



Assessment of Estrogen and Progesterone Level in the Dromedary Camels after Ovulation Induction during Non-Breeding Season

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Abstract

The aim of the present study was to determine the serum Estrogen and Progesterone concentrations after ovulation induction using GnRH and hCG in the dromedary camel. A total number of (19) dromedary camels used in this experiment was divided into three groups during the non-breeding season: Group A (N=7), animals were intramuscular injected by 2 ml GnRH, group B (N=6), animals were intravenous injected by 3 ml hCG. Group C (N=6) control group, animals were intramuscular injected by 1 ml distilled water. Blood samples were collected at alternate days throughout the defined experimental periods (0 hours = time of injections, 48 hours = after 48 hours, Week 1 = after 7 days, and Week 2 = after 14 days), to determine estrogen and progesterone levels using ELISA. Results showed that serum estrogen concentrations (pg / ml) did not differ significantly ($P \leq 0.05$) between the all groups in 48 hours and week 2. In contrast in 0 hours and week 1 which were higher significant ($P \leq 0.05$) between the treated groups (GnRH and hCG groups) compared with control group, while there was no significant difference between the treated groups throughout the alternate periods. Serum progesterone concentrations (ng / dl) did not differ significantly ($P \leq 0.05$) among the all groups in 0 hours and week 2. The serum progesterone concentration in 48 hours were significant higher ($P \leq 0.01$) in the (A) group compared to the (B) and the control groups respectively. Also the levels of hormone were higher significant ($P \leq 0.01$) in the (B) group compared to the (A) and control groups respectively. It can be concluded that the hormonal analysis of estrogen and progesterone in case of follicular ovarian wave indicated a large individual variation.

Keywords: Estrogen, progesterone, ovulation induction, dromedary camels.

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Introduction

The Old World Camelids comprise two species: namely, *Camelus dromedarius* (Dromedary or one – humped camel) and *Camelus bactrianus* (Bactrian or two – humped camel). The term dromedary is derived from the Greek word "Dromados" (run) and in the strict

sense is used for riding camels (Higgins, 1986). Camels are an important livestock species in the arid and semi-arid zones; it contributes significantly to the livelihood of the pastoralists and agro-pastoralists living in the fragile environments of the desert and semi-desert of Asia and Africa (Ishag, 2011).

Many authors were mentioned that camels reproduction is handicapped by different constrains as semen Characteristics, long gestation period, late sexual puberty and maturity, limited breeding season and the mechanism of Oestrous cycle and ovulation of she-camel (Deen, 2008; EL-Hassanien *et al*, 2010). Females show higher breeding activity during winter and spring than summer and autumn seasons. Determination of the blood circulating levels of different reproductive hormones has allowed good chances in the diagnosis of physiological status and pathological conditions in animals (Abdel Hadi and Wasfi, 2001).

Progesterone hormone level in the females 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 (CL). Plasma progesterone level remains very low throughout the follicular wave in the absence of mating and ovulation (Ismail *et al.*, 1998). Progesterone concentration starts to rise after mating and during pregnancy and falls just before parturition. Outside the breeding season, mating activity ceases and the ovaries are inactive or only have a few small follicles (Zeidan, 2011). Progesterone and estradiol hormonal profiles during different reproductive conditions have been characterized in many domestic animals. However, only few reports have studied the hormonal profiles of progesterone and estradiol in camels. Skidmore *et al*, (1996a) studied the ovarian follicular wave patterns ultrasonographically in camels and found that the follicular cycle was divided into a growth phase (10 ± 0.5 days), a mature phase (7.6 ± 0.8 days) and regression phase (11.9 ± 0.8 days). They found that serum estradiol – 17 β

concentration reached a peak value of 39 pg / ml at estrus when the dominant follicle reached a mean diameter of 1.7 cm, and after ovulation, mean serum concentration of progesterone reached a peak value of 2.6 ng / ml on day 8. Elias and Yagil (1984) measured estradiol-17 β concentrations at monthly intervals in pregnant camels and found that the concentrations remained constant at 50 – 100 pg / ml during the first 10 months of gestation before rising to a peak during the 12th month. The same observation was reported by Skidmore *et al*, (1996b), where estradiol-17 β concentrations increased around day 50 to about 100 pg /ml, then remained relatively constant until day 300.

The aim of the present study was to determine the serum Estrogen and Progesterone concentrations after ovulation induction by GnRH and hCG in the dromedary camel

Materials and Methods

Study area: This experiment was conducted during the non-breeding season from May to June, 2014. At the Camel Reproduction Centre (CRC), Nakhlee, which is located 48 km east the centre of Dubai, UAE.

Animals and Management: A total of 19 Mature Dromedary she – Camels, aged 6-8 years were used in this study (average weight between 500-600 kg). They were maintained in groups in fenced pens, each of 0.002-0.004 Km² area. They were fed a diet of commercially formulated camel rations mixed concentrates and Lucerne hay twice a day to provide their requirements. Water was permanently available, and exposed to natural day length and ambient temperatures. The animals were identified by Neck Tags (Necklace neck).

Protocol of ovulation Induction:

Camels were divided into three groups for ovulation induction protocols as follows: Group (A) set as a treatment group (N=7), all animals were intramuscular injected by 2 ml GnRH / Head, group (B) set as a treatment group (N=6), all animals were intravenous injected by 3 ml hCG / Head. While group (C) set as control group without any hormonal treatment (N=6), all animals were intramuscular injected 1 ml / Head by water for injection.

Blood sampling: Blood samples (8 ml) were collected in non-heparinized tubes at alternate days throughout the defined experimental periods (0 hours, 48 hours, after 7 days, and after 14 days) by jugular venipuncture. They were kept at room temperature (19-23°C) for 1-2 hours before being centrifuged at 3000 rpm for 5 minutes for serum separation (ROTINA 380) – Type: 170, Germany. The serum was decanted and stored at -20°C until assayed for progesterone, estrogen.

Hormones assay: Serum estrogen and progesterone concentrations were measured using Enzyme Linked Immunosorbent Assay (ELISA) (Sunrise produced by TECAN, Type: Sunrise, REF: F 039300 SN: 03930005626, Made in Austria) and the analysis done by the software called Magellan (Document part No: I 117519 - 2013 – 12, Version No: 4.2).

Assay Protocol: Each sample from the 19 camel was calculated according to the type of hormone (25, 50 microliters) for the estrogen, and progesterone, respectively. The standards (containing different levels of diluent) were dispensed into assigned wells of a micro plate coated with purified anti-hormone antibodies. This was followed by adding different reagents and solutions, and

incubated for a certain period. Then, finally the absorbance was read in each well at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) using a micro plate reader (Sunrise produced by TECAN, Type: Sunrise, REF: F 039300 SN: 03930005626, Made in Austria), analyzed by a software called Magellan (Document part No: I 117519. 2013, Version No: 4.2).

Statistical analyses: All values expressed as means \pm SD. The data were analyzed using SPSS statistical software version 16 for windows (SPSS, 2015). The experiment parameters which includes, Hormones profile of estrogen, progesterone concentrations between groups and within groups were compared by One way ANOVA. While the multiple differences in Estrogen, Progesterone between groups and within groups were compared by multiple comparison LSD.

Results

Serum estrogen concentration (pg / ml) were not differ significantly ($P \leq 0.05$) between the all groups in 48 hours and Week 2. In the 0 hours and week 1 estrogen concentration were significantly higher ($P \leq 0.05$) between the treated groups (GnRH and hCG groups) compared with control group. There was no significant difference between the treated groups throughout the alternate periods (Table 1).

Serum progesterone concentrations (ng / dl) were not differ significantly ($P \leq 0.05$) in the all groups in 0 hours and Week 2. While the serum progesterone concentration significantly higher ($P \leq 0.01$) in the GnRH group compared to the hCG and the control group respectively in 48 hours. The serum progesterone concentrations during week 1 significantly higher ($P \leq 0.01$) in the

hCG group compared to the GnRH and control groups respectively (Table 2).

Table 1: Estrogen concentration (pg/ml) (Means±SD) during alternate periods in dromedary camels

Groups	N	E2 Con. 0 hrs.	E2 Con. 48 hrs.	E2 Con. Week1	E2 Con. Week 2
GnRH	7	74.69 ± 17.83	77.37 ± 12.77 ^a	67.24± 6.04 ^a	104.31 ± 42.62
hCG	6	67.36 ± 20.38	72.56 ± 13.32 ^a	72.35 ± 24.45 ^a	106.71 ± 76.88
Control	6	57.27 ± 8.52	53.87 ± 20.02 ^b	48.49± 7.62 ^b	58.13 ± 15.11
P. Value		0.196	0.039	0.030	0.202

N = Number of Animals E2 Con = Estrogen Concentration
 0 hrs = the day of Injection 48 hrs = after 48 hours from treatment
 Week 1 = after 7 days from treatment Week 2 = after 14 days from treatment
 Different superscript letters within the same column means significant differences ($P \leq 0.05$).

Table 2: Progesterone concentration (ng / dl) (Means ± SD) during alternate Periods in dromedary camels

Groups	N	P4 Con. 0 hrs	P4 Con. 48 hrs	P4 Con. Week 1	P4 Con. Week 2
GnRH	7	0.86 ± 0.29	1.15 ± 0.21 ^a	1.64 ± 0.06 ^b	1.44 ± 0.76
hCG	6	0.97 ± 0.29	0.89 ± 0.12 ^b	4.19 ± 3.15 ^a	1.03 ± 0.26
Control	6	0.86 ± 0.18	0.76 ± 0.15 ^b	0.82 ± 0.09 ^b	0.77 ± 0.12
P. Value		0.69	0.003	0.011	0.073

N = Number of Animals * P4 Con= Progesterone Concentration
 0 hrs = the day of Injection 48 hrs = after 48 hours from treatment
 Week 1 = after 7 days from treatment Week 2 = after 14 days from treatment
 Different superscript letters within the same column means significant differences ($P \leq 0.05$).

Discussion

In present study concentrations of estrogen were correlated positively with increasing in the follicle diameter (1.0 to 1.9 cm) in diameter. These results agree with that mentioned by Skidmore (2011) (1.0 to 1.7 cm). Anouassi and Tibary (2013) reported that the follicles become responsive when they reach 9 mm which is equal 0.9 cm. On the other hand the concentrations of serum estrogen (pg / ml) were increased significantly ($P \leq 0.05$) before the ovulation which was due to the growing of the follicles, from 74.69 ± 17.83 (pg / ml) to 77.37 ± 12.77, 67.36 ± 20.38 to 72.56 ± 13.32 (pg / ml) in the GnRH, hCG groups compared to the control group respectively. Similar results were observed by Skidmore (2011). Hegazy *et al* (2004), in their results revealed level of Estrogen from 1.24 to 67.23 pg / ml.

But the present findings disagree with Ismail *et al*, (2008), they noticed that the Estrogen concentrations were nearly constant throughout the experimental period. In this work, the concentrations of serum P4 (ng / dl) after ovulation significantly ($P \leq 0.05$) increased slowly and then raised steadily after the first week of the ovulation, from 1.15 ± 0.21 to 1.64 ± 0.06, 0.89 ± 0.12 to 4.19 ± 3.15 in the GnRH, hCG groups compared with the control group (0.76 ± 0.15 to 0.82 ± 0.09) respectively. These results are similar with that reported by Skidmore, (2011) who found progesterone concentrations remain low for the first 3-4 days after ovulation and then rise steadily to a peak of around 2.7 ng / ml on day 8 or 9 before falling sharply again on days 10-11 to reach mean values of 0.5 ng / ml by days 11 or 12. The progesterone concentration was

increased after the treatment with GnRH. This is in agreement with that recorded by Ismail *et al.*, (2008) and Anouassi and Tibary (2013), who reported that progesterone levels start to increase 2–3 days after ovulation and reach high levels (>2 ng/ml) by day 5 after ovulation. In the present study the increase of mean serum progesterone concentrations were coincided with the decrease of serum estrogen levels at the same periods. This may indicate the changing of the follicular structures into luteal structures as a result of GnRH and hCG treatments. The findings are similar to previous report (Ismail *et al.*, 2008).

Conclusions

From the present study it could be concluded that:

1. Ovulation can be induced in non-breeding season by using hormonal protocols.
2. The hormonal analysis of estrogen, and progesterone in case of follicular ovarian wave indicated a large individual variation.

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تقييم مستويات الإستروجين و البروجستيرون في الإبل وحيدة السنام بعد حث التبويض خارج موسم التزاوج

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المستخلص:

الهدف من هذه الدراسة هو تقييم تراكيز الإستروجين و البروجستيرون في السيرم بعد حث التبويض بواسطة GnRH و Hcg في الإبل وحيدة السنام. 19 رأس من الإبل وحيدة السنام تم إستخدامها في هذه التجربة، قسمت عشوائياً إلى ثلاث مجموعات خارج موسم التزاوج كما يلي: المجموعة أ (العدد = 7)، كل حيواناتها حقنت في العضل ب 2 مل من GnRH. المجموعة ب (العدد=6)، كل حيواناتها حقنت في الوريد ب 3 مل من hCG. المجموعة ج (العدد=6) أعتبرت مجموعة تحكم، وكل حيواناتها حقنت في العضل ب 1 مل ماء مقطر. جمعت عينات دم في أيام متفاوتة خلال فترة التجربة المحددة (صفر ساعة = زمن بداية الحقن، 48 ساعة = بعد 48 ساعة من بداية المعاملة، الأسبوع الأول = بعد 7 أيام من بداية التجربة، الأسبوع الثاني = بعد 14 يوم من بداية التجربة). لتحديد مستويات الإستروجين والبروجستيرون أستخدمت الـ ELISA. أظهرت النتائج أن مستويات الإستروجين (بيكوجرام / مل) في السيرم لم يختلف معنوياً عند مستوى الإحتمال (0.05) بين كل المجموعات عند 48 ساعة، والأسبوع الثاني. على النقيض كان في صفر ساعة والأسبوع الأول حيث كان هنالك فرق معنوي عالي عند مستوى الإحتمال (0.05) بين مجموعات المعاملات عند مقارنتها بمجموعة التحكم. بينما لم يكن هنالك أي فرق معنوي بين مجموعتي المعاملات الهرمونية خلال كل التجربة. لم تكن هنالك فروقات معنوية عند مستوى الإحتمال (0.05) في مستويات هرمون البروجستيرون في السيرم (نانوجرام / مل) في كل المجموعات عند صفر ساعة والأسبوع الثاني. بينما كان هنالك فرق معنوي عالي جدا عند مستوى الإحتمال (0.01) في مستوى البروجستيرون في السيرم في المجموعة (أ) بالمقارنة مع المجموعة (ب) و مجموعة التحكم على التوالي عند 48 ساعة. أيضاً الأسبوع الأول أظهر أن مستوى البروجستيرون في السيرم فرقا معنوياً عالياً عند مستوى الإحتمال (0.01) في المجموعة (ب) مقارنة بالمجموعة (أ) و مجموعة التحكم على التوالي. يمكن استنتاج أن التحليل الهرموني للإستروجين و البروجستيرون يشير لاختلافات فردية في موجات النشاط المبيضي الحويصلي.