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Metabolic Changes in the Blood of Dromedary Camel at Early Post-partum

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Abstract

Postpartum resumption of ovarian activity under environmental stressors is highly important for determining subsequent fertility in camel. The current study aimed to investigate the relationship of relevant biochemical and hormonal parameters in the blood serum with regard to ovarian resumption of dromedary camel at early post-partum. Blood samples were collected from female dromedary camel weekly during the postpartum period for 9 successive weeks. Serum and plasma samples were assayed for the following biochemical and hormonal constituents, glucose, cholesterol (CH), triglycerides (TG), total protein (TP), urea, insulin-like growth factor-1 (IGF-1), non-esterified fatty acids (NEFA), lactate dehydrogenase (LDH), cortisol and triiodothyronine (T3). Glucose concentrations ranged between 92.6 and 96.2 mg/dL for the first 6 weeks postpartum, and increased to 106.8 mg/dL at week 7 postpartum ($P < 0.01$). The concentrations of CH followed almost the same manner during the first 7 weeks (ranged between 27.5 and 30.4 mg/dL) and increased to 38.2 mg/dL at week 8 postpartum ($P < 0.01$). Likewise, total protein concentrations decreased from 6.86 g/dL week2 to 6.45 g/dL at week 3 ($P < 0.01$) with no significant changes thereafter. Similarly, triglycerides decreased from 24.5 mg/dL at week 2 to 13.00 mg/dL at week 3 ($P < 0.01$) with no significant changes up to week 9. The concentrations of NEFA decreased significantly at week 3 postpartum ($P < 0.01$) and then maintained at low level throughout the study period. Similarly, cortisol and IGF-1 concentrations were the lowest at week 3 postpartum (5.7 µg/dL and 93.7 µg/mL for cortisol and IGF-1, respectively, $P < 0.01$) and then increased gradually with some little variations of IGF-1. However, the levels of urea followed a characteristic increasing and subsequently decreasing patterns while reached a plateau at weeks 8 and 9 postpartum. Similarly, the concentrations of T3 followed the same pattern and tended to increase at week 9 postpartum. Concentrations of LDH showed a significant decrease at weeks 4 to 6 postpartum and increased thereafter ($P < 0.01$). Throughout the study period, changes of glucose, cortisol, CH, TP, IGF-1, NEFA and LDH had a clear trend, i.e. decreasing at early postpartum period followed by increasing patterns thereafter. The results of the present study suggest that the typical metabolic patterns that are found in blood serum of dromedary camel at postpartum may be reflected in the follicular fluid and, therefore, may use as an indication of reproductive resumption.

Keywords: blood metabolites, hormones, dromedary camel, fertility, postpartum

1. Introduction

In dairy cattle, postpartum negative energy balance (NEB) is directly related to reduced fertility (Beam & Buttler, 1999; Lucy, 2008). Additionally, heat stress provokes NEB by reducing appetite, widening the energy gap between intake and expenditure through maintenance and milk production (Shehab-El-Deen et al., 2010). In camel, postpartum NEB is also found to be associated with delaying in uterine involution, resulting in delaying onset of postpartum ovarian cyclicity (Derar et al., 2014). In this regard, Merkt et al. (1990) reported that

follicles in the ovaries could develop late in the gestation period, and this could be the reason for the signs of estrus observed early post-partum in camels. Although the literature on post-partum ovarian activities in camel is very limited, some studies reported that the post-partum estrus has been happened between 14 to 42 days following parturition (Elias et al., 1984). However, some research work has reported that the first post-partum estrus was delayed until the next breeding season (Musa & Makawi, 1984). Therefore, it is speculated that the variation in post-partum estrus related to follicular development will be reflected in the biochemical components of the blood serum in camel since the changes in blood metabolites were related to developmental competence of oocytes in dairy cows (Leroy et al., 2004; Shehab-El-Deen et al., 2010).

The biochemical profiles of blood serum have widely been used to assess problems associated with the health status of animals (Lee et al., 1978; Hagawane et al., 2002). The assessment of blood biochemical profile is also used to investigate a lot of patho-physiological and metabolic disorders in animals (McDowell, 2003). For instance, a significantly higher levels of serum protein was detected in cyclic cows than in non-cyclic cows (El-Azab et al., 1993). Similarly, Burle et al. (1995) also reported low concentrations of serum cholesterol in anoestrus cows than in cycling cows. It is well-known that the developmental competence of the follicular growth is retarded and requires more time to become dominant follicle for ovulation under NEB conditions (Beam & Buttler, 1998, 1999). If NEB is accompanied with heat stress the developmental competence of the dominant follicle is more adversely affected (Shehab-El-Deen et al., 2010; Ferreira et al., 2011). It is not known yet however, whether these NEB-heat stress associated changes present in the blood metabolites at post-partum are reflected in the reproductive cyclicity in camel. A comprehensive understanding on post-partum biochemical indices is needed for further improvement of camel reproduction. Therefore, the aim of this study was to investigate the relationship of relevant biochemical and hormonal parameters in the blood serum with regard to ovarian resumption of dromedary camel at early post-partum.

2. Materials and Methods

2.1 Animals and Sample Preparation

Ten post-partum she camel (dromedary), aged 10-12 years were used for this study. The animals had no history of dystocia or birth related problem during parturition. Blood samples were taken into three tubes (unheparinized, gel-clot activator tube; EDTA tube; sodium fluoride (NaF) tube) (Xinle Medical®, KSA) in each week up to 9 weeks post-partum. Blood samples were cooled at 4 °C, and transported on ice (4 °C) to the laboratory. The blood samples were centrifuged (1400×*g*, 30 min) within 2 h after collection and the serum or plasma was separated.

2.2 Biochemical Analyses

In each sample, the concentrations of triiodothyronine (T3), cortisol, glucose, cholesterol, triglycerides, urea, total protein, LDH, IGF1 and NEFA were measured. All analyses were performed at the Department of Animal Production and Breeding, College of Agriculture and Veterinary Medicine, Qassim University, Saudi. The metabolite levels in blood serum were done using Autoanalyser (random-access analyzer; Humastar 100). All measurements were carried out according to the manufacturers' instructions. Triiodothyronine (T3), cortisol (Human Diagnostics, KSA) and IGF1 (BioSource Europe S.A.) hormones were measured using specific enzyme-linked immunosorbent assay (ELISA) kits. The measurement was carried out according to the manufacturer's instructions.

2.3 Statistical Analysis

Results are expressed as means±S.E.M. The overall mean concentration±S.E.M. of each metabolite was calculated for blood in all she camel. The concentrations of each metabolite in the blood compared among 9 weeks postpartum.

Hypothesis testing was performed with the MIXED procedure of JMP (2013), using a model with repeated measures (time postpartum) with animals as a random effect. Two random errors were defined: 1) the variance between animals (subjects) within time postpartum; 2) the variance between measurements within animals (residual). The second random error was used to test the effects of time postpartum in the model. A value of $P < 0.05$ was considered statistically significant.

3. Results

The results of different metabolites and hormones during postpartum period from first week of parturition until 9th week are shown in Figures 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. The glucose concentrations stayed relatively stable (93-96 mg/dL) until 6 weeks post-partum followed by a sharp increase (107 mg/dL) at week 7 postpartum and then again maintained at steady levels (105 mg/dL) during the study period ($P < 0.01$). Cholesterol followed the

same pattern as of glucose levels except the relatively steady levels (28-30 mg/dL) maintained up to 7 weeks post-partum. Followed by a significant increase (38.2 mg/dL and 36.6 mg/dL) at weeks 8 and 9 post-partum, respectively. The serum concentration of triglycerides dropped significantly at week 3 postpartum and remained low up to week 9 ($P < 0.01$). NEFA also followed the same pattern as of triglycerides while its concentration during first 2 weeks was around 0.27 mmol/L and later come about 0.15 mmol/L ($P < 0.01$). Urea concentrations in serum followed a characteristic pattern where the urea levels dropped at 2 weeks followed by increased and subsequently decreased until 7 weeks and significantly increased at week 8 postpartum ($P < 0.01$). There was a marked decrease in total protein from week 3 postpartum followed by gradual decrease up to 6 weeks and then again gradual increase at week 9 postpartum ($P < 0.01$). LDH followed almost the similar trend that observed in case of total protein where marked decreased was noticed at 4 weeks post-partum and gradual increase thereafter ($P < 0.01$). The concentration of IGF-1 also followed a trend of LDH while a marked declined was evident at week 3 postpartum followed by gradual increase which reached at peak at week 7 post-partum ($P < 0.01$). The level of cortisol dropped from week 2 followed by gradual increase throughout the study period ($P < 0.01$). Triiodothyronine (T3) concentrations in blood serum insignificantly decreased from week 2 postpartum, remained almost steady levels until 6 weeks, and tended to increase throughout the study period up to 9 weeks.

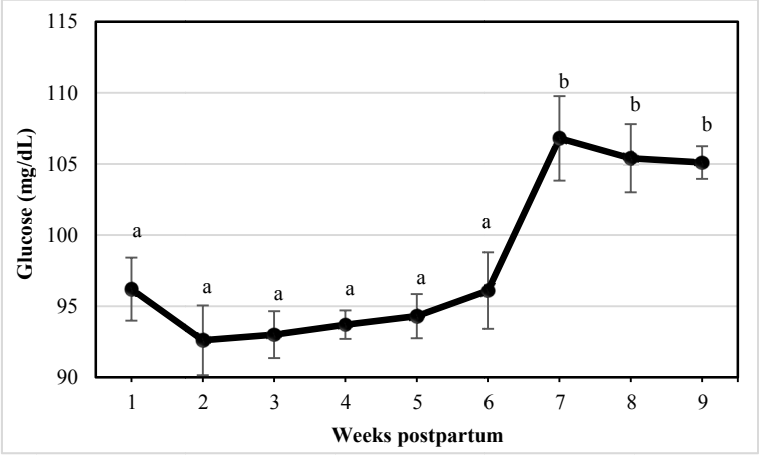

Figure 1. Concentrations of glucose (mg/dL) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

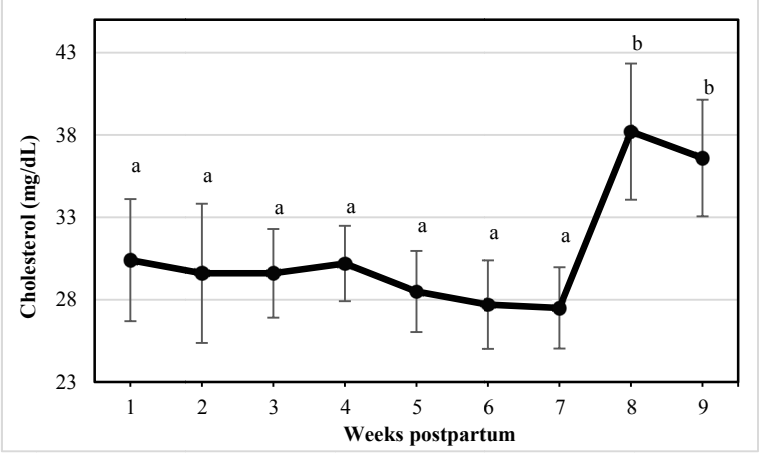

Figure 2. Concentrations of cholesterol (mg/dL) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

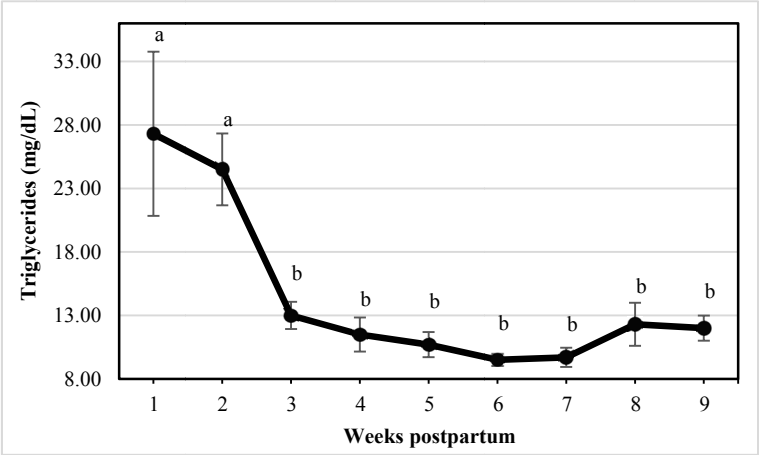

Figure 3. Concentrations of triglycerides (mg/dL in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

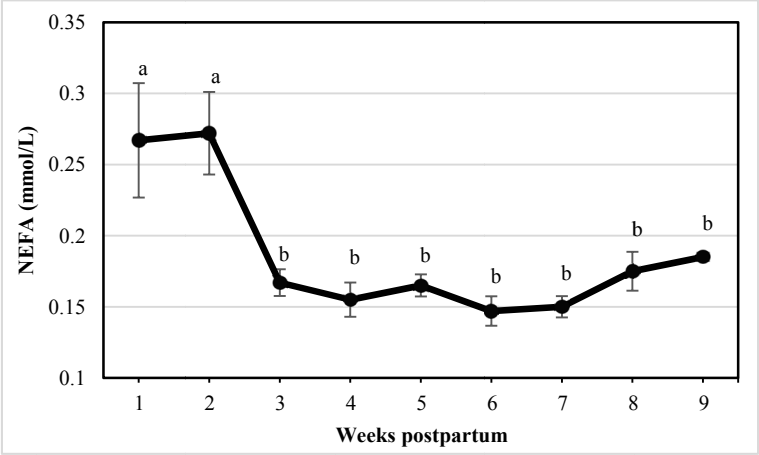

Figure 4. Concentrations of NEFAs (mmol/L) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

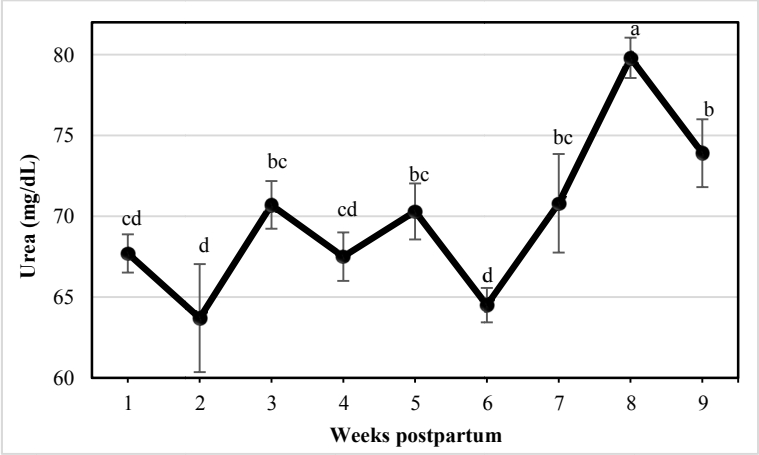

Figure 5. Concentrations of urea (mg/dL) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

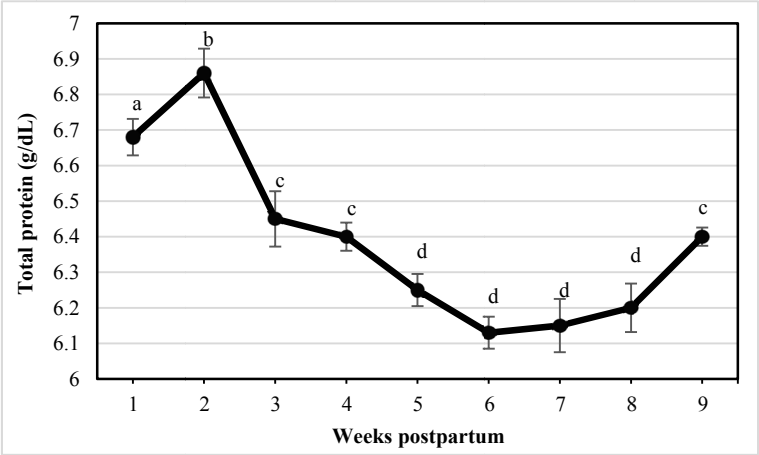

Figure 6. Concentrations of total protein (g/dL) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

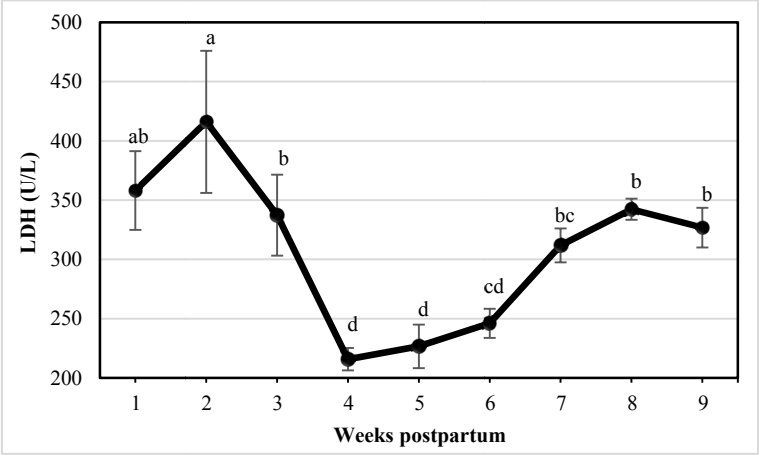

Figure 7. Concentrations of lactate dehydrogenase (LDH; U/L) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

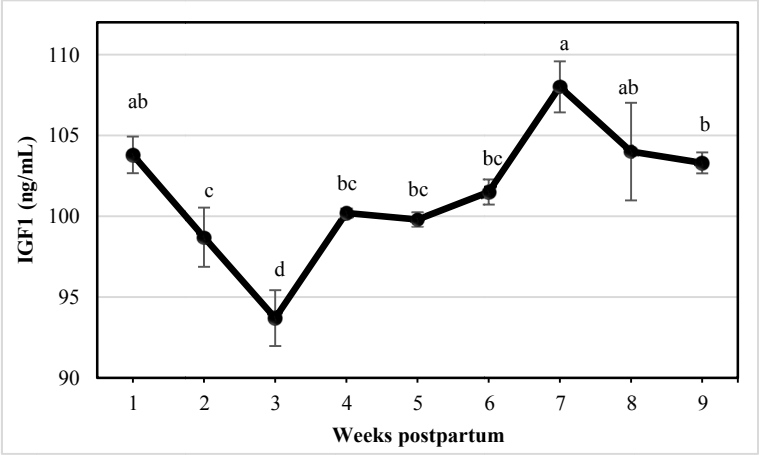

Figure 8. Concentrations of IGF-1 (ng/mL) in blood serum during the postpartum period in female dromedary camel ($P < 0.01$)

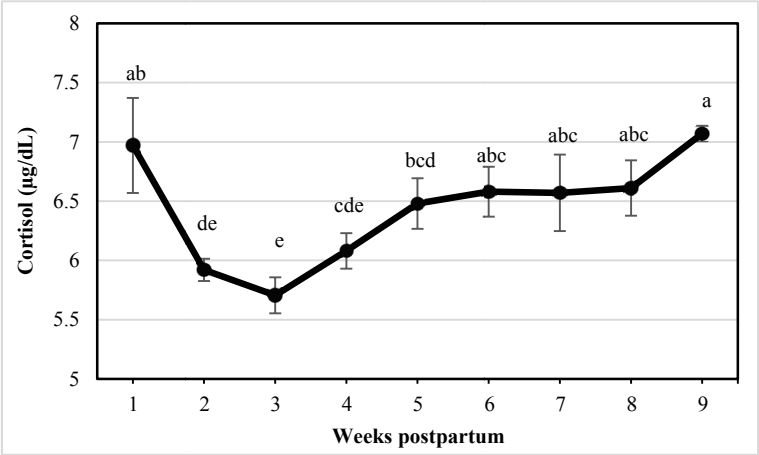

Figure 9. Concentrations of cortisol (µg/dL) in blood during the postpartum period in female dromedary camel ($P < 0.01$)

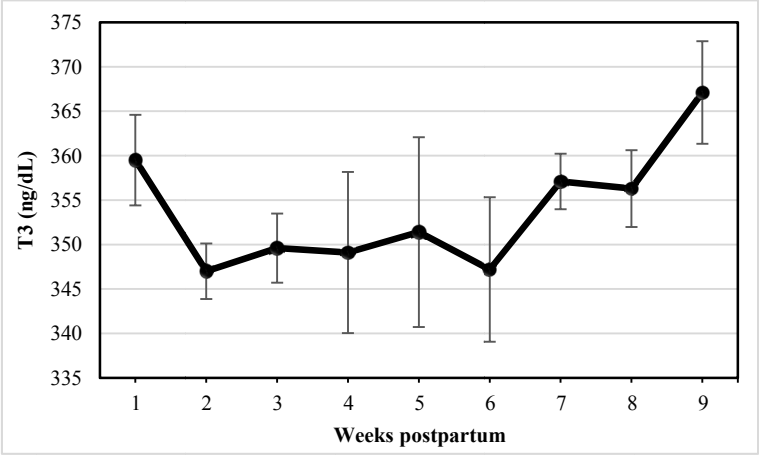

Figure 10. Concentrations of Triiodothyronine (T3; ng/dL) in blood serum during the postpartum period in female dromedary camel. (No significant effect of time postpartum were found)

4. Discussion

Blood metabolites are considered to be the major sources of follicular fluid components required for follicular growth and development which may determine the post-partum ovarian cyclicity. To the best of our knowledge, this is the first study where biochemical changes in blood serum are used as an indirect indication of ovarian resumption at early post-partum in camel.

The concentration of glucose in the blood serum showed a slight decrease at first 2 weeks post-partum followed by a gradual increase up to 6 weeks. Importantly, there is a sharp increase in glucose level at 7 weeks post-partum followed by no significant decrease or increase the period thereafter. In high yielding dairy cows, it is well-known that the glucose concentrations in blood serum are decreased during the period of negative energy balance and this low concentration of glucose in serum is closely related with follicular glucose levels (Butler, 2000; Leroy et al., 2004). While the NEB is associated with heat stress, the level of glucose declined very steadily (Shehab-El-Deen et al., 2010). In our study, we also observed glucose concentrations at basal level until 6 weeks post-partum followed a sharp increase, possibly indicating resumption of ovarian cyclicity.

Urea concentrations were relatively high and showed a characteristic decrease followed by increase pattern in the subsequent weeks until 7 weeks post-partum. However, at week 8 the pattern has been changed and urea concentrations increased exponentially followed by decreased significantly although still at high level. A very high concentrations of urea in blood serum or in follicular fluid was observed in mares and cattle's during early postpartum period (Kenny et al., 2002; Vanholder et al., 2005). A lot of studies concluded that the diet is responsible for elevated levels of urea and has a possible adverse effect on granulosa cells and oocyte

competence (De Wit et al., 2001; Dawuda et al., 2002). Our results also indicate that elevated serum urea levels may be reflected in the follicular fluid of the dominant follicle and hence, may have adverse effect on granulosa cells and oocyte quality.

The level of total protein (TP) in the blood serum increased at 2 weeks post-partum possibly due to supply of higher amount of TP content following parturition. Afterwards the gradual decrease in TP in the succeeding weeks is possibly due to the effect of maintenance and milk production (Shehab-El-Deen et al., 2010). Our data contradicts with the finding in Holstein cows at early post-partum observed by Leroy et al. (2004) which may explain due to NEB associated with HS in camel. However, an increase in TP concentrations at 9 weeks post-partum is possibly an indication of resumption post-partum cyclicity which needs to be studied further at follicular fluid levels.

It is well-known that during the period of NEB, lipolysis causes an increase of NEFA concentrations in blood serum during the first week post-partum. This is in fact reflecting in our study where serum NEFA levels were relatively higher during the first 2 weeks post-partum. This finding supports the data of previous studies on cows subjected to an acute dietary restriction in order to mimic a period of NEB (Comin et al., 2002; Jorritsma et al., 2003). However, the sudden drops of NEFA levels at week 3 followed by maintaining almost a steady levels until the study period. It is anticipated that the lower levels of blood serum NEFA may be reflected the lower levels of NEFA in follicular fluid which is necessary to protect the oocyte and the granulosa cells from high NEFA concentrations since it is found toxic in culture condition in vitro (Yanase et al., 2001; Vanholder et al., 2005). The levels of triglycerides also follow almost the same pattern as of NEFA. This is probably because of elevation of very low-density lipoprotein (VLDL) triglycerides is closely related to elevation of NEFA in blood serum (Reaven & Greenfeld, 1981; Yki-Jarvinen & Taskinen, 1988).

Serum IGF-1 concentrations decreased very sharply during the first 3 weeks post-partum. A sharp decline in IGF-1 is directly related to the intense negative energy balance associated with heat stress (Shehab-El-Deen et al., 2010). Certain levels of IGF-1 in the follicular fluid are crucial for optimal follicular development (Beam & Buttler, 1999) and oocyte quality (Adamiak et al., 2005). Both IGF-1 and glucose have beneficial effects on follicular growth, whereas, glucose is a primary source of energy for the ovary (Rabiee et al., 1997) and IGF-1 stimulates cell growth, cell development and differentiation (Jones & Clemmons, 1995; Rosen & Pollak, 1999). It has been well documented that the decrease in serum concentrations of glucose and IGF-1 in heat stressed cows is due to reduced dry matter intake (Abilay et al., 1975; De Rensis & Scaramuzzi, 2003). The present study results support the findings of the above literature where we observed IGF-1 is markedly reduced during early 3 weeks post-partum followed by gradual increase that reached at plateau at 7 weeks, indicating the resumption of ovarian activities around 7 weeks post-partum along with the increasing concentrations of glucose.

The levels and role of lactate dehydrogenase (LDH), cortisol and triiodothyronine (T3) in post-partum were not studied before. In our previous study, we wanted to make a correlation of LHD concentration between follicular fluid and blood serum and observed significantly less concentration of LDH in follicular fluid, indicating the animals are free from diseases at least in part reproductive diseases (Shehab-El-Deen et al., 2019). A little decrease in LDH activity has also been reported in pregnant camels (Khadjeh, 2002; Saeed et al., 2009). In the present study, we observed a marked decrease in LDH levels at 4 weeks post-partum followed by gradual increase but less than periparturient levels, indicating the animals are approaching to reproductive soundness.

Cortisol elicits physiological adjustment that enables animal to tolerate stressful conditions. In agreement, we observed a sudden decline of cortisol at 3 weeks post-parturition followed by gradual increase in the subsequent weeks, indicating that the animals were in stress early post-partum that are recovered well at 9 weeks and thereafter. In an *in vitro* study, it has also found that the blastocysts developed from oocytes matured in cortisol-supplemented medium expressed relatively higher levels of glucose transporter 1 (GLUT1), fatty acid synthase (FASN), and heat shock protein 70 (HSP70) (Costa et al., 2016). This *in vitro* study elucidated a positive role of cortisol in the acquisition of bovine oocyte competence that is proved by increased embryonic development rates with elevated embryonic transcripts. Furthermore, cortisol has an important role in glucose and lipid metabolism, as well as the cellular reaction to stress. Although the role of Triiodothyronine (T3) in camel reproduction is very scares, the supplementation of T3 in bovine in vitro maturation medium has been found beneficial on the kinetics of embryo development (Costa et al., 2013). In a recent study, Singh *et al.* (2017) also reported that supplementation of T3 in bovine culture medium increased embryo cleavage rate. In our previous study, we observed significantly lower level of T3 in all follicle classes compared to blood serum. The present study also indicates that the level of cortisol was lower at 3 weeks post-partum followed by gradual increase in the weeks thereafter, indicating the possible resumption of ovarian cyclicity, which needs to be studied further. In conclusion, the results suggest that the glucose, cortisol, CH, TP, IGF-1 and NEFA patterns

that are found in blood serum of dromedary camel at post-partum may be reflected in the follicular fluid and, therefore, may use as an indication of reproductive resumption.

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