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Research Article

Utilization of Hormonal Biomarkers for Early Pregnancy Diagnosis in Marecha Camel under Semi-Intsensive Management System

Asim Faraz[1], Muhammad Yaqoob[2], Nasir Ali Tauqir[3], Hafiz Muhammad Ishaq[1], Ayman Balla Mustafa[4], Amir Ismail[5], Muhammad Arslan Akbar[6], Abdul Waheed[1]* and Muhammad Shahid Nabeel[7]

[1]*Department of Livestock and Poultry Production, Bahauddin Zakariya University Multan, Pakistan*
[2]*Tilad Veterinary Center, Riyadh, Kingdom of Saudi Arabia*
[3]*Department of Animal Nutrition, The Islamia University of Bahawalpur*
[4]*Therapeutic Nutrition Department, Faculty of Nursing and Health Sciences, Misurata University, Misurata, Libya*
[5]*Institute of Food Science and Nutrition, Bahauddin Zakariya University Multan, Pakistan*
[6]*Department of Breeding and Genetics, Cholistan University of Veterinary Sciences, Bahawalpur, Pakistan,*
[7]*Camel Breeding and Research Station Rakh Mahni, Punjab, Pakistan*

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AF and MSN conducted research trials and wrote the paper. AW, NAT helped in analysis. MY, HMI, AI helped in write-up. MAA helped in completion of draft manuscript writing. AMB reviewed the paper.

Keywords
Camel, Early pregnancy diagnosis, Progesterone, Estrogen, Pastoral, Physiological Condition, Management system

Abstract | Present study was designed to use hormonal biomarkers in terms of progesterone and estradiol as early pregnancy indicators in female dromedary camel. A total of 44 female Marecha camels were selected in Thal desert of Pakistan during breeding season (December-March) and kept under semi-intensive management system (SIMS). Selected animals were divided into two comparable groups of 22 animals in each to determine the hormonal profile. G1 was composed on lactating non-pregnant while G2 had newly bred she camels. Blood samples from G1 was collected at first day of trial, while G2 animals blood collection was done at day 14th and 21st day post mating. All the blood samples were centrifuged at 5000 rpm for five min; serum was collected and stored at – 18°C for laboratory analysis. Commercially available ELISA kits were used to measure serum progesterone and estradiol levels. Progesterone and estradiol levels were found significantly high (P<0.05) with the value of 3.46±0.25 ng/ml at day 14th and 4.45±0.34 ng/ml at day 21st in G2 (Pregnant animals), while in non-pregnant group serum P4 concentration was recorded to be 1.05±0.29 ng/ml which is considered as basal level of progesterone in dromedary camel. Mean serum estrogen concentration (P<0.05) was found to be 25.28±2.71, 54.80±2.52 pg/ml at 14th and 21st day post mating respectively in pregnant group of animals, while in lactating non pregnant animals E2 level was recorded as 10.38±1.52 pg/ml. Hormonal biomarker values were found to be significantly higher in G2 than G1. Present findings are suggestive of serum progesterone and estrogen levels as biomarkers of early pregnancy detection in female dromedary camel.

Novelty Statement | The study has significant values as it talks about the hormonal levels related to early pregnancy diagnosis in lactating Marecha she-camels, a contribution towards modern approaches in research of camel reproduction. As the Marecha camel is the main breed of Pakistan, playing very important role in rural economy of desert areas of the country, this specific study will be used to build the country's data base for future research.

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Corresponding Author: Abdul Waheed
drabdulwaheed@bzu.edu.pk

Introduction

Pregnancy is very critical condition in mammal's life. Certain biochemical and endocrinological changes occur during this period. Particularly estrogen and progesterone maintain not only pregnancy but also ensure viability of fetus, growth of uterus and preparation of reproductive tract for parturition. Camel reproduction is distinct from other animals because it is an induced ovulater not of spontaneous type so the reproductive hormonal levels are highly variable and difficult to interpret in these species.

To achieve efficient reproductive performance of a camel herd in short breeding season and higher economical investments to get a calf from a winner she camel specially in middle east camel racing industry results in a demand for reproductive scientists to find accurate methods of early pregnancy detection. There are several methods used for pregnancy diagnosis in camel, starting with tail cocking (Bedouin method), rectal palpation, changes in cervical mucus and ultrasonography which is considered as the most reliable source of pregnancy diagnosis. The study has significant values as it tells about the hormonal levels related to early pregnancy detection in Marecha she-camels, definitely new something to be distinguished and support baseline data of camel area. As the Marecha she-camel is main breed of Pakistan, very important regarding food provision to cameleers, this specific study about it will be used to build the country's data base for upcoming studies.

There are 1.1 million heads of camel in Pakistan which constitutes about 26% of the total camel population in Asia (GOP, 2020-21). Majority of the population is found in sandy deserts (Thal, Cholistan and Thar) and south western mountainous region of Bolochistan (Faraz *et al.*, 2019a). However, a large segment of the camel population is hosted in irrigated plans of Punjab and Sindh provinces (Faraz *et al.*, 2019b).

The camelids are inhabitant at the extreme weather conditions on the globe and their increase in progeny is dependent by the available sources of protection and food for the newborn, subsequently their reproduction is controlled by these factors. Camel milk can be a wonderful source of food in drought areas for the human population. During severe drought season; goats, sheep and cattle may be affected while the camel stands comparatively unaffected. The soil of five rivers maintains about 33.51% of Pakistani camel population (Faraz *et al.*, 2019c).

The estrogen level is highly variable and difficult to interpret in these species, after mating carpus-luteum develops and progesterone starts to increase in serum and attains the level of 2ng/ml up to day 6 of post-mating. Another speculation is present regarding estrogen in camels, levels of estrogen are not critical for surge release of LH as in other species (Martin, 2004). Corpus-luteum has normally shorter life span in non-pregnant animals. As camels are induced ovulator, breeding/mating is the factor which induced ovulation. While unbred animals remain un-ovulated and their reproductive cycle bears only follicular phase (Deen *et al.*, 2007).

The steroid hormone release pattern also varies in this valuable animal. In other farm animals, steroid hormone level during pregnancy is well documented. But camels are neglected species in this regard in Pakistan. Hence, this study was planned which covers hormonal parameters (progesterone and estrogen) in reference to lactation and pregnancy in semi-intensive management system (SIMS) which will be a valuable progress to build the data base of country for future researches of this study area.

Materials and Methods

Meteorological conditions of research site

The Camel Breeding and Research Station (CBRS) Rakh-Mahni are located in desert Thal in district Bhakkar having tiny strips of sandy points and sandbanks. The climate is arid to semi-arid subtropical and continental with average temperature varied between 45.6°C in summer and 5.5 to 1.3°C in severe winter. The average rainfall is 150-350 mm annually (Rahim *et al.*, 2011).

Management of experimental animals

The experiment was conducted at CBRS Rakh Mahni. Forty-four Marecha she-camels of 5-12 years of age, reared under semi-intensive management system (SIMS) having mean body weight of 555±95 kg was divided into two groups, 1st group (G1) of lactating non-pregnant she-camels in 2 to 8 month of lactation while 2nd group (G2) of newly bred pregnant she-camels. All animals were carefully examined for health condition before the start of experiment. Only physically healthy camels were included in the trial. Animals were dewormed by injection of 1% Ivermectin @ 1ml/50 kg body weight, before one month of starting trial. All camel sheds were sprayed with Ecofleece solution @ 2cc/liter of water to kill ectoparasites in environment. Vaccination against Trypnosomiasis by was done two weeks before study with injection of Isometamide chloride hydrochloride powder (Trypamidium-Samorine 1 g sachet) by Merial France.

Feeding management

Feed of all animals was same with quality and quantity of ration and provided same trial conditions. Concentrate offered to the animals were @ 2-3 kg/day. The animals were sent 3-4 h for browsing/ grazing in jungle daily. Gram straw (*Cicer arientinum*) was offered to animals at *adlib* as manger feeding in rest of time. Water was offered two

times a day. Different salt blocks were provided to animals in manager while Di Calcium Phosphate (DCP) powder was offered @ 100 g per female camel daily. The chemical composition and different components of concentrate is mentioned in Table 1.

Table 1: (a) Ingredients of experimental ration (b) chemical composition of experimental ration.

(a) Ingredients (%)	Exp-Ration	(b) Parameters (%)	Exp Ration
Maize grain	9	DM	90.32
Wheat bran	24	CP	18.06
Cotton seed cake	25	NDF	29.09
Rape seed cake	6	ADF	14.41
Corn gluten 30%	20	TDN	70
Molasses	14	ME (Mcal/kg DM)	2.41
DCP	1		
Salt	1		

Table 2: MEAN±SEM values of hormonal concentration of Marecha camel at CBRS Rakh Mahni Bhakkar, Punjab.

Parameter	G-1 (Day 1)	G-2 (Day 14)	G-2 (Day 21)
Estrogen (pg/ml)	10.38±1.52[a]	25.28±2.71[a]	54.80±2.52[b]
Progesterone (ng/ml)	1.05±0.29[a]	3.46±0.25[b]	4.45±0.34[b]

The forage species available in grazing area at the research station were *Acacia nilotica*, *Acacia modesta*, *Ziziphus mauritiana*, *Albizia labbek*, *Prosopis cineraria*, *Tamarix aphylla*, *Cenchrus ciliaris*, *Suaeda fruticosa*, *Cymbopogon schoenanthus*, *Kochia indica*, *Tribulus terrestris*, *Capparis spinosa*, *Haloxylon salincornicum*, *Calligonam polygonoides*, *Capparis decidua* and *Haloxylon recurvum*.

Blood sampling and laboratory analysis

Blood samples were taken from G1 (Non-Pregnant) animals at first day of trial to measure serum progesterone and estrogen concentrations. While in G2 animals, samples were taken twice with one week interval. First blood sample were collected at 14th day and second blood sample were taken at 21st day after mating. Proper retraining and antiseptic protocols were followed throughout sampling period. All the blood samples were centrifuged @ 5000 rpm for 5 min. Serum was stored in Eppendorf tubes at -18° C for further laboratory analysis. The estrogen and Progesterone concentration in serum was measured by commercially available.

Statistical analysis

The data for analysis of variance (ANOVA) computation was performed by using software SPSS (SPSS, 2008). Tukey's test was applied at 0.05 level of significance for comparing the treatment's differences among their means (Steel *et al.*, 1997).

Ethical approval

Feeding of camels in the current study was in accordance to the routine farm practices taking browsing/grazing and stall feeding was also available. Animals were offered clean, fresh water and different loops of salt. Proper vaccination and deworming of camels were done according to technical recommendations. All national and institutional guidelines for use and care of trial animals were followed.

Results and Discussion

The mean values (P<0.05) of Progesterone were 3.05±0.33, 4.45±0.34 in G2 at day 14th and 21st respectively. While P4 value in G1 was recorded as 1.05±0.29 ng/ml. Similarly mean serum estrogen concentration (P<0.05) was found to be 25.28±2.71, 54.80±2.52 pg/ml at day 14th and 21st respectively in pregnant group of animals, while in lactating non pregnant animals E2 level was recorded as 10.38±1.52 pg/ml. A significant increase in serum progesterone and estrogen level was observed in lactating pregnant camels.

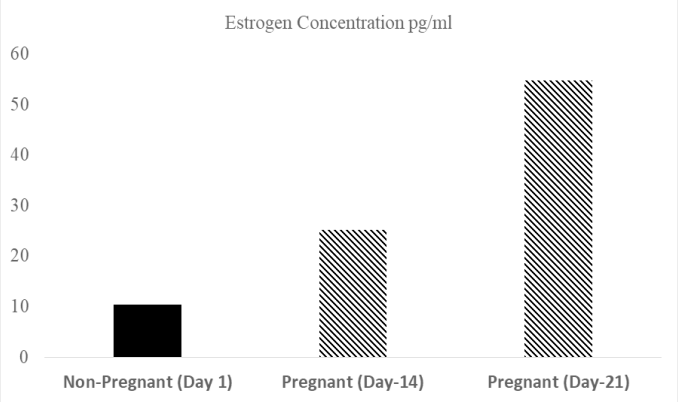

Figure 1: Graphical representation of MEAN±SEM Values for serum estrogen values in both group of camels.

In present study serum progesterone level at 14th day post mating was recorded as 3.46±0.25 which resembles to a previous study conducted by (Skidmore *et al.*, 1996) that progesterone concentration was 3.4 ng/ml at 8th day after ovulation. The constant level of progesterone during all gestation length is due to no change in corpus-luteum of pregnancy after 60 days. Serum progesterone continues to increase steadily until day 8 or 9, then falls sharply on day 11.5±1, exhibit wide variations amongst females. Deen *et al.* (2007) reported average progesterone concentration in pregnant camels 3-4.14 ng/ml in 1st half of gestation, while in 2nd half of gestation progesterone level was 2.88 to 5.09 ng/ml.

In this experiment serum estradiol level at day 14th and 21st was recorded to be 25.28±2.71, 54.80±2.52 pg/ml respectively, while in non-pregnant group its value was

10.38±1.52 pg/ml. According to another study, estradiol continues to increase at 20-25 days and it reaches to its highest level of 100 pg/ml till 100 days of gestation (Skidmore *et al.*, 1996). According to author this increase in estrogen level is because follicular activity in dromedary camel doesn't stop even up to 6 months of gestation. The main source of estradiol is embryonic vesicle and placenta also because extra embryonic membrane in camel has the capacity of aromatization, just after 10 days of fertilization, along with ability of endometrium to conjugate free estrogen. Reported estrogen concentration was 1.32 to 8.74 and 72.5±21.68 pg/ml in 1st and 2nd half of gestation respectively (Deen *et al.*, 2007) while similar results were also observed by Ayoub *et al.* (2003).

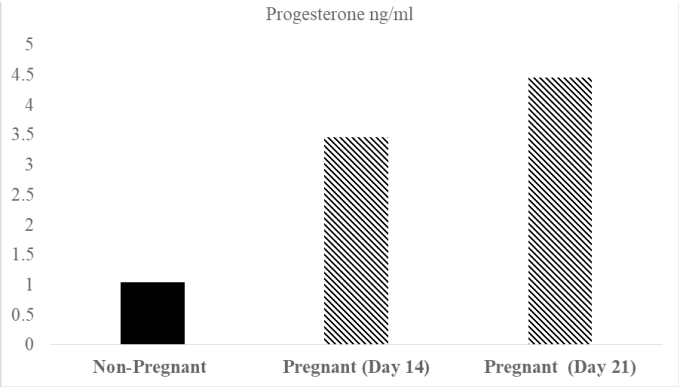

Figure 2: Graphical representation of MEAN±SEM Values for serum progesterone values in both group of camels. CBRS, camel breeding and research station; G-1, Non pregnant lactating she camel; G-2, Newly pregnant she camel; Day 1, First day of trial; Day 14, 14th day post mating; Day 21, 21st day post mating; E2, Estrogen; P4, Progesterone; Means having different superscript in rows are significantly different (P<0.05).

Some of the authors reported a significant increase in estrogen level occurs during last 70-80 days of gestation, while in this duration a considerable increase in foetal fluid volume, foetal size and weight is also noted which indicates placental estrogen to play crucial role in foetal development (Elwishy *et al.*, 1981). Tibary and Anouassi, (1997) reported the values of estradiol that ranged between 9-110 pg/ml. Ismail *et al.* (1998) found in their study that during ovulation, 34.20±6.04 pg/ml was the concentration of estradiol. Their reports showed that the concentration of 17β-estradiol during the anestrous phase was 32.16±9.9 pg/ml, in serum and during estrus; 94.4±7.2 (Cristofori and Quaranta, 1993), during follicular development phase values noted were 44.4±2.7 pg/ml and Ayoub *et al.* (2003) reported its lower values that were during estrus; 29.7±7.3 pg/ml and a week after was 5.72±4.55 pg/ml.

The concentration of serum estradiol was not significantly changed with various sizes of follicles during the growing follicular phase such as follicle sizes 1.6-2.1, 1.1-1.5, and 2.2-2.5 cm in concentrations such as 211.1±31.9, 228.6±32 and 210.1±30.0 pg/ml respectively (El-Bahr *et al.*, 2015). These findings are alike to the findings of Ghoneim *et al.* (2013). Average values relevant to the season were described by El-Harairy *et al.* (2010) and were noted as its high significant values during winter season and value was 62.18±1.16 pg/ml and spring season (56.15±1.25 pg/ml) compared with autumn and summer seasons (28.16±1.31 pg/ml and 20.13±1.02 pg/ml respectively). These seasonal differences were also studied by Khaldoun (1993). Furthermore, high concentration of estradiol was observed during spring and winter season in the similar prepubertal female camels as studied by Al-Saiady *et al.* (2012).

Reports of the study of Muhammad *et al.* (2011) showed the comparison of non-pregnant to pregnant camels and was not found significant differences; 19.8 vs 27.03 pg/ml, respectively. Tibary and Anouassi (1997) reported estradiol concentrations increased after fecundation and showed irregular differences during the first two trimesters of pregnancy and Abdulkareem *et al.* (2015) reported its value ranged between 34.5 and 45.8 pg/ml. Mean concentrations of estradiol reported by Cristofori and Quaranta (1993) as its level was 60.0±5.4 pg/ml during the 1st trimester of pregnancy, 71±9.0 pg/ml level was during 2nd trimester, and 95±3.3 pg/ml values during 3rd trimester. Skidmore *et al.* (1996), reported that serum concentrations of estrogen was found distinct fluctuations during the starting 100 days of gestation period. A notable surge in plasma estradiol begins from 2 to 4 days before parturition and reached its peak in 2 h before delivery in excess of 200 pg/ml, (Ayoub *et al.*, 2003).

Plasma concentrations of progesterone in camel were measured both during pregnancy and estrus cycle but high variability among individual were published by El-Basheir *et al.* (2001). GnRH during ovulation stimulation increased the concentration of progesterone in plasma from basal level up to a peak level (0.32±0.05 ng/ml, 7.15±0.97 ng/ml, respectively) on the 7th day after ovulation and its level was reduced up to 0.9±0.4 ng/ml by 11 days as reported by Rawy *et al.*, (2012). Concentration of progesterone ranged according to season between 0.07-0.226 ng/ml as higher value in autumn period in prepubertal female camel (Al-Saiady *et al.*, 2012).

Progesterone level had not been changed during the first week of post-ovulation in pregnant and non-pregnant female camels, while it was decreased if pregnancy didn't occur and the corpus-luteum was maintained in pregnant female camel as it was more value than 2ng/ml along the gestation period. During the first month, its values were reached up to 3-9 ng/ml (Nagy *et al.*, 2015) and a higher specific difference was noted by Vyas *et al.*, (2010). Cristofori and Quaranta, (1993) reported that in the pregnant female, the concentration of plasma progesterone

was 10 times higher than in non-pregnant on average: (3.20±5.40 ng/ml vs 0.35±0.02 ng/ml respectively). Muhammad *et al*. (2011) explained that the mean values of progesterone in pregnant and non-pregnant and female camels were 4.23 and 1.39 ng/ml respectively in Nigeria.

To diagnose early pregnancy, camel farmers used a traditional way of tail curling but possibility of false-positive detection might be there. Deen (2008) got achievement in a monitoring among camels showing the behavior of tail curling (assumed to become pregnant), concentrations of plasma progesterone in 68% had more values than 1 ng/ml which confirm their pregnant position. The progesterone level was continued same up to the 5th month after the first month of pregnancy, and then decreased slowly until the parturition. Skidmore *et al*. (1996), reported different pattern as fluctuations in level from 2 to 5.5 ng/ml arise with the pregnancy in all the cases. Tibary and Anouassi (1997) explained that these fluctuations were associated to the blood volume or to the secretory functions of corpus-luteum. Furthermore, Agarwal and Khanna (1993) reported that the concentration level of plasma progesterone could be dependent on the sex of the fetus as it had high values in those animals with a male as compared to a female: 5.0±0.6 vs 3.6 ± 0.30 ng/ml, respectively. Another study conducted by Derar *et al*. (2014) showed that the concentration of progesterone at peak level was determined on the 9th day of postpartum as observed at 3.2±3 ng/ml and it was reduced slowly to a basal concentration.

Shujait *et al*. (2011) reported progesterone (ng/ml) and estradiol (pg/ml) mean concentrations as 0.98±0.26 and 41.42±5.44 in Pakistani dromedary she-camel. Kamoun and Jemmali (2014) reported plasma progesterone concentration as 0.38, 0.01 and 0.13 g/L respectively, in lactating, non-lactating and empty pubertal Tunisian dromedary camels. El-Maaty *et al*. (2014-15) reported progesterone (ng/ml) and estradiol (pg/dl) mean concentrations as 1.43±0.31, 349.22±1.64 and 4.60±0.40, 362.87±21.55 in non-pregnant and pregnant Sudanese dromedary camels. Kelanemer *et al*. (2015) reported progesterone (ng/ml) and estradiol (pg/dl) mean plasma concentration as 6.02±0.17, 106.60±7.24 and 0.26±0.17, 33.60±7.24, respectively, at 30 days ante-partum and post-partum in pregnant dromedary camels.

Shahooth (2015) reported serum mean concentration of progesterone (ng/ml) and estradiol (pg/dl) as 0.724±0.080, 31.33±2.533; 0.641±0.090, 35.75±2.019; 0.608±0.116, 31.17±2.160; 1.309±0.514, 31.83±3.094 respectively, in September, October, November and December in dromedary camel. Reported progesterone and estrogen levels in dromedary camels in UAE were to be 0.86±0.18 ng/ml and 57.27±8.52 pg/ml, respectively (Ahmed and Mustafa, 2016). Abd-El-Rahman *et al*. (2017) reported progesterone (ng/ml) and estradiol (pg/ml) mean concentrations as 5.50±0.08, 17.60±0.28; 7.30±0.23, 23.50±0.38; 6.150±0.15, 160.9±3.54, respectively, in 1st, 2nd and 3rd trimester in Egyptian dromedary camels. Reported serum progesterone concentrations were to be 0.89±0.02 and 1.61±0.14 ng/ml respectively, in follicular and luteal phase of Nigerian dromedary camel (Majama *et al*., 2018).

Reported serum mean concentration of progesterone was to be 2.5±0.15 ng/ml in Egyptian dromedary camel (Mohamed *et al*., 2019). Ebissy *et al*. (2019) reported progesterone (ng/ml) and estrogen (pg/ml) concentration as 3.86±0.10, 202.8±22.5 and 4.73±0.17, 303.3±22.6 respectively, at 28 and 14 days before parturition in Egyptian dromedary camels. In another study about Egyptian dromedary camels, El-Maaty *et al*. (2019) reported progesterone (ng/ml) and estrogen (pg/ml) concentration as 1.97±0.14, 53.66±2.46 and 1.15±0.09, 45.86±3.17 respectively, at peak and low breeding season. Moussa *et al*. (2019) reported mean serum progesterone (ng/ml) and estrogen (pg/ml) concentration as 2.68±2.31, 19.96±3.37 and 3.67±2.13, 5.91±0.46, respectively, during estrous and met-estrus period in Mali dromedary camels.

Conclusions and Recommendations

Serum progesterone and estrogen levels was significantly higher in pregnant she camels. Current study is suggestive to use serum progesterone and estrogen levels as biomarkers of early pregnancy detection in female dromedary camel.

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Conflict of interest

The authors have declared no conflict of interest.

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