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Comparing three superovulation protocols in dromedary camels: FSH, eCG-FSH and hMG

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Summary

The objective of this study was to compare three superovulation protocols in dromedary camels. Follicular waves of dromedary camel donors (n=6) were synchronized using two GnRH injections. Superovulation was initiated 2 days after the second injection (day 0 of experiment). The experiment was conducted using change over design, where all females received three superovulation treatments one after the other with a resting period of one month in between. Superovulation was carried out for all donors using 390 mg FSH, 1000 IU eCG and 330 mg FSH, or 16.5 ampule hMG (75 i.u. FSH and 75 i.u. LH per ampoule). FSH and hMG were injected twice daily, indecreasing doses, over 5.5 days. Thirty-six h after the last FSH or hMG injection, donors were mated once and received an intravenous injection of the GnRH analogue. The diameter and number of ovarian follicles ≥4 mm on day 4 after superovulation and the total number of corpora lutea and follicles ≥9 mm on day 7.5 after mating were evaluated by ultrasound examinations. No significant differences were found between the total number of corpora lutea among FSH (13.8 ± 2.65), eCG-FSH (15 ± 2.60) and hMG (10.8 ± 2.30) and the number of expanded hatched blastocysts in FSH (5.7 ± 2.32), eCG-FSH (8.8 ± 2.10) and hMG (5.8 ± 2.40) treated donors. This study showed that all three superovulatory protocols could be used successfully and interchangeably in dromedary camels.

Key words: Camels, eCG, FSH, hMG, Superovulation

Introduction

Camels are seasonal breeders that produce the first calf around 5-6 years of age following a long gestation period of nearly 12.5 months (Merkt *et al*., 1990). Their calving intervals could be as long as 18 to 30 months (Tibary *et al*., 2005). Hence, the number of offspring produced by an elite female camel is not sufficient for genetic intervention (Musa *et al*., 1993).

Multiple ovulation and embryo transfer (MOET) helps shorten the generation interval and increases the number of progenies from desirable females. Camel embryo transfer has been used since 1992 (McKinnon and Tinson, 1992; Skidmore *et al*., 1992); however, there is variation in superovulatory responses to different gonadotropins in camels. Previous literature reported wide ranges of transferable embryos following superovulation by FSH (0-36; Vyas *et al*., 1998; Tinson *et al*., 2000; Anouassi and Tibary, 2013), eCG (0-19; Anouassi and Ali, 1990; Cooper *et al*., 1990; McKinnon *et al*., 1994; Vyas *et al*., 1998; Tinson *et al*., 2000; Anouassi and Tibary, 2013), and eCG-FSH (0-5.4; Skidmore *et al*., 2002; Skidmore and Billah, 2011).

Several problems have been associated with eCG treatment including the development of two different generations of follicles, premature luteinization of follicles, failure of ovulation and the development of large anovulatory follicles (Anouassi and Tibary, 2013). Additionally, the repeated use of eCG and/or FSH is speculated to induce a refractoriness to the treatment (Anouassi and Tibary, 2013). Human menopausal gonadotropin (hMG) has been used successfully for superovulation in cattle (Lauria *et al*., 1982a, b; McGowan *et al*., 1985). However, there is no evidence regarding its use in camels.

The objective of this study was to investigate superovulatory responses following FSH, eCG-FSH and hMG treatments in dromedary camels.

Materials and Methods

Experimental animals

This study was conducted at the Camel Advanced Reproductive Technologies Center (CARTC, Margham, UAE), from December 2015 to April 2016. Six dromedary camel donors that were between 6-14 years old and 553.87 ± 18.09 kg and were free from uterine and ovarian abnormalities detected by rectal palpation and/or ultrasound examination, were used in this experiment. Each camel received alfalfa hay (4 kg), mixed dried hay (3 kg), mixed concentrate (1 kg; Zabeel B-Mix, Zabeel Feedmill, Dubai, UAE), dry dates (300 g), and wheat bran (700 g) on daily basis. They had free access to water and mineral blocks.

Experimental design

Follicular waves of elite dromedary camel donors were synchronized using two intramuscular injections of

a GnRH analogue (Alarelin, 25 µg, Vetaroline®, Aburaihan, Iran; Moghiseh *et al*., 2008) 12-15 days apart. Ultrasound scanning was performed prior to the second GnRH injection (between 12-14 days after the first GnRH) to ensure the presence of a mature follicle (12-18 mm in diameter). The experiment was implemented using change over design in which two days after GnRH administration, all six females received three superovulation treatments one after the other with a resting period of one month in between. In order to ease the embryo recovery procedure, the experiment was carried out in 6 replicates, each consisting of 3 females that received different superovulation protocols. Accordingly, all donors received either FSH (390 mg, Folltropin-V®, Bioniche, Canada; intramuscular), eCG (1000 IU, Pregnecol®, Bioniche, Canada)-FSH (330 mg), and hMG (16.5 ampules, 75 IU FSH and 75 IU LH; Menogon®, Ferring GmbH, Germany), intramuscularly. FSH was administered to all donors twice daily in decreasing doses (80, 50, 30, 20, 10 mg) between days 0 and 4, as well as a single injection of FSH on day 5 (10 mg) after superovulation. In the eCG-FSH treatment, donors received a single intramuscular injection of eCG (1000 IU) on day 0 of superovulation, followed by two daily injections of FSH in decreasing doses (60, 40, 30, 20, 10 mg) and a single injection of 10 mg FSH on day 5 after superovulation. In the hMG treatment, donors received hMG (4, 2, 1, 0.5, 0.5 ampules) twice daily between days 0 and 4, and a 0.5 ampule on day 5 after superovulation. On day 4 after superovulation, camels received 2 doses of prostoglandin $F_2\alpha$ analogue (25 mg Dinoprost tromethamin; Lutalyse®, pfizer, Belgium; intramuscular), 12 h apart and the diameter and number of ovarian follicles (≥4 mm) were recorded for each ovary. Thirty-six h after the last FSH or hMG injection, each donor was mated with a dromedary camel bull of proven fertility, and received an intravenous injection of a GnRH analogue (40 µg buserelin acetate, Gestar®, Over, Argentina). On day 7.5 after mating (the day before embryo recovery), the total number of corpora lutea and follicles ≥9 mm in diameter was recorded. On day 8.5 after mating (7.5 days after ovulation = the day of embryo recovery), the total number and diameter of expanded hatched blastocysts were recorded.

Ultrasonography

Camels were restrained in a standing position in a stanchion equipped with a belt placed under the neck and under the caudal abdomen. Routine ultrasound examinations of ovaries and uterus were conducted using a real time ultrasound scanner (Aritta V60, Hitachi Aloka, Japan) equipped with a 7.5 MHz intraoperative convex transducer (UST-9132IWP). Ovarian structures including follicles (≥4 mm) and corpora lutea were measured with an internal caliper.

Embryo recovery

The uterus was flushed non-surgically 8.5 days after mating. Donors were restrained in a standing position, sedated with xylazine hydrochloride (50 mg, IV, AnaSed®, Shenandoah, lowa, USA) and received caudal epidural anesthesia (100 mg lignocaine hydrochloride, ilium Lignocaine 20®, Troy Laboratories, Australia). After cleaning the perineal area with betadine surgical scrub (Purdue Products, USA) and 70% ethanol spray, a silicone 2-way foley catheter (66 cm; 20 FR, 30 CC; Vetoquinol, USA) with stylet was introduced into the vagina and directed toward the external opening of the cervix. The catheter was then directed through the cervix via rectal manipulation, where the balloon was inflated (25-30 ml) in front of the external opening of the cervix. Flushing of the uterus was performed several times (total 2000 ml of flushing media) with warm Ringer serum (37°C; Ringers Injection, Egypt Otsuka Pharmaceutical Co.) supplemented with BSA (2 g/L; Bovine Serum Albumin, Sigma-Aldrich, USA), penicillin G Na (100 IU/ml; Pen® Potassium, Jaberebn Hayyan Pharmaceutical, Tehran, Iran) and streptomycin sulfate (100 µg/ml; Streptocin®, Jaberebn Hayyan Pharmaceutical, Tehran, Iran). Expanded hatched blastocysts were later recovered by passing the used flushing media through a filter (90 µ pore size; Em Con filter, Kruuse, Germany) and examining the residues on a large square gridded dish (100 × 15 mm; Reproduction Provisions LLC, USA) using a stereomicroscope (OLYMPUS, SZ51, Japan, magnification: ×10-40). Each expanded hatched blastocyst was then evaluated and measured (longitudinally and vertically) by another stereomicroscope (OLYMPUS, SZX16, Japan) equipped with a digital camera (OLYMPUS, DP26, Japan) and special software associated with the internal caliper (CellSens software, Olympus, Japan).

Statistical analysis

Between group differences were compared using Proc GLM followed by a Tukey test in SAS (2014). When the assumptions of parametric tests were not met, the non-parametric ANOVA for single factors (Kruskal Wallis one way ANOVA) was used. Data were presented as means±SEM.

Results

For the first and second GnRH analogue injections, the sizes of the ovarian follicles were 15 ± 0.56 (12-18 mm) and 13.4 ± 0.53 (11-17.6 mm), respectively. On day 4 after superovulation, the total number of follicles ≥4 mm, follicles ≤6 mm and follicles >6 mm were not significantly different between the three treatments (P>0.05; Table 1). On day 7.5 after mating, there was no significant difference in the number of corpora lutea between the three treatments (FSH: 13.8 ± 2.65; eCG-FSH: 15 ± 2.60; hMG: 10.8 ± 2.30; P>0.05; Table 1). However, the total number of follicles ≥9 mm was larger in the hMG (13.2 ± 2.73) treatment compared to the FSH (0.7 ± 0.33) treated groups (P<0.05) and was comparable to the eCG treatment (7.7 ± 2.10; P>0.05; Table 1).

The total number of recovered expanded hatched blastocysts was not different between the three treatments [FSH: 5.7 ± 2.32 (2-17); eCG-FSH: 8.8 ± 2.10

Table 1: Superovulatory responses including: follicles ≥4 mm on day 4 of superovulation, corpora lutea and follicles ≥9 mm on day 7.5 after mating and expanded hatched blastocysts (number and diameter) recovered on day 8.5 after mating following FSH, eCG-FSH and hMG treatments in dromedary camels

Groups	Follicles on day 4 of superovulation			Day 7.5 after mating		Embryo	
	≤6 mm	>6 mm	Total	Corpus luteum	Follicle ≥9 mm	Number	Diameter (µm)
FSH	4.5 ± 1.82[a]	15.2 ± 2.20[a]	17.7 ± 3.40[a]	13.8 ± 2.65[a]	0.7 ± 0.33[a]	5.7 ± 2.32[a]	738.1 ± 37.02[a]
eCG-FSH	4.3 ± 0.84[a]	19.8 ± 2.81[a]	25.8 ± 4.84[a]	15 ± 2.60[a]	7.7 ± 2.10[ab]	8.8 ± 2.10[a]	793.9 ± 23.65[a]
hMG	7.8 ± 1.90[a]	19.8 ± 2.81[a]	27.7 ± 3.15[a]	10.8 ± 2.30[a]	13.2 ± 2.73[b]	5.8 ± 2.40[a]	886.2 ± 57.97[a]

[ab] Values within columns with different superscript differ (P<0.05). Data are presented as mean±SEM

(1-17); hMG: 5.8 ± 2.40 (0-16); P>0.05; Table 1]. In addition, no difference was found between the diameter of the expanded hatched blastocysts in the FSH [738.1 ± 37.02 (393-1167 µm)], eCG-FSH [793.9 ± 23.65 (419-1099 µm)] and hMG [886.2 ± 57.97 (300-1571 µm); P>0.05] treatments. No unfertilized ova were recovered from the donors in this study.

Discussion

The present study investigated superovulatory responses following the administration of FSH (390 mg), combined eCG (1000 IU) and FSH (330 mg) and hMG (16.5 ampules) in dromedary camels. We found no significant difference in the total number of corpora lutea and expanded hatched blastocysts among the three superovulation protocols. Previous research on different superovulatory regimens for dromedary camels suggests a single injection of eCG (1500 to 6000 IU) to be the first and easiest choice to superovulate camels resulting in 0 to 19 embryos (Anouassi and Ali, 1990; Cooper *et al*., 1990; McKinnon *et al*., 1994; Vyes *et al*., 1998; Tinson *et al*., 2000; Anouassi and Tibary, 2013). The variation in superovulatory responses following the eCG treatment could be attributed to individual refractoriness to the hormone. Accordingly, approximately 20-30% of superovulated females do not develop any follicles, possibly due to their immunization against eCG. The high incidence of follicle luteinization, possibly caused by LH activity, is another problem of using this hormone (Anouassi and Tibary, 2013). Another disadvantage may be attributed to eCG's long half-life, resulting in excessive follicular development followed by ovulation failure. The half-life of glycoproteins such as eCG has been associated with the sialic acid content of these molecules (Morell *et al*., 1971). Accordingly, the eCG plasma half-life is about 6 days in horses (Cole *et al*., 1967), 50-120 h in cow (Schams *et al*., 1978) and 21 h in sheep (McIntosh, 1974), whereas, the half-life of FSH is 94 ± 21, 334 ± 41, 118 ± 16, 301 ± 23 min for rats, rabbits, ewes and cows, respectively (Laster, 1972). Because of the drawbacks of eCG, FSH alone was used instead of eCG with promising results (McKinnon *et al*., 1994; Vyes *et al*., 1998). In a comparative study, the FSH protocol (n=68) resulted in 3.84 embryos, while the eCG treatment (n=84) produced 2.3 embryos (McKinnon *et al*., 1994). Similar results were reported using FSH (400 mg; n=176; 8.2 ± 6.1 embryos) and eCG (3000 IU; n=7.1 ± 4.3 embryo; Anouassi and Tibary, 2013). In order to take advantage of the long half-life of eCG and benefit from FSH, a combination of both has been applied for the superovulation of camels (Skidmore *et al*., 2002; Nowshari and Ali, 2005; Skidmore and Billah, 2011). In an earlier research, the combination of FSH (400 mg) and eCG (2500 IU) was associated with 5.8 embryos (Skidmore *et al*., 2002). In another study using nearly the same protocol (FSH: 400 mg and eCG: 2000 IU), a smaller number of embryos (3.4 embryos) were produced (Skidmore and Billah, 2011). In yet another comparative study using FSH alone (400 mg) or a combination of eCG (2000 IU), 5.1 ± 3.7 and 1.8 ± 3.6 embryos were produced, respectively (Nowshari and Ali, 2005). In the present work, the response to the eCG-FSH treatment (8.8 ± 2.10 expanded hatched blastocysts) seemed to be better than previous studies. Besides, the amounts of eCG (1000 IU) and FSH (330 mg) were comparatively less and the induction of ovulation and mating were performed at a fixed time on day 6 after superovulation.

Alternative medicine (hMG; 16.5 ampule, twice daily for 5.5 days) was used for the superovulation of dromedary camels in the present study. Superovulatory responses in the hMG treatment (corpora lutea: 10.8 ± 2.30 and expanded hatched blastocyst: 5.8 ± 2.40) were similar to FSH (corpora lutea: 13.8 ± 2.65 and expanded hatched blastocysts: 5.7 ± 2.32) and eCG-FSH treatments (corpora lutea: 15 ± 2.60 and expanded hatched blastocysts: 8.8 ± 2.10; P>0.05). Comparable results using FSH or hMG in the suprovulation of dairy (Lauria *et al*., 1982a) and beef cows (McGowan *et al*., 1985) have been reported previously. In the hMG treatment of the present study, the number of follicles ≥9 mm detected on day 7.5 after mating (13.2 ± 2.73; the day before embryo recovery) was significantly higher than that of FSH (0.7 ± 0.33; P<0.05). This could be caused by the greater number of follicles stimulated to grow by hMG and detected (follicles ≤6 mm) on day 4 after superovulation. Although the total number of expanded hatched blastocysts using hMG was comparable to that of the FSH treatment (considering the greater number of follicles ≥9 mm in the hMG treatment) superovulatory responses in the former could be improved by increasing the interval from the last injection to the time of mating.

In the present study, we used a change over design method with a rest period of one month, so that each donor camel received three treatments. We showed that repeated superovulation with different treatments may not affect superovulatory responses in dromedary camels.

In conclusion, three superovulatory protocols; FSH,

eCG-FSH and hMG could be used successfully and interchangeably without affecting superovulatory responses in dromedary camels.

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