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The prolonged reproductive response to immunization against inhibin and manipulating ovarian hyperactivity for timed ovulation in camels

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ABSTRACT

The current study aimed: **a)** to investigate the extended effects of immunization against inhibin on dromedary camel ovarian function and **b)** to determine the efficiency of manipulating ovarian hyperactivity in the actively-immunized camels on synchronization of ovulation time compared to conventional hormonal superovulation. Sixteen pluriparous camels, *Camelus dromedarius*, were used during the breeding season (January-February), and were divided into 3 groups: **immunized camels** (n = 5) were previously immunized against inhibin (100 μg recombinant bovine inhibin-A subcutaneously followed by two boosters of 50 μg/dose each at 2-week intervals) during the former transition period (August–September), and was further subjected to a synchronization of ovulation regimen (5000 i.u. hCG intramuscularly (i.m) (1st ovulation induction, day 0) followed by 789 μg Cloprostenol acetate i.m. seven days later, and then inducing ovulation on day 17) five months after immunization; **eCG-treated camels** (n = 6) were superovulated by 2500 i.u. eCG (i.m.) after administration of progesterone for 13 consecutive days; **control** (n = 5) received 1 ml saline (i.m.). Ovarian dynamics were monitored by transrectal ultrasonography throughout the experimental period, and the changes in reproductive hormones were determined. At the peak of the mature phase of follicular development, total no. of follicles was higher ($P < 0.05$) in both immunized (9.0 ± 1.0) and eCG-treated camels (7.0 ± 0.6) compared to that of control (3.0 ± 0.7), whereas no significant difference was observed in dominant follicle size. Meanwhile, FSH concentration recorded higher ($P < 0.05$) mean values in the immunized camels than in both eCG-treated and control camels. Further, both of circulating inhibin and E_2 concentrations were higher ($P < 0.05$) in immunized and eCG-treated camels compared to the control. Triple ovulation rates were higher ($P < 0.05$) in both immunized and eCG-treated camels compared to that of control with values 100, 100 and 0%, respectively. These results imply that, after 5 months from initial immunization, active immunization against inhibin originated extended ovarian hyperactivity, which could be effectively controlled for timed ovulation in camels. The results also demonstrate the efficiency of this technique as a sufficient alternative to traditional hormonal superovulation in dromedaries.

1. Introduction

As in other farm animals, embryo transfer in camels implicates efficient means to generate a reliable superovulatory response in donors. To date, several conventional gonadotropin-based regimens have been developed to induce superovulation in dromedaries (reviewed by Anouassi and Tibary, 2013). In this regard, the treatment is initiated by inhibiting follicular development with a progesterone prime followed by stimulation of ovarian activity, and then elimination of preovulatory follicle(s) (Nowshari and Ali, 2005). However, hormonal superovulation is usually associated with many constraints; i.e., the amount of time and labor involved, high incidence of retained follicles, substantial individual variations in ovarian response and quality of recovered embryos (Holtz, 2005). In addition, repeated superovulation brings about an apparent refractoriness to the treatment due to the formation of antibodies against exogenous gonadotropin. Furthermore, there is a lack in knowledge as to how much time is required for a superovulated animal to reinstitute its hormonal balance before a renewed superovulatory treatment is considered (Holtz, 2005; Anouassi and Tibary, 2013). Therefore, several studies have been conducted to establish alternatives to this conventional technique.

Since the discovery of inhibin (McCullagh, 1932), its initial isolation from bovine follicular fluid (De Jong and Sharpe, 1976), and

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the recognition of its role as a classical endocrine hormone (Findlay, 1994); a specific increasing interest has been raised in developing inhibin-based fecundity vaccines to induce multiple ovulation in different species (Han et al., 2007; Padilla et al., 2008; Wang et al., 2009). These include steroid-free follicular fluid, native/purified inhibin, synthetic inhibin-α peptides and recombinant inhibin-α subunits (reviewed by Bhardwaj et al., 2012).

Immunization against endogenous inhibin, either actively or passively, has been reported to induce superovulation, increase ovulation rates and embryos collected in sheep (Bingol et al., 2012; Zhu et al., 2013), goats (Kandiel et al., 2008; Wang et al., 2009), cows (Takedomi et al., 2005), buffalos (Li et al., 2011), and mares (Meyers-Brown, 1995). In camels, Tibary and Anouassi (1997) briefly described the efficiency of immunizing camels against the N-terminal sequence of inhibin-A on the ovarian activity. Most recently, active immunization against inhibin was sufficiently used to alter the reproductive hormones pattern, hence, improved camel ovarian activity off the breeding season (Rateb et al., 2015). However, to date, no publications addressed the long-term effects of this technique on dromedary ovarian function. Thus, the present investigation aims: **a)** to elucidate whether active immunization against inhibin during the transition period (August-September) has a prolonged superovulatory effect during the subsequent breeding season (January-February) and **b)** to establish an efficient hormonal regimen to control the immunized camels' ovarian activity for timed ovulation.

2. Materials and methods

2.1. Animals and management

This investigation was conducted during the breeding season (January-February) on sixteen adult non-lactating camels, *Camelus dromedarius*, with an average body weight of 425 ± 15.2 kg, body condition score 2.5 ± 0.5 (Faye et al., 2011) and aged from 6 to 13 years. The animals were housed in an open paddock with fenced area belonging to the Artificial Insemination Lab., Maryout Research Station (Latitude 31° 00′ N; Longitude 29° 47′ E), Desert Research Center, Egypt. The camels were allowed to graze daily 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 animals were clinically examined and were found free of disease or reproductive disorders. All procedures and experimental protocols were conducted in conformity with the European Union Directive for the protection of experimental animals (2010/63/EU). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

2.2. Experimental design

The camels were divided into 3 experimental groups. The first group (n = 5) was previously immunized against inhibin, and was further subjected to synchronization of ovulation after 5 months from initial immunization. The second group (n = 6) was subjected to a gonadotropin-based superovulation protocol. The third group (n = 5) served as control and received 1 ml saline intramuscularly.

2.3. Immunization protocol

Camels of the first group were actively immunized against inhibin-A during the transition period (August-September), and the experiment was carried out during the subsequent breeding season (January–February). The immunization protocol was conducted according to the method described recently (Rateb et al., 2015). Briefly, each female received a subcutaneous initial immunization shot of 100 μg recombinant bovine inhibin-A (Cusabio; Cat. no. CSB-EP011718BO) emulsified in Freund's adjuvant (Sigma-Aldrich). Afterwards, starting 14 days from initial immunization, each female received two subcutaneous boosters of 50 μg/dose each at 2-week intervals.

2.4. Superovulation and synchronization of ovulation protocols

A hormonal protocol was established to synchronize ovulation in actively-immunized camels during the breeding season. At the beginning of the experiment (day 0), transrectal ultrasonography revealed presence of multiple mature follicles in all previously-immunized camels. Accordingly, each female received 5000 i.u. hCG (Chorimon, Institute Biochimique S.A., Switzerland) intramuscularly (i.m.) to induce ovulation (1st ovulation induction, day 0). Seven days later, each camel was treated with 789 μg Cloprostenol acetate (Estrumate®, Agro-pharm Inc., Willow dale, Ontario, Canada) i.m. to induce luteolysis of the formed corpora lutea (day 7). Starting four days from the $PGF_{2\alpha}$ treatment, ovarian dynamics were daily monitored by transrectal ultrasonography, and then the camels were subjected to induction of ovulation when the follicles reached the optimum size for ovulation (2nd ovulation induction, day 17) as previously illustrated.

In the meantime, camels of the second group were hormonally superovulated according to our most recently described protocol (Khalifa et al., 2016). Initially (day -13), a Controlled Intra-vaginal Drug Releaser (CIDR 1.38 g, Pfizer®, Italy) was inserted vaginally in each animal for 13 consecutive days. One day before CIDRs removal, each camel was treated with 789 μg Cloprostenol acetate intramuscularly. Immediately after CIDR withdrawal, each female received a single shot of 2500 i.u eCG (Folligon®, Intervet Corp., Canada) to stimulate ovarian activity. Starting from day 4 after the superovulation treatment, ovarian dynamics were monitored by transrectal ultrasonography at 2-day intervals until reaching the optimum time for induction of ovulation (day 12), where each camel received 5000 i.u. hCG intramuscularly.

Simultaneously, the normal pattern of ovarian activity was monitored in the control group at 4 day intervals after receiving 1 ml saline intramuscularly. At the peak of the mature phase of follicular development ovulation induction was performed as formerly shown.

Induction of ovulation in all groups was carried out when at least one ovarian follicle reached the size of 1.3 to1.8 cm in diameter (Manjunatha et al., 2012). Ovulation rates were recorded by transrectal ultrasonography at 72 h following induction of ovulation.

2.5. Ultrasound examination

Ovarian dynamics in all camels were monitored 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 according to the time scale of each protocol. Ovarian structures were categorized as; I: Follicular recruitment (multiple small follicles < 0.4 cm in diameter arranged at the periphery of the ovary), II: Growing follicles (≥ 0.5–1 cm), III: Mature preovulatory follicles (black circles protruding on ovarian surface ≥1–2 cm in diameter) and IV: Anovulatory follicles (≥2 cm) (Manjunatha et al., 2012). The number and size of different types of follicles were recorded throughout the period of the study. In camels bearing multiple follicles, mean values of mature preovulatory follicles on both ovaries were considered when calculating average values of dominant follicles size. Ovulation was confirmed