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SALIVARY SEX STEROID HORMONES IN FEMALE BACTRIAN CAMELS (*Camelus bactrianus*) DURING DIFFERENT REPRODUCTIVE STAGES

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ABSTRACT

The study aimed to verify the usage of salivary sex steroid hormones monitored in captive bactrian camels (*Camelus bactrianus*) as a new non-invasive method in this species. Saliva of 5 adult female camels housed in the Prague zoological garden were sampled for more than 1 year in maximum interval of 10 days and concentrations of progesterone (P_4) and oestradiol (E_2) were measured. The concentrations of P_4 (n = 312) and E_2 (n = 310) were both significantly ($p < 0.0001$) affected by pregnancy status of animals. Mean (±SE) P_4 concentrations in non-pregnant stages were 2.234 (±0.220) nmol/l, while during the 2nd third of pregnancy it was 5.105 (±0.858) nmol/l. E_2 concentrations differed significantly between non-pregnant stages with mean value 0.037 (±0.005) nmol/l and during the 3rd third of pregnancy when reached 0.098 (±0.012) nmol/l. The seasonal differences in non-pregnant female camels were also evaluated. While no significant seasonal deviations were found in E_2 concentrations, P_4 values were significantly higher in summer than in spring. The study concluded that salivary P_4 and E_2 measurements are suitable for monitoring different reproductive stages in half-tamed female camels. The autumn and winter seasons seemed to be the best for pregnancy diagnosis in camels bred in Europe.

Key words: Camelid, oestradiol, pregnancy, progesterone, saliva

All studies focused on hormonal changes in camels were carried out so far only from blood, e.g. study of Elias *et al* (1984), Zhao *et al* (1994), Skidmore *et al* (1996a, 1996b), or from milk (Abdel Rahim and Elnazier, 1987; Abdel Rahim, 1989). The hormonal changes in serum or plasma have been already well documented in female camels. Oestradiol, also reported as oestradiol-17β, (E_2) and progesterone (P_4) concentrations were often used for monitoring the reproductive cycle and pregnancy in dromedary camels (*Camelus dromedarius*) (Skidmore *et al,* 1996a, 1996b; Ayoub *et al,* 2003; Muhammad *et al,* 2011) but also in bactrian camels (*Camelus bactrianus*) (Zhao *et al,* 1994; Zhao *et al,* 1998) with similar results. Nevertheless, camels are seasonal breeders (Al-Hazmi, 2000, Nowshari and Ali, 2005; Ali *et al,* 2008; El-Harairy and Attia, 2010; El-Harairy *et al,* 2010) and hormonal changes in non-pregnant animals can also be influenced by a season of the year (Agarwal and Khanna, 1993; Al-Qarawi *et al,* 2000; El-Harairy and Attia, 2010). The breeding season depends on area of distribution, climate, temperature, humidity, day light length and rainfalls (Bono *et al,* 1989; El-Harairy and Attia, 2010). The average gestation period is between 370 and 385 days in dromedary camels (Skidmore *et al,* 2000; Musa *et al,* 2006; Al-Sobayil, 2008) and 402.22 ± 11.5 days in bactrian camels (Zhao *et al,* 1994).

Camels are able to produce large amount of saliva (Haberová *et al,* 2012; Wemmer and Murtaugh, 1980; von Engelhardt *et al,* 2006) and monitoring of reproduction through saliva has been already confirmed in other ungulate species, e.g. in buffaloes (*Bubalus bubalis*) (Qureshi *et al,* 1999), black rhinoceros (*Diceros bicornis*) (Czekala and Callison, 1996) or Indian rhinoceros (*Rhinoceros unicornis*) (Gomez *et al,* 2004).

The aim of the study was to verify the potential of steroid hormones monitoring in camels from saliva as a new non-invasive method in bactrian camels and evaluate the seasonal changes in non-pregnant female camels kept in European zoological garden.

Materials and Methods

Animals and husbandry

Five adult female bactrian camels (*Camelus bactrianus*) housed in the Prague zoological garden, Czech Republic, aged between 14-24 years were included in the research. Females were kept together

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with male for the whole time and these were able to mate freely, so the time of conception was not known. Each third of pregnancy was defined retrospectively from parturition and it is represented according to respective days based on overall gestation length. The term of parturition was used for data analysis. At least ⅓ out of every detected pregnancy (n =5) was monitored during the sampling period. Non-pregnant stages were noticed in 4 females.

Animals were fed by meadow hay, fresh vegetable, pellets for herbivores, and oats for the majority of year. Fresh fodder was provided during some parts of spring. Animals were trained for human contact, enabling touching and feeding from hand but another type of manipulation was not possible.

Sample collection and hormonal assay

Samples were collected from 3rd February 2011 to 27th February 2012. The interval between samplings was 2 or 3 days during the period between December and March, 7 days during April, May and November, and 10 days between June and October.

The whole herd was lured close to the fence by showing a bucket with dried bread. Usually, whole herd ran up to the sampling place immediately and animals were showing their willingness to cooperate. The sampling was carried out through the bar fence. In selected animal, the production of saliva was stimulated by feeding a piece of dried wheat or wheat-rye bread without any pieces of salt or cereals. When the camel was trying to get to the bread, saliva was wiped off from the tongue by the handle of a disposable plastic spoon. Saliva was collected in the crease of the spoon handle and was removed to the polypropylene centrifuge tubes of volume 15 ml. The process was repeated several times until the amount of foamy saliva reached 5 ml. The animal was rewarded by piece of bread.

The test tubes with saliva were stored in temperature between 4 – 8°C and transported to a freezer (-20°C) within 12 hours after the sampling. The concentrations of P_4 and E_2 were measured in the Institute of Endocrinology (Prague, Czech Republic). The total unconjugated 17β-oestradiol was measured by the RIA Spectria Estradiol kit® (Orion Diagnostica Oy, Finland) and P_4 by the Progesterone RIA kit (Immunotech, A Beckman Coulter Company, France). The salivary samples were processed according to the guidelines of the kits' producers and by the same methods as samples of serum.

All animals included in the research cooperated of their own free will and they were not forced to sampling. The research was approved by the management of the Prague zoological garden and also by the veterinarian of this institution.

Data analysis

Stages of pregnancy were counted from the term of parturition. One pregnancy ended by abortion and the term of possible parturition was counted from Crown Vertebral Rump Length (CVRL) of the aborted calf (Elwishy *et al,* 1981; Bello *et al,* 2012; Hena and Sonfada, 2012). The thirds of camel pregnancy were based on the study of Bello *et al* (2012): 130 days or less to parturition were classified as 3rd ⅓, 131 to 260 days to parturition as 2nd ⅓ and 261 days or more as 1st ⅓.

Periods of the year were classified according to meteorological reckoning: spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February) (Barnett and Dobson, 2010).

The statistics were performed using software Statistica CZ 12 (Stat Soft, Inc.). The data distribution was not normal ($p < 0.01$; Lilliefors test and Shapiro–Wilk test) and the non-parametric statistics (Kruskal-Wallis test, Multiple comparison of p-values, Mann–Whitney U test) were used to analyse the results. The significance level was accepted at $p < 0.05$.

Low, undetectable concentrations of P_4 (n=16) were obtained mainly (75.0%) in non-pregnant animals. E_2 concentration under detectable level was noticed only in one sample. These samples were analysed as zero concentrations. Two samples overreached a maximum measurable E_2 concentration and these samples were excluded from the analyses.

Results

312 samples of camel saliva were collected and successfully analysed. The concentrations of P_4 (n = 312) and E_2 (n = 310) were both significantly ($p < 0.0001$) affected by pregnancy status of animals (Kruskal-Wallis test: H (4, N = 313) = 27.1809 and H (4, N = 311) = 39.3600, respectively). Mean P_4 and E_2 levels during different stages of reproduction are provided in Table 1.

Hormonal changes during pregnancy

Mean P_4 concentrations are shown in Fig 1. P_4 concentrations fluctuated during the pregnancy and the peak, averaging 6.06±2.90 nmol/l, was reached between the 150th and 126th day before parturition (BP). The mean P_4 concentrations fell to 3.61±0.75 nmol/l by the last 50 days of pregnancy.

As shown in Fig 2, the E_2 concentrations increased rapidly 175 days BP and reached the 1st

peak, averaging 0.115±0.060 nmol/l, between the 150th and 126th day BP. The E_2 increased significantly (Mann-Whitney U Test, U = 447, $p < 0.01$) again 50 days BP and reached the values of 0.120±0.022 nmol/l in the last 25 days of pregnancy.

Hormonal changes in non-pregnant animals during the year

In non-pregnant animals, the P_4 values were significantly affected by the season (Kruskal-Wallis test: H (3, N = 114) = 10.2520, p = 0.0165). P_4 concentrations were significantly higher in summer than in spring (Multiple comparison of p-values, p = 0.0345) and as shown in Fig 3, similar trend visible for the winter season (Multiple comparison of p-values, p = 0.0522). The concentrations during autumn and winter stayed always under 6.5 nmol/l.

P_4 concentrations differed significantly also between months (Kruskal-Wallis test: H (11, N= 114) = 27.0522, p = 0.0045) and the highest mean concentrations were reached in July and August (4.248 and 5.055 nmol/l). The lowest mean P_4 concentrations were noticed during February (1.159 nmol/l).

However, no significant seasonal or monthly differences ($p > 0.05$) were found in E_2 concentrations.

Discussion

The study is the first report of salivary sex steroid hormone monitoring in camels and also the first study focused on hormone monitoring in camels bred in Europe. Despite the fact that the number of studies focused on hormonal analysis from saliva in animals is limited and concentrations of hormones in saliva are fluctuating (Hofman, 2001; Kobelt *et al*, 2003), the presented results showed that salivary P_4 and E_2 are suitable for monitoring of different reproductive stages in half-tamed female camels similar as in other ungulate species, e.g. study of Czekala and Callison (1996), Moriyoshi *et al* (1996), Qureshi *et al* (1999) and Gomez *et al* (2004).

Mean P_4 and E_2 concentration changes during the pregnancies in presented study had similar pattern as in study of Skidmore *et al* (1996a) or Zhao *et al* (1998) dealing with blood samples of camels. A positive correlation between salivary and serum

Table 1. Salivary progesterone and oestradiol concentrations in female camels during different phases of reproduction.

	Progesterone (nmol/l)		Oestradiol (nmol/l)	
	Mean ± SE	**Range**	**Mean ± SE**	**Range**
Non-pregnant (n = 114)	2.2339±0.2197[a]	0.0000 - 14.4100	0.0374±0.0049[a]	0.0010 - 0.3620
Post-partum period (n = 14)	1.5193±0.3057[a,b]	0.0000 - 3.3600	0.0500±0.0099[a,b]	0.0120 - 0.1630
1st third of pregnancy (n = 66)	2.5527±0.3346[a,b]	0.0000 - 11.6400	0.0564±0.0086[b]	0.0000 - 0.3680
2nd third of pregnancy (n = 29/28)	5.1045±0.8584[c]	0.3600 - 23.3100	0.0693±0.0181[a,b]	0.0060 - 1.6500
3rd third of pregnancy (n = 90/89)	4.3357±0.5348[b,c]	0.0000 - 26.2700	0.0978±0.0118[b]	0.0078 - 0.6149

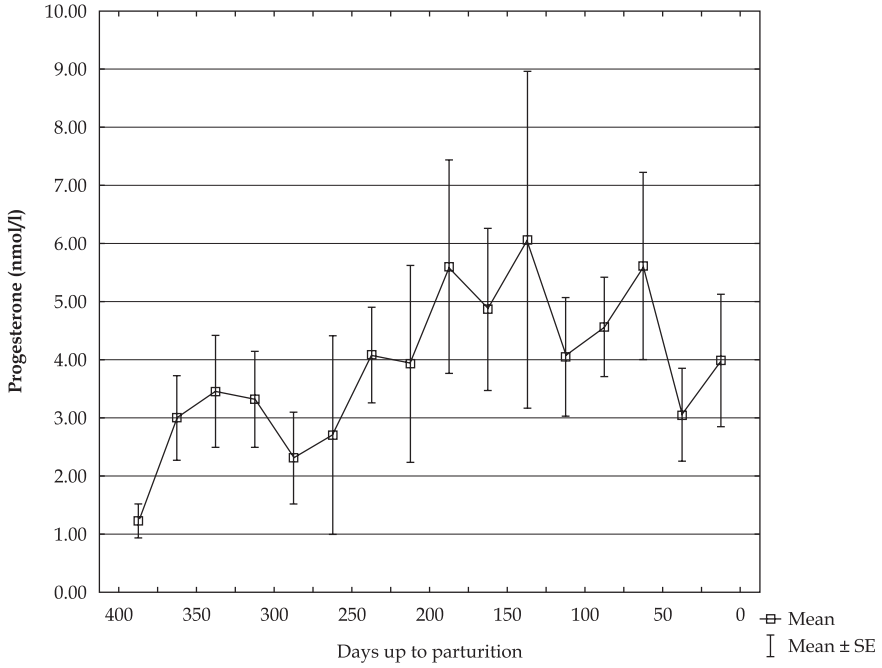

Fig 1. Mean ± SE progesterone concentrations in camel saliva during pregnancy.

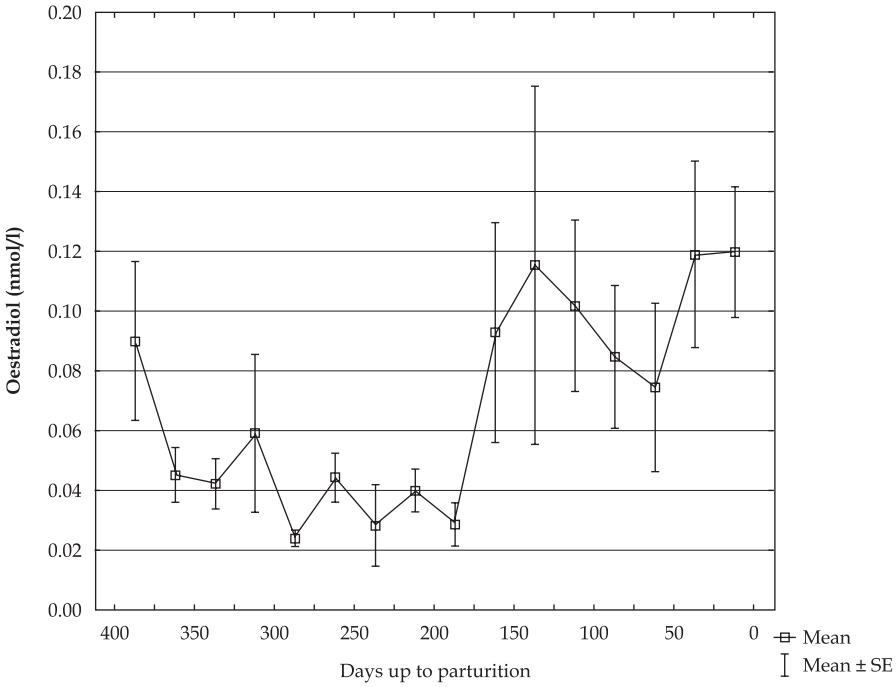

Fig 2. Mean ± SE oestradiol concentrations in camel saliva during pregnancy.

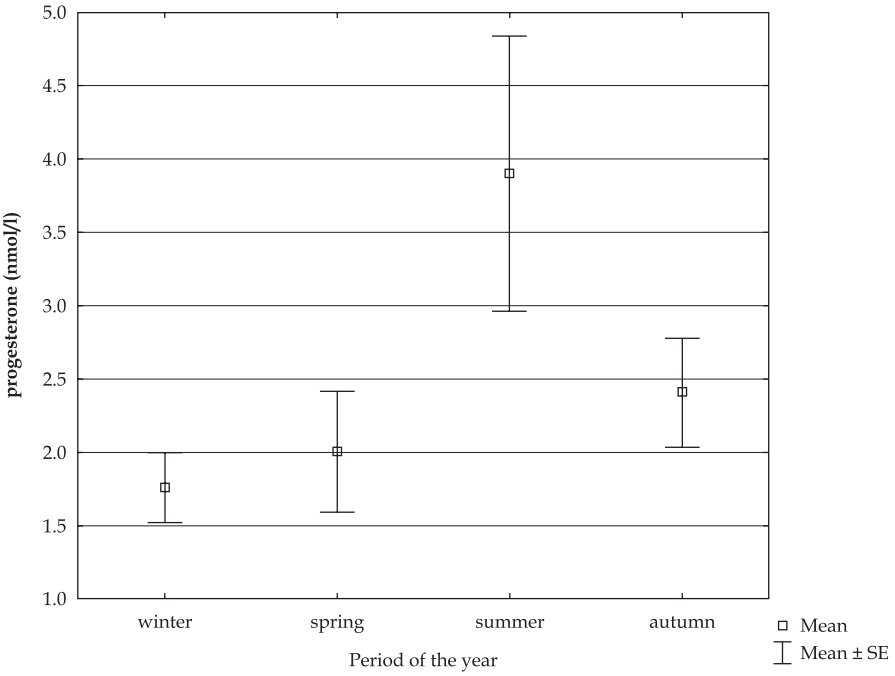

Fig 3. Mean ± SE progesterone concentrations in saliva of non-pregnant female camels during different periods of the year.

oestradiol was already reported by Qureshi *et al* (1999) in buffaloes and the fact that serum hormonal concentrations correlated with concentrations in saliva was well proved in human (Hofman, 2001).

Salivary sex steroid hormones concentrations seemed not to be suitable for early pregnancy diagnosis in camels because no significant differences between pregnant and non-pregnant animals were found. However, with repeated sampling, it could be possible to determine pregnancy during the 2nd or 3rd third of pregnancy. Contrary to research of Volkery *et al* (2012) carried out on alpacas, this study confirmed the significant differences between P_4 concentrations at these stages of pregnancy and non-

pregnant animals. Some results evaluated in the first 2 months of pregnancy could also be misrepresented because it was not possible to determine exact time of conception. Higher E_2 concentrations noticed in the beginning of pregnancy (Fig 2), between 375th and 400th day before parturition, were also probably caused by the impossibility to know the exact day of conception and some animals could be in oestrus in that time.

Contrary to Hussein *et al* (2008), seasonal difference in P_4 concentrations were proved non-pregnant animals. Higher P_4 concentrations during the spring and summer could be caused by unrecognised early embryonic losses that are common in camels (Nagy and Juhasz, 2008); some animals could conceived during spring but they did not give a birth due to embryonic loss and they could be incorrectly assessed as non-pregnant.

The majority (89.4%) of parturitions of camels kept in the Prague zoological garden occurred between February and May (Haberová and Fedorov, 2012), so autumn or winter periods belongs usually to 2nd or 3rd third of pregnancy in camels. This fact, together with the finding that P_4 stayed at lower concentrations during autumn and winter in non-pregnant animals instigate to the conclusion that these 2 periods of the year seem to be the best for pregnancy diagnosis in camels in Europe or temperate regions of northern Hemisphere.

Present study did not prove any significant variation in mean values of E_2 in non-pregnant animals between different months or seasons similar like Hussein *et al* (2008) but the maximum fluctuations were reached during February, March, June and December. This feature could be caused to the presence of heat in camel females (Skidmore *et al*, 1996a) which was not possible to detect in presented study and selected breeding facility.

In conclusion, (1) hormonal analyses of saliva are suitable for reproductive status monitoring in female camels and more detailed research at this topic can be recommended. (2) Both salivary steroid hormones, oestradiol-17β and progesterone, can be recommended for pregnancy diagnosis during the 2nd and 3rd third of camel pregnancy. (3) Autumn or winter seems to be the best periods for pregnancy diagnosis in camels in Europe.

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