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The Effect of Treatment with Gonoadotrophins on the Ovarian Response and Ovulation Rate in Alxa Bactrian Camels (*Camelus bactrianus*) During the Breeding Season

[1, 5]B.P. Wang, [1, 5]D. Zhang, [1, 5]Y.Y. Liu, [1, 5]F. Wang, [1, 5]S.Y. Wang, [1, 5]L.D. Han, [1, 5]C.Y. Liu, [1, 5]C.X. Liu, [2]J.P. Liu, [1, 5]J. Pan, [3]W.B. Zhang, [3]Tuo Ya, [3]Zhaori Getu, [3]Daolema, [4]C.H. Huang, [4]J.L. Han, [4]Suya, [6]L.G. Zhang, [1, 5]H.M. Zhou and [1, 5]L. Zhang
[1]College of Life Science, [2]College of Animal Science and Animal Medicine, Inner Mongolia Agricultural University, 010018 Hohhot, China
[3]Institute of Camel Science of Alxa League, 750300 Alxa Left County, China
[4]Inner Mongolia Livestock Improvement Station, 010010 Hohhot, China
[5]Inner Mongolia Key Laboratory of Bio-Manufacture, 010018 Hohhot, China
[6]Ulanqab Livestock Improvement Station, 012000 Ulanqab, China

Abstract: The current study investigated the ovarian response to gonadotropin for establishing a suitable protocol of superovulation in Bactrian camel. Fifteen female camels were randomly divided into 4 groups to compare 4 different superovulation protocols during the natural breeding season. Each camel in 4 groups was injected with FSH at 80, 80, 60, 60, 60, 60, 40 and 40 mg, respectively total dosage of 480 mg, for consecutive 4 days at 12 h intervals. The camels in group 2-4 were naturally mated and subsequently injected 300 IU LH 48 h after the last injection of FSH, the camels in group 1 received the same procedure exception of LH injection. Ovarian follicles and corpora luteas were observed through synchronistic laparotomy 7-9 days after natural mating. The results indicated that there is no significant difference in average ovulation rate ($p>0.05$) among group 1-3 nor among group 2-4. However, there is considerable difference between group 1 and 4 in average ovulation rate ($p<0.05$), among which the value is highest (81.38±6.44%) for group 4 but lowest (16.89±7.98%) for group 1. Furthermore, the average number of follicles has yet no obvious difference ($p>0.05$) among group 1-3 nor among group 2-4 but significant difference ($p<0.05$) between group 1 and 4. Comparatively the animals in group 1 yielded highest average number of follicles (9.80±1.50). Conclusively, the protocol 4 had a best superovulatory effect with an average corpora lutea 9.33±1.45 and therefore it can be used for superovulation in camels.

Key words: Superovulation, ovarian response, corpora luteas, follicles, Alxa Bactrian camel

INTRODUCTION

The Bactrian camel (*Camelus bactrianus*) is a very important domestic animal adapted to the desert and the largest population lives the steppe in China and Mongolia (Novoa, 1970). This species in China possesses 3 various breeds namely Alxa, Xinjiang and Sonid camel (Zhao, 1998), amongst which Alxa camel raised in Alxa desert steppe of Northwestern China is dominant in numbers.

Traditionally, Bactrian camels are kept for utilizing in transportation and production of milk, hair, hide and meat as well as entertainment. However, the camel population has been sharply decreasing because of the substitution of machinery for the animal power. Furthermore, the reproductive efficiency of camels under their natural pastoral conditions is low due to the short breeding season, the late age of reaching puberty and the long gestation period of 13 months, usually resulting in just one calf every 2 years (Nawito *et al*., 1967; Skidmore *et al*., 1992). These hindered the pace of improvement and breeding of the camel.

Unlike the Bactrian camel, since the assisted reproductive techniques were successfully developed estrus cynchronization, superovulation, artificial insemination, *in vitro* fertilization, embryo transfer were commercially applied in cow, sheep, pig and horse production which greatly promote the amelioration and production of the domestic animals and benefit the breeders and producers. In addition, ovulatory induction,

Corresponding Author: L. Zhangm, College of Life Science, Inner Mongolia Agricultural University, 010018 Hohhot, China

embryo recovery and transfer have been attempted in dromedary camels (Vyas *et al*., 2004; Ismail *et al*., 1993; McKinnon *et al*., 1994; Skidmore *et al*., 2009; Skidmore and Billah, 2011; Ismail *et al*., 2008; Wani and Skidmore, 2010). But for Bactrian camels most previous researches were aimed at dairy production (Fukuda *et al*., 2010), anatomy (Xu *et al*., 2010), physiology (Qi *et al*., 2011; Skidmore *et al*., 1992), immunology (Xu *et al*., 2012) and only a few on embryo production (Zhou and Guo, 2006b; Nikjou *et al*., 2008; Niasari-Naslaji *et al*., 2009) or cloning (Zhou and Guo, 2006a). So far the assisted reproductive technology in Alxa Bactrian camel has not been established and again the essential information of ovarian response to stimulation and embryo physiology remain unknown. Overall, the reproductive techniques in Bactrian camels are far behind that of other livestock in China.

In this study, therefore, researchers primarily investigate the ovarian response to the various gonadotropins and compare the effects of four different approaches on superovulation for subsequently founding a suitable superovulation protocol in Alxa Bactrian camel.

MATERIALS AND METHODS

Experimental location and animals: These experiments were conducted during the natural breeding season (from November to March) in local area (Alxa league, latitude 37°21'~42°C47'N, longitude 97°10'~106°52'E; sea level altitude 900~1400 m) and the procedures were approved by the ethical committee of Animal Science. A total of 15 healthy female Bactrian camels aged 6-14, weighed 400-500 kg, were stochastically divided into four different groups for four various experiments. The animals were freely kept in desert grassland (Fig. 1a).

Animal superovulation

Group 1: Five female Bactrian camels numbered 1-5 were used in group 1. The oestrous cycles were synchronized with the aid of Controlled Internal Drug Release Dispensers (CIDR; Phamacica and Upjohn, Auckland, New Zealand), the day of CIDR insertion was taken as day 0. Then, each camel was treated with purified porcine pituitary FSH (pFSH, Folltropin-V: Bioniche, Canada) at 80, 80, 60, 60, 60, 60, 40 and 40 mg, respectively, total dosage of 480 mg, for 4 consecutive days at 12 h intervals from day 9. Concurrently at the 5th injection of FSH, camels were injected with 0.5 mg prostaglandin (Estrumste, Schering Plough, USA) on day 11. The CIDR was removed on day 10 and detection of oestrous was performed at 8 hourly intervals until mating occurred. Each camel was mated on day 14, 48 h after the last pFSH injection and the ovarian response observed via surgical laparotomy 7-9 days after mating (Fig. 1a). This protocol was carried out by the standard procedures on sheep/cattle in China (Zhang *et al*., 2008).

Group 2: Four (No. 6-9) camels were used in this trail and the oestrus cycles were also synchronized with the aid of CIDR 2 days after intramuscularly injected with 300 IU Lutropin (Ningbo, China). From day 7 after the CIDR was inserted each female was injected with pFSH twice daily for 4 days as described for group 1. On day 9, at the same time as the 5th injection of pFSH, the animal was injected with 0.5 mg prostaglandin (Estrumste, Schering Plough, USA). The CIDR was removed on day 10 and detection of oestrous was performed at 8 hourly intervals until mating occurred. Each camel was mated on day 14, 48 h after the last pFSH injection was injected with 300 IU LH simultaneously and the ovarian response observed via surgical laparotomy 7-9 days after mating (Fig. 1b).

Group 3: This group contained 3 camels (No. 10-12) which were received injection of 300 IU LH on the 0 day. The camels, from day 7 were treated with pFSH twice a day for 4 days, total dosage of 480 mg (decreasing doses as group 1). Oestrous detection was performed 8 h intervals on day 11. The animals in this group were mated 48 h after the last injection of pFSH, followed with 300 IU LH injection. Ovarian response was observed surgically after mating 7-9 days (Fig. 1c).

Group 4: Each female camel (total 3 camels, No. 13-15) received injection of 300 IU LH on the 0 day. The camels, from day 7 were treated with pFSH twice a day for 4 days, total dosage of 480 mg (decreasing doses as group 1). Specially, on day 7 each camel was then treated with a combination of 2000IU Pregnant Mare Serum Gonadotropin (PMSG, sigma, USA) intramuscularly injected at the same time as the first pFSH and a total dose of 480 mg of pFSH given in declining doses over a period of 4 days from day 7-10 (as described for group 1). Oestrous detection was performed 8 h intervals after the last injection of FSH. The animal were treated LH 300 IU while were mating with virile stud on day 12 (Fig. 1d). Ovarian response was observed surgically after mating 7-9 days (Fig. 2d).

Ovarian response: Ovarian response was observed through surgical laparotomy 7-9 days after natural mating. The camels were restrained in sternal recumbent posture with all the four legs tied with ropes. Each camel was given intramuscularly with 10 mL xylidinothiazoline

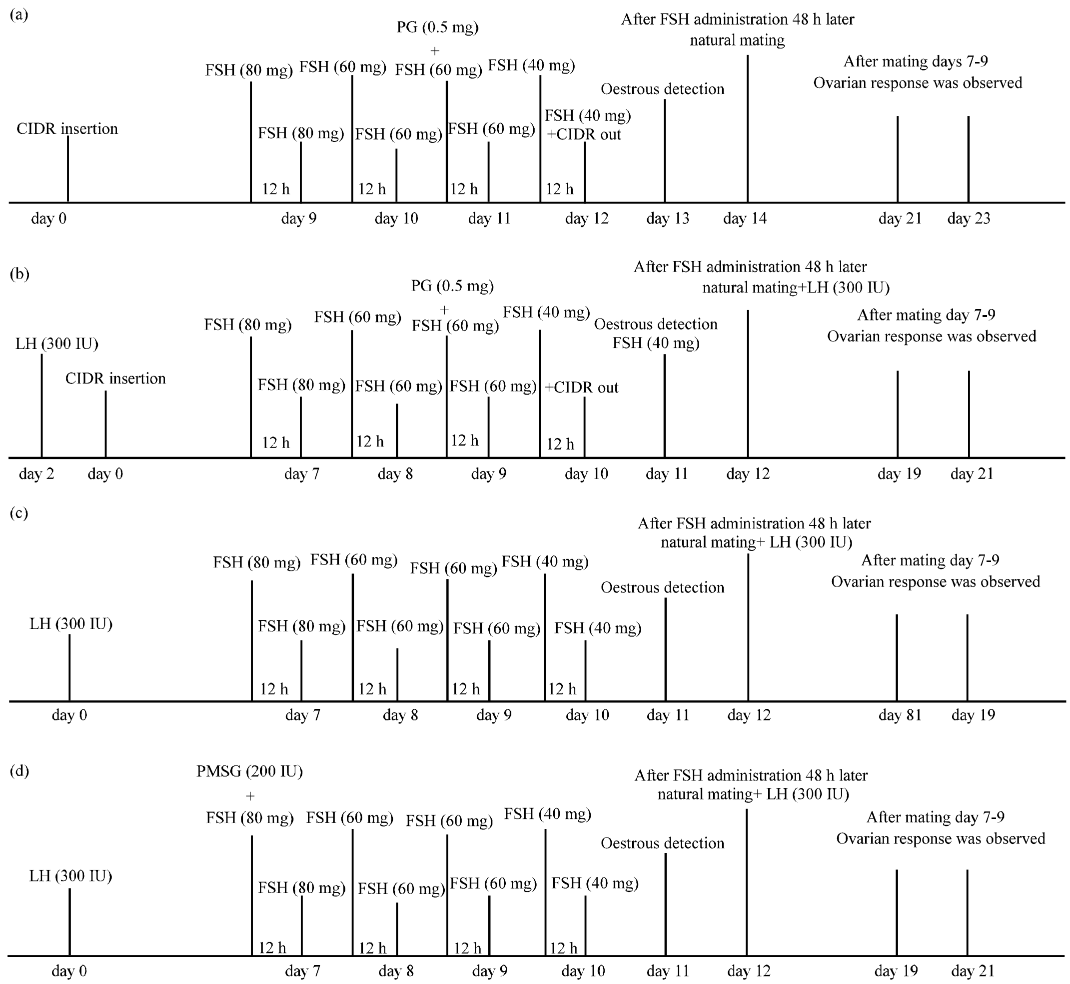

Fig. 1: a) Schematic representation the group 1 of superovulation; b) schematic representation the group 2 of superovulation; c) schematic representation the group 3 of superovulation; d) schematic representation the group 4 of superovulation

injection (Lanzhou, China). A paramedian incision was made cranial to the udder to access the reproductive tract. All calculated follicles were 0.8-2.5 cm in diameter. The number of Corpora Lutea (CL) was counted. Ovarian response was performed on day 7-9 after first mating in the four groups, respectively. Ovulation rate was expressed as (Ismail *et al*., 2007):

$$\text{Ovulation rate} = \frac{\text{No. of CLs}\times 100}{\text{No. of CLs+No. of follicles}}$$

Statistical analysis: All data were analyzed with SPSS Statistical Software (Version 17.0). The effect of various protocols on the superovulation response was compared by one-way ANOVA and means were also subjected to multiple comparisons by LSD Method. Results were indicated as mean±SEM.

RESULTS AND DISCUSSION

Superstimulatory response: In group 1, the camels No. 1, 2 and 5 produced between 6 and 14 small follicles (4-5 mm), however then did not ovulate well as only a few CL (1, 0 and 1) were found. This indicated that they did not respond to the ovulation treatment (Table 1).

So two of five Bactrian camels used responded to the superstimulatory treatment in the first group while the other groups all experimental camels were comprehensive (100%) responded to the current disposal of superovulation by developing more than two corpora luteas for the individual in the experiments (Table 1). In the 4 groups there is no significant difference (Table 2, $p>0.05$) in average ovulation rate among group 1-3 nor among group 2-4. However, there is considerable difference between group 1, 2 and 4 in average ovulation rate (Table 2, $p<0.05$), among which the value is highest (81.38±6.44%) for group 4 but lowest (16.89±7.98%) for group 1. Furthermore, the average number of follicles has yet no obvious difference (Table 2, $p>0.05$) among group 1-3 nor among group 2-4 but significant difference (Table 2, $p<0.05$) between group 1 and 4. Comparatively the animals in group 1 yielded highest average number of follicles (9.80±1.50).

In the experiments, we firstly determined the difference of superovulatory response among 4 protocols (Fig. 2a and b) in Alxa Bactrian camels. The result indicated that protocol 4 had the best superovulatory response with an average 9.33±1.45 corpora luteas. Protocol 1 treatment without LH had the lowest numbers of corpora lutea (2.60±0.93) and many follicles (Fig. 2c). Meanwhile, the combination treatment with LH and FSH (protocol 2 and 3) obtained little more or less mean and total number of corpora lutea than the treatment just protocol 1 or 4. In other words there is no significant difference between the group 1-3 for the average numbers of corpora lutea ($p>0.05$) but the average numbers of CL in the protocol 1 was significantly lower than that of the protocol 4 ($p<0.05$). Described above in accordance with the Table 1 and the number of ovulations (9 CLs) was given by camel No. 13 (Fig. 2d).

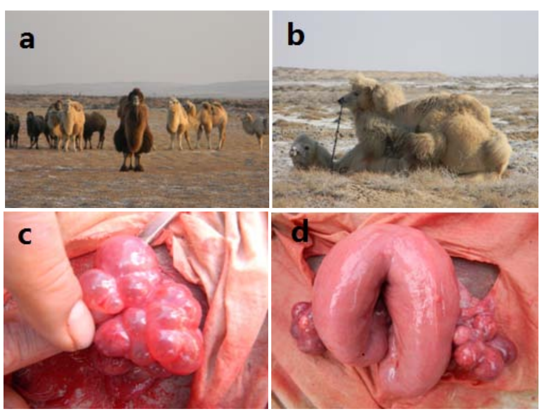

Fig. 2: a) The camels were freely kept in desert grassland; b) The camels were naturally mating; c) Ovaries of camel number 1 in group 1 containing 1 corpora lutea and many follicles; d) Ovaries of camel number13 in group 4 containing 9 corpora lutea

Unlike most large domestic species, camels have some unique reproductive features that present a challenge to the development of advanced breeding techniques. Previously, no attempt has been performed to superovulate Alxa Bactrian camels during the breeding season. However, there are analogous reports on superovulation of the reproductive processes in the dromedary (Nowshari and Ali, 2005; Ismail *et al*., 2007; Skidmore and Billah, 2011), Bactrian camels (Nikjou *et al*., 2008) and other domestic animal species. Also, some studies have used exogenous administration of gonadotropins which including different hormones used to increase the number of follicles available for aspiration, the combination of eCG with LH (Van de Leemput *et al*., 1999; Vos *et al*., 1994), FSH and GnRH to bovine. In the study, protocol 1 was carried out by the standard procedures on sheep/cattle in China (Zhang *et al*., 2008) and the result was that two of five Bactrian camels used responded to the superstimulatory treatment and developed more follicles than camels in other groups, while the number of CLs was significantly less than the camels in protocol 4. It suggested that the standard procedures on sheep/cattle not suitable for Bactrian camels.

Table 1: The ovarian response of the experimental camels

	Protocols		
Experiments	Animal number	No. of corpora follicles	No. of lutea
1	1	12	1
	2	7	0
	3	14	4
	4	6	5
	5	10	1
2	6	8	3
	7	9	2
	8	4	9
	9	4	7
3	10	10	2
	11	5	6
	12	4	11
4	13	2	9
	14	3	7
	15	1	12

Table 2: The ovarian response of the experimental camels (Mean±SEM)

Protocols	No. of (response) camels	Average of ovulation rate (%)	Average (total) No. of follicles	Average (total) No. of Corpora Lutea (CL)
1	5 (2)	16.89±7.980^{a}	9.80±1.50 (49)^{a}	2.20±0.97 (11)^{a}
2	4 (4)	44.58±12.80^{ab}	6.25±1.31 (34)^{ab}	5.25±1.65 (21)^{ab}
3	3 (3)	48.18±16.66^{ab}	6.33±1.86 (19)^{ab}	6.33±2.60 (19)^{ab}
4	3 (3)	81.38±6.440^{b}	2.00±0.577 (6)^{b}	9.33±1.45 (28)^{b}

Values in the same column with different superscript letters (a, b) are significantly different at $p<0.05$

Understanding the pattern of follicle development in Bactrian camels is increasingly important for designing improved methods to manipulate reproduction in this species. In Bactrian camels, Chen and Yuan (1980) reported a mean follicular wave duration of 19 days with a range of 14-21 days and they are seasonally induced ovulators. Ovulation does not occur in the absence of a sufficient stimulus such as copulation, artificial insemination or intramuscular injection of seminal plasma (Chen *et al*., 1980; Xu *et al*., 1985). In the absence of a male, female alpacas may remain in estrus for up to 36 days with only occasional periods of anestrus that last no longer than 48 h (San-Martin *et al*., 1968). Some studies suggest that LH produced after mated in Bactrian camels (Chen *et al*., 1985), some research imply that ovulation failure is caused by inadequate LH release in response to copulation in female camels (Skidmore *et al*., 2009). Other research reported that ovulation can be induced in dromedary and Bactrian camel by a single injection of LH, GnRH (Ismail *et al*., 2008; Skidmore *et al*., 2009; Wani and Skidmore, 2010), human Chorionic Gonadotropin (hCG) (Marie and Anouassi, 1986; Anouassi and Ali, 1990) or eCG (Ismail *et al*., 1993; McKinnon *et al*., 1994; Skidmore *et al*., 2009; Skidmose and Billah, 2011). In this study, the camels in protocol 2-4 were injected with 300 IU LH before treated with exogenous gonadotrophin and while mating with virile stud. The results show that all experimental camels were comprehensive (100%) responded to the current disposal of superovulation by developing more than two corpora luteas for the individual. It suggested that LH is curial factor for the superovulation in Bactrian camels that injection of 300 IU LH while mating with virile stud induced successful ovulation and that injection of 300 IU LH before treated with exogenous gonadotrophin stimulated effective ovaries follicular synchronization.

All of gonadotrophin treatments in previous studies were just after the insertion of the Progesterone Releasing Intravaginal Device (PRID), CIDR or daily progesterone injection (Skidmore *et al*., 1992; Cooper *et al*., 1992; McKinnon *et al*., 1994; Ismail *et al*., 1993, 2008). And similar findings were recorded about successful superovulation in the study center on Alxa Bactrian camel. However, the results of protocol 2 (with CIDR) and 3 (without CIDR) showed that there were no significant difference on average numbers of corpora lutea, follicles and average ovulation rate ($p>0.05$). It suggested that superovulation efficiency little affected by CIDR in Bactrian camel.

Superovulation of the camel (*Camelus dromedarius*) was concerned on the superstimulatory response and ovulation rate of the promising results in the current study. Previous studies proved that eCG has been successfully used various doses to stimulate the ovaries for the production of multiple follicles in camels. One group (McKinnon *et al*., 1994) administered a single injection of 3000-6000 IU eCG, the results showed that more follicles developed and therefore more embryos were recovered from those treated with eCG (118/52; 227%); other teams has been also successfully used in camels to stimulate the ovaries for the production of multiple follicles (Marie and Anouassi, 1986; Skidmore *et al*.,1992). While another method for superstimulation in camel is the use of ovine FSH in a split dose regime over 3-6 days (Cooper *et al*., 1992; Skidmore *et al*., 1992) and the result revealed that more embryos were recovered from those treated with FSH than with eCG (McKinnon *et al*., 1994). PMSG reduces the variability of the interval between progestogen withdrawal and ovulation thus improving the synchronization of ovulation and fecundity. Many researchers have indicated the importance of administering PMSG to obtain a more predictable and compact estrous or ovulation. Koyuncu and Alticekic (2010)'s results suggested that it was possible to induce synchronization of estrous, fertile estrous, successful pregnancy and lambing, to improve fecundity by administration PMSG. Dafalla *et al*. (1987)'s team reported that the camels responded excellently to the treatment with PMSG. So, the camels were treated with pFSH combined with PG or PMSG for superstimulation in this experiment. The result showed that the protocol 4: LH, FSH and PMSG without CIDR which resulted in highest average of ovulation rate ($81.38\pm6.44\%$) and average numbers of corpora lutea (9.33 ± 1.45).

CONCLUSION

LH, FSH and PMSG injection without CIDR was found to be an efficient method for superovulation in Alxa Bactrian camels during the breeding season and the high ovarian response (100.0%) was obtained in the present study. It has demonstrated that it possible to establish superstimulatory in Alxa Bactrian camels. However, the optimum of the superstimulatory in Alxa Bactrian camels requires further investigation.

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