DOI : 10.5958/2277-8934.2015.00039.9 *Vol 22 No 2, p 239-245*

HAEMATOLOGY, BIOCHEMISTRY AND BLOOD GAS ANALYSIS IN HEALTHY FEMALE DROMEDARY CAMELS, THEIR CALVES AND UMBILICAL CORD BLOOD AT SPONTANEOUS PARTURITION

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ABSTRACT

This study was carried out to investigate the haematological, biochemical, acid-base and blood gas parameters in 12 healthy pregnant dromedary camels, their calves and umbilical vein blood at spontaneous parturition. At parturition, blood samples were collected from each camel, their calves and umbilical cord. A complete blood count (CBC) and serum concentrations of calcium, phosphorus, magnesium, blood urea nitrogen (BUN) and glucose were measured. The serum activity of cardiac troponin I (cTnI), γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK) and alkaline phosphatase (ALP) were also measured. None of the female camels showed any evidence of clinical disease at parturition. In calves, total white blood cells (WBCs) count was significantly higher than in dam or cord blood, but total WBCs count did not change significantly between mother and cord blood. Neutrophil count was significantly higher in calf blood than in dam and cord blood, but did not differ significantly between dam and cord blood. The RBCs count, haemoglobin concentration and haematocrit per cent were significantly higher in calf compared to dam and cord blood. The serum activity of AST was significantly higher in dam than in calf and cord blood. On the contrary, the serum activity of GGT was significantly lower in dam than calf and cord blood. The serum concentration of cTnI was significantly higher in calf than dam and cord blood The blood pH was significantly lower in calf than in dam and cord blood and it was significantly higher in cord than in dam blood. On the contrary, PCO_2 was significantly higher in calf than in dam and cord blood, but no significant difference was found between cord and dam PCO_2. The serum concentration of blood HCO_3^- and base excess (BE) value were significantly lower in dam than calf and cord blood. This study showed reference values for normal haemato-biochemical and blood gas parameters in dam, calf and cord blood during spontaneous parturition.

Key words: Biochemistry, blood gases, camel, haematology, parturition

During normal parturition, significant changes occur in physiological parameters because of pain, anxiety and uterine contraction (Song *et al,* 2004). Uterine contractions induce an increase in circulating blood volume as blood restricted to the uterus is released into the general circulation, and as parturition reaches near to completion, there is reduced compression of the caudal vena cava which allows an increase in cardiac output and arterial blood pressure (Chamchad *et al,* 2007). Obstetrical interventions may increase the stress and pain of parturition and may induce further haemodynamic or vascular changes (Lucio *et al,* 2009).

Parturition can be stressful for both the dam and for the neonate. In the cow, survival of the calf at or shortly after birth can be compromised leading to high death losses and a serious impact on net income for the cattle producer (Bellows, 1997). Labour induces a massive catecholamine surge in the foetus, which helps to preserve blood flow to the brain, heart and adrenal glands and to promote post-natal adaptive circulatory changes (Kredatusova *et al,* 2011). The stress of labour also leads to the release of maternal cortisol and catecholamines, which may prolong labour and impair placental flow. Disturbances of respiratory gas exchange are common in the perinatal period and are associated with alterations in both arterial blood gas tensions and cardiovascular function (Gardiner, 1980). Therefore, at present, in veterinary practice the main emphasis is on the prevention of asphyxia of calves at birth (Szenci, 2003).

Stress hormones also bring about lipolysis with the release of free fatty acids (readily transferable

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across the placenta) and hyperglycaemia, which will exacerbate foetal hypoxia. All these changes tend to intensify foetal metabolic acidosis, which indeed becomes progressively more severe as labour advances (Reynolds, 2010; Kredatusova *et al,* 2011). Direct and indirect asphyxia is suggested as the cause of death because in 73 to 75% of the calves that died in the perinatal period, no pathological changes were detected. The occurrence of asphyxia in calves dying perinatally was estimated to be 58.3%. As a result of disturbances in the utero-placental circulation occurring during parturition, due to the rupture of foetal membranes and uterine contractions, all calf foetuses develop more or less severe hypoxia and consequently acidosis (Szenci, 2003). Monitoring the acid-base balance in the calf at birth is also important, so that the dangers threatening the foetus can be recognised early, and averted (Szenci, 2003).

The biochemical analysis of umbilical vein or artery could provide valuable clinical information and facilitate appropriate medical and surgical treatments or allow the proper and timely administration of oxygen and warmth to mother and newborn pup (Groppetti *et al,* 2010). Measuring of lactate levels, in the cord blood are best suited to long-term survival prognosis (Kredatusova *et al,* 2011). The present study was carried out to investigate these variables in healthy female dromedary camels, their calves and umbilical vein blood at spontaneous parturition.

Materials and Methods

Animals

Twelve healthy multiparous pregnant female dromedary camels with mean body weight 450 kg (range: 380-560 kg) and mean age 8.9 years (range: 7.5-11.0 years) were maintained in a free-stall barn and kept under the Laboratory Animal Control Guidelines of Qassim University, which basically conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA (NIH publications No. 86 to 23, revised 1996).

Blood sampling

At parturition, 3 blood samples, 10 ml each, were collected from each camel, their calves and umbilical cord blood out of which 2 ml in EDTA tubes was for haematological analyses and 2 ml in heparinised tubes used for the determination of blood gas parameters. The remaining 6 ml in plain tubes was centrifuged at 1200 × g for 10 min and the obtained serum samples were immediately stored at -20°C for determination of cTnI and other biochemical analysis.

Determination of haematological and biochemical parameters

A complete blood count (CBC) [total and differential leukocytic count, erythrocyte count, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] was carried out on the EDTA sample using the VetScan HM5, Abaxis, California, USA.

An automated biochemical analyser (VetScan VS2, Abaxis, California, USA) was used to determine the serum concentrations of calcium, phosphorus, magnesium, blood urea nitrogen (BUN) and glucose. The serum activity of γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK) and alkaline phosphatase (ALP) were also measured.

Cardiac troponin I assay and validation of cTnI intra-assay repeatability

Cardiac troponin I was measured in serum using a commercial available test (I-stat, cTnI, VetScan, Abaxis, CA, USA), using a two-site enzyme-linked immunosorbant assay. The assay had been proven effective for the detection of camel cTnI (Tharwat, 2012; Tharwat *et al,* 2013a,b,c; Tharwat and Al-Sobayil, 2014a). The lower limit of detection of cTnI for this assay was 0.02 ng/ml. The i-STAT cTnI test reports 0.00 to 50.00 ng/ml. Samples above the reportable range will yield ">50.00 ng/ml" on the analyser display screen. However, the performance characteristics of the i-STAT cTnI measurement have not been established for cTnI values above 35.00 ng/ml. Values < 0.02 ng/ml cannot be discriminated. Although, the analyser provides a specific point estimate of 0.00, 0.01 or 0.02 ng/ml. All results are expressed as ng/ml with inter-assay coefficient of variance (CV) of less than 5%. The intra-assay repeatability was performed using sera from 27 healthy camels and 9 camels with various disorders (cardiomyopathy (n=3), renal abscess (n=2), ruptured urinary bladder (n=2), intestinal obstruction (n=1) and ruptured urethra (n=1) susceptible to increase basal cTnI levels (Tharwat *et al,* 2013b). Each sample was assessed twice during the same day. The range of cTnI values were from 0.00 ng/mL to 4.87 ng/ml with intra-assay CV of 5.64% (r = 0.9997) (Tharwat *et al,* 2013b).

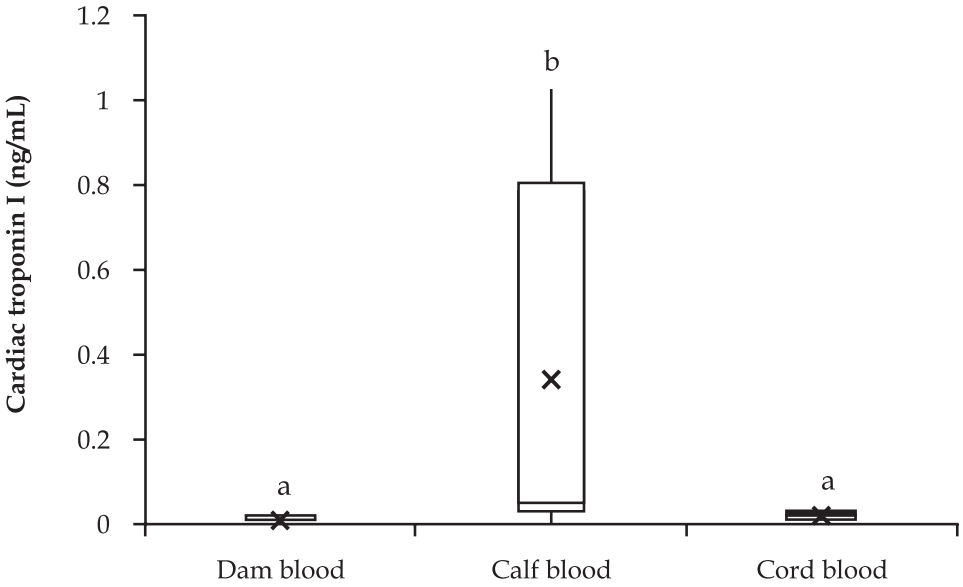

Fig 1. Box and whiskers plots of serum cardiac troponin I in camels, their calves and umbilical cord (n=12) blood at parturition. Values with different letters differ significantly (P>0.5).

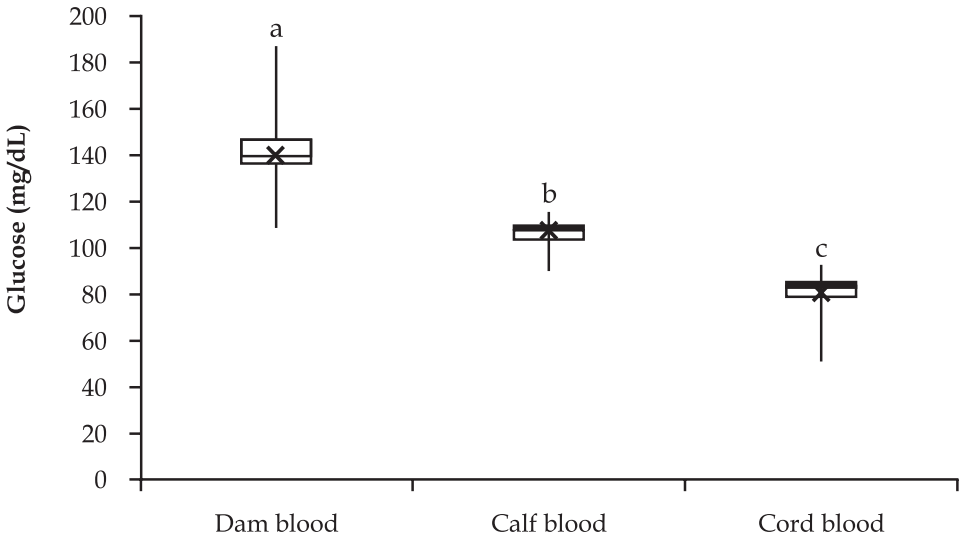

Fig 2. Box and whiskers plots of serum glucose in camels, their calves and umbilical cord (n=12) blood at parturition. Values with different letters differ significantly (P>0.5).

Blood gas analyses

The heparinised blood samples were used immediately to analyse the acid-base and blood gas parameter values in dam, calf and cord blood *in situ* using a portable clinical veterinary analyser (I-STAT®, Abaxis, California, USA). In this way, blood pH, partial pressure of carbon dioxide (PCO_2), oxygen partial pressure (PO_2), bicarbonate (HCO_{-3}), total carbon dioxide (TCO_2), base excess (BE), oxygen saturation (SO_2), lactate, sodium, potassium and chloride were analysed immediately in order to prevent changes in the concentrations of these parameters (Gokce *et al,* 2004; Tharwat and Al-Sobayil, 2014b).

Statistical analysis

Data are presented as means ± SD and were analysed statistically using the SPSS statistical package, version 18, 2009, with Fisher's protected least significant difference (LSD) as the post-ANOVA test. The Duncan test was used to calculate multiple comparisons. The level of significance was tested at $P<0.05$.

Results and Discussion

During parturition, significant changes occur in physiological parameters because of pain, anxiety and uterine contraction (Song *et al,* 2004). During normal parturition, uterine contractions induce an increase

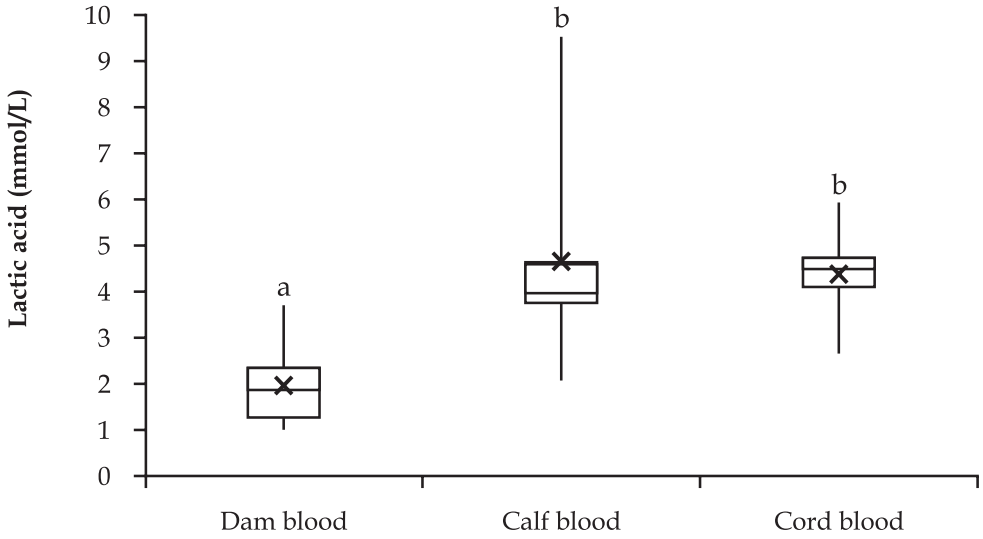

Fig 3. Box and whiskers plots of serum lactic acid in camels, their calves and umbilical cord (n=12) blood at parturition. Values with different letters differ significantly (P>0.5).

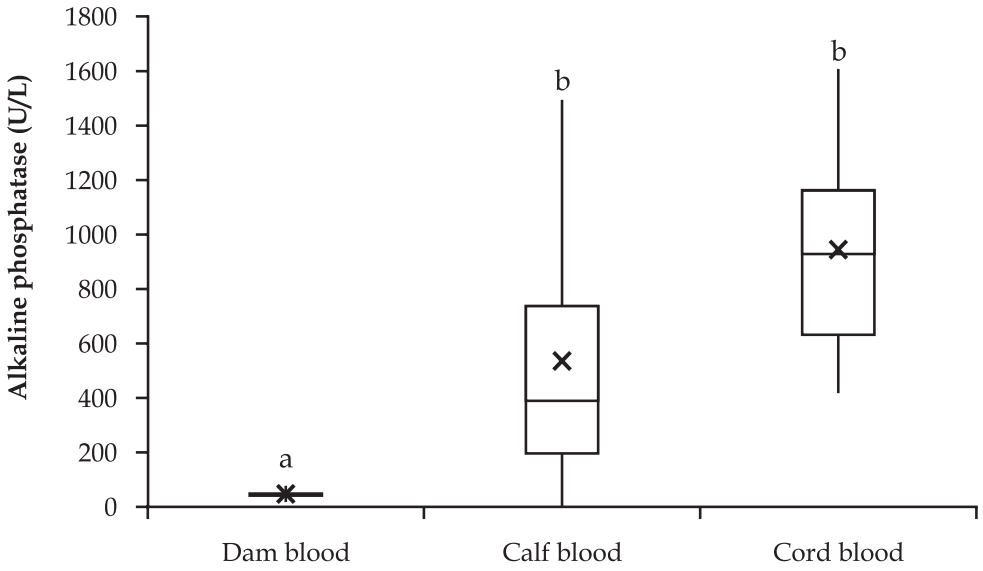

Fig 4. Box and whiskers plots of serum alkaline phosphatase in camels, their calves and umbilical cord (n=12) blood at parturition. Values with different letters differ significantly (P>0.5).

in circulating blood volume as blood restricted to the uterus is released into the general circulation, and as parturition reaches nears completion, there is reduced compression of the caudal vena cava which allows an increase in cardiac output and arterial blood pressure (Chamchad *et al,* 2007). A high heart rate is a result of the combination of stress and contractions (uterine and abdominal) during parturition (Kredatusova *et al,* 2011).

For the neonate, labour represent the most critical phase contributing to the first minutes after birth (Indrebo *et al,* 2007). Early recognition of foetal distress and dystocia are crucial to the successful management of labour and for optimal neonatal health. The total length of parturition and the time required for puppy expulsion are commonly considered the most important parameters affecting neonatal viability (Groppetti *et al,* 2010; Kredatusova *et al,* 2011).

To the authors' knowledge, this is the first study to investigate the haematological, biochemical, acid-base and blood gas parameters in healthy female camels, their calves and umbilical vein blood at spontaneous parturition. We have recently reported the haematobiochemical variables in adult female camels during the periparturient period (Tharwat *et al,* 2015a) and in camel calves during the first month of age (Tharwat *et al,* 2015b).

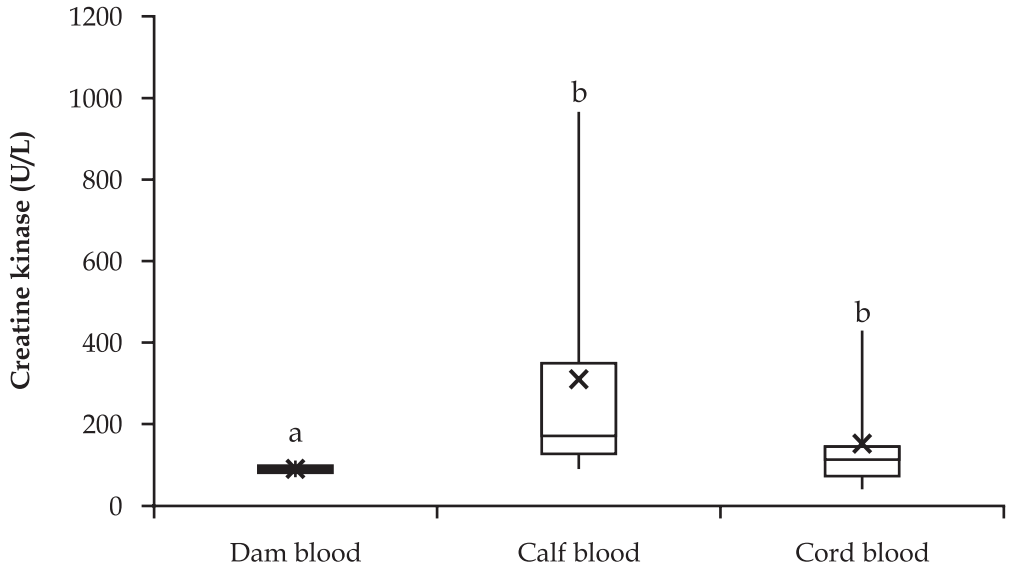

Fig 5. Box and whiskers plots of serum creatine kinase in camels, their calves and umbilical cord (n=12) blood at parturition. Values with different letters differ significantly (P>0.5).

In this study, none of the female camels showed any evidence of clinical disease at parturition. All the camel calves (6 females and 4 males) were born at term and by spontaneous parturition and no signs of disease were observed in any calf. Table 1 summarises the haematological variables in female camels, their calves and umbilical cord blood at parturition. In calves, total WBCs count was significantly higher (P=0.0004) than in dam or cord blood, but total WBCs count did not change significantly between mother and cord blood (P=0.61). Lymphocyte count was significantly higher (P=0.03) in calves and cord blood than in dams, but did not change significantly between calf and cord blood (P=0.25). In the cord blood, monocyte count was significantly lower (P<0.0001) than in dam and calf blood. Neutrophil count was significantly higher (P=0.0002) in calf blood than in dam and cord blood, but did not differ significantly between dam and cord blood (P=0.77). The RBCs count, haemoglobin concentration and haematocrit per cent were significantly higher (P<0.05) in calf compared to dam and cord blood. The MCV, MCH and MCHC values did not show any significance among dam, calf and cord blood (P>0.05). The increases in most of the recorded haematological parameters in calf blood compared to dam or cold blood could be attributed to calf responses to stress during parturition (Cappel *et al,* 1998; Kredatusova *et al,* 2011).

The biochemical variables in female camels, their calves and umbilical cord blood at parturition are also summarised in table 1. The serum activity of AST was significantly higher (P=0.009) in dam than in calf and cord blood. The increased serum activity of AST in dams at parturition is physiological as recently reported (Tharwat *et al,* 2015a). On the contrary, the serum activity of GGT was significantly lower (P=0.003) in dam than calf and cord blood. The serum concentration of calcium was significantly lower (P=0.001) in dam than in calf and cord blood, and the serum concentration of calcium was significantly higher (P=0.014) in cord than calf blood. In dams, the serum concentration of inorganic phosphorus was significantly lower (P=0.003) in dam than in calf and cord blood. The serum concentration of magnesium was significantly lower (P=0.0008) in calf than in dam and cord blood. The serum concentrations of BUN did not change significantly among dam, calf and cord blood (P>0.05).

The serum concentrations of cTnI, glucose, lactic acid, ALP and CK in dams, their calves and umbilical cord blood at parturition are illustrated in Figs 1 to 5, respectively. The serum concentration of cTnI was significantly higher (P=0.02) in calf than in dam and cord blood; however, no significant difference (P=0.10) was detected between dam and cord blood cTnI. The serum concentration of glucose was significantly higher (P<0.0001) in dam than in calf and cord blood; the calf blood glucose was significantly higher (P<0.0001) than in cord blood. The serum concentration of lactic acid was significantly lower (P=0.037) in dam than in calf and cord blood; no significant differences (P=0.82) were detected between lactic acid in calves and cord blood. The serum activity of ALP was significantly lower (P=0.0004)

Table 1. Haematological parameters (mean ± SD) in female dromedary camels, their calves and cord blood at parturition (n=12)

Parameter	Dam blood	Calf blood	Cord blood
White blood cells ($\times 10^9$/L)	20.8±6.7[a]	30.1±2.3[b]	22.5±8.1[a]
Lymphocytes ($\times 10^9$/L)	1.9±0.8[a]	2.4±6.4[b]	2.8±0.8[b]
Monocytes ($\times 10^9$/L)	0.6±0.8[a]	0.9±0.6[a]	0.17±0.04[b]
Neutrophils ($\times 10^9$/L)	18.2±5.8[a]	26.8±2.0[b]	19.2±9.1[a]
Red blood cells ($\times 10^{12}$/L)	8.8±2.1[a]	10.6±0.8[b]	8.9±1.5[a]
Haemoglobin (g/dL)	13.4±2.8[a]	16.1±0.9[b]	14.7±1.3[a]
Haematocrit (%)	21.9±4.7[a]	25.9±2.5[b]	23.9±1.7[a]
Mean corpuscular volume (fl)	24.8±2.0[a]	24.7±1.4[a]	25.9±1.4[a]
Mean corpuscular haemoglobin (pg)	15.3±1.1[a]	15.3±0.5[a]	15.8±0.5[a]
Mean corpuscular haemoglobin concentration (g/dL)	61.7±7.0[a]	62.5±3.5[a]	61.3±2.4[a]
Aspartate aminotransferase (U/L)	75±7[a]	61±17[b]	46±16[b]
γ-glutamyl transferase (U/L)	9±2[a]	15±2[b]	18±6.7[b]
Calcium (mmol/L)	2.4±0.1[a]	2.9±0.5[b]	3.6±0.4[c]
Phosphorus (mmol/L)	1.5±0.2[a]	2.0±0.2[b]	1.9±0.4[b]
Magnesium (mmol/L)	1.1±0.1[b]	0.7±0.4[a]	1.6±1.3[b]
Blood urea nitrogen (mmol/L)	10.3±1.3[a]	10.3±1.6[a]	11.8±3.7[a]

a,b,c Differ significantly.

in dam than in calf and cord blood; no significant difference (P=0.17) was found between calves and cord blood ALP. The serum activity of CK was significantly lower (P=0.001) in dam than in calf and cord blood; no significant difference (P=0.08) was detected between calves and cord blood CK.

In humans, the cardiac biomarker tropnin I (cTnI) appears in serum within a few hours of myocardial injury and peaks at 12 to 18 h (Bassand *et al,* 2007). The serum concentration elevates after acute myocardial injury because of leakage from the damaged myocardial cells (O'Brien *et al,* 2006). In pediatrics, there is growing interest in the use of cTnI and its value in assessing cardiac injuries in children (Kanaan and Chiang, 2004; Shastri *et al,* 2012). In normal children, the cTnI concentrations are higher in the first year of life, being highest after birth (as found in this study), and gradually decreasing to adult concentrations towards the end of the first year. In neonates, the use of cTnI, especially in relation to perinatal asphyxia has been very well studied (McAuliffe *et al,* 2004). Perinatal hypoxemia is associated with progressive organ failure, and thus has implications for cardiac function that can be expressed as an increase of cTnI.

Table 2 summarises the acid-base, blood gas and electrolyte parameters in female camels, their calves and umbilical cord blood at parturition. The blood pH was significantly lower (P<0.0001) in calf than in dam and cord blood, was significantly higher (P<0.0001)

Table 2. Blood gas, acid-base and electrolyte parameters (mean ± SD) in female dromedary camels, their calves and cord blood at parturition (n=12).

Parameter	Dam blood	Calf blood	Cord blood
pH	7.371±0.003[a]	7.292±0.034[b]	7.477±0.043[c]
PCO_2 (mmHg)	36.24±1.79[a]	62.60±4.76[b]	35.4±2.2[a]
Base Excess (mmol/L)	- 4±0.9[a]	3.2±0.8[b]	2.9±2.5[b]
HCO_3 (mmol/L)	21.0±0.9[a]	30.2±0.6[b]	26.2±1.9[b]
TCO_2 (mmol/L)	22.2±1.0[a]	32.0±0.8[b]	27.5±1.9[c]
Anion Gap (mmol/L)	17.4±0.6[a]	13.8±1.8[b]	12.9±1.7[b]
Sodium (mmol/L)	163±2[a]	156±1[b]	153±1.4[b]
Potassium (mmol/L)	3.8±0.2[a]	4.4±2.2[b]	4.7±0.5[a]
Chloride (mmol/L)	127±2[a]	116±1[b]	118±2[a]

PCO_2, partial pressure of carbon dioxide; HCO_3, bicarbonate; TCO_2, total carbon dioxide.
[a,b] Differ significantly.

in cord than in dam blood. On the contrary, PCO_2 was significantly higher (P<0.0001) in calf than in dam and cord blood, but no significant difference (P=0.18) was found between cord and Dam PCO_2. The serum concentration of blood HCO_3 and BE value were significantly lower in dam (P<0.0001) than calf and cord blood. The TCO_2 value was significantly higher (P<0.0001) in calf than in dam and cord blood and was significantly higher (P<0.0001) in cord than

in dam blood. The anion gap value was significantly higher (P<0.0001) in dam than in calf and cord blood. The serum concentration of sodium was significantly higher (P<0.0001) in dam than in calf and cord blood and was significantly higher (P<0.0001) in calf than cord blood. In dam blood, the serum concentration of potassium was significantly lower (P=0.0001) than in calf and cord blood. However, in calf blood, the serum concentration of chloride was significantly lower than in dam (P<0.0001) and cord blood (P=0.03).

In conclusion, this study showed reference values for normal haemato-biochemical and blood gas parameters in dam, calf and cord blood during spontaneous parturition.

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