The effect of LH and GnRH analogues on induction of ovulation in Bactrian camel (*Camelus bactrianus*)

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Summary

Ovarian follicle response and corpus luteum formation following induction of ovulation using gonadotropin-releasing hormone (GnRH) analogues and luteinizing hormone (LH) in Bactrian camel were characterized. Bactrian camels with a mature follicle (13-19.6 mm) received: 1) natural porcine LH (25 mg, IV, n = 4), 2) Buserelin (20 µg, IV, n = 4) and 3) Alarelin (25 µg, IM, n = 4). Daily ultrasonography and blood samplings were conducted between day -3 and +15 of the experiment (day 0 = Induction of ovulation). Data were analyzed by univariat analysis with repeated measures analysis included in the model. Following treatment, mature follicle ovulated within 2 days and a new follicle wave emerged after 2-3 days. New mature follicle reached a size of 13.5 ± 0.14 mm by day 12. Corpus luteum was detected on day 6 and reached the maximum size of 19.73 ± 0.81 mm on day 9. Progesterone concentration initiated to increase on day 5, reached maximum concentration on day 9 and decreased significantly on day 11. In conclusion, due to the lack of significant difference among treatment groups (P>0.05), Alarelin may be considered as a drug of choice for inducing ovulation in Bactrian camel because of its effectiveness, simple route of administration (IM vs. IV), lower price, and local availability.

Key words: Bactrian camel, Induction of ovulation, New follicle wave emergence

Introduction

Efficient methods to induce ovulation in camel facilitate the use of reproductive technologies such as artificial insemination and embryo transfer in this species (Cooper *et al*., 1992). In Bactrian camel, ovulation can be induced by deep intravaginal deposition of whole semen or sperm-free seminal plasma (Chen *et al*., 1985), as well as intramuscular injection of seminal fluid (Zhao *et al*., 1990). In Dromedary camel, ovulation can be induced by mating with an intact or vasectomized male (Marie and Anouassi, 1987), whereas, manual stimulation of the cervix or intrauterine injection of whole semen, seminal plasma, water or prostaglandin $F_2\alpha$ analogue, does not stimulate the release of sufficient luteinizing hormone (LH) from the pituitary to cause ovulation (Musa and Abusineina, 1978; Sheldrick *et al*., 1992). The ovulatory response in camel can be multifactorial including a chemical factor in the seminal plasma named as "gonadotropin-releasing hormone (GnRH)-like factor" (Zhao *et al*., 1992; Pan *et al*., 2001; Adams *et al*., 2005), neuro-hormonal responses to the mechanical stimuli of coitus and the male effect (Marie and Anouassi, 1987; Anouassi *et al*., 1992; Moslah *et al*., 1992; Skidmore *et al*., 1996). Although, mating to the vasectomized male and inseminating or injecting seminal plasma were found to be effective in inducing ovulation in camel, they are not routinely, recommended due to the difficulty of collecting semen from male camels and risk of spreading venereal diseases.

Ovulation can be induced in Dromedary and Bactrian camel by a single injection of

LH, GnRH or human chorionic gonadotropin (hCG) (Chen *et al*., 1985; Marie and Anouassi, 1987; Anouassi *et al*., 1992; Sheldrick *et al*., 1992; Skidmore *et al*., 1996). Skidmore *et al*. (1996) characterized ovulatory response in Dromedary camel. The purpose of this study was to characterize ovulatory response and luteal activity following LH, Buserelin and Alarelin injections in Bactrian camel.

Materials and Methods

Experimental location

The investigation was conducted at the Bactrian Camel Research Center, Jahadabad, Meshkinshahr, Ardabil province, Iran (latitude: 38' 23" N; longitude: 47' 40" E; altitude: 1568.5 m) during January and February 2007.

Experimental animals

Twelve female Bactrian camels, 6–14-year-old and 547.6 ± 38.66 kg live weight, free from any uterine and ovarian abnormalities detectable by rectal palpation and ultrasonography, were selected. The females were housed separately from males 20 m apart, but they could see males and hear their sound. Each camel received alfalfa hay (*ad lib*) and 1.5 kg wheat straw mixed with 2.5 kg concentrate including barley (67.8%), cottonseed meal (12%), wheat barn (17.1%), molasses (1.9%), vitamins and minerals (1.2%) on a daily basis.

Ovarian ultrasonography

All procedures were performed on standing camels, restrained in a stanchion equipped with a belt placed under the neck and the caudal abdomen. Ovarian examinations were performed daily using a real time ultrasound scanner (Aloka 500; Japan) equipped with a 5 MHz linear array transrectal transducer. During each examination, the location and diameter of the individually identified follicles (≥4 mm in diameter), and corpus luteum (CL) were recorded using the internal electronic calipers. New follicular wave emergence (initiation of growing phase) was defined as the day at which the dominant follicle had, retrospectively, a diameter of 4-5 mm. Initiation of the mature (dominant) phase was defined when follicular diameter reached 13 mm. The follicle was defined responsive (to GnRH) when its diameter was between 13-19.6 mm. This was based on our previous trial characterizing the mature phase in Bactrian camel (Nikjou, 2008; Nikjou *et al*., 2008). The time at which the mature follicle disappeared was considered as the day of ovulation. The initiation of morphological CL regression was considered when there was a sudden decrease in CL diameter. The interval between the initiations of two consecutive mature phases was defined as the interwave interval.

Blood sampling and progesterone assay

Jugular vein blood samples were collected into vacutainers (Vacutainer systems, Becton Dickson, UK) every morning and kept at 4°C for less than 3 h prior to centrifugation. Following centrifugation of blood samples at 1100 g for 10 min, the serum was decanted and stored at -20°C until assayed for progesterone concentration. Progesterone concentration was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (demeditec Progesterone ELISA: Demeditec Diagnostics GmbH, Germany). The sensitivity of the assay was 0.2 ng/ml and the intra- and inter-assay coefficients of variation were 4.68 and 12.5%, respectively. The suitability of the assay for use with camel serum was tested as described previously (Skidmore *et al*., 1996). In summary, the suitability of the assay for use with camel serum was tested by comparing the parallelism of the standard curve produced by standards made up in charcoal-stripped male camel serum and the human serum provided in the assay kit. In addition, a serum sample of pregnant female camel with high progesterone concentration was diluted serially in the male camel serum and human serum run against the standard curves in the assay. All the curves were parallel with the kit standard curve indicating that camel serum did not interfere with binding in the assay.

The initiation of functional CL

regression was considered when there was a sudden and significant progressive decrease in progesterone concentrations.

Experimental design

Daily ovarian ultrasound examinations were conducted until a mature follicle of 13-19.6 mm in diameter was detected. Then, the camels (n = 12) were randomly divided into three groups and received one of the following treatments (day 0 = injection day): LH: 25 mg, natural porcine LH (Lutropin-V®, Bioniche, Canada; IV); Buserelin: 20 µg, GnRH analogue (Receptal®, Intervet, Holland; IV) and Alarelin or Luliberin-A: 25 µg, GnRH analogue (Vetaroline®, Aburaihan, Iran; IM). Daily blood sampling and ultrasound examinations were performed from day -3 through day +15 of the experiment.

Statistical analysis

Changes in the progesterone concentration and the size of ovarian follicles and CL over time were analyzed by univariat analysis with repeated measures analysis included in the model (Littell *et al*., 1996). Single-point measurements for follicular, CL characteristics were compared using analysis of variance (ANOVA) followed by Tukey's Honestly significant difference (SAS, 2001). Analyses were used where variance-covariance structures over time did not conform to the ANOVA assumptions, using sphericity test in SAS (SAS, 2001). Data were presented as mean ± SEM and p-value <0.05 was considered statistically significant.

Results

Ultrasound examination of the ovaries

There were not significant differences in the size of ovulating follicle, ovulation response, emergence day of new follicle wave, and maturation day of new follicle wave among treatment groups (P>0.05). Also, there were no differences among groups on the days at which CL was detected, reached its maximum size and regressed. Therefore, data for all experimental groups were pooled. Ovulation was induced when mature follicle reached a diameter of 16.0 ± 0.68 (13.9-19) mm, ovulation occurred within 2 days after treatment. New follicular wave emergence was detected on days 2 (LH and Buserelin) and 3 (Alarelin). The dominant follicle derived from this wave reached the mature size of 13.5 ± 0.14 (13-14.5) mm in diameter on day 10 (LH) or 12 (Buserelin and Alarelin) after inducing ovulation (Fig. 1). The growth rate of new follicles was not significantly affected by LH (1.11 ± 0.35 mm/day), Buserelin (0.9 ± 0.41 mm/day) or Alarelin (1.5 ± 0.27 mm/day) treatment. The interwave interval between two consecutive mature phases was 13 ± 0.83 days. Corpus luteum was detected on day 6 at the size of 15.2 ± 0.66 (12.2-22) mm in diameter and continued to grow until day 9, when it reached the maximum size of 19.7 ± 0.81 (15.6-28) mm in diameter. It then started to regress on day 12 (P = 0.04; Fig. 2).

Progesterone assay

There were no differences in progesterone concentration among experimental groups after inducing ovulation. In all groups (Fig. 2), progesterone concentration started to increase on day 5 (P = 0.006), reached maximum concentrations of 2.4 ± 0.3 (LH), 3.8 ± 0.03 (Buserelin) and 2.0 ± 0.24 (Alarelin) on day 9 and decreased significantly on day 11 (P<0.0001). Progesterone concentrations were more than 1 ng/ml on days 7-11, 6-10 and 8-10 in LH, Buserelin and Alarelin groups, respectively.

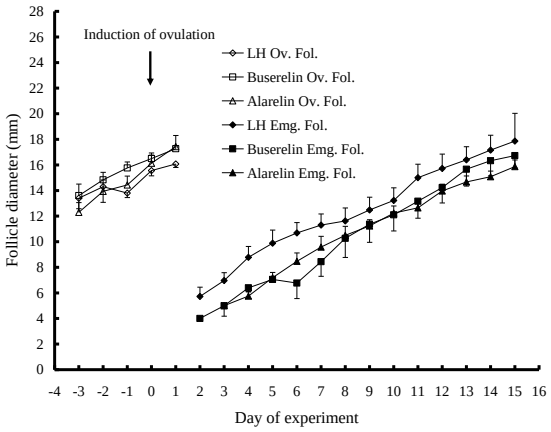

Fig. 1: Follicular dynamics after injection of GnRH analogues (Buserelin and Alarelin) or a natural LH in Bactrian camel

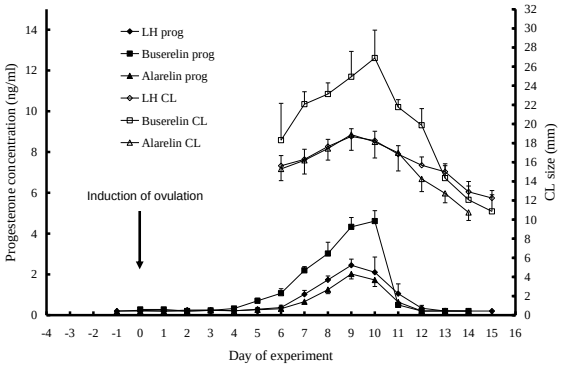

Fig. 2: Corpus luteum (CL) growth and regression, and serum progesterone profile after injection of GnRH analogues (Buserelin and Alarelin) or a natural LH in Bactrian camels

Discussion

Ovulatory response, corpus luteum growth, the time of new wave emergence and serum progesterone concentration were not affected by treatments.

All mature follicles ovulated in response to natural LH, Buserelin, and Alarelin treatments which confirm previous observations (Chen *et al*., 1985; Marie and Anouassi, 1987; Anouassi *et al*., 1992; McKinnon and Tinson, 1992; Sheldrick *et al*., 1992, Alfuraiji and Mousa 1995; Skidmore *et al*., 1996). Chinese researchers used LH (300 IU in 4 ml saline; IM), hCG (1000-2000 IU in 4 ml saline; IM), and LHRH analogue (250-500 µg in 2-4 ml saline; IM) to induce ovulation (follicle size >13 mm in diameter), when camel did not respond to intravaginal insemination of seminal plasma (Chen *et al*., 1985). In the later study, 66% of Bactrian camels ovulated within 36 h after insemination and the remainder ovulated within 48 h. In Dromedary camel, serum LH concentration increased after 1 h, reached a maximum concentration within 2-3 h, and started to decrease about 6 h after mating (Marie and Anouassi, 1987). In Bactrian camel, LH reached its maximum concentration by 4 h but returned to its basal level by 24 h after mating (Xu *et al*., 1985).

In the present study, ovulation was successfully induced when mature follicle was 16.0 ± 0.68 (13.9-19) mm in diameter. Skidmore *et al*. (1996) found that ovulation rate reached 85% in natural mating, 81% with 20 µg Buserelin, and 67% with 3000 IU hCG, when the growing follicle was 0.9-1.9 cm in diameter in Dromedary camel. A marked reduction in the effectiveness of induction of ovulation was observed when the diameter of the dominant follicle exceeded 2.0 cm, and optimal response was achieved between 13 and 17 mm diameter (Skidmore *et al*., 1996).

The interwave interval was 13.0 ± 0.83 days. We found that interwave interval in Bactrian camel during natural follicle wave cycle is 19.1 ± 0.59 days (unpublished data). The reduction in the interwave interval, observed in this study, is in agreement with the previous reports in Dromedary camel in which mating and subsequent ovulation shortened the interwave interval from 18.2 ± 3.8 days in non-mated camels to 13.8 ± 3.3 days in mated animals (Skidmore *et al*., 1996). These observations also rationalized previous strategies for reproductive management in which female camel was ready to mate with fertile bulls on 12-13 days after a sterile mating (Chen and Yuen, 1984; Marie and Anouassi, 1987). Following induction of ovulation, the new follicular wave started to emerge during the luteal phase, and the follicle became mature and ovulatory once luteolysis occurred. Rapid decline in progesterone secretion as a result of luteolysis enhances the growth of the newly recruited follicle and thus reduced the interwave interval (Skidmore *et al*., 1996). Using these findings, both in Dromedary and Bactrian camels, it may be hypothesized that two injections of ovulating agents, about 13-14 days apart may be used to control and synchronize follicle wave cycles in camel (Nikjou *et al*., 2008).

The new follicular wave emerged on day 2-3 and reached the mature size of 13.5 ± 0.14 mm on day 12 after injections. In Dromedary camel, Skidmore *et al*. (1996) have shown that new follicles became visible about 5 days after mating and took about 8 days to become mature follicle with the diameter of 13 mm. The pattern of CL growth and regression was similar to the previous findings for Dromedary camel (Marie and Anouassi, 1987; Skidmore *et al*., 1996), Bactrian camel (Chen *et al*., 1985), and llamas (Adam *et al*., 1989; Adams *et al*.,

1991).

Progesterone concentration started to increase on day 5, peaked at 2.7 ± 0.2 ng/ml on day 9 and started to decrease on day 11 after induction of ovulation. In comparison with other farm animals, CL regressed earlier and progesterone declined immediately after reaching maximum concentration in either Dromedary or Bactrian camel.

In conclusion, ovulation can be induced by natural LH, Buserelin or Alarelin when the growing follicle is between 13 and 19 mm in diameter in Bactrian camel. Alarelin may be a drug of choice for inducing ovulation in Bactrian camel because of its effectiveness, simple route of administration (IM vs. IV), lower price and local availability.

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