

Development of Some Fetal Measurements and Ovarian Hormones during Gestation Period in Maghrebian She-Camels under Egyptian Conditions

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Abstract : This work aimed to study the development in some fetal measurements, including crown-rump length (CRL), body trunk (TD), bi-parietal (BPD) and eye ball (EBD) diameters, and steroid hormone profile (progesterone and estradiol $17\text{-}\beta$, during gestation period of Maghrebian camels. A total of 14 pregnant Maghrebian camels 6-12 years, 400- 460 kg LBW and with known conception date) taken from the Center of Studies and Development of Camel Production, Marsa Matrouh Station, Marsa Matrouh Governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Centre, Egypt. All animals were kept under similar environmental conditions. Real-time, B-mode diagnostic ultrasound equipment was used for sequential estimations of fetal measurements and pregnancy diagnosis. On each day of ultrasonographic examination, blood samples were collected to determine progesterone (P4) and estradiol concentration in blood serum. Results showed that CRL gradually increased from 3 up to 14 wk of gestation. The exponential regression equation gave the best fit ($R^2 = 0.985$) for the unique measurement of gestational age ($CRL = 0.431e^{0.197\text{fetal age (wk)}}$). TD showed gradual increase from 6 up to 17 wk of gestation. The exponential regression equation gave the best fit ($R^2 = 0.973$) for the unique measurement of gestational age ($TD = 0.438e^{0.148\text{ fetal age (wk)}}$). BPD showed marked increase from 57 up to 220 days of gestation. The exponential regression equation gave the best fit ($R^2 = 0.970$) for the unique measurement of gestational age ($BPD = 0.545e^{0.081\text{fetal age (wk)}}$). EBD showed continued increase from 12 up to 49 wk of gestation. The linear regression equation gave the best fit ($R^2 = 0.997$) for the unique measurement of gestational age [$EBD = 0.075\text{ fetal age (wk)}$]. The correlation between gestational age and fetal measurements (CRL and EBD) were higher than those of TD and BPD, and the highest correlation was between CRL and gestational age in camels. There were no association between gestational age and concentration of P4 or E2, but both hormones levels showed somewhat positive relationships with TD, BPD and EBD, not with CRL. Profile of P4 and E2 showed marked increase with advancing gestation period, particularly at late stage of pregnancy. In conclusion, the overall data indicated the value of ultrasonographic fetal characteristics in camels for the evaluation of fetal development, estimation of gestational age during the entire length of pregnancy period or as a relatively easy means for observing the events of pregnancy for research purpose and a practical and economic tool for breeders.

Keywords: Camel, fetal age, crown-rump length, trunk, bi-parietal, steroid hormones.

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I. Introduction

Gestational age estimation is considered to be essential for maintaining a high level of reproductive efficiency in camels (Vyas et al., 2002) for allowing producers to group camels based on their nutritional needs (Bach, 2012) and application of correct management of pregnant camels at the appropriate times.

Pr-partum ultrasound examinations have been used to measure some fetal parameters and to predict the gestational age in several animal species. Pre-partum ultrasonography can provide sequential measurements of the conceptus throughout different developmental stages. Specific fetal parameters have been used to determine the gestational age, including thoracic, abdominal, and umbilical diameters in cattle (Hunnam et al., 2009), aortic systolic and bi-parietal diameters, eye volume, femur length, and kidney cross-sectional area in equine (Renaudin et al., 2000), crown-rump length (CRL), amniotic vesicle, bi-parietal (BPD), and eye ball diameter (EBD) in buffalo (Ali and Fahmy, 2008), CRL, chest depth, abdominal, BPD, and EBD, and ruminal length

(RUL) in sheep (Ali and Hayder, 2007) and goats (Amer, 2010), and gestational sac diameter, BPD, thorax height, femoral, tarsus-hoof, tibial, and fronto-occipital lengths in alpacas (Ferrer et al., 2013).

Many authors suggest a correlation between fetal and extra-fetal measurable parameters and fetal gestational age during pregnancy (Kutzler et al., 2003; Milani et al., 2011). Inner chorionic cavity (ICC) and outer diameter uterine (ODU), measured during the first half of pregnancy, and body diameter (BD), CRL, BPD, and deep portion of telencephalic vesicle (DPTV), measured in the second half pregnancy, were the parameters most correlated to the gestational age of canine fetuses (Milani et al., 2011).

In dromedary camels, majority of studies were conducted on fetuses obtained from slaughtered dams with unknown breeding dates (Manal et al., 2006). In Kingdom of Saudi Arabia, Ali et al. (2013) determined the biometric threshold of different fetal parts and organs, including CRL, BPD, abdominal diameter, RUL and EBD in dromedary camels using ultrasonography. In Egypt, rare data are available on fetometry of dromedary camels and the choice of these parameters depended on their feasibility in ultrasound examinations at different gestational stages. Further investigations on conceptuses of known breeding dates are needed to give more exact data on the development of the camel conceptus and to enable the accurate estimation of gestational age (Hussein et al., 1991).

Therefore, the present paper aimed to study the development in some fetal measurements, including crown-rump length (CRL), body trunk (TD), bi-parietal (BPD) and eye ball (EBD) diameters, and steroid hormone profile (progesterone and estradiol 17- β , during gestation period of Magrebian camels.

II. Materials and Methods

Animals and management

A group of 14 pregnant she-camels (*Camelus dromedarius*) aged 6-12 years, weighing 400-460 kg and with known conception date were kept in the Center of Studies and Development of Camel Production, Marsa Matrouh Station, Marsa Matrouh Governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Centre, Egypt.

Pregnancy was diagnosed by ultrasonographic examination starting at 4 weeks post-mating. Pregnancy was anticipated through observation of anechogenic fluid in the uterine horn and by the presence of CL on the ovarian surface. Animals were housed in closed pens.

All animals were fed twice daily on a ration consisting of concentrate feed mixture (CFM, 12.24% CP), berseem hay (BH) during dry feeding period or fresh berseem (FB) during green feeding period, and rice straw (RS), based on live body weight according to APRI. Both CFM and BH or FB were given twice daily, while RS and water were offered all daytime. During the gestation period with lactation, animals were hand milked twice daily.

Ultrasonographic examination and fetal measurements

Ultrasound machine: Sonosite M-Turbo-with linear endorectal Prop (6-8MH).Fujifilm-Sonosite Co.USA.) was used for sequential estimations of some fetal measurements at various intervals of the pregnancy period. Pre-examination, each animal was fixed and the ultrasonographic examination was performed by one operator.

The fetal measurements, including crown-rump length (CRL), bi-parietal diameter (BPD), were carried out according to Ali et al. (2013) included the following parameters: crown- rump length (CRL) as a straight line between the fetal crown and the origin of the tail, bi-parietal diameter (BPD) of the head as the widest distance between the outer borders of the cranium at an angle of 90° to the falx cerebri, trunk diameter (TD) as maximum diameter of the abdomen at the insertion of the umbilical cord, and eye ball diameter (EBD) as the longest dimension of the vitreous body from medial sclera to lateral sclera).

Blood sampling

On day of ultrasonographic examination, blood samples were taken at the morning before feeding via the jugular vein from each animal into evacuated tubes (10 ml). Serum was separated by centrifugation of blood at 2500 rpm for 15 min and stored at -20 °C until use later for progesterone (P4) and estradiol (E2) assay. Determination of progesterone in serum samples was carried out using radioimmunoassay kit (catalog No. 1188, Immunotech, France) as described by the manufacture.

Statistical analysis

Based on known date of conception 4 weeks post-mating, different fetal measurements were considered as being dependent on gestational age. Trend line of each measure at different gestation times with regression equation of the highest R-squared values was done for evaluating the type of relationship between the gestational age and each of fetal measurement studied. In this study, 3 models i.e. exponential, linear, and logarithmic were attempted for best fit for calculation of fetal age and all models showed marked differences.

Data of P4 were statistically analyzed using the General Linear Model (GLM) procedure of SAS (1999). Data analyzed obtained were tested by analysis of variance with one way design (effect of pregnancy week-interval) on P4 level according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$; where: Y_{ij} = observed values, μ = overall mean, T_i = effect of time and e_{ij} = Random error. Values were given as mean \pm standard error. The Differences were subjected to Duncan's Multiple Range Test (Duncan, 1955) and all significant differences were set at $P < 0.05$.

III. Results and Discussion

Crown-rump length

Fetal crown-rump length (CRL) gradually increased by advancing early gestation period from 3 up to 14 wk (Fig. 1 and Plate 1). Similar trend was reported by ultrasonography in camel conceptus. In this respect, CRL was estimated to be 1.23, 1.95 and 4.7 cm at fetal age of 5, 6 and 8 weeks (Ali et al., 2013) and 2.7 cm on days 40-45 of fetal age (Al-Rawi, 2014).

This is in consistent trend reported on slaughtered camels, whereas some fetal measurements (CRL) increased steadily (Elwishy et al., 1981; Hussein et al., 1991; Manal et al., 2006). The fetus almost doubled its weight during the last 45 days of pregnancy (Elwishy et al., 1981).

The exponential, linear and logarithmic functions were derived to explain the fetal age-CRL relationship in dromedary camel and the respective R^2 values were 0.985, 0.940 and 0.817, respectively. The exponential regression equation gave the best fit ($R^2 = 0.985$) for the unique measurement of gestational age. It is quite evident from the analysis and curve fitting that the exponential function explained CRL in camel fetus for the entire pregnancy period to the extent of 98.5%, hence the equation $y = 0.431e^{0.197x}$ can reliably be utilized for the prediction of CRL of camel fetus at calving, where $y = \text{CRL (cm)}$ and $x = \text{fetal age (wk)}$.

Similarly, Ali et al. (2013) observed exponential regression of CRL in camel during the interval of the 3rd to 9th wk of pregnancy period. In slaughtered camels, Elwishy et al. (1981) suggested an accurate equation to estimate the gestational age through CRL estimation. However, greatly different estimates of conceptus age from the actual age, were given for fetuses with CRL of < 8 cm by this equation (Hussein et al., 1991). In comparing with camel, Ferreira et al. (2012) found the highest linear regression and correlation coefficients in buffaloes was between CRL and gestational age, where R^2 for this relationship was 0.989. In bovine, Somnuk et al. (2017) found that the relationship between fetal age and CRL showed strong positive correlation coefficient ($R^2 = 0.950$) in quadratic regression.

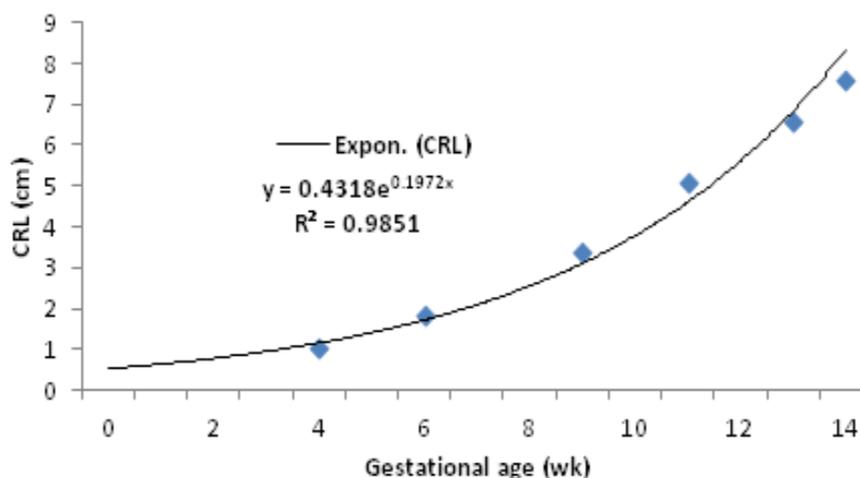


Fig. 1. Growth curve of fetal crown-rump length (CRL) at consecutive gestational ages (day).

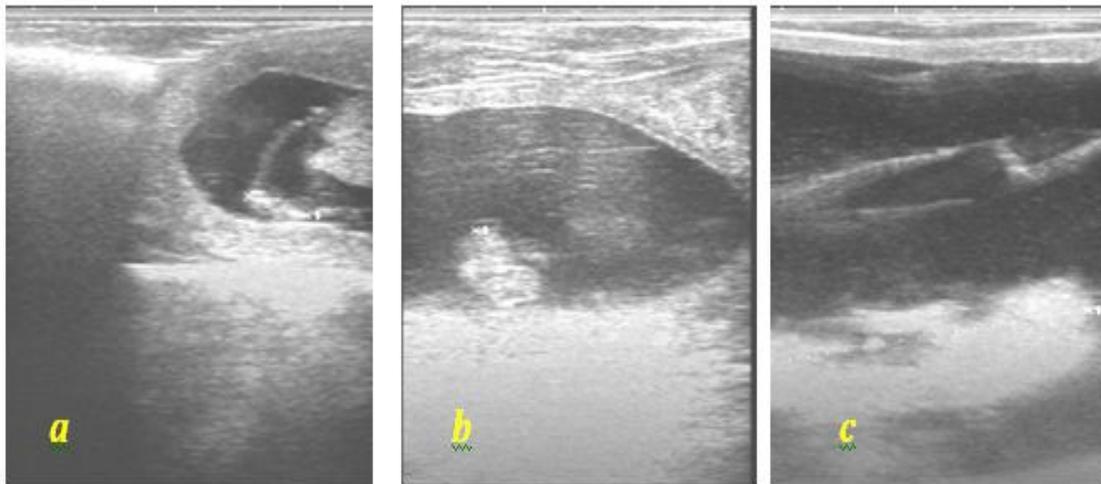


Plate 1. Ultrasonographic images of She-camel showing crown-rump length at 30, 46 and 91 days of pregnancy, respectively.

In camels, organization of the embryo into head, body, and limb buds was first observed between the 6th and 7th week of pregnancy. Fetal head, neck, abdomen, and individual limb buds could be easily identified on day 55 of pregnancy (Skidmore, 2000). Therefore, the embryo was easily recognizable as having identifiable features by day 40 post-mating (Vyas et al., 2002). In the study of Ali et al. (2013), ossification of the head, ribs, and vertebrae was first detected at an interval from 7 to 9 weeks of pregnancy period. In bovine, CRL can be used Between 31-83 d of gestation (Somnuk et al., 2017).

Trunk diameter

The fetal trunk diameter (TD) was within the scope of ultrasound scanning during the first trimester of pregnancy. During this interval, TD showed gradual increase by advancing gestation period from 6 up to 17 wk (Fig. 2 and Plate 2). Al-Rawi (2014) reported that trunk was clearly visible between days 40 and 45 post-mating. This was indicated in the present study, whereas trunk was within the scope of ultrasound scanning during the first trimester of pregnancy. Similar trend was reported on camels by Ali et al. (2013) by estimating abdominal diameter between the 8th and 15th week of pregnancy in camel, being 2.46 cm at 12 weeks, while Al-Rawi (2014) found that the diameter of the camel embryo was about 10 mm on day 35 post-mating

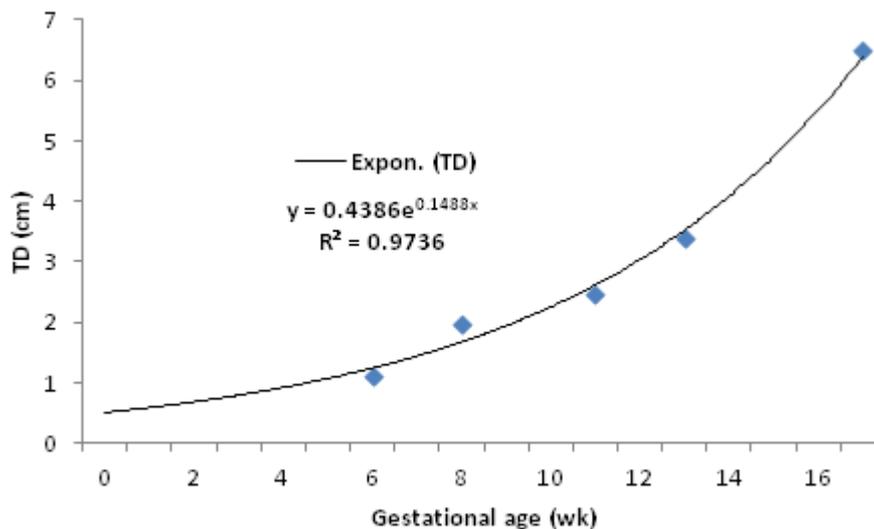


Fig. 2. Growth curve of fetal trunk diameter (TD) at consecutive gestational ages (day).

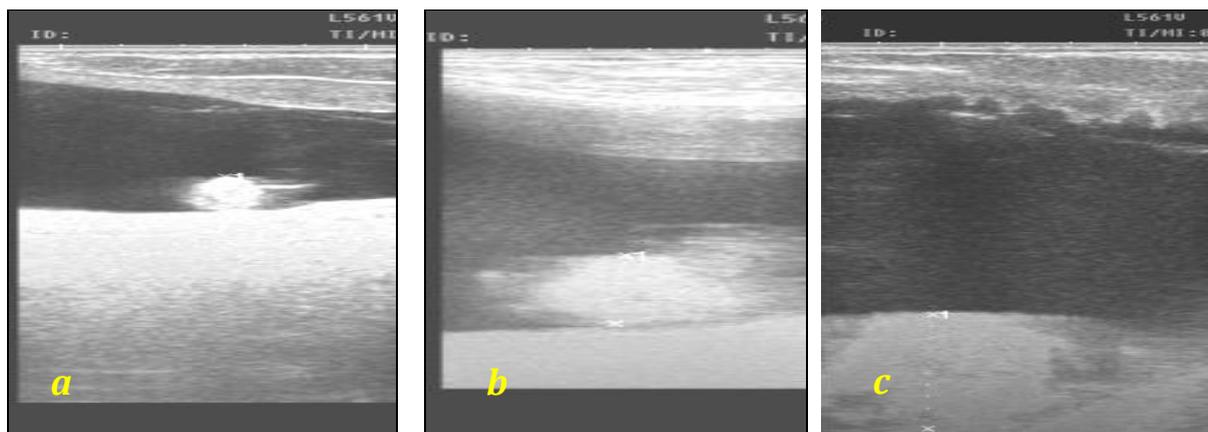


Plate. 2. Ultrasonographic images of She-camel showing trunk diameter at 45, 76 and 121 days of pregnancy, respectively.

The exponential, linear and logarithmic functions were derived to explain the fetal age-TD relationship in dromedary camel and the respective R^2 values were 0.973, 0.756 and 0.836, respectively. The exponential regression equation gave the best fit ($R^2 = 0.973$) for the unique measurement of gestational age. It is quite evident from the analysis and curve fitting that the exponential function explained TD in camel fetus for the entire pregnancy period to the extent of 97.3%, hence the equation $y = 0.438e^{0.148x}$ can reliably be utilized for the prediction of TD of camel fetus at calving, where $y = TD (cm)$ and $x = fetal\ age (wk)$. In comparable with the present results, **Ali et al. (2013)** found that the abdominal diameter developed linearly, being in high correlation with fetal age during the interval from 8 to 14 wk of fetal age, but the accessibility for abdominal diameter in camels during the total gestational period was 12.8%. Also in bovine, trunk width significantly increased in linear pattern throughout 2nd and 3rd trimesters of gestation (**Lazim et al., 2016**) and trunk width had positive correlation with fetal age in other ultrasonographic studies that used trans-rectal (**Kähn, 2004**) and trans-abdominal (**Hunnam et al., 2009**) transducer.

Bi-parietal diameter

From the 1st up to the 2nd trimester of pregnancy (8-31 wk), BPD showed marked increase by advancing gestation period from 57 up to 220 days (Fig.3 and Plate 3). Similar trend was reported by **Ali et al. (2013)** by estimating BPD between the 8th and 15th week of pregnancy in camel, estimating 3.62 cm at 15 weeks of pregnancy period. On days 40 and 45, head length was about 0.8 cm (**Al-Rawi, 2014**).

The exponential, linear and logarithmic functions were derived to explain the fetal age-BPD relationship in dromedary camel and the respective R^2 values were 0.970, 0.867 and 0.885, respectively. The exponential regression equation gave the best fit ($R^2 = 0.970$) for the unique measurement of gestational age. It is quite evident from the analysis and curve fitting that the exponential function explained BPD in camel fetus for the entire pregnancy period to the extent of 97%, hence the equation $y = 0.545e^{0.081x}$ can reliably be utilized for the prediction of BPD of camel fetus at calving, where $y = BPD (cm)$ and $x = fetal\ age (wk)$.

In different breeds of sheep, the linear regression function explained BPD and analysis of data showed a high correlation between BPD and gestational age with a R^2 of 0.75-0.88 in dependence of the sheep breed (**Vencato, et al., 2013**). In buffalo, **Ferreira et al. (2012)** observed the highest regression and correlation coefficients between CRL and gestational age, being also in linear function ($R^2 = 0.971$).

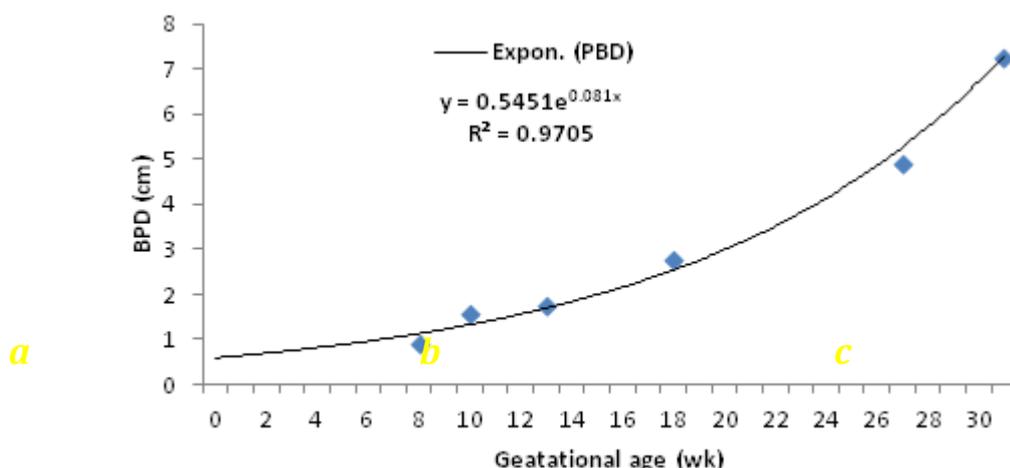


Fig. 3. Growth curve of fetal bi-parietal diameter (BPD) at consecutive gestational ages (day).

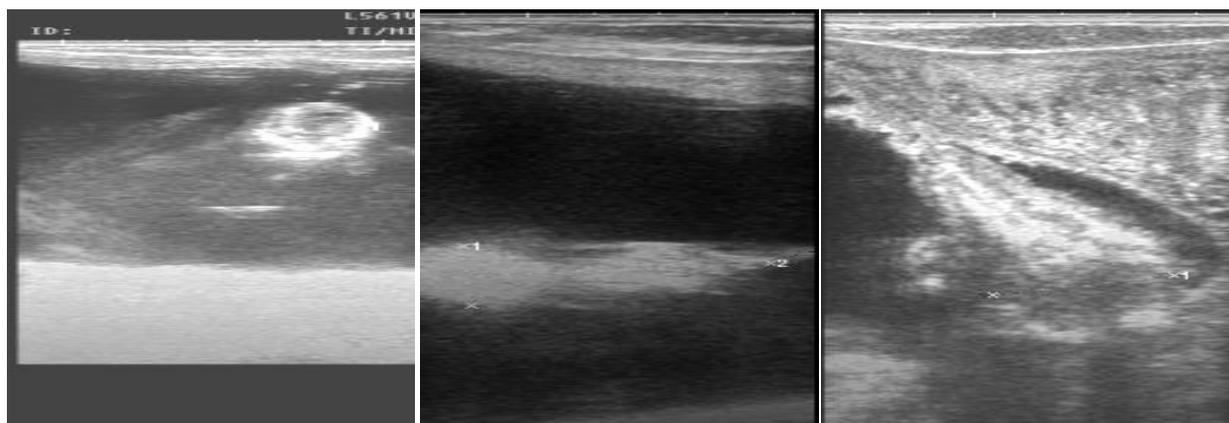


Plate 3. Ultrasonographic images of She-camel showing bi-parietal diameter at 72, 94 and 128 days of pregnancy, respectively.

Eye ball diameter

During the 1st and 3rd trimester up to of pregnancy, EBD showed continued increase by advancing fetal age from 12 up to 49 wk (Fig. 4 and Plate 4). According to **Al-Rawi (2014)**, eye ball appears as anechoic spot within the head on days 40 and 45 post-mating. Similar trend was reported by **Ali et al. (2013)** by estimating BPD between the 8th and 15th week of pregnancy in camel. The optic area in camel fetus was first identified on day 40 post-mating. The optic area was a non-echogenic spherical structure. This area was surrounded by an echogenic bony socket. The nonechogenic structure might perhaps have been due to the presence of the optic lens (**Vyas et al., 2002**).

The exponential, linear, logarithmic and functions were derived to explain the fetal age-BPD relationship in dromedary camel and the respective R^2 values were 0.977, 0.983 and 0.969, respectively. The linear regression equation gave the best fit ($R^2 = 0.997$) for the unique measurement of gestational age. It is quite evident from the analysis and curve fitting that the linear function explained EBD in camel fetus for the entire pregnancy period to the extent of 98.3%, hence the equation $y = 0.075x$ can reliably be utilized for the prediction of EBD of camel fetus at calving, where $y = \text{EBD (cm)}$ and $x = \text{fetal age (wk)}$. EBD at which a high accuracy level in the estimation of gestational age using ultrasonography is realizable in camels as reported by **Ali et al. (2013)**, who indicated liner regression of EBD during the interval from 10 to 18 wk or from 35 to 55 wk of the gestation period. The highest correlation was found with EBD during the third trimester of pregnancy. Accessibility during the total gestational period was 38.3% for EBD 2.

Based on the results of the present study, CRL, TD, BPD and EBD were reliable parameters to predict gestational age in Maghrebian camels. In the current study CRL and EBD had strong positive correlation ($R^2 = 0.985$ and 0.983) for pregnancy interval of 4-14 wk and 12-49 wk, respectively. Based on these findings, CRL as a fetal measure appeared as the most reliable predictor of the gestational age at the 1st stage of pregnancy and may be used the scientists, veterinarians and animal breeders for all practical purposes. By ultrasonographic estimation, **Ali et al. (2013)** reported that CRL was accessible between the 3rd and the 9th week of gestation,

thereafter it became beyond the scope of the transducer and was unable to be monitored completely on the screen. Accessibility during the total gestational period of camels was 35/329 (10.6%) for CRL. A high correlation was also found between gestational age and CRL during the first trimester of pregnancy. Also, EBD estimation during the 3rd trimester of pregnancy could provide a relatively easy means for the prediction of gestational age in camels.

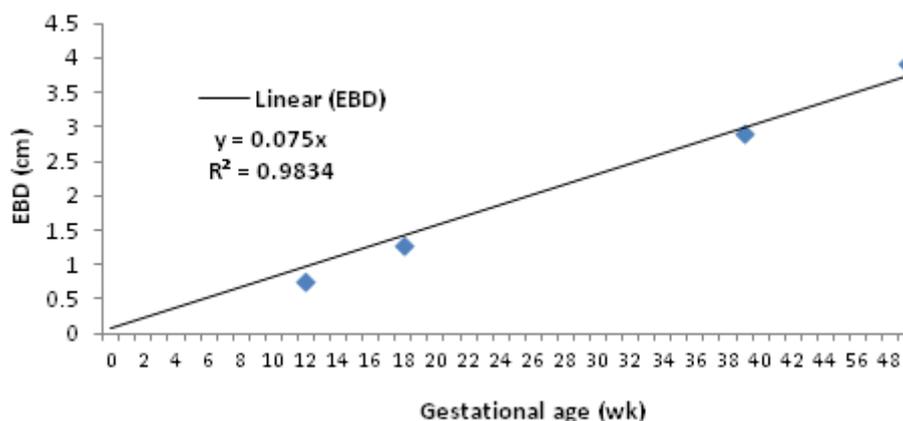


Fig. 4. Change in eye ball diameter (BPD) at early and late gestational ages (day).

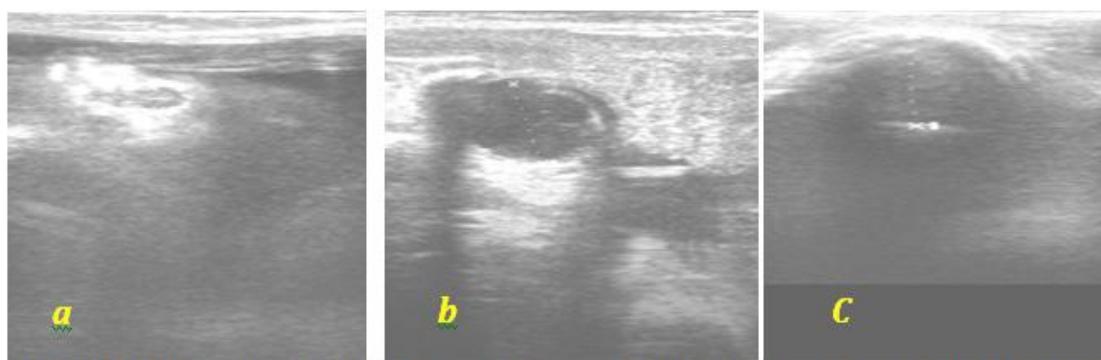


Fig. 4. Ultrasonographic images of She-camel showing eye ball diameter at 88, 268 and 345 days of pregnancy, respectively.

Progesterone (P4) and estradiol-17β (E2) profile

Results revealed different trends of change between both of serum P4 and E2 levels and each fetal measurement, being inconsistent for CRL estimate (Fig. 5a), more parallel with TD (Fig. 5b) and BPD (Fig. 5c), and slightly parallel with EBD (Fig. 5d).

Regarding change in steroid hormones during different gestation week from 4 up to 560 wk, serum P4 concentration was significantly ($P < 0.05$) lower at gestational interval from 4-6 up to 13-14 wk (1st trimester), moderate at 17-18 wk, increased significantly ($P < 0.05$) at 27-38 wk, showing the highest values at 50 wk (end of the 3rd trimester). However, serum E2 level showed significantly ($P < 0.05$) the 1st increase at 8-10 wk, showing insignificant change up to 13-14 wk, then significantly ($P < 0.05$) increased, being the highest at 50 wk of gestation period (table 1).

Steroids are produced by fetal ruminant ovaries (Tanaka et al., 2001). Studies on P4 concentrations during pregnancy indicated that CL is the primary source of P4 in 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). The placental origin of E2 seems more likely as it has been shown that extra embryonic membranes of the camel conceptus possess considerable aromatizing capacity from as early as day 10 post-ovulation and the endometrium also has the ability to conjugate the free E2 (Skidmore et al., 1985).

The present results indicated positive relationship between P4 concentration and gestational age, but no significant association between fetal age and P4 secretion in bovine (Yang and Fortune, 2008). In accordance with the present results, plasma P4 concentration in camels increased from 1.5 to 3.68 ng/ml during the interval from 5 to 55 days post-partum (Mostafa et al., 2017), and to 2 ng/ml during early months of pregnancy, and to 5.8 ng/ml over a period of 8 mo, but followed by a strong decrease during the last two months before calving

(Bakheit et al., 2012). Also, average P4 concentration increased during pregnancy period, being 5.87-12.07 ng/ml from the 23rd wk to the end of gestation and 2.88-5.09 ng/ml between 23rd to 32nd wk of gestation, then (Deen et al., 2007). Moreover, serum P4 concentration was 3.4 ng/ml at 3-8 days post-ovulation, and ranged between 3 and 5 ng/ml for the first 90-100 days of gestation, then slightly decreased to 2-4 ng/ml until day 300 of pregnancy. A further slight decrease then occurs over the next 70-80 days followed by a rapid drop to <1 ng/ml on the day before or at parturition (Skidmore et al., 1996). These findings may suggest the presence of a lutetrophic factor of embryonic origin (Maria do Carmo Feliciano et al., 2003).

Although, Yang and Fortune (2008) reported a significant negative correlation between E2 and gestational age in bovine, the present results showed a positive relationship between E2 and gestation period in camels. In agreement with the obtained trend of change in E2 level, average daily E2 profiles in camels were found to be low or basal (0.69-8.24 pg/ml) during pregnancy period. Average concentration of E2 was low up to 38th weeks of gestation, then started to increase slowly and steadily after the 39th weeks, being more than 50, 100, 250, 300 and 375 pg/ml at the 42nd, 45th, 47th, 49th and 52nd weeks of gestation, respectively (Deen et al., 2007). Also, serum E2 concentrations showed a first definite increase around day 20-25 post-ovulation and continue to rise until concentrations of 100 pg/ml on days 60-70 in humped camels, (Elias et al., 1984). This increase in rate of E2 secretion could be ovarian or placental in origin.

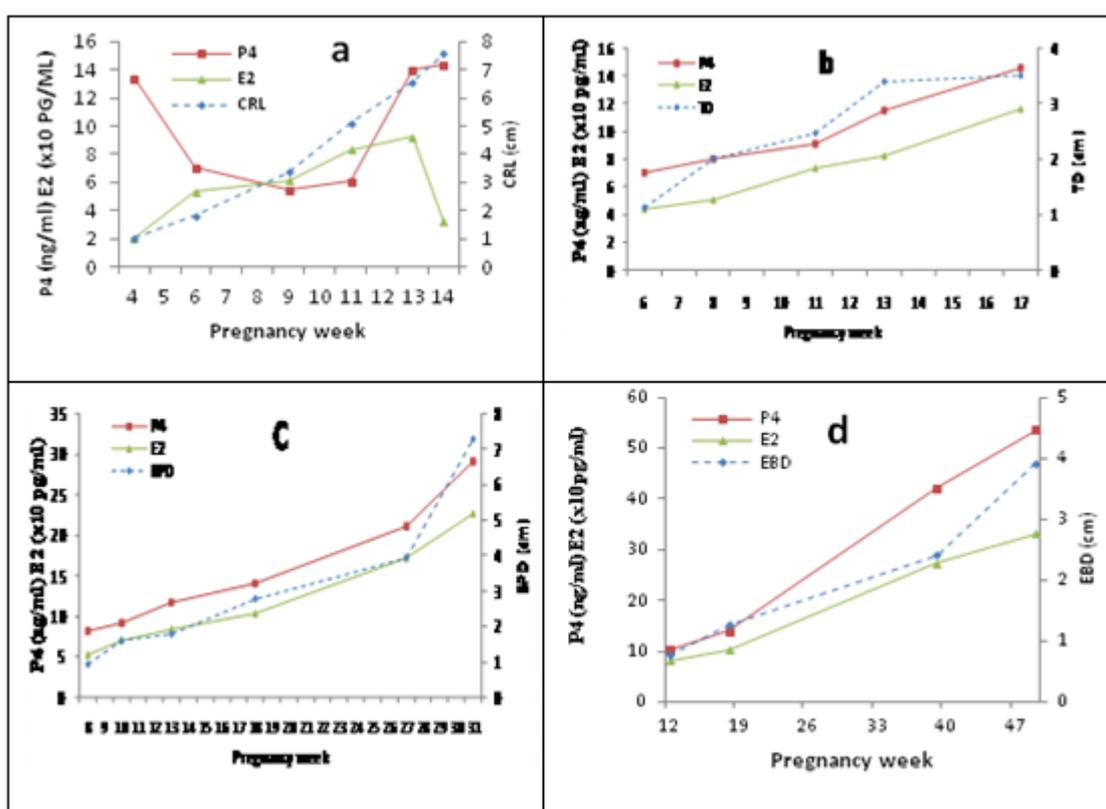


Fig. 5. Relationship between P4 or E2 level and fetal measurements including CRL (a), TD (b), BPD (c) and EBD (d) throughout consecutive gestation week interval.

Table 1. Concentration of progesterone and estrogen in blood serum of Maghrabian She-camels at different week intervals of pregnancy period.

Pregnancy week interval	P4 (ng/ml)	E2 (pg/ml)
4-6	9.15±1.5 ^d	30.91±6.8 ^c
8-10	7.69±0.56 ^d	50.89±3.5 ^d
11-12	9.85±1.18 ^d	80.27±2.8 ^d
13-14	12.5±0.64 ^d	60.64±12.1 ^d
17-18	14.2±0.14 ^c	100.8±2.9 ^c
27-38	30.9±4.31 ^b	220.4±20.6 ^b
50	53.6±0.21 ^a	330.2±9.7 ^a

Means denoted within the same column with different superscripts are significantly different at $P < 0.05$.

An ovarian origin could be justified by the fact that follicular activity in the dromedary is not inhibited until 6 mo of pregnancy although mature follicles (<10 mm) are not generally found beyond 105 days of pregnancy (El-Wishy et al., 1981; Musa and Abusineina, 1978). The timing of E2 increase in the final months in the pregnant dromedary coincides with the important period of increase in fetal weight and fluid volume between 9 and 12.5 mo (El-Wishy et al., 1981) giving rise to the possibility of placental E2 being important for fetal growth.

IV. Conclusion

The correlation between gestational age and fetal measurements (CRL and EBD) were higher than those of TD and BPD, and the highest correlation was between CRL and gestational age in camels. There were no association between gestational age and concentration of P4 or E2, but both hormones levels showed somewhat positive relationships with TD, BPD and EBD, not with CRL.

In clinical practice it is useful to predict gestational age in order to organize pregnancy assistance or to obtain the expected date of calving after fetal completion. Ultrasonography monitoring the pregnant camels can be used to follow fetal development using serial measurements of somatic measurements such as CRL during the 1st trimester, and EBD during the 3rd trimester, while TD or BPD are appropriate fetal measurements during the 2nd trimester of pregnancy period in camels. Feed supplementation may be suggested to ensure a sufficient nutritional contribution to sustain the fetal growth, so important information during different stages of pregnancy period, including fetal measurements may allow giving adjusted nutritional requirements during the last trimester of pregnancy, could be very important in the management of the reproduction, and to estimate the delivery date.

In conclusion, the overall data indicated the value of ultrasonographic fetal characteristics in camels for the evaluation of fetal development, estimation of gestational age during the entire length of pregnancy period or as a relatively easy means for observing the events of pregnancy for research purpose and a practical and economic tool for breeders.

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