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The influence of active immunization against inhibin on dromedary camel ovarian and hormonal dynamics

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ABSTRACT

The efficiency of neutralizing endogenous inhibin on promoting camel ovarian dynamics during the transition period in Egypt (mid-August–mid-December) was investigated. Ten pluriparous dromedary camels, *Camelus dromedarius*, were divided into 2 groups of five animals each. Animals of the first group were actively immunized against recombinant bovine inhibin-A, whereas animals of the second group served as control. Ovarian follicular activity was monitored by transrectal ultrasonography throughout the entire experimental period. Changes in hormonal profiles of estradiol 17-β (E2), follicle stimulating hormone (FSH) and circulating inhibin-A (INH-A) were also determined. The results showed that on day 28 following initial immunization (mid-September), mean values of total no. of follicles, FSH and E2 concentrations in the immunized camels were 3.5, 3.3 and 2.6 times higher than those of control, respectively. Furthermore, the immunized group exhibited improvement in ovarian follicular activity throughout the transition period, whereas ovarian dynamics in the control group remained limited until the onset of the breeding season (mid-December). Additionally, the immunized camels recorded a positive correlation ($P<0.05$) between serum E2 concentrations and each of FSH ($r=0.77$), circulating inhibin-A ($r=0.56$), total no. of follicles ($r=0.75$) and diameter of dominant follicles ($r=0.69$). These results elucidate that active immunization against inhibin may provide a sufficient method to enhance ovarian activity and can override seasonal anestrus in dromedary camels prior to the onset of the breeding season.

1. Introduction

In mammals, several hormones work in a finely-orchestrated harmony to regulate reproduction. In addition to typical sex steroids, mammalian ovaries produce a large family of non-steroid hormones; specifically the transforming growth factor-β (TGF-β) superfamily (Kingsley, 1994). The TGF-β family of cytokines comprises over 40 identified members among which is inhibin (Robertson et al., 2004). Inhibin is a disulphide-linked heterodimeric glycoprotein composed of two dissimilar subunits designated as alpha and beta, where dimerization of these subunits produces two forms of inhibin namely inhibin-A and inhibin-B (Campbell and Baird, 2001; Zhu et al., 2013). Although both types of inhibin show similar bioactivities (Ling et al., 1986), inhibin-B has been reported to exhibit 15–20% the activity of inhibin-A *in vitro* (Robertson et al., 1997). Further, the dominant form of dimeric inhibins shifts from inhibin-B to inhibin-A with follicular development (Ohshima et al., 2002). Thus, inhibin-A is considered the principal isoform responsible for inhibin bioactivity (Robertson et al., 1996).

In the ovaries, the follicular fluid is a rich source of inhibin since it is secreted from both granulosa and theca cells to predominate other non-steroid hormones (Knight, 1996). Through endocrine, autocrine and paracrine actions (Knight and Glister, 2001), inhibin suppresses folliculogenesis in females by selectively and potently inhibiting follicle stimulating hormone (FSH) secretion from the pituitary (Medan et al., 2007).

Different types of inhibin-based immunogens were used to promote ovarian follicular development mediated by an increase in plasma FSH concentration. These include steroid-free follicular fluid, native/purified inhibin, synthetic inhibin-α peptides and recombinant inhibin-α subunits (reviewed by Bhardwaj et al., 2012). Suppression of the regulatory effect of inhibin, either actively or passively, has been reported to directly stimulate ovarian follicular development in sheep (Bingol et al., 2012; Naqvi et al., 2009), goats (Medan et al., 2003; Wang et al., 2009), cattle (Takedomi et al., 2005), buffalo (Li et al., 2011) and horses (Mckinnon et al., 1992; Meyers-Brown, 1995). In camels, however, only one brief description of an unpublished preliminary trial has been reported (Tibary and Anouassi, 1997). The objective of this

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investigation is to evince the efficiency of active immunization against inhibin-A on promoting camel ovarian activity during the transition period in Egypt.

2. Materials and methods

2.1. Animals and management

This investigation was carried out at the Artificial Insemination Lab. in Maryout Research Station (Latitude 31°00′N; Longitude 29°47′E), which belongs to Desert Research Center, Egypt. Ten healthy adult non-lactating one-humped camels, *Camelus dromedarius*, aged from 6 to 13 years and with an average body weight of 420 ± 17.3 kg were used during the transition period (mid-August–mid-December, 2013). The animals were maintained in an open paddock with fenced area and were allowed a daily free-grazing period from 0800 to 1400 h, and Egyptian clover (*Trifolium alexandrinum*) hay was offered *ad libitum*. Fresh water was presented once daily after returning from the pasture. Prior to executing the experiment, all selected animals were clinically examined and were found free of disease and reproductive disorders. All procedures and experimental protocols were conducted in accordance with the "Guidelines on the Care and Use of Animals for Scientific Purposes" developed by the National Advisory Committee for Laboratory Animal Research (NACLAR), Singapore (2004).

2.2. Experimental design and immunization protocol

The camels were randomly divided into 2 groups of five animals each. The first group was actively immunized against inhibin-A following a regimen adapted from that described earlier in mares (McCue et al., 1992). Each female was initially immunized by a subcutaneous dose of 1 ml inhibin vaccine, equivalent to 100 μg recombinant bovine inhibin-A (Cusabio; Cat. no. CSB-EP011718BO). The recombinant protein was dissolved in PBS solution, and then was emulsified with an equal volume of Freund's incomplete adjuvant (Sigma–Aldrich). The preparation of this water-in-oil mixture was done according to the manufacturer's instructions. Afterwards, starting 14 days from initial immunization, each female received two subcutaneous boosters of 50 μg/dose each at 2-week intervals. Simultaneously, each of the control camels was treated subcutaneously with 1 ml saline emulsified in 1 ml Freund's incomplete adjuvant at times paralleling to that of the treated camels.

2.3. Ultrasound examination

Ovarian follicular dynamics in all camels were weekly monitored throughout the period of the study starting one week before initial immunization. The examination was carried out using a Dynamic Imaging, concept MLV scanner ultrasound device (Eickemeyer Magic 5000 Digital), integrated with a dual frequency (5.0–7.5 MHz) linear transducer. The ovaries were examined for presence of different structures as follows; I: Follicular recruitment (multiple small follicles <0.4 cm in diameter), II: Growing follicles (≥0.5–1 cm), III: Mature preovulatory follicles (≥2 cm) and IV: Anovulatory follicles (≥2 cm). Non-echogenic structures with increased echogenicity within their cavity were considered regressing follicles (Tibary and Anouassi, 2000). In camels bearing multiple follicles, mean values of mature preovulatory follicles on both ovaries were considered when calculating average values of dominant follicles size. The number and size of different types of follicles were regularly recorded using a printer (Sony) connected to the ultrasound.

2.4. Blood samples

Blood samples were collected from the jugular vein of all animals before access to food and water, paralleling ultrasound examinations, throughout the period of the study. The samples were withdrawn into non-heparinized tubes, and then were transferred to a refrigerator at 5 °C. Blood serum was separated by letting the collection tubes stand oblique for 24 h, then serum was aspirated and stored at −20 °C until analyses.

2.5. Reproductive hormones analyses

Blood serum estradiol 17-β (E2) was analyzed using competitive solid phase enzyme immunoassay kits (BioVendor—Laboratorní medicína a.s., Czech Republic) with 7.7% intra- and 8.7% inter-assay CV's. Additionally, hormonal profile of follicle stimulating hormone (FSH) in blood serum was analyzed using competitive enzyme immunoassay kits specified for determination of bovine FSH. The kits were obtained from Cusabio Biotech Co., Ltd. (Cat. no. CSB-E15856B) with intra and inter assay CV's <8% and <10%, respectively. Similarly, circulating inhibin-A (INH-A) was analyzed using ELISA kits for determination of bovine inhibin alpha chain (Cusabio; Cat. no. CSB-EL011718BO), with intra and inter-assay CV's <8% and <10%, respectively. All analyses procedures were performed following instructions provided by the manufacturers.

2.6. Statistical analysis

Initially, normal distribution was checked using the Shapiro–Wilk test, and when the distribution was not normal data were $\log_{10}$-transformed to improve the approximation of normality. The changes in ovarian dynamics and reproductive hormones concentrations in control and vaccinated camels, throughout the transition period, were statistically analyzed by repeated measures analysis of variance (ANOVA) to determine the fixed effects of group, day and group by day interaction on total number of follicles, diameter of the dominant follicles, as well on estradiol 17-β, follicle stimulating hormone and inhibin-A concentrations. The differences between means were detected using Duncan's Multiple Range Test (Snedecor and Cochran, 1956). The correlations among reproductive hormones, as well as among ovarian dynamics criteria and hormone levels were determined by Spearman correlation coefficient. The statistical significance threshold was set at 5% and data were analyzed using IBM-SPSS statistics program (IBM-SPSS, 2013). The data are expressed as means ± standard error (SE).

3. Results

The results showed that follicle numbers (FNs) in the control group were not significantly affected throughout the transition period. However, the immunized camels recorded low ($P < 0.05$) mean FNs until day 14 (1.17 ± 0.3), then increased ($P < 0.05$) starting from day 21 (early September) thereafter to record the highest ($P < 0.05$) total number of follicles in mid-December (day 119) with values 3.10 ± 0.5 and 5.1 ± 0.3, respectively. These values were higher ($P < 0.05$) than those recorded in the control group with corresponding values 1.0 ± 0.0, 1.0 ± 0.0 and 1.25 ± 0.2, respectively (Fig. 1). In the meantime, average diameter of preovulatory follicles (≥1–2 cm) in immunized camels started to increase ($P < 0.05$) from day 91 (1.23 ± 0.2 cm) to reach maximum diameters on day 119 (1.9 ± 0.5 cm). These values were higher ($P < 0.05$) than those of control with respective values 0.98 ± 0.2 and 0.51 ±0.2 cm for the same days, respectively (Fig. 1). Collectively, immunization against endogenous inhibin successfully improved ovarian follicular activity during the transition period (Fig. 2).