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Trace Elements Profiles of Pregnant Camels (*Camilus dromedaries*), Fetus, and Amniotic Fluid at Birth and their Associations with Calf Birth Weight

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ABSTRACT

Amniotic fluid is a dynamic complex mixture that carries components contributing to the regulation of fetal development. The present study aimed to measure the levels of trace elements, such as Fe, Zn, Cu, Mg, Se, and Mn in amniotic fluid, maternal serum, and venous umbilical cord serum at delivery. The study further investigated the relationships among levels of elements in amniotic fluid (AF), maternal serum (MS), and venous umbilical cord serum (VUCS) in order to assess the possibility of monitoring abnormal fetal growth. Blood samples were obtained from 30 pregnant female camels at delivery and the corresponding VUCS and AF were examined. The Fe, Zn, Cu, Mg, Se, and Mn were analyzed on the atomic absorption spectrophotometer. Concentrations of trace elements, Fe, Zn, Cu, Mg, Se, and Mn in VUCS were significantly higher, compared to MS or AF. The trace elements, Fe, Zn, Cu, Mg, Se, and Mn were present in significantly lower concentrations of AF than in MS or VUCS. Neonatal birth weight did not correlate with MS levels of the trace elements. However, neonatal weight correlated positively with venous cord serum Fe, Se, and Zn levels. There was a scarcity of correlation between maternal and fetus trace elements in the pregnant camels. In conclusion, AF could even be the result of simple filtration of maternal blood. Evaluation of selected trace element levels in MS did not appear to be useful within the assessment of fetus growth. The findings of this study indicated an active transport for Fe, Zn, Cu, Mg, Se, and Mn between pregnant camels and fetus.

Keywords: Calf birth weight, Dromedary camel, Placental barriers, Pregnancy, Trace element

INTRODUCTION

Newborn calves of dromedary camels show weakness and low birth weight in a high proportion, leading to economic loss. Calf birth weight was very variable, and depended on the gender of the fetus, breed, parity (Freetly et al., 2000; Nagy and Juhász, 2019) and the nutritional state of the mother (Zachara et al., 1986), especially in the late pregnancy period that fetal requirements increase for energy, protein and minerals. Usual fetal development depends on suitable store of trace elements, including iron (Fe), zinc (Zn), and copper (Cu), magnesium (Mg), and selenium (Se) and manganese (Nandakumaran et al., 2016). The viable role of trace minerals in fetal development and their growth was recently suggested as well as their relationship to calf birth weight (Graham et al., 1994). Deficiency of trace minerals such as Fe and Zn can retard the fetal growth (Mitchell et al., 1998). Likewise, Se (Black, 2001; Mitchell et al., 1998) and Cu deficiency (Mills and Davies, 1979) were implicated as possible factors that could impair fetal development. Lower levels of the trace minerals such as Cu, Fe, and Zn were stated in aborted fetus, suggesting a possible role for these minerals in fetal growth and development (Graham et al., 1994). Pregnancy placed a considerable burden on the homeostasis of trace elements in mammals (Black, 2001), whereby the physiology of pregnant animals, and the requirements of the growing fetus changed (Faye and Bengoumi, 1994). There is evidence that camels were susceptible to trace element disorders similar to those of other ruminants (Faye et al., 1992). There were several reports of clinical mineral defects in camels (Faye et al., 1992; Faye and Bengoumi, 1994; Zong-Ping et al., 1994; Chuka Ozegbe, 2005), and their prevalence and importance were likely to be misjudged as signs of subclinical deficiency may not be detected over a long period of time.

In all mammalian species, amniotic fluid (AF) builds up early, and then decreases with the growth of the embryo (Suliburska et al., 2016). The role of the trace elements contained in AF is not clear. It was suggested that this fluid could be an important source of fetal nutrition (Abdelrahman and Kincaid, 1993). Each fetus was completely dependent on its mother via the placenta for the supply of trace elements (Perveen et al., 2002). The

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sensitivity of the fetus to the absorption of trace elements was not only strongly influenced by the uptake by the mother, the stage of pregnancy and the placental transport, but also by the inherent ability of the fetal organs to accumulate reserves (Black, 2001). The transport of trace elements from the pregnant animal to the fetus varied during pregnancy, and it gradually increased during this period of time (Tibary and Anouassi, 1997). Some trace elements were transported across the placenta in an active process, while others appeared to be transported passively (Romeu et al., 1986). Camels have an epitheliochorial type of placenta in which the fetal membranes do not invade the endometrial layer of the uterus (Tibary and Anouassi, 1997). The mechanisms involved in transplacental transport of trace elements from the pregnant camel to the fetus are still not well known. In order to more fully understand the transfer of trace elements from dam to fetus; concentrations of the trace elements in maternal, venous umbilical cord serum and amniotic fluid were investigated. Although some data exist for a number of species, including rabbits (Kriesten Schmidtmann et al., 1986), rats (Romeu Alemany et al., 1986), mice (McArdle and Erlich, 1991), sheep (Langlands Bowles et al., 1982), cattle (Van Wouwe et al., 1991) and humans (Hurley, 1976; Zhou et al., 2019), references were few for camels. The purpose of present study was to determine the element concentrations of Fe, Zn, Cu, Mg, Se and Mn in maternal, fetal cord blood and amniotic fluid at birth. Moreover, correlations between elements in fetal cord serum, amniotic fluid and maternal blood were established. Present study aimed to be a contribution for a better understanding of the mechanism of trace element transport from the pregnant dam to the fetus, and of the effect of certain elements on birth weight.

MATERIALS AND METHODS

Ethical approval

All institutional and national guidelines for the care and use of animals were followed according to the guidelines approved All procedures involving the care and the use of the animals were approved by the ethics committee of the faculty of veterinary medicine, institutional animal care and use Committee, Aswan University, Egypt.

Animals and study area

The present study was carried out on 30 pregnant camels (camels' dromedaries) during September 2016 to February 2018 in a private farm in Daraw village, Aswan province, Egypt. Camels were free from brucellosis and tuberculosis (with average age: 7 to 10 years; weight: 450 to 550 kg), which were vaccinated against Camel pox, Brucella and Rift Valley Fever. All the animals were raised under the semi-intensive system in which they were fed with barely and alfalfa hay with very limited grazing, and with common salt supplements, and had free access to drinking water. The camels were housed in an open yard. The pregnant camels were selected in consultation with cameleer who recorded their mating history, and the pregnancy was confirmed by rectal palpation.

Sampling

Ten milliliters of blood was collected from each pregnant camel immediately after delivery via jugular vein and from the umbilical cord vein of newborn while the placenta was still not separated. The umbilical cord vein contained a serum which was enriched with all necessary nutrients from the maternal blood within the placenta to supply the fetus. The blood samples were allowed to clot, and centrifuged at 3000 round per minute (rpm) for 15 minutes; the serum was separated and stored at – 20 °C for further analysis. During the first stage of labor, the amino-chorionic sac (transparent, a vascular amnion) appeared within the vulva with the fetus parts were visible within the water bag, a ten- milliliter syringe fitted with a twenty-gauge needle was wont to collect a sample of amniotic fluid which was turbid, yellowish and watery by penetrating the amnion, taking care to avoid contamination from blood or allantois fluid. Following rupture of the amnion, a sample of cord blood was obtained from umbilical cord vein. Calf birth weights were measured before colostrum was taken. All the collected blood serum and amniotic fluid samples were analyzed for Fe, Zn, Cu, Mg, Se and Mn by using an atomic absorption spectrophotometer (Shimadzu, Model AA-6601, Japan).

Statistical analyses

All obtained data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25 (Armonk, NY: IBM Corp). Differences in the element contents in maternal, venous umbilical cord serum and amniotic fluid were statistically analyzed with Analysis of variance (ANOVA). Pearson's correlation was used to examine the relationships between each of trace elements and calf birth weight. Likewise, correlation of trace element levels among maternal, venous umbilical cord serum and amniotic fluid were analyzed. Mean values in the same row with different letters were statistically significant, and the highest values were represented with the letter (a). Statistical significance was declared at the $p \leq 0.05$ level and the data were presented as the mean ± Standard Error (SE).

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RESULTS

Trace element contents in maternal, venous umbilical cord serum and amniotic fluid

Iron

As shown in table 1, the mean levels of iron were 0.66 ± 0.12 parts per million (ppm) in maternal serum, 0.37± 0.09 ppm in amniotic fluid and 2.98 ± 0.66 ppm in venous umbilical cord serum, respectively. The venous umbilical cord serum iron levels were significantly higher than those in maternal serum ($P < 0.05$) and amniotic fluid; and, the maternal serum iron levels were also significantly higher than those in amniotic fluid ($P < 0.05$).

Zinc

Zinc levels in the maternal serum, venous umbilical cord, and amniotic fluid were 0.78 ± 0.10 ppm, 1.08 ± 0.29 ppm and, 0.53 ± 0.09 ppm respectively. The venous umbilical cord had a significant higher concentration of zinc than maternal serum and amniotic fluid ($P < 0.05$). Beside, zinc levels were also significantly higher in the maternal serum than in the amniotic fluid ($P < 0.05$).

Copper

The mean copper levels in maternal and cord serum and amniotic fluid were 0.75 ± 0.08 ppm, 0.91 ± 0.14 ppm and 0.61 ± 0.09 ppm; respectively. There was a significant difference between copper levels in maternal, cord and amniotic fluid ($P < 0.05$).

Magnesium

The mean levels of magnesium in maternal and venous umbilical cord serum and amniotic fluid were 7.21 ± 0.75 ppm, 8.42 ± 0.46 ppm and 6.78 ± 0.44 ppm respectively. The mean magnesium levels in both maternal and venous umbilical cord serum were significantly higher than those in amniotic fluid ($P < 0.05$).

Selenium

As shown in table 1, the mean levels of selenium were1.06 ± 0.24 ppm, 0.79 ± 0.15ppm and 0.66 ± 0.06 ppm in venous umbilical cord serum, maternal serum and amniotic fluid, respectively. The content of selenium in amniotic fluid was significantly lower than that in both maternal and venous cord serum ($P < 0.05$); and maternal serum selenium levels were also significantly lower than those in venous umbilical cord serum ($P < 0.05$).

Manganese

The mean manganese levels in maternal serum, amniotic fluid and venous umbilical cord serum were 0.27 ± 0.15ppm, 0.18 ± 0.04 ppm and 0.52 ± 0.13ppm respectively (Table 1). Mean manganese levels in maternal serum were significantly lower than that in venous umbilical cord serum ($P < 0.05$). Besides, magnesium levels were also significantly higher in the maternal serum than in the amniotic fluid ($P < 0.05$).

The correlation between maternal serum and amniotic fluid trace element contents

Several elements in AF were effectively influenced by those in maternal serum (Table 2). Concentrations of Fe, Cu, Mg, Se and Mn in AF were positively correlated with those in maternal serum (r: 0.09, 0.08, 0.01, 0.41 and 0.12), respectively. In contrast, there was a negative correlation of Zn contents between AF and maternal serum (MS) with r: - 0.19.

The correlation between maternal and venous umbilical cord serum trace element contents

Maternal serum concentrations of Fe, Cu and se were negatively correlated with those in venous umbilical cord serum (VUCS) (r: - 0.04, - 0.11 and - 0.07), respectively, but there were positive correlations of Zn, Mg and Mn contents between MS and VUCS with r: 0.01, 0.07 and 0.08 respectively (Table 3).

The relationship of trace elements levels in maternal serum, Venus umbilical cord serum and amniotic fluid with calf birth weight

The mean birth weight of the camels was 25.5±0.08 kg. Pearson correlation showed a significant positive correlation ($P < 0.05$) amongst VUCS levels of Fe, Zn and Se and calf birth weight (Table 4). Fe level in the AF showed a significant negative correlation (table 5) with calf birth weight ($P < 0.05$). No significant correlation ($P > 0.05$) could be shown between calf birth weights and levels of trace elements in maternal serum samples (Table 6).

Table 1. Element levels in maternal sera, venous umbilical cord sera and amniotic fluid of camels at parturition

Parameter (ppm)	Maternal serum (n=30)	Amniotic fluid (n=30)	Cord serum (n=30)
Fe	0.66 ± 0.12^b	0.37 ± 0.09^c	2.98 ± 0.66^a
Zn	0.78 ± 0.10^b	0.53 ± 0.09^c	1.08 ± 0.29^a
Cu	0.75 ± 0.08^b	0.61 ± 0.09^c	0.91 ± 0.14^a
Mg	7.21 ± 0.75^b	6.78 ± 0.44^c	8.42 ± 0.46^a
Se	0.79 ± 0.15^b	0.66 ± 0.06^c	1.06 ± 0.24^a
Mn	0.27 ± 0.15^b	0.18 ± 0.04^c	0.52 ± 0.13^a

Data are expressed as mean values ± Standard error (SE); the number of studied samples in each fluid is shown in parentheses. [a,b,c] Mean± SE in the same row with different superscripts are significantly ($P<0.05$) different. n: the number of studied samples.

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Table 2. Correlation between concentrations of trace elements in maternal sera versus amniotic fluid of camels at parturition

Parameter (ppm)	Pearson correlation Coefficient (r)	p Significant correlation at p= 0.05
Fe	0.09	0.65
Zn	-0.19	0.31
Cu	0.08	0.69
Mg	0.01	0.97
Se	0.41	0.03
Mn	0.12	0.53

Table 3. Correlation between concentrations of trace elements in maternal sera versus venous umbilical cord sera of camels at parturition

Parameter (ppm)	Pearson correlation Coefficient (r)	Significant correlation at p= 0.05
Fe	-0.04	0.84
Zn	0.01	0.94
Cu	-0.11	0.55
Mg	0.07	0.70
Se	-0.07	0.71
Mn	0.08	0.60

Table 4. Relationship between trace elements concentrations in maternal serum and calf birth weight

Elements (ppm)	Pearson correlation Coefficient (r)	Significant correlation at p= 0.05
Fe	-0.23	0.23
Zn	0.11	0.57
Cu	-0.09	0.76
Mg	-0.15	0.43
Se	0.11	0.56
Mn	-0.02	0.92

Table 5. Relationship between trace elements concentrations in amniotic fluid and calf birth weight

Parameter (ppm)	Pearson correlation Coefficient (r)	Significant correlation at p= 0.05
Fe	-0.43	0.02
Zn	0.07	0.70
Cu	0.12	0.58
Mg	0.19	0.32
Se	-0.35	0.87
Mn	0.15	0.44

Table 6. Relationship between trace elements concentrations in venous umbilical cord sera and calf birth weight

Parameters (ppm)	Pearson correlation Coefficient (r)	Significant correlation at p= 0.05
Fe	0.37	0.04
Zn	0.37	0.04
Cu	-0.24	0.21
Mg	-0.11	0.56
Se	0.48	0.01
Mn	0.24	0.20

DISCUSSION

To the best of knowledge, the current study is first study applied This was often the primary study to characterize the trace elements profiles in MS, VUCS and AF of pregnant dromedary camel , and to investigate the correlations between the concentration of the elements levels in MS, VUCS and AF, and calf birth weight. whether the correlations between element levels in MS, VUCS and AF exists or not. There were two main reasons to study the physiological relationships between blood trace elements profiles in the dams and the body weight of their newborn. First, a dam that calves a heavy calf had a greater risk of dystocia. Second, a calf which was too weak at delivery may have more problems of vitality. For good intrauterine development, the fetus needs a sufficient amount of nutrients and trace elements that can only be obtained from the mother's blood via the placenta (Rossipal et al., 2000). In the present study, the function of placenta as a fetal-maternal barrier in the transfer of some minerals from dam to fetus during gestation was well investigated. In this study, the significant

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higher concentration of Zn and Mn in VUCS than in MS was proved. Present study was in agreement with previous ones comparing the levels of metal elements in maternal and cord blood (Baig et al., 2003; Nandakumaran, 2016; Zhou et al., 2019) in human. It was supposed that Zn and Mn may cross the placenta via active transport, and the fetus exerts specific demand for Zn and Mn. However, other clarifications for the higher Mn concentrations in cord blood was also suggested, such as lower or restricted removal of Mn by fetus or inability of the fetus to use Mn (Widdowson et al., 1974).

The demand for trace elements increased rapidly during gestation, and this may result in a decline in maternal and/or fetal stores (Al-Saleh et al., 2004). In the present study, venous umbilical cord serum Fe, Cu, Se and Mg concentrations were significantly higher than those in the corresponding dam serum.

Iron was the most vital element within the blood which contributes to hemoglobin composition, and was additionally essential for the successful development of the fetus. In the present study, levels of Fe in VUCS were higher than those in MS. This finding was in accordance with that reported previously in human (Rallis and Papasteriadis, 1987) and in ovine (Gooneratne and Christensen, 1989), and indicated active transport of this element across camel placenta. This trend of changes during gestation in camel appeared too compatible with the observations in the pregnant cattle, where a decrease in placental transfer of Fe appeared to occur in the last third of pregnancy (Richards, 1999).

There was constant increase in Cu deposition throughout the fetal period and, therefore, an increasing demand for Cu by the fetus (Eltohamy et al., 1986). In this study, the level of Cu in VUCS was higher than that in MS, whereas another study showed inverse results (Zhou et al., 2019).This interaction implied that fetus has a capacity to sequester maternal Cu, even when the dam is Cu deficient (Graham et al., 1994). According to Seboussi et al. (2010) and Eltohamy et al. (1986), Cu concentration in serum decreased at the end of pregnancy due to active transfer from hepatic storage of the dam to its fetus. A significant correlation between Se and Cu was observed in camels receiving a selenium supplementation (Koller et al., 1984).

In the present study, the levels of Se in VUCS were higher than that in MS. This may be due to that selenium level which may readily crosses the camel placenta as observed in bovine (Wooten et al., 1996) and human (Baig et al., 2003). As shown in the present study, Mg levels in VUCS were significantly higher than that in MS, which was in accordance with the recent study in human (Nandakumaran et al., 2016). The results showed that the concentration of trace elements in AF was found to be lower than MS. These data suggested that trace elements in AF may be derived from blood. The role of trace elements contained in AF was not clear. Because AF was swallowed by the fetus, it was suggested that this fluid may be an important source of certain trace elements for fetal nutrition (Wooten et al., 1996).

In the current study, we did not observe a correlation between the concentration of the various elements under investigation in the maternal serum and the birth weight of the calf. Maternal serum levels of the various elements studied did not correlate positively or negatively with calf birth weight. Birth weight of the calf was one of the basic issues to judge pregnant camel management, and to expect the possibility of newborn thickness or mortality. According to various earlier studies (Barhat et al., 1979; Al Mutairi, 2000; Bissa, 2002; Nagy and Juhász, 2019), birth weight of camel calves varied from 19 to52 kg. The calf birth weight in the present study ranged between 18 and 45 kg, and the absence of a correlation of trace element levels with calf weight led to assume that the levels of these elements in MS were not useful values for the assessment of fetal weight. However, Fe levels in the AF were found to correlate negatively with calf birth weight while levels of other elements did not reveal any significant correlation. Interestingly, Fe, Zn and Se levels in the VUCS were found to have a positive correlation with calf birth weight. Amongst the variation factors, breed, parity and weight of the pregnant dam, sire, and the year and month of birth were reported to effect calf birth weight significantly (Barhat et al., 1979; Al Mutairi, 2000; Bissa, 2002; Nagy and Juhász, 2019). To the best of knowledge, no study was demonstrated an association between trace elements concentrations and camel calf birth weight. In cow, Graham et al. (1994) reported that fetal size increased as fetal Cu increased, and was less than or equal to maternal Cu. Gooneratne and Christensen (1989) showed that neither maternal nor fetal Mn were correlated with fetal size. Clearly, further studies are necessary to examine the effects of these trace elements on calf birth weight.

Several elements in AF and VUCS were effectively influenced by those in MS (Table 2 and 3). For the VUCS, the results showed a positive correlation between concentrations of Zn, Mg and Mn in VUCS and those in MS. In contrast, there were negative correlations of Fe, Cu and Se contents between VUCS and MS. On the other hand, concentrations of Fe, Cu, Mg, Se and Mn in AF were positively correlated with those in MS, while, a negative correlation of Zn contents between AF and MS was reported. There was no significant correlation between all minerals in MS and VUCS or AF. Unfortunately, there were no many previous studies reported regarding this aspect in camel. Mitchell et al. (1998) reported a positive correlation among maternal and fetal Cu, maternal and fetal Mn, and maternal and fetal Zn. In contrast, there was a lack of correlation between maternal

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and fetal Fe in cow. A positive correlation between maternal and fetal trace elements suggested fetal dependence on the dam for its supply of nutrients, including trace elements. A lack of correlation suggested fetal independence. Mechanisms regulating interactions between nutrients were still poorly described Mechanisms regulating retention, excretion or interactions between nutrients at sites of cell transport or storage proteins were needed further investigations, but studies such as presented here can direct future research toward biochemical descriptions of nutrient interactions.

CONCLUSION

The present results indicated a lively placenta transport of Fe, Zn, Mg, Mn, Cu and Se appear to be exchanged actively between dam and fetus. Fe, Zn, Cu, Mg, Se and Mn exchanged passively between dam and amniotic fluid. Evaluation of Fe, Zn, Cu, Mg, Se and Mn in maternal serum did not appear to be useful within the assessment of fetal growth**.**

DECLARATION

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Competing interests

The authors declare that they have no conflict of interest exists.

Author`s contribution

Walaa M. Essawi collected the samples, designed the experiment, performed laboratory analyses and participated in the preparation of the manuscript. Hagar F. Gouda contributed to data analysis and prepared the manuscript (writing and revision). All authors approved the final version of manuscript before publication.

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