Egypt. J. Vet. Sci. **Vol. 45-46** pp. 41-53 (2014 - 2015)

Thyroid and Sex Hormones in Serum of Pregnant and Non Pregnant Camels *(Camelus dromedaries)*

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THIS STUDY describes difference in thyroid hormones and sex hormones in pregnant and non pregnant camels. Female camels (n=12) of different ages brought from Sudan and belonged to a private camel farm underwent rectal ultrasound scanning for detection of early pregnancy. Male and females were kept together in the same barn during the breeding season. Blood was collected and subjected to hormonal analysis. Estradiol, progesterone, testosterone, T_3, and T_4 Insulin were assayed. Results revealed that levels of T_3 and T_4 were significantly high in pregnant camels. While, low significant T_4:T_3 was observed in pregnant camels. Progesterone levels significantly increased in pregnant camels and its values ranged from 2.83 to 6.89 ng/ml. Both testosterone and estradiol slightly increased during early pregnancy. In conclusion, progesterone still the most important confirmatory hormone for detecting pregnancy in camels even thought after the advent of ultrasound examination because pregnancy can't be detected by either trans-rectal ultrasound or rectal palpation beyond 90 days of gestation. It appeared that thyroid hormones play important role during camel's gestation.

Keywords: Pregnancy, Insulin like growth factor-I, Thyroid hormones, Steroid hormones, Camels.

Light colored camels in Egypt are one humped. Most light colored camels are imported from and through Sudan (El-Wishy, 1988). The one humped camel (*Camelus dromedarius*) is a seasonal breeder with a relatively short breeding period, when pre-ovulatory size follicle is exhibited (Tibary and Anouassi, 1997). The breeding season starts from December to August every year in Egypt (Wilson, 1989). Regarding reproduction, all camelids are induced ovulators, usually ovulating only after mating, and if the camel does not conceive the corpus luteum has a very short lifespan of only 8 – 10 days (Marie & Anouassi, 1987 and Skidmore, 2005).

Ultrasound is being used in she-camel for studying ovarian dynamics (Skidmore *et al.,* 1995 and Tibary & Anouassi, 1996) and detection early pregnancy (Skidmore *et al.,* 1992).

Hormones, enzymes and hemograms have been described in the blood of female camels during different reproductive statuses including the estrous cycle (Elias *et al.,* 1984 a,b and Homeida *et al.,* 1988), pregnancy (Skidmore *et al.,*1996 and Zhao *et al.,* 1998) and postpartum period (Agarwal *et al.,* 1992).

The thyroid gland predominantly secretes the pro-hormone T_4 but also produces smaller amounts of the active hormone T_3. However, T_4 must be activated by deiodination to T_3 in order to initiate thyroid hormone action. Most plasma T_3 is derived from peripheral conversion of T_4. This reaction is catalyzed by the type 1 and 2 iodothyronine deiodinases (Bianco *et al.,* 2002). Luteal cells of bovine mature corpora lutea are also involved in the synthesis of thyroid hormones, which may modulate progesterone synthesis, acting in an autocrine and paracrine way (Mutinati *et al.,* 2010).

Human placenta secretes transthyretin, which is a serum transport protein for T_4, into maternal and fetal circulations that can be taken up by trophoblasts and translocated to the fetal circulation (Mortimer *et al.,* 2012).

Studies on circulating levels of Insulin Like Growth factor-I were not available in dromedary camels so the purpose of this study was to characterize its levels in blood serum of early pregnant and non pregnant camels in addition to thyroid and ovarian hormones and find out the relation between them.

Material and Methods

Animals

Twelve female camels of different ages brought from Sudan and belonged to a private camel farm underwent this study. Females were kept with males in open yards and subjected to natural day light and temperature. Camels were fed in groups and clean water was available *ad libitum*. Camels were scanned with endorectal ultrasound for detection of pregnancy in squatting position.

Blood sampling

Blood samples from the jugular vein of 9 pregnant from days 30 to 90 of gestation with each ultrasound examination and from 3 non-pregnant camels previously examined by ultrasound were collected in plain vacationers and were transported to laboratory. Then blood sera was harvested and stored at -20°C until hormonal assay.

Hormonal assay

T_3 and T_4 (Monobind, Lake Forest, CA 92630, USA) were assayed using ELISA diagnostic kits. The limit of sensitivity, intra- and inter-assay coefficients of variation was 0.04ng/ml, 3.9%, 8.9%for T_3 (Kozwich *et al.,* 1991), 0.4µg/dl, 4.4%, 8.3% for T_4 (Charkes, 1996). Chemiluminescence Enzume immunoassay (CLIA) was used to assay estradiol (E2, Ratcliffe *et al.,*1988), quantitative solid phase enzyme linked immunosorbent assay (Immunospec corporation, 7018 Owensmouth Ave, Suite 103 Canoga Park, CA, 91303) was used to measure progesterone (P4, Kakabakos and Khosravi, 1992) and total Testosterone

(Sanchez-Carbayo *et al.*, 1998). Sensitivity, intra- and inter- assay coefficients of variation were 0.05 ng/ml, 6.4% and 10.9% for P4 and 5.0 pg/ml, 3.9% , 10.1% for E2 and 0.1 ng/mL, 11% and 13 % for total testosterone.

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Statistical analysis of the data was performed using SPSS® software (SPSS, 2007). Data was subjected to Independent sample t-test.

Results

Pregnancy was detected from 30 to 90 days with ultrasound. Pregnancies >90 days were confirmed by absence of detectable non pregnant uterus and progesterone >2ng/ml. T_3 levels are significantly (P=0.036) high in pregnant camels (3.51±0.23) compared to non pregnant camels (2.16±0.52). As well as T_4 levels are significantly (P=0.025) high (10.24±0.31) in pregnant camels compared to non pregnant ones (8.34±0.37). T_4: T_3 are significantly (P=0.049) high in non pregnant camels. Mean estradiol (E2) and testosterone (Testo) are not significantly different between pregnant and non pregnant camels, but their levels slightly increased in pregnant camels (Table 1). Progesterone (P4) levels increased significantly (P=0.007) in pregnant camels (4.60±0.40) and its values ranged from 2.83 to 6.89 ng/ml.

TABLE 1. Mean concentrations of IGF-I, T3, T4, Progesterone (P4) , estradiol (E2) and testosterone (Testo) in serum of pregnant and non pregnant camels.

Hormone	Non pregnant			Pregnant		
	Mean ±SEM	Min.	Max.	Mean ±SEM	Min.	Max.
IGF-I(ng/mL)	597.52±45.26	552.26	642.78	475.71±61.17	269.34	725.96
T_3(ng/mL)*	2.16±0.52	1.65	2.68	3.51±0.23	2.71	4.61
T_4(µg/dl)*	8.34±0.37	7.97	8.71	10.24±0.31	8.90	11.68
T_4:T_3*	4.04±0.79	3.25	4.85	3.00±0.16	2.22	4.02
P4(ng/mL)**	1.43±0.31	1.12	1.74	4.60±0.40	2.83	6.89
E2 (pg/mL)	349.22±1.64	347.59	350.86	362.87±21.55	273.35	488.41
Testo (ng/mL)	0.31±0.19	0.07	0.35	0.59±0.23	0.05	1.96

Minimum (Min.), Maximum (Max.), Standard error of mean (SEM), * P<0.05, ** P<0.01

Correlation coefficients (Table 2) showed that levels of T_3 directly correlated with T_4 and this correlation is highly significant (r=0.74, P=0.006). Both T_3 and T_4 inversely correlated with estradiol and directly with both progesterone and testosterone but these correlations are considered weak and insignificant. Progesterone is directly correlated with estradiol (r=0.64, p=0.02). Progesterone and estradiol are directly correlated with testosterone but these correlations are also not significant.

TABLE 2. Correlation coefficients between different hormones.

	T3	T4	P4	E2	T4
IGF-1	0.13	0.05	-0.55	-0.54	0.44
T3	1	0.74	0.15	-0.30	0.21
T4		1	0.24	-0.31	0.39
p4			1	0.64	0.21
E2				1	0.28

* correlation is significant at $P<0.05$, ** correlation is significant at $P<0.01$.

Discussion

The dromedary or one-humped camel is a ruminant but it has a diffuse epitheliochorial placenta like that of pigs and horses (van Lennep, 1963, Shalash and Nawito, 1963).

It is induced ovulator (Chen *et al.,* 1985, Anouassi *et al.,* 1992) and the corpus luteum that develops after a sterile mating has a life span of only 8-10 days (Musa and Abusineina, 1978, Marie and Anouassi, 1987, Sumar, 2000). The ovulation and luteal phases are induced only in bred females, while un-bred females remain unovulated and display follicular phase only (Martin, 2004).

Sex hormones

Up till now progesterone hormone level is a very useful tool to monitor pregnancy in camels (Alfuraiji, 1998). The primary source of progesterone in the female camel is the corpus luteum. The placenta does not contribute to progesterone secretion, and all camelids depend entirely on progesterone from the corpus luteum to maintain their pregnancy (Skidmore, 2005).

The high levels of progesterone in the serum observed in the present study indicated ovulation in nine female camels due to successful mating. The present findings indicated that serum progesterone levels in the early pregnant camel remain higher than2.0 ng/ml (2.83 to 6.89) and coincide with the ultrasound findings. Ultrasound results could only be interpreted as pregnant and non pregnant since males and females were kept together and the precise mating date was not recorded. Moreover, Camel pregnancy was not subjected to intensive research using ultrasound so no equation for determining gestational age in camels in all invented ultrasound scanners. In accordance to other studies, in dromedary camels, during the first month after mating progesterone levels ranged from 3 to 7 ng/mL (Agarwal *et al.*, 1987 and Elias *et al.*, 1984a) and in the early pregnant llama (3 to 4.5 ng/mL) and the last month of pregnancy (7.4 to 9.2) compared to non-pregnant llamas where it ranged from 0.9 to 1.4 ng/ml (Adam *et al.*, 1989). As well as plasma progesterone concentrations increased by 5 days after mating and remained elevated in pregnant llamas (>2.0 ng/ml) throughout most

of pregnancy (Leon *et al.,* 1990). Similar to the dromedary camels of the present work, serum progesterone during pregnancy in Bactrian camel increased 15 days after insemination and remained elevated throughout most of gestation with means 3.06± 0.49 to 8.51±4.80ng/mL during early or late gestation (Zhao *et al.,* 1998).

Concentrations of progesterone remained <1ng/ml throughout the estrous cycle in non pregnant dromedary camels (Homeida *et al.,* 1988). As well as level of progesterone remains low in the absence of mating and ovulation (Ayoub *et al.,* 2003, Skidmore, 2005, Ghazi, 2007 and Babiker *et al.,* 2011). Recently, Ali *et al.* (2010) reported low values (1.7ng/ml) of progesterone in serum of normal non pregnant camels compared to those affected with ovarian reproductive disorders. Moreover, low levels of serum progesterone (1.2±2.0 ng/ml) were recorded in serum of non pregnant camels though high progesterone levels were observed in both bursal (1.7±0.9 ng/ml) and follicular (4.3±1.0 ng/ml) fluid in camels affected with ovarian hydrobursitis (Ali *et al.,* 2011). Recently, all classes of ovarian follicles shared in pregnancy maintenance in pregnant camels by secreting more progesterone concentrations than follicles in non pregnant camels and the increase in follicle size the more progesterone concentration measured (El-Shahat *et al.,* 2013).

During pregnancy, progesterone is probably produced by the corpus luteum and that the oestrogens are probably produced by the trophoblast (Skidmore *et al.,* 1994, 1996).The increase in estradiol in pregnant camels of this study compared to non pregnant ones was reported also in llama (Leon *et al.,* 1990) and is referred to the capacity for oestrogen production by the trophoblast (Wooding *et al.,* 2003). Estradiol increased significantly from 11 month of pregnancy till parturition (Zhao *et al.,* 1998). The contribution of ovarian follicles in secreting estradiol during pregnancy was lower than in non pregnant camels during their breeding season (El-Shahat *et al.,* 2013).

Though testosterone is considered a male hormone, a small amount of testosterone is produced by the ovaries and the adrenal cortex. Testosterone levels are usually produced at low levels during pregnancy even in female camels. Testosterone, concentrations were significantly (P<0.05) high during winter than other seasons of the year in non-pregnant camels (El-Harairy *et al.,* 2010). It was reported that testosterone levels in non pregnant camels were found to follow the same variations as estrogen. Increased size of the follicle is accompanied by an increase in testosterone levels. Plasma testosterone levels increase from 50 pg/ml, then declines with the regression of the follicle (Homeida *et al.,* 1988). However within follicular fluid, the increase in follicle size was associated with a decrease in testosterone concentration whatever camels were pregnant or not (El-Shahat *et al.,* 2013). Small ovarian follicles in pregnant camels had high testosterone levels compared to those of non pregnant ones (El-Shahat *et al.,* 2013). Contrary to camels, high testosterone levels were

recorded in non pregnant mares that failed to conceive (Abo El-Maaty *et al.,* 2012). The equine species is characterized by its ability to convert strongly androgen-to-estrogen, principally by aromatase localized in follicular theca interna and granulosa cells (Sirois *et al.,* 1991) and in the corpus luteum (Silberzahn *et al.,* 1983). Testosterone the main endogenous anabolic androgen, is produced by the ovary of the cycling mare (Silberzahn *et al.,* 1983) and by the corpus luteum in early pregnant mares (Daels *et al.,* 1996).

Thyroid hormones

Since the main changes in thyroid function associated with the pregnant state are increased thyroid hormone requirements so these increased requirements can only be met by a proportional increase in hormone production that directly depends upon the availability of dietary iodine (Glinoer, 2003). In agreement with the results of the present work, triiodothyronine (T_3) and thyroxin (T_4) concentrations increased throughout pregnancy in dromedary camels (Heshmat *et al.,*1984 and Agrawal *et al.,* 1989) and in Llama (Leon *et al.,* 1990). However, lowest T_3 and T_4 values were observed during the tenth month in camels (Agrawal *et al.,* 1989). In contrast to result of this study, T_4:T_3 ratio showed minor, non significant fluctuations during pregnancy (Agrawal *et al.,* 1989). Total T_4 and T_3 concentrations increased markedly during the first and second trimesters in women (Chan *et al.,* 1975) with a significant decrease in free T_3 and T_4 in women at both weeks 14 and 32 of pregnancy compared to pre-pregnancy levels (Lof *et al.,* 2005). During camel pregnancy, small and medium ovarian follicles had more T_4 levels than large follicles but large follicle of non pregnant camels had more T_4 than other follicle classes and more than large follicles of pregnant camels (El-Shahat *et al.,* 2013).

Significant low T_4 level (14.7±1.3 µg/dl) was recorded in normal non pregnant dromedary camels compared to others with vaginal adhesions (Ali *et al.,* 2010). In contrast to camels, thyroid hormones increased only during late pregnancy in the cow (Soliman *et al.,* 1963, 1964). As well as, T_4 concentrations did not vary substantially in pregnant goats then declines to a minimum concentration 1 day before parturition (Agrawal *et al.,* 1985). In mares, neither thyroxin levels increased during pregnancy nor stage of pregnancy had significant effect (Katovich *et al.,* 1974).

Although, Dixit *et al.* (1970) have reported a decrease in protein bound Iodine levels of camels with age, but there still numerous questions that needs clarification. Age had no significant effect on the serum concentration of T_3 and T_4 in camels from 1to >8 years (Agarwal *et al.,* 1986) and Turkoman horses (Nazifi *et al.,* 2003). Moreover, Wasfi *et al.* (1987) and Agarwal *et al.* (1989) reported no correlation between thyroid hormones and age. Season (Khurana, & Madan, 1986 and Yagil *et al.,* 1978) but neither fetal sex (Agarwal *et al.,* 1985) nor age of the dam (Shoda and Ishii, 1976) affected thyroid status. Similarly, thyroid hormone levels were not affected by failure of conception or by abortion

(Agarwal *et al.,* 1989). On the other hand, absence of any significant change in thyroid hormones of non pregnant camels or those that have aborted confirms that the fetal load does not alter the thyroid status of the animal to diagnostic levels (Agarwal *et al.*,1989).

The increase in thyroid hormones during early pregnancy observed in camels of this work may refer to the involvement of thyroid hormones in progesterone synthesis (Spicer *et al.,* 2001, Nishimura *et al.,* 2004) and lead us to suggest that luteal cells of mature corpora lutea may be involved in the synthesis of thyroid hormones, which may modulate progesterone synthesis, acting in an autocrine and paracrine way as in bovine (Mutinati *et al.,* 2010).

The inverse correlation between thyroid hormones and estrogen observed here was also recorded in mares (unpublished data) and referred to that estrogens can alter the secretion rate and dynamics of thyroid hormones (Boccabella & Alger, 1964, Yamada *et al.,* 1966 and Chen & Walfish, 1978). It seems that fluctuations in thyroid activity might be due to interactions with varying concentrations of estrogens and progesterone during pregnancy (Elias *et al.,* 1984a).

Conclusions

Camels are not only differing from ruminant in their type of placenta but they are also mysterious in their endocrine profiles during pregnancy and are nearly similar to equine. Thyroid hormones play an important role during pregnancy in camels.

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(*Received* 21/ 8/2014;
accepted 4/ 10/2015)

هرمونات الغدة الدرقية والجنسية فى مصل اناث الابل (النوق) الحوامل والجلد (غير حامل)

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هذه الدراسة تصف الفروق فى مستوى هرمونات الغدة الدرقية والهرمونات الجنسية فى اناث الجمال (النوق)الحامل والغير حامل. أجريت الدراسة على عدد ١٢ من اناث الابل (النوق) فى اعمار متفاوته واردة من السودان باحد المزارع الخاصة وتم فحصها بالسونار لتشخيص الحمل فى المراحل المبكرة. توجد الاناث مع الذكور فى نفس العنبر اثناء موسم التلقيح. تم تجميع عينات الدم لتقدير مستوى هرمونات الاستراديول والبروجستيرون والتستوستيرون والثيروكسين والترای ايودوثيرونين والانسيولين.

أشارت النتائج الى ارتفاع معنوى فى مستوى هرمونى والثيروكسين والترای ايودوثيرونين وان النسبة بينهما كانت منخفضة T4:T3 فى اناث الابل الحوامل مقارنة بالاناث الجلد. كما اشارت النتائج الى ارتفاع فى مستوى هرمون البروجستيرون فى النوق الحوامل حيث بلغت ٢,٨٣ الى ٦,٨٩ نانوجرام/مللى . مع حدوث ارتفاع طفيف فى كل من التستوستيرون والاستراديول اثناء الحمل فى المراحل الجنينية المبكرة .

يستخلص من هذه الدراسة ان تقدير مستوى البروجستيرون فى الدم مايزال الفيصل الاكيد فى تحديد الحمل على الرغم من تقنية الموجات فوق الصوتية (السونار) الحديثة.