (left, TLO and right, TRO) increased ($P<0.05$) in estrus stage compared with non-estrus stage. Furthermore, overall means of diameter follicles in both ovaries (left, DLO and right, DRO) increased ($P<0.05$) in estrus compared with non-estrus stage during the follicular phase of the cycle status (Table 2; Fig. 3). There were clearly negative correlations ($P<0.05$) between cyclic status and each of GLC, P_4 , TLO and DLO ($r=-0.78$, -0.81 , -0.92 and -0.79 , respectively). On contrarily, positive correlations ($P<0.05$) were recorded between cyclic status and level of AST, E_2 and DLO ($r=0.68$, 0.93 , and 0.84 , respectively).

Reproductive Efficiency

Conception and pregnancy rates were recorded 77.22%. However, twinning rate recorded 7.96%. The survival rate of calve camels recorded 85.71%, while

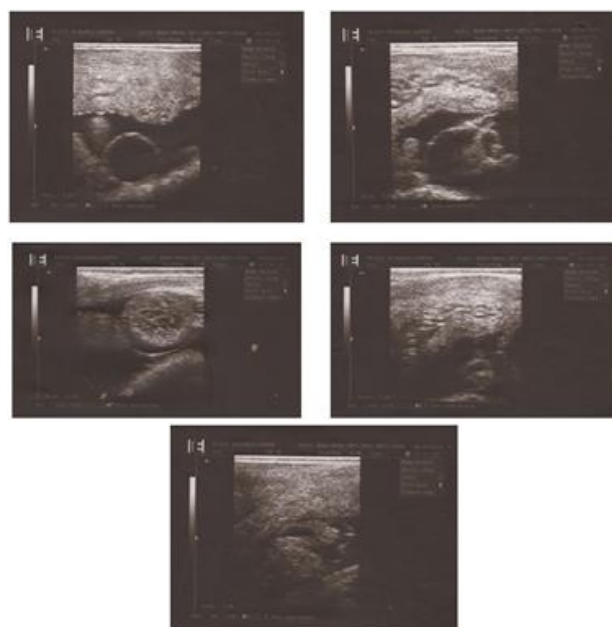


Fig. 1: Trans-rectal ultrasonic images for pregnancy after 30 days of natural mating in one-humped she-camels during early pregnancy stage.

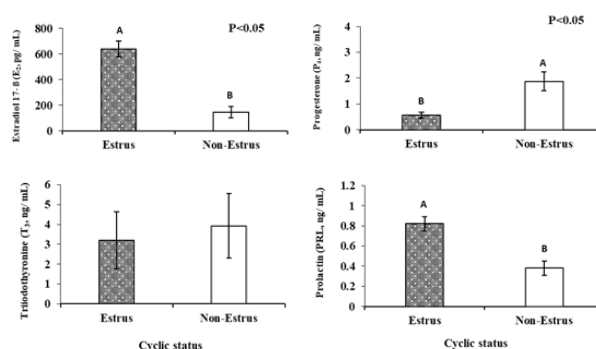


Fig. 2: Changes in plasma hormonal profiles in one-humped she-camels during cyclic status in the North western Coast of Egypt.

mortality rate of calve camels was 14.28%. Of the 14 calve camels born of she-camels 42.9% males vs. 57.1% females were recorded (Fig. 4 and 1).

Changes in Blood Biochemical Constituents and Hormonal Profiles during Pregnancy Period

Plasma TP, ALB concentrations were not affected by pregnancy period, while plasma GLC level was significantly affected, the lowest ($P<0.05$) value was recorded in first trimester, then increased gradually in second and third trimesters of pregnancy. Overall means of plasma CHOL and TG concentrations were not significantly changed during different pregnancy trimesters. Plasma level of ALT activity was higher ($P<0.05$) in second and third trimesters than first trimester of pregnancy. Contrariwise, plasma level of ALP activity was higher ($P<0.05$) in first and second trimesters compared with third trimester of pregnancy. While value of AST activity did not differ significantly among different pregnancy trimesters (Table 3).

Overall means of antioxidant enzymes activities included TCA, MDA and NO levels did not differ significantly among different pregnancy periods. However,

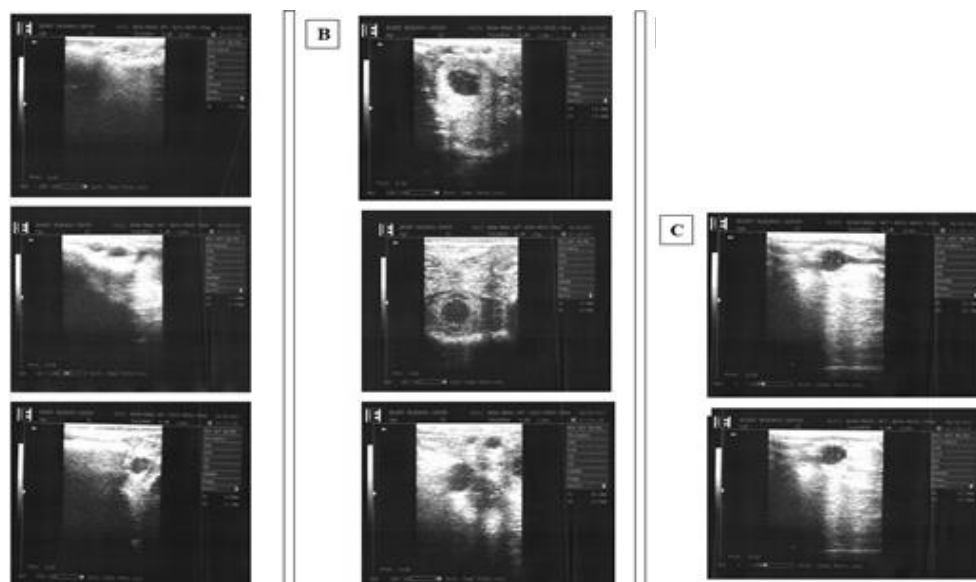


Fig. 3: Ultrasonography monitoring of ovarian activity in one-humped she-camels during cyclic status [A] small follicles [B] diameter of dominant follicles on left ovary (DLO), [C] diameter of dominant follicles on right ovary (DRO), prior to natural mating.

Table 1: Changes in plasma biochemical constituents, antioxidant enzymes activities in one-humped she-camels during cyclic status in the North western Coast of Egypt

Items	Cyclic status	
	Estrus	Non-Estrus
Blood biochemical parameters		
Total protein (TP, g/dL)	6.01±0.08	6.06±0.12
Albumin (ALB, g/dL)	4.06±0.05	3.97±0.07
Glucose (GLC, mg/ dL)	97.56±7.07 ^B	118.45±3.86 ^A
Cholesterol (CHOL, mg/ dL)	1.03.24±1.54	107.88±4.48
Triglycerides (TG, mg/ dL)	85.83±2.20	89.69±4.79
Alanine aminotransferase (ALT, IU/L)	68.06±6.36	78.96±11.17
Aspartate aminotransferase (AST, IU/L)	182.54±4.85	171.97±2.58
Alkaline phosphatase (ALP, IU/L)	23.26±3.11	23.80±1.05
Antioxidant enzymes activities		
Total antioxidant capacity (TCA, mM/L)	0.67±0.09	0.77±0.06
Malondialdehyde (MDA, nM/mL)	5.15±0.14	5.56±0.38
Superoxide dismutase (SOD, U/mL)	5.75±0.33	6.47±0.28
Hydrogen peroxide (HP, mM/L)	0.14±0.01	0.15±0.04
Nitric oxide (NO, µM/L)	23.32±1.27	26.05±2.06

^{A-B} Values (Mean±SE) within the same row with different letters differ significantly (P<0.05).

Table 2: Changes in ovarian activities in one-humped she-camels during cyclic status in the North Western Coast of Egypt

Items	Cyclic status	
	Estrus	Non-Estrus
Follicles number		
Total follicles of left ovary (TLO)	3.20±0.20 ^A	1.20±0.20 ^B
Total follicles of right ovary (TRO)	2.00±0.44 ^A	1.20±0.58 ^B
Follicles diameter (cm)		
Diameter follicles of left ovary (DLO)	2.03±0.43 ^A	0.59±0.07 ^B
Diameter follicles of right ovary (DRO)	1.22±0.12 ^A	0.40±0.06 ^B

^{A-B} Values (Mean±SE) within the same row with different letters differ significantly (P<0.05).

value of SOD was higher in first more than second trimester, while the lowest value was recorded in third trimester of pregnancy period. Value of HP was (P<0.05) higher in first trimester compared with second or third trimesters of pregnancy (Table 3).

Our results showed that overall means of plasma E₂ was not significantly affected by different trimesters of pregnancy. The lowest value was recorded in first trimester while higher values were recorded in second and third trimesters of pregnancy. This trend was reversed in the P₄ level where the lowest value was recorded in third trimester. On the other hand, plasma T₃ concentration did not differ significantly among pregnancy trimesters. The

lower values were recorded in second and third trimesters while the highest value was recorded in first trimester of pregnancy. Overall means of plasma PRL was non-significantly affected by pregnancy. Plasma PRL level was started from (0.72±0.15ng/mL) in first trimester and continue to increase slightly during second and third trimesters of pregnancy (1.02±0.10 and 1.20±0.25ng/mL) respectively (Fig. 5).

Negative correlations (P<0.05) were recorded between pregnancy status and levels of GLC, ALP, HP and SOD (r=-0.39, -0.37, -0.42 and -0.83, respectively). While, positive correlations (P<0.05) were noted in TG and ALT levels (r=0.28 and 0.36, respectively).

Table 3: Changes in plasma biochemical constituents, antioxidant enzymes activities and hormonal profiles in one-humped she-camels during pregnant period in the North Western Coast of Egypt (Mean \pm SE)

Items	Trimesters of Pregnancy		
	1 st Trimester	2 nd Trimester	3 rd Trimester
Blood biochemical parameters			
Total protein (TP, g/dL)	5.63 \pm 0.10	5.91 \pm 0.19	5.82 \pm 0.15
Albumin (ALB, g/dL)	4.54 \pm 0.11	4.68 \pm 0.16	4.35 \pm 0.12
Glucose (GLC, mg/ dL)	103.53 \pm 4.63 ^B	123.91 \pm 6.31 ^{AB}	134.61 \pm 11.51 ^A
Cholesterol (CHOL, mg/ dL)	125.67 \pm 4.66	122.98 \pm 3.50	122.21 \pm 5.62
Triglycerides (TG, mg/ dL)	89.05 \pm 2.82	95.21 \pm 2.42	96.92 \pm 1.95
Alanine aminotransferase (ALT, IU/L)	68.96 \pm 2.32 ^B	83.16 \pm 3.72 ^A	84.65 \pm 4.19 ^A
Aspartate aminotransferase (AST, IU/L)	178.20 \pm 4.69	179.54 \pm 4.61	172.00 \pm 3.16
Alkaline phosphatase (ALP, IU/L)	31.13 \pm 1.72 ^A	31.19 \pm 2.22 ^A	21.95 \pm 2.55 ^B
Antioxidant enzymes activities			
Total antioxidant capacity (TCA, mM/L)	0.62 \pm 0.04	0.61 \pm 0.02	0.61 \pm 0.03
Malondialdehyde (MDA, nM/mL)	5.35 \pm 0.33	5.52 \pm 0.18	5.82 \pm 0.27
Superoxide dismutase (SOD, U/mL)	5.92 \pm 0.34 ^A	4.42 \pm 0.27 ^B	1.7 \pm 0.15 ^C
Hydrogen peroxide (HP, mM/L)	0.19 \pm 0.01 ^A	0.18 \pm 0.01 ^{AB}	0.16 \pm 0.01 ^B
Nitric oxide (NO, μ M/L)	24.56 \pm 1.10	23.04 \pm 0.45	22.96 \pm 0.91

^{A-C} Values (Mean \pm SE) within the same row with different letters differ significantly (P<0.05).

Table 4: Changes in plasma biochemical constituents, antioxidant enzymes activities and hormonal profiles in one-humped she-camels during lactation period in the North Western Coast of Egypt

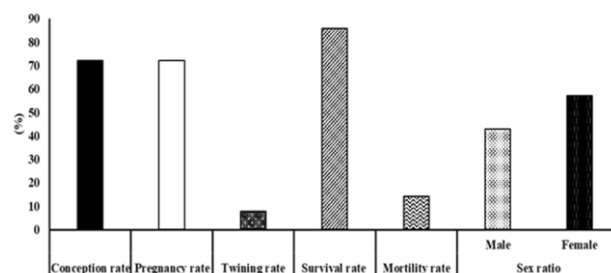
Items	Trimesters of lactation		
	1 st Trimester	2 nd Trimester	3 rd Trimester
Blood biochemical parameters			
Total protein (TP, g/dL)	5.64 \pm 0.28	5.91 \pm 0.19	5.83 \pm 0.12
Albumin (ALB, g/dL)	4.13 \pm 0.13 ^B	4.08 \pm 0.09 ^B	4.43 \pm 0.11 ^A
Glucose (GLC, mg/ d, L)	97.39 \pm 5.34	95.07 \pm 2.71	98.01 \pm 2.65
Cholesterol (CHOL, mg/ dL)	135.76 \pm 3.08 ^A	119.97 \pm 2.13 ^B	108.66 \pm 3.87 ^C
Triglycerides (TG, mg/ dL)	93.83 \pm 2.28 ^A	82.31 \pm 1.95 ^B	73.44 \pm 1.92 ^C
Alanine aminotransferase (ALT, IU/L)	70.88 \pm 1.93 ^B	96.91 \pm 2.41 ^A	101.46 \pm 1.06 ^A
Aspartate aminotransferase (AST, IU/L)	178.08 \pm 5.27	171.07 \pm 1.64	173.74 \pm 3.01
Alkaline phosphatase (ALP, IU/L)	21.34 \pm 2.55 ^B	30.19 \pm 2.60 ^A	30.79 \pm 2.12 ^A
Antioxidant enzymes activities			
Total antioxidant capacity (TAC, mM/L)	0.59 \pm 0.02	0.66 \pm 0.03	0.70 \pm 0.01
Malondialdehyde (MDA, nM/mL)	5.90 \pm 0.22	6.60 \pm 0.36	6.39 \pm 0.24
Superoxide dismutase (SOD, U/mL)	1.07 \pm 0.27 ^B	2.48 \pm 0.57 ^A	1.10 \pm 0.10 ^B
Hydrogen peroxide (HP, mM/L)	0.20 \pm 0.01 ^A	0.16 \pm 0.01 ^B	0.17 \pm 0.01 ^B
Nitric oxide (NO, μ M/L)	24.62 \pm 0.72	23.59 \pm 0.71	24.65 \pm 0.69

^{A-C} Values (Mean \pm SE) within the same row with different letters differ significantly (P<0.05).

Changes in Blood Biochemical Constituents and Hormonal Profiles during Lactation Period

Mean value of plasma TP and GLC levels did not differ significantly among different trimesters of lactation periods. However, plasma ALB value increased (P<0.05) in third trimester compared with first and second trimesters of lactation. Furthermore, plasma CHOL concentration increased (P<0.05) in first trimester and decreased gradually through second and third trimesters of lactation. The same trend was observed for of plasma TG concentrations. Mean value of alanine ALT activity was higher (P<0.05) in second and third trimesters of lactation than in the first trimester. The activity of AST was not affected by lactation periods. Mean value of ALP activity was higher (P<0.05) in second and third trimesters of lactation than during the early stage (Table 4).

Mean values of TCA and MDA were not affected with trimesters of lactation, while mean value of SOD was higher (P<0.05) in second (2.48 \pm 0.57U/mL) more than first (1.07 \pm 0.27U/mL) or third (1.10 \pm 0.10U/mL) trimesters of lactation period. Mean value of HP was (P<0.05) higher in first trimester, then declined in second and third trimesters of lactation period. Also, the results showed that mean value of HP was not affected by different trimesters of lactation period (Table 4).

**Fig. 4:** Reproductive performance of She-camels following natural mating in the North Western Coast of Egypt.

Overall means of plasma E₂ concentrations did not differ significantly among different lactation periods. Conversely, means of plasma P₄ concentrations were affected (P<0.05) by lactation periods. The highest concentration of progesterone was recorded in first trimester of lactation period, then declined during second and third trimesters of lactation period. Overall means of plasma T₃ did not differ significantly among different lactation periods. While overall means of plasma PRL concentrations the highest (P<0.05) value was recorded in first trimester (5.13 \pm 2.3ng/mL) followed by second (1.54 \pm 0.12ng/mL) and third (1.24 \pm 0.45ng/mL) trimester of lactation period (Fig. 6).

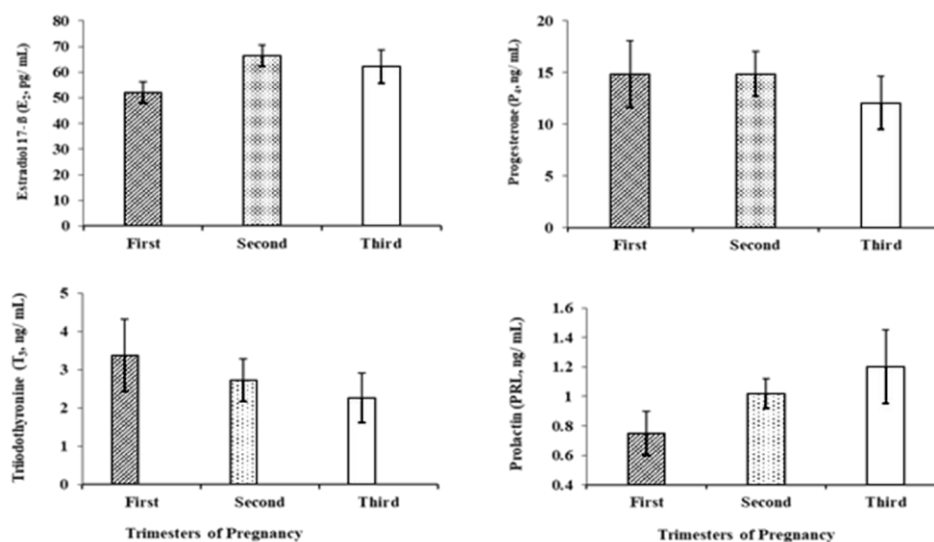


Fig. 5: Changes in plasma hormonal profiles in one-humped she-camels during pregnancy period in the North western Coast of Egypt.

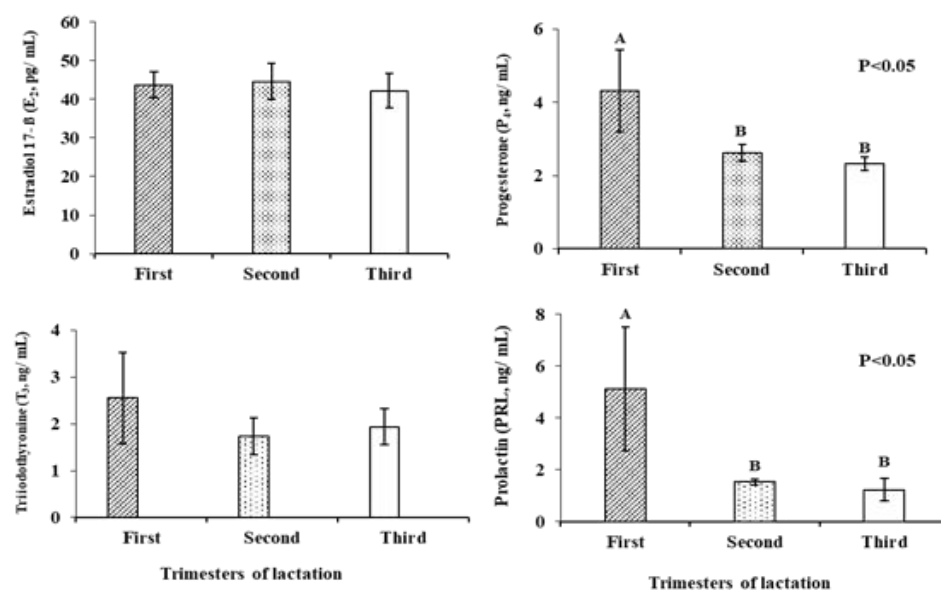


Fig. 6: Changes in plasma hormonal profiles in one-humped she-camels during lactation period in the North western Coast of Egypt.

The results showed that positive correlations ($P < 0.05$) were recorded between lactation and levels of CHOL, ALB, ALP, ALT and TAC ($r = 0.26, 0.30, 0.28, 0.70$ and 0.30 , respectively). While, negative correlations ($P < 0.05$) were recorded between lactation and level of P₄ and PRL ($r = -0.39$ and -0.41 respectively).

DISCUSSION

Changes in Blood Biochemical Constituents and Hormonal Profiles during Cycle Status

Our results showed that plasma concentrations of TP, ALB, CHOL, TG, ALT, AST and ALP were not significantly changed among estrus status. It is worthy of mentioning that our values were within the reference values of few available reports (Abdalmula et al. 2019; Islam et al. 2019) during cyclic stages in she-camels (*Camelus Dromedarius*). While GLC level decreased in estrus status, compared with non-estrus. These data are in agreement with those reported by Abd El-Hamid et al. (2015) where

serum GLC concentration decreased at days 10 and 20 of induction of ovulation period in dromedary she-camels during breeding season. The same results were reported in several species, in goats, plasma GLC level decreased during ovarian dynamics and estrus activities (Rateb et al. 2019). This reduction in plasma GLC with ovarian activities and increased of E₂ hormone, due to uptake GLC by ovarian tissues to accommodate ovarian activities (Rabee and Lean 2000). These results are supported by the data presented in Fig. 2.

Also, plasma antioxidant enzymes activities including TCA, MDA, SOD, HP and NO were not affected by different cycle status. Agree with these results, (Kandiel et al. 2016) reported that no significant difference was recorded in level of biochemical markers included (SOD, GPX, MDA, NO) during estrus cycle stages in Barki sheep. Colakoglu et al. (2017) suggested that blood antioxidant enzymes activities such as GSH-Px and MDA would be useful in evaluation the fertility of dairy cows under seasonal variations. Fewer reports were presented about

relationship between oxidative status and ovarian activity especially in camels. However, many studies were presented about relationship between oxidative status and pregnancy or per-parturient status in camels (Abd-El-Rahman et al. 2017). The considered balance between generation reactive nitrogen species and elimination reactive oxygen species (ROS) is an important role in gene expression. So, it is required for every metabolic function and biological activities in mammals (Shkolnik et al. 2011). The ROS are produced as by-products of aerobic metabolism, as results of many factors such as cellular metabolites and as a response to climate changes and toxicity of the surrounding environment (Shedeed et al. 2019). ROS exert both negative and positive effects in mammalian ovaries from maturation follicles to fertilization (Fujii et al. 2005). Several factors such as macrophages, leukocytes, and cytokines, which present in the follicular fluid, are considered major sources of ROS, which are involved in follicular growth by regulating angiogenesis and maturation by increasing the metabolic function of granulosa cells and ovarian steroid biosynthesis (Du et al. 2006). They also support the LH hormone in induction ovulation (Khalifa et al. 2020). In contrast, the excessive of ROS resulting in blood flow alterations and eventual follicle rupture (Wang et al. 2017). These results are supported by the data of ovarian activities (Fig. 2 and 3) and reproductive performance (Fig. 4).

Plasma E₂ concentration was significantly higher in estrus cycle compared with non-estrus cycle during the follicular phase. While plasma P₄ concentrations decreased significantly in estrus cycle compared with non-estrus during the follicular phase of the cycle status. However, value of plasma T₃ was not affected throughout follicular phase of the cycle status. This finding is in agreement with previous reports on dromedary camels (Ali et al. 2011; Abd El-Hamid et al. 2015) as they reported that, the increased of E₂ concentration due to the found of dominant follicle size (1.2-1.8cm) during mature ovarian phase (Atigui et al. 2014), the concentration of E₂ secretion is around (317.8-444.4pg/mL), whereas P₄ concentration remains below (1ng/mL) during the follicular phase. Additionally, in dromedary she-camels, a higher significant correlation was found between E₂ levels and follicular size during mature phase. This led to exhibit behavioral estrus patterns to mating reported by Padalino et al. (2016). These results are supported by the data presented in Fig. 2.

Plasma PRL value increased during estrus cycle (Fig. 2). In cattle and ewes, plasma PRL levels higher peaks in the estrus cycle were tied in with releasing amount of luteinizing hormone (Davis et al. 1971) reported that serum PRL levels increased at 2–3-day intervals during the natural cycle. In ewe's, plasma RPL level increased during natural estrus and a positive correlation between fertility and plasma PRL level was found (Rhind et al. 1980). In mares, a significant increase in plasma PRL levels was recorded at the first ovulation during reproductive season (Besognet et al. 1995).

Changes in Ovarian Follicular Activities

In she-camels, follicular wave can be divided into three phases: 1] the growth phase: about 3 to 6 follicles grow until reaching approximately (0.8cm) in diameter, 2] the mature phase: where one or two growing follicles

become dominant and continue to develop until the diameter reaches from (1.2 to 1.8cm), in the absence of ovulation, 3] the mature phase is followed by a regression phase, reaches in which anovulatory follicles from 2 to 4cm in diameter (Monaco et al. 2015). Our ovarian data in the present study showed that total number follicles (TNF) and diameter follicles in both ovaries left and right (DLF, DRF) increased significantly during estrus cycle (Table 2). Similar results have been previously obtained in she-camels (Rawy et al. 2014) where maximum of dominant follicular diameter in the ovulatory phase was found to be (1.82±0.2cm) with reach to higher production, of estrogen. These results are supported by the data of estrogen (Fig. 2). El-Hairry et al. (2010) reported that the histological examination in the left and right ovaries of the dromedary she-camel revealed that, camel's ovary showed higher activity during breeding seasons (winter) than in summer and autumn. Furthermore, (Zeidan et al. 2008) concluded that the left ovary contains (growing and mature follicles) was more active than the right one in she-camels during breeding season from December to April in Egypt.

Reproductive Efficiency

The reproductive performance in she-camels' herds is extremely low as compared with that of other domestic livestock due to several factors such as age, breeds, infectious diseases, management, long gestation period and nutrition (Bakheit et al. 2016). In this study, the overall of conception/ pregnancy rates were recorded 77.22%. However, the twinning and survival rates of calve camels were recorded 7.96 and 85.71%, respectively. While, the mortality rate of calve camels was recorded 14.28%. The sex ratio of calve camels was 42.9% males vs. 57.1% females (Fig.4). Agreement with these results, (Mostafa et al. 2018; Ali et al. 2018) they reported that the conception and pregnancy rates were 79.8 and 76.2% in herd camels, in order. On the other hand, (Mohammed and Al-Mutairi 2012) reported that the overall mean of conception rate ranged from 47 to 57.5% during breeding season. Quzy et al. (2013), reported that pregnancy rates were recorded 65%, 61% when hormonal protocols Ovsynch and PG were applied during the natural breeding season in she-camels. The average of mortality rate of calve camels were recorded between 13.5-27% reported by (Kaufmann 2005). Three cases of twin pregnancies were recorded in 30.0 gravid uteri of slaughtered camels (Tinson et al. 2001), this was due to multiple ovulations (Khalifa 2012).

Changes in Blood Biochemical Constituents and Hormonal Profiles during Pregnancy Period

Our results showed that overall of plasma TP, ALB, CHOL, TG and AST were not affected by pregnancy trimesters. These results were consistent with previous results in pregnant she-camels (Abd El-Hamid et al. 2015; Abd-El-Rahman et al. 2017).

Plasma GLC concentration lowered significantly in first trimester compared with second and third of pregnancy period. These data are in agreement with those reported by (Ayoub et al. 2003) in pregnant she-camels. The decrease in plasma GLC of pregnant she-camels may be due to highly bearing embryo consumption which reflected the greater demands from glucose and uptake it from muscle and fat (Firat and Ozpmnar 2002).

In the present study, plasma level ALT elevated significantly in second and third trimester of pregnancy period, also value of ALP was significant higher in first and second of pregnancy (Table 3). Tharwat et al. (2015) reported that serum activity of ALT and ALP in the female camels increased significantly during 2nd and 3rd trimesters of pregnancy. Similar findings were reported in cows (Tharwat et al. 2012) during pregnancy stages. This increase in transaminases activities are not considered a pathological condition, but rather is due to impairment in some muscle and liver cells due to rapid gluconeogenesis associated with pregnancy (Saeed et al. 2009). In our study, overall means of SOD and HP enzymes activities were significant higher in first trimester but declined in second and third trimesters of pregnancy. These results agreed with (Garrel et al. 2010; Mohebbi-Fani et al. 2012) reported that SOD and HP levels declined gradually during placental growth in pregnant ewes. The SOD and HP are a major antioxidant defense of reactive oxygen species (ROS) in both animal and human. Higher SOD₂ and HP activities lead to more of production of H₂O₂ and propagation of ROS in the mitochondria and cytoplasm which leads to imbalance between production of ROS and antioxidant systems, thus the occurrence of pregnancy disorders (Al-Gubory et al. 2010).

Overall means of TCA, MDA and NO levels were not affected by different trimesters of pregnancy. Agree with our results, Abd-El-Rahman et al. (2017) concluded that TCA values did not differ between 1st and 2nd trimesters of pregnancy, while MDA values evaluated during the three trimesters of pregnancy in dromedary She-Camel. In dairy cows, Sharma et al. (2011) reported that the value of TCA enzyme activity did not changed among late pregnancy and early lactation stages. Castillo et al. (2006) concluded that no changed was recorded in MDA levels in dairy cows during late pregnancy. In this study, the balance in producing malondialdehyde and nitric oxide refers to the efficiency consumption of oxygen and neutralization of free radicals and this can be linked to improve of total antioxidant capacity.

Plasma E₂ was markedly affected by different pregnancy period, where the lowest value was in first trimester, and then increased with the advanced of pregnancy. Agreement with our results, Abo El-Maaty et al. (2015) reported that plasma E₂ concentration increased in pregnant camels and is referred to the capacity for estradiol production by the trophoblast (Wooding et al. 2003). Plasma E₂ is a good indicator to predict the time of parturition in she-camel.

On the other hand, the trend of P₄ level was significantly affected by pregnancy where the higher values were recorded in first and second trimesters, and started to decline during third trimester of pregnancy. Serur et al. (2006) noted a steady increase in plasma P₄ during early and mid-gestation, followed by a decrease in late gestation returning to basal levels during early post-partum period. Furthermore, Agrawal et al. (1996), observed higher values of progesterone ranging from 1.0 to 7.5ng /ml on day 7 post-mating. They concluded that this elevation in progesterone concentration occurred as a consequence of ovulation and development of CL in camels. Similarly, Skidmore (2005) reported that serum progesterone levels, in pregnant camel, were higher in early (2.83 to

6.89ng/mL) and the last month of pregnancy (7.4 to 9.2ng/mL).

Plasma T₃ concentration was not significantly changed among different pregnancy trimesters. The higher value was recorded in first trimester, and there were slight declines in second and third trimesters of pregnancy. Our findings are in agreement with those reported by (Abo El-Maaty et al. 2015; Abd-El-Rahman et al. 2017) where that value of T₃ was higher in 1st trimester and gradually decreased at, 2nd and 3rd trimesters in pregnant she-camels. Thyroid gland plays an important role in many metabolic activities (Khan et al. 2014) such as lipoprotein metabolism (Duntas 2002), and its hormonal activity has a role crucial to sustain the reproductive performance, healthy pregnancy, and brain development in fetus of animals (LaFranchi et al. 2005). Reduction of thyroid hormones during pregnancy could be due to, increase their production of thyroid-binding globulin from (TBG) hepatocytes. High TBG with high estradiol concentration during pregnancy lead to a reduction of thyroxine hormones during pregnancy of camels (Leung and Farwell 2010).

Overall means of plasma PRL was no significantly affected by pregnancy period where it increased steadily from first trimester and continued in second and third trimester of pregnancy. These results were in agreement with previous results in she-camels (Ibrahim et al. 2017). Dopamine is known to regulate the secretion of PRL through a long feedback mechanism (Gill-Sharma 2009) and is particularly important for females after labor (Delitala, 2009). High level of E₂ leads to stimulate the growth of pituitary and promote (PRL) release (Perez 1986).

Changes in Blood Biochemical Constituents and Hormonal Profiles during Lactation Period

Mean value of plasma TP did not differ significantly among lactation periods. However, plasma ALB level was significantly higher in third trimester of lactation period. Dowelmadina et al. (2012) and Joshi et al. (2017) reported that total TP and GLC concentrations were similar in all three stages of lactation (Early-mid-late), while the ALB concentration was significant increased from early to late stage of lactation period in camels. Plasma proteins play a crucial role in maintaining homeostasis, and a decline in their concentration may suggest imply fat infiltration into the liver (Faramarzian et al. 2016). In this study protein fractions levels recorded during lactation stages may reflect the more catabolism of protein for milk synthesis and providing immunoglobulins, additionally the albumin has the water property and higher incentive in camels, likely, show particular adjustment for the desert environment (Salman and Afzel 2004).

Abd El-Salaam et al. (2018) concluded that serum GLC concentration was not significantly changed at 2 months before calving and 1st, 2nd and 3rd months of post-partum period in Maghrebian she-camels.

Results of the present study indicated that lipid metabolites including CHOL and TG concentrations increased in first trimester and gradually decline in second and third trimesters of lactation period. Similar results were previously observed in lactating camels (Abd El-Salaam and Arafa 2018), in dairy cows (Kessler et al. 2014). In this study, the rise of CHOL and TG concentrations at first of

lactation period are most likely due to the increasing need for synthesis of milk fat and milk fat content such as long chain fatty acids and chylomicrons (Faramarzian et al. 2016), in addition to the need of the ovaries for synthesis of steroid hormones (Turk et al. 2015).

The present study revealed that ALT activity increased significantly during second and third trimesters of lactation period. However, AST activity was not affected. Transaminases were reported as essential enzymes in biological processes due to their role in catabolism and biosynthesis of amino acids. They are found mainly intramuscularly, in liver and heart muscles, and to a certain degree in skeletal muscles, kidney and red blood cells. The change in their level reflects the function of these organs, and for that reason they are specific indicator of liver dysfunction (Harper and Redwell 1977). In accordance with the present results, Khalifa (2012) reported that plasma ALT enzyme activity increased during post-partum months in camels, while AST enzyme activity did not change. Saeed et al. (2009) reported that the changes in transaminases might be due to gluconeogenesis enhancement in some muscle and liver cells, but the observed changes in plasma ALT activity in this study are within in normal range of camel (Abd El-Hamid et al. 2015). Also, in this study plasma ALP activity increased significantly during second and third trimesters of lactation period. Agree with those results, Tharwat et al. (2015) reported that ALP enzymes activity increased after parturition in camels. The increase in plasma ALP activity may also be explained by the stress of lactation and increased placental production of this enzyme during pregnancy period (Tharwat et al. 2015).

Mean values of TCA, MDA and HP activities did not differ among different lactation stages, while mean value of SOD concentrations was higher in the mid stage compared with early and late stages of lactation period. Moreover, value of HP activity was significant higher in early stage. Konvicna et al. (2015) and Shedeed et al. (2019) recorded that SOD and HP concentrations were significantly higher during lactation period. The oxidative stress continues to be a problem in lactating animals and it causes many other diseases such as mastitis, retention of fetal membranes due to several factors such as calving which has a greater effect on this imbalance (Sordillo et al. 2007), in addition to unbalance of diets content from the minerals and vitamins (Sharma et al. 2011). This leads to activate some enzymes (protein kinase C, xanthine oxidase, cyclooxygenase, ascorbic acid oxidase, lipoxigenase, ATPase, and cAMP phosphodiesterase), which lead to the production of ROS species (Kurek-Gorecka et al. 2014). The performance of lactating animals can be optimized to a certain extent by supplementing diets with optimal levels of neutral additives to enhance the antioxidant defense mechanism (Sordillo et al. 2009).

The present results showed that levels plasma E₂ and T₃ levels did not differ among different lactation period, while plasma P₄ concentrations were significantly affected. The higher concentration of P₄ was recorded in first trimester, and then declined through second and third trimesters of lactation period. These findings are in agreement with previous reports in camels (Kamoun and Jemmali 2014), where P₄ level decreased sharply at parturition and continue to decline with advanced of

lactation. It seems that this characteristic drop of P₄ at parturition may be due to the destruction of the corpus luteum of pregnancy as a sequel of luteolysis. In addition, the lactation status significantly effects on decrease of serum P₄ level (El-Zahar et al. 2017).

The same trend was found in the levels of plasma PRL. The prolactin hormone secreted by the anterior pituitary plays an important role in establishment of lactation and milk production, where it works on promoting the transcription of casein mRNA, stimulating synthesis of alpha-lact albumin and increasing lipoprotein lipase activity in the mammary gland. Prolactin exists in three heterogenic forms, varying in biological activity, the monomer is a principal consider active biological form, represents the greatest quantity from prolactin. The level of prolactin increases during late pregnancy and decreases with advance of lactation period as result to the proportion of the monomer in circulation decreases in response to selective uptake it by the mammary gland, In addition to a shift in the ratio of monomer active to less active forms (Ostrom, 1990).

Conclusion

In conclusion, the present study brings knowledge on the values of hormonal, blood biochemical and biomarkers of antioxidants parameters in she-camels (*Camelus dromedarius*) throughout various physiological statuses such as estrus, pregnancy and lactation. These values are necessary for diagnostic interpretation of pathological, and might have important roles in adjusting the different functions throughout different physiological statuses under arid environmental conditions. Also, from the aforementioned results we can get the knowledge of the pattern of ovarian dynamics and critical reproductive periods with oxidative stress and reproductive performance for she-camel herds.

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Authors' Contributions

Conceptualization: Ibrahim S Abd El-Hamid. Conduct all stages the research and blood plasma biochemical analysis in DRC complex laboratories and statistical analysis of the results and drafting the manuscript.

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