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Study on Some Physiological Markers for Early Embryonic death in Pregnant She-camels Under Egyptian Conditions.

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Abstract: *This study aimed to evaluate levels of some metabolites, minerals, P4 and E2 in blood of She-camels (Camelus dromedarius) as affected by reproductive status (non-pregnant, embryonic loss and pregnant camels). During the breeding season, camels showing estrus (n=21) were naturally mated with virile studs. Ultrasonography on day 20-90 post-mating (PM) revealed 12 pregnants (G1), 4 animals with embryonic loss (G2) and 5 non-pregnants (G3). She-camels were hand milked and ultrasonographic examination was used for pregnancy diagnosis on days, 20, 35, 45, 55, 75 and 90 post-mating. All animals were bled on days 5, 20, 35, 45, 55, 75 and 90 post-mating for determining concentration of total proteins (TP), albumin (AL), glucose, total cholesterol (TC), total lipids (TL), urea and creatinine, activity of asprtate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) and concentration of progesterone (P4**)** and estrogen (E2) in blood serum. However, both concentration of globulin (GL) and albumin: globulin ratio (AL: GL) were calculated. Results showed that conception rate was 76.2% on day 20 PM, while 23.8% were non-pregnant. Between day 20 and 75 PM, embryonic loss rate was 25% and calving rate was 57.1%. Concentration of TP, AL, GL, AL: GL ratio was not affected by reproductive status, post-mating day and their interaction. Concentration of glucose and TL was not affected by reproductive status. Serum TC concentration was lower (P<0.05) in G1 than in G2, while G3 showed insignificant differences in comparing with both groups. Serum glucose concentration decreased (P<0.05) between 5 and 20 day PM. Serum TC concentration increased (P<0.05) between 5 and 35 day PM, then insignificantly decreased up to 90 days PM. Concentration of TL was not affected by post-mating day. The effect of interaction between reproductive status and post-mating day on concentration of glucose, TC and TL was not significant. Serum urea and creatinine concentration was higher (P<0.05) in G2 than in G1, while G3 showed insignificant differences as compared to both groups. Serum urea and creatinine concentration showed fluctuated trend of change (P<0.05). Creatinine concentration increased during the first 20 days post-mating, and then insignificantly decreased up to 90 day PM. Only creatinine concentration was lower in G1 than in G2 and G3 on most post-mating days. Serum AST and ALP activities was not affected by reproductive status, post-mating day and their interaction. Activity of ALT was lower (P<0.05) in G1 than in G2 and G3, increased (P<0.05) during the first 20 days, decreased at 35 day and then showed insignificant change up to 90 day PM. Content of P was the highest (P<0.05) in G2; Zn and Cu contents were the highest (P<0.05) in G3, while G1 showed the lowest (P<0.05) values of P, Zn and Cu contents and insignificantly the lowest Ca content. Only P content was the highest (P<0.05) on days 45 and 90 PM and the lowest (P<0.05) on day 5 post-mating. On days 35 and 75 PM, G3 and G2 showed the highest values of Cu versus lower values in G1. Concentration of P4 was higher (P<0.05) in G1 than in G2 or G3, increased (P<0.05) from 5 up to 45 day PM, then decreased on day 55 and again increased on days 75 and 90 PM. Concentration of E2 was not affected by reproductive status or post-mating day. There was an opposite trend of change in both P4 and E2 concentration on different post-partum days in G1 as compared to G2 or G3 on most post-mating days.*

In conclusion, embryonic loss rate in camels was about 25% between 20 and 75 days post-mating. No remarkable changes in most blood biochemicals and minerals as well as enzyme activity were detected in camels with embryonic loss as compared to pregnant or non-pregnant camels. Level of P4 on days 15 and 35 post-mating may be use as physiological indicator for pregnancy or early embryonic loss.

Keywords: *Camels, embryonic death, blood biochemicals, ultrasonography, progesterone.*

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I. Introduction

Despite, the low reproductive performance of camels, reproductive disorders can lead to economic losses in terms of reduced fertility, low life time production, culling of the animal from the farm longer calving interval and increased expenses on medication in farm animals. Reproductive disorders are one of the most

common important pathological conditions and/or diseases in camels in UAE (Al-Juboori and Baker, 2012). Poor reproductive efficiency has been described as a major problem in camelids. Alpacas have a mean annual fertility of 50% (Fernande-Baca *et al*., 1970) and llamas a mean birth rate of 46% (Condorena *et al*., 1988). Low fertility in alpacas is probably due to the high (50 to 58%) embryonic loss before 30 days of gestation (Bravo and Sumar, 1985). In camel, the reproductive rate varies between 25 and 80% depending on the level of management and veterinary care provided (Tibary and Anouassi, 1997).

A single pregnancy diagnosis in She-camel is not sufficient to guarantee a birth, especially if done at a very early stage, this is due to in part to errors in diagnosis, but is also due to the high incidence of early embryo loss seen in these species (Skidmore, 2000). Transrectal ultrasonography has been used to diagnose and monitor early pregnancy in dromedary camels (Pratap *et al*., 2012).

In a clinical survey study, high rate of embryonic death up to 35% was reported in dromedary camels (Tibary and Anouassi, 1997). Embryonic death in camelidae may be attributed to genetic factors, CL insufficiency or hostile uterine environment; however, no single factor can be manipulated to improve embryo viability. Early pregnancy loss between day 20 to 90, post breeding was insignificantly lower in primiparous (5.7%) than in multiiparous (16.9%) She-camels. The pregnancy loss during embryonic stage and early fetal stage was 10.8 and 6.9% in multiparous, whereas no early fetal loss had occurred in primiparous She-camels (Pratap *et al*., 2012). Similarly, Lopez-Gatius *et al*. (2005) reported that the right horn pregnancy was lost within 45 to 60 days post-breeding in all the twin pregnancies, while the left horn pregnancy continued in 10 of 13 animals.

An early pregnancy loss is probably one of the most important factors resulting in the reduction of reproductive efficiency in camels. At present, there is no practical way to reduce embryonic loss in camels, however, recognizing the occurrence and incidence of embryonic loss may be instrumental in application of new reproductive technologies to increase service rate in a herd. Although, the specific factors responsible for early embryonic loss in dairy cows are not known, they may be similar to those factors responsible for reduced conception rates. Various uterine disorders have been described in camelids and may play an important role in reduced fertility in these species (Tibary and Anouassi, 1997). In bovine, pregnancy loss contributes to reproductive inefficiency in lactating animals because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss (Fricke *et al*., 1998). Metabolism of P4 could produce impaired embryonic development and potentially increased pregnancy loss (Fricke, 2004).

There are a few reports in the literature regarding early embryonic losses in dromedary camels and no reports regarding the non-hormonal factors responsible for the incidence of early embryonic loss (EEL) in She-camels. Therefore, the present study was aimed to evaluate the level of some metabolites and minerals beside the hormonal level of P4 and E2 in blood of Maghrabian She-camels (*Camelus dromedarius*) exhibiting EEL by ultrasonography in comparing with pregnant or non-pregnant She-camels.

II. Materials And Methods

This study was carried out at the Camel Research Station, Marsy Matrouh, belonging to Animal Production Research Institute, Agricultural Research Center during the breeding season.

This study was conducted during the breeding season on 21 clinically healthy and sexually adult dromedary She-camels with live body weight averaged 380-560 kg, 5-17 years of age and within 1 - 9 parities. All experimental She-camels were daily fed basal diet in similar amounts, being 4.5 kg concentrate feed mixture (CFM); 2 kg berseem hay (BH) and 2 kg bean straw (BS) per animal during pre-partum and 3.5 kg CFM, 2.5 kg BH and 2 kg BS per animal during post-partum period. The CFM used in feeding all the experimental animals was composed of 25% wheat bran, 25% yellow corn, 9% uncorticated cotton seed meal, 20% barely, 15% bean straw, 3% molasses, 2% premix and 1% common salt. Chemical analysis of CFM, BH and BS is shown in Table (1).

Feeds were offered to all animals twice daily, while clean water was available all day time. Animal were housed individually and fed one of the three experimental diets and water was offered all day time.

Table 1. Chemical analysis (on DM basis) of concentrate feed mixture (CFM), berseem hay (BH) and bean straw use for feeding all the experimental She-camels.

Item	CFM	BH	BS
DM (%)	88.6	89.0	89.3
Chemical analysis (%):			
OM	92.3	87.7	86.3
CF	8.14	30.5	37.5
CP	12.25	11.30	5.30
EE	4.95	3.20	1.40
NFE	66.96	37.70	42.00
Ash	7.70	12.3	13.70

Experimental groups:

During the breeding season, She-camels showing estrus behavior (n=21) were naturally mated with virile studs. The first examination of the uterus for the She-camel by ultrasonography in this experiment was done at the interval from 20 to 90 days post-mating. The 1st ultrasonography examination revealed that conception was indicated in 16 out of 21 animals, while 5 animals were non-pregnant (23.8%). On day 90 post-mating, 4 out of 16 conceived animals were non-pregnant and classified as early embryonic loss (25%), while only 12 out of 21 animals (64%) were pregnant and calved at the end of gestation period as shown in the Table 2.

Table 2. Determination the pregnant, non pregnant and early embryonic loss in she-camel by ultrasonography during the experimental period.

Experimental group	Number of the experimental she-camels			
	Start of experiment	On day 20 post-mating	On day 90 post-mating	At calving
G1(Non-pregnant)	-	5	5	-
G2(EEL)	-	-	4	-
G3(Pregnant)	-	16	16	12
Total	21	21	21	21

Milking and milk samples:

Milk yield was measured after the calves were allowed to suckle colostrum from their dams for the first seven days. Hand milking of the animals was done twice daily in the morning and evening.

Pregnancy diagnosis:

Ultrasonographic scanner Vetson-color machine (Kontron Medical, France) with endo-rectal ultrasound multi-frequency probe 5 LV (2-7 MHZ). was used for pregnancy diagnosis in all groups on day 20, 35, 45, 55, 75 and 90 post-mating. The rectum was evacuated of all feces before insertion of the transducer which was lubricated with gel and inserted through anal ring. The urinary bladder was recognized as homogenous nonechoic, and taken as land mark for genital tract. As the transducer face was moved cranially beyond the urinary bladder, the uterus was imaged and by changing the angle of the probe, the uterine horns were imaged. The female genital tract of these camels were scanned ultrasonographically for the detection of early pregnancy and the growth and anatomical features (the embryonic vesicle, embryo proper, heartbeats, fetal membrane, the head, eye bole, neck, trunk, and limb bad), the animals were restrained in a suitably designed crate, in standing position, for scanning the uterine horns and ovaries. The transducer probe was positioned dorsal to genital tract and advanced cranially. The dorsal and lateral surface of each uterine horn was scanned for signs of pregnancy. Pregnancy was confirmed by the presence of fluid of varying amounts (embryonic vesicle) and visualization of an echogenic mass (embryo) in the lumen of the uterine horn, plus the presence of CL on the ovarian surface. Pregnancy was indicated at calving date by incidence of normal calves.

Blood sampling:

Animals in each experimental group were bled 5, 20, 35, 45, 55, 75 and 90 days post-mating. Bleeding was done before morning feeding, and blood was collected from each animal by jugular vein-puncture into test-tube. The test-tubes and their contents were allowed to stand for about six hours, and the serum which had separated from cells was carefully decanted into serum vials.

Serum samples were stored in deep freezer at -20°C before being analyzed for total proteins, albumin, glucose, cholesterol, total lipids, urea, and creatinine. Also, activity of asprtate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as well as hormonal concentration of progesterone (P4**)** and estradiol-17β (E2) was determined in blood serum. However, both concentration of globulin and albumin: globulin ratio were calculated.

Statistical analysis:

Data obtained in this study were statistically analyzed by ANOVA (factorial design), using the General Linear Model (GLM) procedures of the statistical Analysis Systems (SAS, 1999, version 8.0) to test group differences (1….3), sampling time (1….. 7) and their interaction. The significant differences among least squares means were declared at $P< 0.05$ using Duncan's Multiple Range Test (Duncan, 1955).

III. Results And Discussion

Fertility data and embryonic loss:

A total of 21 She-camels showing estrus behavior was naturally mated with virile studs. The ultrasonography examination on day 20 post-mating revealed incidence of pregnancy in 16 out of 21 animals (76.2%), while 5 animals (23.8%) were non-pregnant. On days 35, 45, 55 and 75 post-mating, 4 out of 16

conceived animals (25%, one case on each day) were non-pregnant and considered as embryonic loss cases. On day 90 post-mating, no embryonic loss was detected, while only 12 pregnants out of 21 animals (57.1%) were calved at the end of gestation period (Table 3).

Ultrasonographic examination of the uterine horn of camels showed that the concepts appeared as an accumulation of elongated non-echoic conceptus fluids, regular and round in shape on day 20 of pregnancy (Fig. 1), increased accumulation of more rounded conceptus fluids in cross-section and the echogenic fetus just becomes visible lying on the uterine floor.

Table 3. Rates of conception, incidence of embryonic loss and calving of She-camels

Item	(Number) %
Conception rate on day 20 post-mating	(16/21) 76.2
Conception rate on days 35 and 45 post-mating	(15/21) 71.4
Incidence of embryonic loss (20-45 day)	(1/16) 6.25
Conception rate on day 55 post-mating	(13/21) 61.9
Incidence of embryonic loss (20-55 day)	(3/16) 18.75
Conception rate on day 75 post-mating	(12/21) 57.1
Incidence of embryonic loss (20-75 day)	(4/16) 25.0
Conception rate on day 90 post-mating	(12/21) 57.1
Calving rate	(12/21) 57.1

Also, CL of 15.9 mm in diameter on the ovarian surface on day 35 of pregnancy (Fig. 2), marked size of the fetus and volume of fetal fluids with CL of 28.2 mm in diameter on the ovarian surface on day 40 of pregnancy (Fig. 3), and developmental growth of the head, neck, abdomen and limb buds of the fetus on day 55, 75 and 90 of pregnancy (Figs. 4, 5 and 6), respectively. Ultrasonographic examination of embryonic loss cases reveled absence of fetal fluids (Fig. 7). It is of interest to note that all pregnancy cases were noted in the left uterine horn of pregnant camels. These findings supported by several investigators (Skidmore, 2000; Al-Rawi, 2014).

The breeding season of camels in Egypt extends from October to February (Shalash, 1965). In the current study, She-camels were mated when they were showing an estrous activity during the breeding season. In our study, 5 of 25 (20%) of camels exhibiting estrus and mated did not conceived (G1) on day 20 post-mating. In this respect, Skidmore *et al.* (1996a) reported induce ovulation only in 70-85% of camels following natural mating. This is also due to the time elapsed between mating and ovulation in camel is 24 to 36 hours (Marie and Anouassi, 1986).

The obtained results in our study recorded that the incidence of embryonic loss of 25% in conceived animals during the interval from 20 up to 75 days post-mating. This rate is within a range from 8 to 32% of early embryonic loss in camels (Tibary *et al*., 2006). In Iraq, Al-Rawi (2014) showed 33.3% early embryonic death cases between day 25 to 50 post-mating and the fertility rate of 33.3% in primiparous non-lactating one-humped She-camels.

It is well known that endometritis is considered an important cause of embryonic loss in mares. Ginther *et al.* (1985) related the process of endometritis with high evidence of embryonic loss (18.2%) in pony mares between 11 and 15 days post-ovulation in mares. The authors concluded that these losses were probably due to premature luteolysis secondary to endometritis. They also proposed that the primary luteal insufficiency and failure of the maternal recognition mechanism of gestation could lead to low P4 concentration and embryonic death. Embryonic death in camelidae may be attributed to genetic factors, corpus luteum insufficiency or hostile uterine environment; however, no single factor can be manipulated to improve embryo viability.

Earlier reports suggested that the side of ovulation may have an effect on the early embryonic mortality because most pregnancies are carried on the left horn (Tibary and Anouassi, 1997), and both ovarian sides are equally functioning, but ovulation followed by successful pregnancy is more probable to be from the left ovary (Ali, 2010). In this study calving rate was 64%, being higher than 41% in Egypt (Zeidan, 1999), 40% as an overall calving rate of approximately for 30 herds in Tunisia (Djellouli and Saint-Martin, 1992) and 39.1% in Libya (El-Azab *et al*., 1997).

In this way, Tinson et al. (2001) mentioned some pathological reasons which lead to infertility in camels, and uterine infections were considered to be the most common cause of reproductive failure in female camels (Ali *et al*., 2012). Such findings may suggest somewhat improvement in reproductive performance of dromedary camels under the experimental condition in Egypt.

Generally, an early pregnancy loss is probably one of the most important factors resulting in the reduction of reproductive efficiency in camels. At present, there is no practical way to reduce embryonic loss in camels, however, recognizing the occurrence and incidence of embryonic loss may be instrumental in application of new reproductive technologies to increase service rate in a herd (Pratap et al., 2012).

There were insignificant differences in concentration of total proteins and their fraction as well as in albumin: globulin ratio as affected by experimental group (pregnant, embryonic loss and non-pregnant groups), post-

mating day and their interaction (Table 4). Yadav *et al*. (2006) indicated that high levels of plasma proteins in the late trimester of pregnancy are needed for the optimum secretion of gonadotropin release factors and number of other hormones needed in the culmination of the pregnancy, Yokus and Cakmr (2006) found that the total proteins concentration did not change with reproductive status in cattle and sheep. This finding is in accordance with the tendency of reduction in total proteins concentration in pregnant in comparing with pregnancy loss or non-pregnant camels in the present results, However, Boudebza *et al*. (2014) and Sharma *et al*. (2015) in ewes as well as Bamerny (2013) in goats, found that concentration of plasma total proteins decreased during pregnancy to post-partum phase or early lactation phase. Also, Balikci *et al*. (2007) reported a decrease in total proteins on day 150 of gestation in ewes. In similar trend, Jainudee and Hafez (1994) reported significant decrease in total proteins concentration in sheep on day 120 of gestation, as compared to other stages of gestation and during the whole investigated period.

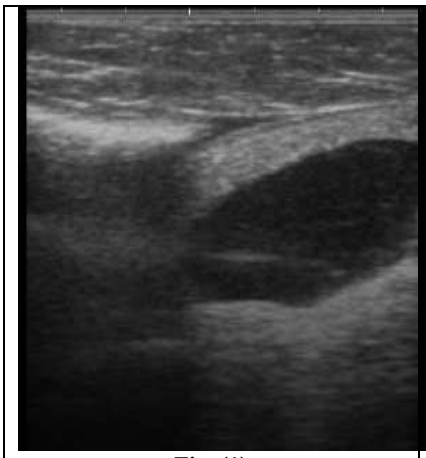
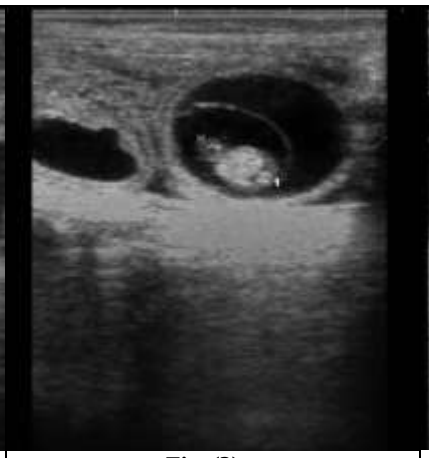


Fig. (1)



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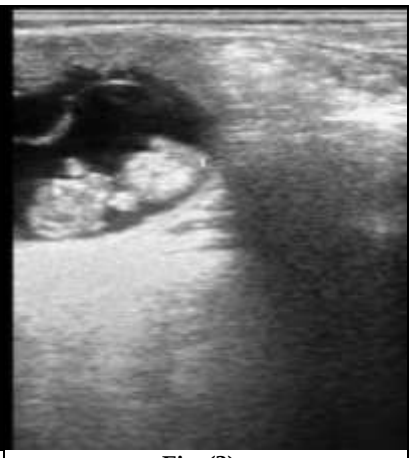

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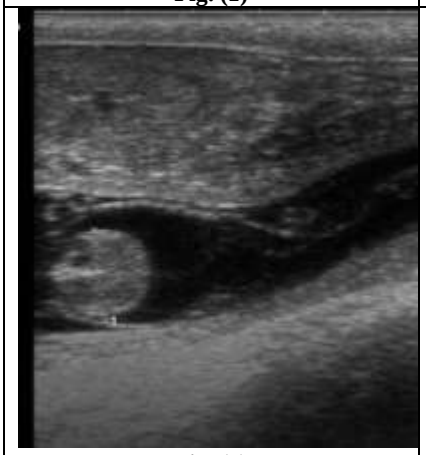

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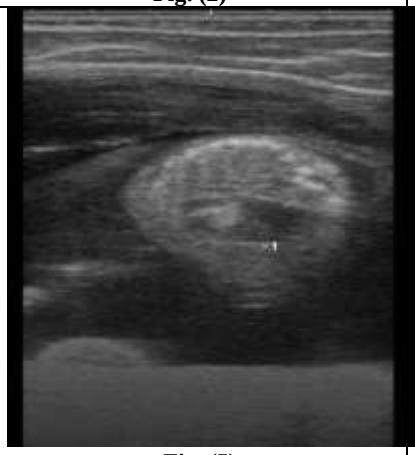

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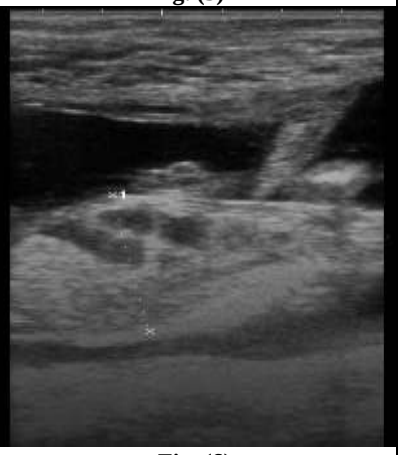

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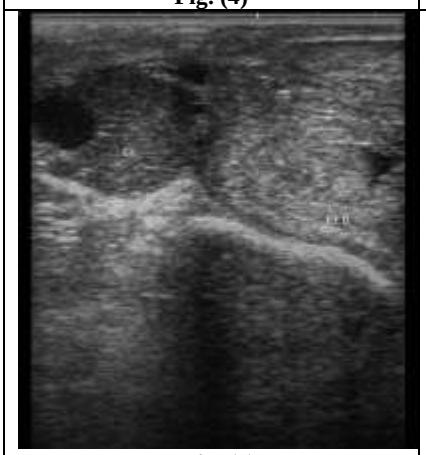

Fig. (7)

<u>Ultrasonographic images of She-camel showing:</u>

Fig. (1): Pregnancy on day 20 with accumulation of elongated non-echoic conceptus fluids.

Fig. (2): Pregnancy on day 35 with more rounded conceptus fluids in cross-section and the echogenic fetus just becomes visible lying on the uterine floor. Also, CL of 15.9 mm in diameter was seen on the ovarian surface.

Fig. (3): Pregnancy on day 45 with marked size of the fetus and volume of fetal fluids. Also, CL of 28.2 mm in diameter was seen on the ovarian surface.

Figs. (4, 5 and 6): Rapid developmental growth of the head, neck, abdomen and limb buds of the fetus on day 55, 75 and 90 of pregnancy, respectively.

Fig. (7): Embryonic loss with CL on the ovary (on day 75 post-mating).

Blood parameters:

Concentration of total proteins and their fractions:

As gestation progresses, the concentration of many biochemical parameters change significantly from those found in the non pregnant state (Balikci *et al*., 2007). By advancing the pregnancy period, there is a difference in serum total proteins concentration as the need of the dam changes (Balikci *et al*., 2007). In this respect, Mir *et al*. (2008) found significant increase in plasma total proteins concentration during mid and late pregnancy as compared to the early pregnancy stage.

Table 4. Concentration of total proteins and their fractions in blood serum of She-camels as affected by reproductive status, post-mating day or their interaction.

Item	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AL:GL ratio
Effect of reproductive status (RS):				
Pregnant	6.32±0.052	3.95±0.039	2.64±0.035	1.71±0.030
Embryonic loss	6.46±0.100	4.12±0.093	2.34±0.059	1.79±0.063
Non-pregnant	6.50±0.114	4.09±0.068	2.41±0.075	1.75±0.053
Effect of post-mating day (PM):				
5	6.41±0.167	3.98±0.092	2.43±0.078	1.68±0.062
20	6.37±0.124	4.00±0.093	2.38±0.096	1.74±0.078
35	6.54±0.112	3.98±0.100	2.56±0.066	1.59±0.057
45	6.33±0.117	3.86±0.070	2.47±0.076	1.59±0.053
55	6.31±0.115	4.07±0.095	2.25±0.059	1.84±0.068
75	6.31±0.089	4.10±0.068	2.21±0.047	1.88±0.048
90	6.38±0.138	4.06±0.079	2.31±0.076	1.79±0.054
Interaction (RS x PM)	NS	NS	NS	NS

NS: Not significant.

The observed reduction in total proteins concentration in our study might be due to the preparation of reproductive system during pregnancy (growth of uterus) which requires large quantity of protein (Sharma *et al*., 2015) and fetus synthesis all its proteins from the amino acids derived from the dam, and growth of the fetus increases exponentially reaching a maximum level, especially in muscles, during late pregnancy (Jainudee and Hafez, 1994).

It is of interest to note that reduced total protein concentration was associated with a tendency of decreased albumin and increased globulin concentration in pregnant camels (Table 4). It is generally accepted that albumin levels are positively related with productive and reproductive performance (Payne and Payne, 1987). In agreement with the present results, plasma albumin level did not seem to vary significantly during pregnancy in pregnant goats (Jankowiak *et al*., 2006) and cows (Yildiz *et al*., 2005). Also, Piccioli *et al*. (1997) and Brzostowski *et al*. (1995) observed that albumin level did not change in late pregnancy and lactation period. However, El-Sherif and Assad (2001) reported that plasma concentration of albumin in pregnant ewes did not decrease. On the other hand, globulin concentration was recorded to increase during pregnancy as compare to postpartum/early lactation phase in ewes (Antunovic *et al*., 2011) and in goats (Bamerny, 2013). Increasing globulin level in pregnant camels might be due to accumulation of proteins especially immunoglobulins (Ig) which occurs as a preparatory step for the bulk secretion of Ig into colostrum after lambing and also might be used for formation of milk protein (Kaneko *et al*., 2008).

Concentration of glucose and lipid profile:

Effect of reproductive status on serum concentration of glucose and total lipids was not significant. However, serum concentration of total cholesterol was affected by reproductive status, being significantly ($P<0.05$) lower in pregnant than in pregnancy loss camel group, while non-pregnant camel group showed insignificant differences in comparing with both groups (Table 5).

As affected by post-mating day, serum concentration of glucose showed significantly ($P<0.05$) marked reduction by advancing post-mating day, in particular, between 5 and 20 day post-mating. However, serum concentration of total cholesterol significantly ($P<0.05$) increase between 5 and 35 day post-mating, then insignificantly decreased up to 90 days post-mating. On the other hand, concentration of total lipids was not affected by post-mating day. It is of interest to note that the effect of interaction between reproductive status and post-mating day on concentration of glucose, total cholesterol and total lipids was not significant (Table 4).

Glucose estimates in blood serum of camels with different reproductive statuses and at various post-partum days ranged between 55.32 and 64.31mg/dl (Table 4). The present glucose values in our study are within the normal range for adult camel (50 to 120 mg/100 ml) according to Faye and Mulato (1991). Also, these values are nearly similar to those previously reported by Al-Sultan (2003) on Mayhem camels in Saudi Arabia, being 58 mg/dl. Blood glucose concentration is affected mainly by feeding system (Al-Saiady *et al*., 2013), although plasma glucose concentrations can be used for the monitorization of the energy status of the female and low concentrations in the post-partum period are associated to a significant decrease in reproductive efficiency (Harrison *et al*., 1990).

Table 5. Concentration of glucose, total cholesterol and total lipids in blood serum of She-camels.

Item	Glucose (mg/dl)	Total cholesterol (mg/dl)	Total lipids (mg/dl)
Reproductive status (RS):			
Pregnant	58.25±2.048	120.33±1.480^{b}	219.29±15.12
Embryonic loss	63.53±4.635	128.36±3.206^{a}	165.72±20.81
Non-pregnant	61.30±4.044	127.08±3.525^{ab}	168.20±12.78
Post-mating day (PM):			
5	60.64±6.569^{a}	114.79±2.889^{b}	241.05±42.17
20	57.15±3.885^{b}	122.55±4.199^{ab}	171.79±31.45
35	62.95±4.283^{b}	127.85±4.150^{a}	234.38±26.87
45	60.40±2.571^{b}	122.22±2.022^{ab}	213.85±28.64
55	55.32±2.843^{b}	124.17±4.180^{ab}	180.38±18.98
75	64.31±3.843^{b}	124.46±2.935^{ab}	160.49±18.97
90	57.15±4.087^{b}	124.70±3.023^{ab}	201.58±22.73
Interaction (RS x PM)	NS	NS	NS

Means denoted within the same column for each factor with different superscripts are significantly different at P<0.05. NS: Not significant.

Cholesterol is the metabolic precursor of P4, which is vehiculated mainly through the diet and reaches the luteal cells associated to HDL and LDL (Juengel and Niswender, 1999). Badawy *et al*. (2008) reported that Total lipids levels ranged between 65.9 and 121.8 mg/dl. The increase in cholesterol and total lipids could be attributed to the increased non-esterified fatty acids and fat catabolism.

Absent of significant differences among camel groups in glucose level and lipid profile at different reproductive statuses may indicate similar nutrition and energy balance for all camel groups.

Concentration of urea and creatinine:

Effect of reproductive status on serum concentration of both urea and creatinine was significant (P<0.05), being higher in pregnancy loss than in pregnant camel group, while non-pregnant camel group showed insignificant differences as compared to pregnant and pregnancy loss camel groups. Also, serum concentration of both urea and creatinine showed significant (P<0.05) changes during different post-partum day. Urea concentration fluctuated trend of change, showing significantly (P<0.05) the highest concentration on day 20 and 45 post-mating and the lowest concentration on day 55 post-mating. However, creatinine concentration increased during the first 20 days post-mating, and then insignificantly decreased up to 90 day post-mating (Table 6).

It is worthy noting that only creatinine concentration of each reproductive status significantly (P<0.05) interacted with post-mating day. In general, creatinine level was almost lower in pregnant camels than in embryonic loss and non-pregnant ones at most post-mating days (Figure 8).

As in ruminants, camels can utilize urea for microbial synthesis of protein and are able to regulate its excretion on the renal tubular level as well as sheep (Badawy *et al*., 2008).

The estimated urea concentration recorded in our study was higher than that reported by Badawy *et al*., (2008) on She-camel in Egypt, being 34.99 and 30.29 mg/dl in summer and autumn and 28.7 and 29.9 mg/dl in winter and spring, respectively. The overall mean value of creatinine level was 0.9 mg/dl (Badawy *et al*., (2008). Also, estimates of creatinine in our study are slightly different from 1.9 mg/dl as reported by Al-Sultan (2003).

In harmony with the obtained results, Trevaskis and Fulkerson (1999) did not find a significant relationship between plasma urea concentrations at insemination time and subsequent pregnancy rate. According to these findings, camels with reproductive disorders (embryonic loss or non-pregnant) are showing normal values of blood biochemicals as found in pregnant ones.

Table 6. Concentration of urea and creatinine in blood serum of She-camels as affected by reproductive status, post-mating day or their interaction.

Item	Urea (mg/dl)	Creatinine (mg/dl)
Reproductive status (RS x PM):		
Pregnant	50.61±1.751[b]	1.20±0.027[b]
Embryonic loss	59.35±4.556[a]	1.48±0.087[a]
Non-pregnant	56.96±3.154[ab]	1.39±0.052[ab]
Post-mating day (PM):		
5	52.59±4.461[abc]	1.22±0.062[b]
20	60.49±3.451[a]	1.52±0.068[a]
35	47.03±3.290[bc]	1.22±0.082[b]
45	61.32±3.933[a]	1.18±0.056[b]
55	42.12±3.083[c]	1.22±0.045[b]
75	53.89±3.561[ab]	1.37±0.075[b]
90	55.51±4.590[ab]	1.26±0.061[b]
Interaction (RS x PM)	NS	*

Means denoted within the same column for each factor with different superscripts are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05.

Figure 8. Change in creatinine level in blood serum of She-camels at different post-mating days.

In the current study, concentrations of most metabolic parameters in blood serum are nearly similar in pregnant, embryonic loss and non-pregnant camels. Therefore, these parameters did not have predictive value for fertility following mating. A possible reason for this is that body condition score fluctuations among females were not evident and serum concentrations of metabolites were within the normal range for all reproductive statuses.

Enzyme activity:

Both AST and ALP activities in blood serum was not affected significantly by reproductive status, post-mating day and their interaction. However, ALT activity was significantly (P<0.05) lower in pregnant than in pregnancy loss and non-pregnant camel groups. Also, ALT activity significantly (P<0.05) increased during the first 20 days, decreased at 35 day and then showed insignificant change up to 90 day post-mating. The effect of interaction between reproductive status and post-mating day on ALT activity was not significant (Table 7).
In comparable with the present activity of transaminases, Badawy *et al*. (2008) estimated values of 13.9-17.5 U/l for ALT and 2.9-3.8 U/l for AST in She-camels.

The importance of ALP enzyme come from its role in food absorption which aid in growth and development of fetus, in addition to liver and skeletal muscles which consider source of this enzyme (Shane and Suzuki, 1974), the present activity of ALP in this study is within the normal activity of ALP in camel serum, ranging from 60 to 140 IU/l (Mostafa *et al*. 2013). In accordance with the present results, Al-Zamely (2011)

found insignificant increase in ALP activity in pre- than in post-partum period in camels. This increase may be due to increase its production from placenta during pregnancy (Pirani *et al*., 1972).

Table 7. Activity of AST, ALT and ALP in blood serum of She-camels as affected by reproductive status, post-mating day or their interaction.

Item	AST (U/l)	ALT (U/l)	ALP (U/l)
Reproductive status (RS):			
Pregnant	38.86±1.022	34.94±0.852^{b}	73.34±1.582
Embryonic loss	40.57±3.206	42.46±2.089^{a}	71.19±2.800
Non-pregnant	39.46±1.902	38.40±1.719^{b}	72.72±2.028
Post-mating day (PM):			
5	37.24±0.638	32.72±1.596^{c}	71.62±2.829
20	46.08±3.367	43.84±2.109^{a}	73.54±3.301
35	35.76±2.390	32.96±1.742^{c}	74.76±2.815
45	39.96±1.460	38.08±1.502^{bc}	67.38±2.555
55	36.40±1.535	34.04±1.894^{c}	71.61±3.330
75	40.56±2.552	36.16±2.354^{bc}	77.49±3.860
90	38.76±3.217	40.04±1.736^{ab}	73.70±2.869
Interaction (RS x PM)	NS	NS	NS

Means denoted within the same column for each factor with different superscripts are significantly different at P<0.05. NS: Not significant.

Mineral contents in blood serum:

Effect of reproductive status was significant (P<0.05) on contents of P, Zn and Cu in blood serum of camels, while Ca content was not significantly affected. Embryonic loss camels showed significantly (P<0.05) the highest P content and non-pregnant camels showed significantly (P<0.05) the highest Zn and Cu contents, while pregnant camels had significantly (P<0.05) the lowest values of P, Zn and Cu contents and insignificantly the lowest Ca content. Effect of post-mating day was significant (P<0.05) only P content, showing fluctuate trend of change, being the highest on days 45 and 90 post-mating and the lowest at early pregnancy on day 5 post-mating (Table 8).

Table 8. Concentration of total proteins and their fractions in blood serum of She-camels as affected by reproductive status, post-mating day or their interaction.

Item	Calcium (mg/dl)	Phosphorus (mg/dl)	Zinc (µg/dl)	Copper (µg/dl)
Reproductive status (RS):				
Pregnant	7.14±0.201	3.82±0.088^{b}	261.90±9.271^{b}	93.68±5.372^{b}
Embryonic loss	6.74±0.288	4.42±0.165^{a}	305.29±26.925^{ab}	119.96±10.982^{ab}
Non-pregnant	6.88±0.254	4.17±0.147^{ab}	316.77±18.199^{a}	115.81±11.058^{a}
Post-mating day (PM):				
5	7.15±0.390	3.69±0.175^{c}	252.82±23.303	115.56±11.324
20	6.32±0.506	3.96±0.198^{bc}	301.38±27.513	109.68±11.233
35	7.84±0.359	3.65±0.200^{c}	270.88±18.232	109.76±15.749
45	6.89±0.242	4.40±0.145^{ab}	256.74±18.295	101.80±12.783
55	7.20±0.418	3.86±0.216^{c}	254.60±15.158	100.16±9.544
75	6.94±0.425	3.87±0.176^{c}	301.63±18.292	87.03±13.394
90	6.80±0.271	4.47±0.137^{a}	320.38±28.254	92.16±8.306
Interaction (RS x PM)	NS	NS	NS	*

Means denoted within the same column for each factor with different superscripts are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05.

Effect of interaction between reproductive status and post-mating day was significant only on Cu contents in blood serum of camels (Table 6). Throughout post-mating period, Cu contents showed nearly similar trend of change, but wide variation in Cu contents was observed on days 35 and 75 post-mating, On these days, non-pregnant and embryonic loss camels showed the highest values versus lower values in pregnant camels, respectively (Fig. 9).

According to the obtained results, the reproductive status has an important influence on blood macro- and micro-mineral concentrations. It was reported that there were differences among macro mineral levels during pregnancy as a result of dam and fetus requirements (Siggurdson, 1988). In accordance with the present results of Ca, Tanritanir *et al*. (2009) reported no statistical differences in Ca levels between, before and after parturition.

Figure 9. Change in copper contents in blood serum of She-camels at different post-mating days.

On the other hand, Also, Abdou (1995) reported insignificant change in Ca level during pregnancy up to the fourth month of pregnancy. In comparable with Ca results, Ca levels (Moghaddam and Hassanpour, 2008) and Ca ion values (Waziri *et al*., 2010) were lower at post-partum than gestation period and increased as gestation progressed, while decreased after parturition (Kadzere *et al*. (1997). Also, Winnicka (2004) reported that Ca concentration during pregnancy remained relatively stable and did not exceed the reference values in goats as well as women and rats (Prentice, 2000; O'Brien *et al.,* 2003). Moreover, Uddin and Ahmed (1984) found that plasma Ca level was significantly higher in pregnancy than in lactation, being significantly higher at early than at late pregnancy and in late than early lactation. Yokus *et al*. (2004) observed that Ca level decreased slightly at late than at early pregnancy and then increased at lactation period in sheep. Also, Azab and Abdel-Maksoud (1999) observed that plasma Ca levels markedly decreased during late pregnancy to reach its minimum level at parturition and continued to decrease for 3 wk post-partum. Adaptive changes in the management of Ca and P occur in the female as soon as gestation begins, long before the period of the highest demand of the fetus for the elements (Dai *et al*., 2001). The requirements of the fetus for total P increases significantly in the second half of gestation and reaches its peak in humans during the 3rd trimester (Prentice, 2003).

Generally, Georgievskii *et al*. (1982) attributed the increased level of Ca in plasma during gestation and lactation to high level of plasma parathyroid hormone in these periods, which activates osteoclasts and increase the level of calcaemia to mobilize skeletal Ca reserves. Mobilization is necessary to meet high Ca demand by the fetus for skeletal formation and for milk formation during lactation (Fredeen and Vankessel, 1990). During lactation in dairy cows, Ca was transferred from the plasma into milk with the result that Ca concentration dropped (Ballantine and Herben, 1989).

The present results of P content are in agreement with Tanritanir *et al*. (2009) who reported that P level significantly increased during late gestation and postpartum as compared to early pregnancy in ewes and goats. Also, Yokus *et al*. (2004) demonstrated in lactation to decrease the level of P when compared to pregnant ewes. Contrary, concentration of inorganic P did not differ between non-pregnant goats and pregnant does over the entire period of gestation (Krajnicakova *et al*., 2003), being near the lower limit of the reference range (Winnicka, 2004). Similarly, plasma P concentration does not change during pregnancy, in women and goats (Prentice, 2000) as well as in cattle (Yokus and Cakmr, 2006).

The macro minerals (Ca and P) have an impact on animal reproduction, especially during dry period (Özlem *et al*., 2000), but their needs increase during pregnancy because both the fetus and mother are in need of them, therefore, during periods of macro mineral insufficiencies, the fetus and reproductive tissue will have first

priority for macro minerals and the dam will mobilize nutrients from her body to meet the fetus macro mineral requirements (Kulcu and Yur, 2003). During late pregnancy, requirements of female to these elements increase due to the rapid growth of the fetus (Ozpmnar, 2002), so these nutrients can greatly affect reproduction (Karademir, 2007). All animals require minerals such as calcium (Ca) and phosphorus (P) for growth, reproduction and lactation, which often affect specific requirements, and serve as catalytic components of enzymes or regulate several mechanism involved just in pregnancy and lactation (Samardzija *et al*., 2011).

Copper is a vital component as cofactor in many enzymatic systems, and is required in the body for the production of the red blood cells as it is essential for absorption, and transport of iron necessary for hemoglobin synthesis (Tuormaa, 2000). It is one of the essential trace metals which is necessary in maintaining the functioning of living organisms. It is required for the function of over 30 proteins including superoxide dismutase, ceruloplasmin, lysyl oxidase, cytochrome c oxidase, tyrosinase, dopamine-β-hydroxylase and hephaestin (Zatta and Frank 2007; Prohaska, 2011). Reproductive performance of animals may be compromised if copper levels are marginal. Copper complexes with GnRH and interact with GnRH receptors and modulate intracellular signaling in the gonadotrope cells of the anterior pituitary (Michaluk and Kochman, 2007). There are many factors that can influence Cu absorption in ruminants.

Hormonal profile:

Effect of reproductive status was significant (P<0.05) only on P4 concentration, being higher in pregnant than in embryonic loss or non-pregnant camels. Also, P4 concentration was affected significantly (P<0.05) by post-mating day, showing dramatic increase from 5 up to 45 day post-mating, then decreased on day 55 and again increased on days 75 and 90 post-mating. On the other hand, E2 concentration was not affected by reproductive status or post-mating day. The insignificant effect of interaction between reproductive status and post-mating day reflected an opposite trend of change in both P4 and E2 concentration on different post-partum days in pregnant than in embryonic loss or non-pregnant camel groups at most post-mating days (Fig. 10 and 11, respectively, Table 9).

Higher P4 concentration observed in pregnant than in embryonic loss or non-pregnant camels in this study was reported by Volkmann *et al*. (2009), who observed marked decrease in the levels of P4 in mares suffered from early embryonic death.

Table 9. Concentration of progesterone (P4) and estrogen (E2) in blood serum of She-camels as affected by reproductive status, post-mating day and their interaction.

Item	Progesterone (ng/ml)	Estrogen (pg/ml)
Reproductive status (RS):		
Pregnant	5.99±0.190^{a}	4.50±1.158
Embryonic loss	3.34±0.878^{b}	6.27±1.184
Non-pregnant	3.13±0.654^{b}	6.59±0.928
Post-mating day (PM):		
5	1.50±0.331^{c}	4.50±0.815
20	3.61±0.817^{bc}	4.45±0.942
35	2.33±0.423^{bc}	4.45±1.185
45	9.16±3.212^{a}	4.62±1.033
55	3.68±0.921^{bc}	4.82±1.161
75	6.02±0.935^{ab}	2.83±1.020
90	8.63±1.059^{a}	10.43±4.864
Interaction (RS x PM)	NS	NS

Means denoted within the same column for each factor with different superscripts are significantly different at P<0.05. NS: Not significant.

This result agrees with those of Kamoun and Jemmali (2014), who found that serum P4 was higher in gravid than in empty She-camels. Plasma P4 level is high during pregnancy and it drop at parturition. Also, Bakheit *et al*. (2012) observed that P4 concentration in pregnant She-camels significantly increases during early months of pregnancy to a value above 2 ng/ml blood. During pregnancy the value is increased to an average value of 5.8±1.45 ng/ml blood over a period of 8 months followed by a strong decrease during the last two months before calving. Moreover, Mobarak and El-Wishy (1971) indicated a significant rise in P4 level two days after successful mating of all young She-camel. It is well known that level of P4 in females is a very useful tool to monitor pregnancy in camels. The increase in plasma P4 concentrations of pregnant females from days 15 to 55 (Fig. 10) may suggest the presence of a lutetrophic factor of embryonic origin (Feliciano *et al*., 2003).

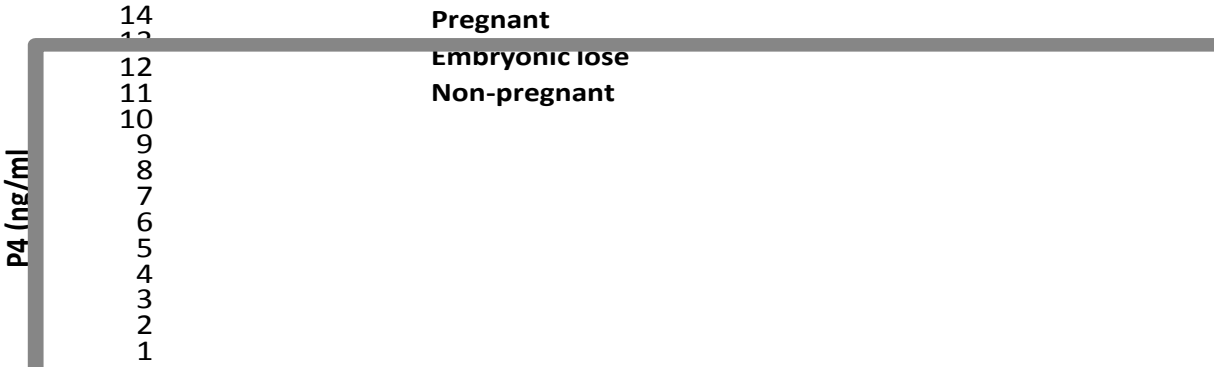

Figure 10. Change in progesterone concentration in blood serum of She-camels at different post-mating days.

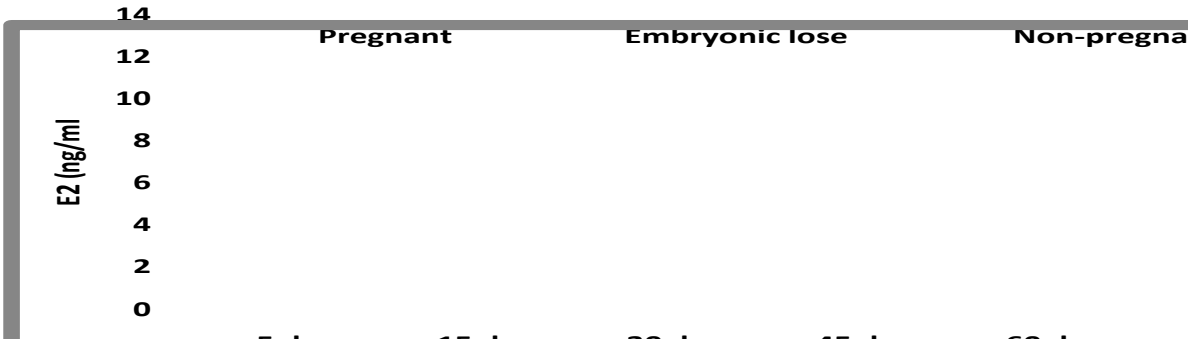

Figure 11. Change in estrogen (E2) concentration in blood serum of She-camels at different post-mating days.

The CL is the primary source of P4 in She-camel. The placenta does not contribute to P4 secretion, and all camelids depend entirely on P4 from the CL to maintain their pregnancy (Skidmore, 2005). Any decrease in P4 levels during pregnancy indicates insufficiency in its production from the CL due to luteolysis. Several factors could lead to luteolysis and a decrease in peripheral levels of P4 during early pregnancy which ends pregnancy. Slight inflammatory reagents in the uterus initiate the production of prostaglandins which induces luteolysis and in turn ends pregnancy in mare (Krakowski, *et al*., 2010). Generally, P4 is critical to embryonic survival, the cause-and-effect relationship between P4 and spontaneous embryonic loss remains unclear (Ball, 1988). In agreement with Shelton *et al*. (1990), the obtained results, there was no early deviation in P4 profiles between pregnant and embryonic loss and non-pregnant camels (Fig. 10). Also, most embryo transfer studies report that pregnant and non-pregnant recipients have similar P4 profiles until the day of embryo transfer, which usually takes place on days 6 to 8 (Chagase Silva *et al*., 2002). Moreover, plasma P4 concentration of inseminated cattle was only significantly different between pregnant and non-pregnant females from day 17 onwards, that is, no early deviation (days 5 to 7) was observed in the P4 profiles of pregnant and non-pregnant cattle (Feliciano *et al*., 2003).

In embryonic loss and non-pregnant camels, serum oestradiol-17β concentrations showed a first definite increase on day 15 post-mating and continue to rise up to day 75 post-mating. Similar trend was reported by Skidmore *et al*. (1996b). However, the timing of the oestrogen increase in the final 70-80 days in the pregnant dromedary coincides with the important period of increase in fetal weight and fetal fluid volume, giving rise to the possibility of placental E2 being important for fetal growth. In dromedaries the oestrogen sulphate concentrations showed two definite peaks in early gestation, the first peak occurs around day 26 and the second around day 70 (Skidmore *et al*., 1996b).

Based on the foregoing results, only P4 level on days 15 and 35 post-mating may be use as physiological indicator for pregnancy or early embryonic loss. In early embryonic loss camels, P4 level decreased to values above 3 µg/ml in pregnant and embryonic loss camels on day 15 post-mating, then decreased to less than 2.0 µg/ml in embryonic loss camels but still ≥3 µg/ml in pregnant camels. However, in non-pregnant camels, P4 level was ≤2 µg/ml on days 15 and 35 post-mating.

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