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## Ultrasound evaluation of ovarian response to photoperiodic control measures in *Camelus dromedarius*

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### ABSTRACT

The present study was carried out with two objectives, (a) the use of ultrasound scanners to study the effect of 'mask on eyes' as a photoperiodic control measure for folliculogenesis in female camels, (b) Use of blood progesterone assays to monitor ovarian changes in camels. Fourteen female camels aged 7-11 years were used for the present experiment. Seven were studied for the effect of a mask over the eyes (for six hours daily) as a photoperiodic control measure on ovarian activity and the remaining seven were kept as controls. Ovaries were examined by ultrasound at weekly intervals for seven weeks during the non-breeding season. Camels were mated with virile stud when a follicle ( $\geq 0.9$  cm diameter, ovulating size) was visible on either of the ovaries. Ovaries were monitored for ovulation up to 48 h post-mating by ultrasound at 12 hourly intervals and at 20, 30 and 40 days post mating to ascertain pregnancy. A commercially available RIA kit was used for serum progesterone assay on samples obtained at 0, 7, 15, 30 and 45 days of mating. No follicle was observed in camels before treatment and in treated (masked) or untreated camels during the first week of treatment. By the third week 100% camels in the treatment group evidenced measurable small follicles (0.5-0.89 cm, 6/7) or follicles of ovulating size ( $\geq 0.9$  cm, 1/7). Follicles of ovulating size were observed in 28.6, 14.3, 14.3 and 14.3 percent camels by 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> week of treatment. Fifty percent (3/6) of the camels became pregnant. The serum plasma progesterone level increased after ovulation and remained higher than 1.0 ng/mL in pregnant camels. In the control group one camel showed a follicle (0.6 cm diameter) at the 5<sup>th</sup> week, which did not reach ovulating size. The results of the present study indicate that protecting eyes from sunlight one or two months ahead of the breeding season stimulates follicular growth in camels and pregnancy can occur in these camels when mated.

**Key words:** ovary, photoperiodicity, progesterone, ultrasound, *Camelus dromedarius*

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## Introduction

The dromedary as a domestic animal is best adapted to living conditions in an arid environment and to the rarity of water and pasture typical of such regions. The reproductive performance of camels is poor, as confirmed by the empirical data and field studies (WILSON, 1989). Low reproductive performance has remained a major obstacle to the growth of populations of dromedaries (PUROHIT and PAREEK, 2000). The factors responsible for the poor reproductive performance of dromedary camels are the limited breeding season, delayed puberty, delayed postpartum estrus and lengthy gestation period (ABOUL-ELA, 1991). Since the mean gestation period of the camel is  $390 \pm 0.99$  days (SAHANI et al., 1997), the female camels are bred in one season, calve in the subsequent breeding season and then remain sexually quiescent until the following breeding season, leading to long inter-calving periods and significant economic losses (WILSON, 1989).

The dromedary and the Bactrian camel are both regarded as seasonal breeders (WILSON, 1984; CHEN and YUEN, 1984). In Israel, the mating season extends from December to March (YAGIL, 1981). The breeding season of camels in India extends from December to March i.e. the period of short day length (MATHARU, 1966). Similar short day breeding seasons have also been reported in Somaliland (MARES, 1954), Pakistan (YASIN and WAHID, 1957), Egypt (SHALASH, 1965) and Sudan (MUSA and ABUSINEINA, 1978). In contrast, continuous breeding season has been reported in countries, which have limited photoperiodic changes as Kenya (WILSON, 1984; WILSON, 1989). Therefore camels are reported to have the potential to breed all year round but are prevented from doing so by environmental causes (SGHIRI and DRIANCOURT, 1999).

Out of season breeding in camels using hormonal therapy has shown limited success (ELIAS et al., 1985; AGARWAL et al., 1997) as has breeding during early post partum period (VYAS and SAHANI, 2000). However no organized attempts to manipulate the onset of the breeding season or to extend it have been reported (MUSA et al., 1993).

Photoperiod regulation of pineal-hypophyseal-gonadal function has been elucidated for enhancement of growth, puberty and out of the season breeding in small ruminants (CHEMINEAU et al., 1988; MALPAUX et al., 1993), horses (PALMER et al., 1982) and mink (GULEWICH et al., 1995). However in the camel, studies on manipulation of reproductive rhythm by photoperiodic control are limited (AGARWAL and KHANNA, 1998). Managing camels in dark rooms is largely impractical due to their large size. The present study was undertaken to study the effect of masks on the eyes as photoperiodic control on ovarian activity during the non-breeding season in dromedary camels.

## Materials and methods

*Experimental animals.* Adult (7-11 yrs), non-pregnant female camels (n = 14), having calved at least once with apparently healthy internal genitalia, belonging to herd of the National Camel Research Centre, Bikaner, Rajasthan, India, situated at 28.3° North latitude and 73.5° East longitude were selected for the present experiment. Seven female camels were kept as controls and seven were kept under treatment. The experimental camels were kept in loose but intensive management conditions during the period of experiment. They were confined in a camel yard surrounded by a 12 foot high wall. They were offered rations once daily and were taken out once daily for watering.

*Photoperiodic control measure.* A mask prepared from thick black canvas cloth was used to blindfold the camels for six hours from 9.30 AM to 3.30 PM (six hours) for eight weeks starting from 1<sup>st</sup> October.

*Follicular activity and breeding.* The follicular activity over the ovaries in both the masked and the control group was monitored by ultrasonography. The ultrasonographic examination of ovaries was performed once before the application of the mask and at weekly intervals thereafter using endovaginal annular array, mechanical sector, and dual frequency (5 MHz, 7.5 MHz) probe of Scanner-200 (Pie Medical, The Netherlands) as described earlier (VYAS and SAHANI, 2000). The female camels in the control group were also monitored. Each camel was restrained in the sternal recumbent posture on sand and sedated with 80-120 mg i.v. Xylazine (Xylazine, Indian Immunologicals limited, Hyderabad, India). Observations were carried out by the same operator, recorded on videotape and were subsequently reviewed to monitor the status of the ovaries. The camels were mated with virile stud camels when a follicular size  $\geq 9$  mm was observed. Ovaries were examined ultrasonographically at 12 h intervals from mating to 48 h post-mating to monitor ovulation. Pregnancy diagnosis was done by ultrasonography using linear array transducer on day 20, 30, and 40 post-mating.

*Serum progesterone assay.* Blood samples were collected at 0, 7, 15 and 30 days of mating. The blood samples were kept at room temperature for 1-2 h before being centrifuged at 3000 rpm for 10 min. The serum was decanted and stored at -20 °C until subsequent assay for progesterone using progesterone direct radio immuno assay kit (DiaSorin s.r.l., 13040 Saluggia, Vercelli, Italy). The radioactivity of the precipitate was measured with the help of I<sup>-125</sup> gamma counter IC 4702 (M/s Electronics Corporation of India Limited, Hyderabad). The sensitivity of assay was below 0.10 ng/mL (0.32 nmol/L) at 95% confidence limit. Coefficient of variation for serum containing 1.1 and 11.2 ng progesterone/mL were 7.2 and 4.6% for intra-assay variability.

*Statistical analysis.* The frequencies of animals with follicle (0.5-0.89 cm diameter) were compared by chi-square (SNEDECOR and COCHRAN, 1968).

## Results

A total of 184 ultrasonographic examinations were performed on the 14 camels to examine ovarian follicular status. Before the start of the experiment the ovaries were quiescent.

Table 1. Periodic appearance of follicles in camels (treatment-masked and control) during the non-breeding season

Time (week)	Percent camels having follicle with diameter						Percent mating
	0.5-0.89 cm		≥ 0.9 cm		Total		
	Control	Treatment	Control	Treatment	Control	Treatment	
0	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-
2	-	14.3 (1) <sup>NS</sup>	-	-	-	-	-
3	-	85.7 (6)	-	14.3 (1)	-	100 (7)	14.3 (1)
4	-	57.1 (4)	-	28.6 (2)	-	-	28.6 (2)
5	14.3 (1)	42.8 (3)	-	14.3 (1)	-	-	14.3 (1)
6	14.3 (1)	28.6 (2)	-	14.3 (1)	-	-	14.3 (1)
7	14.3 (1)	14.3 (1)	-	14.3 (1)	-	-	14.3 (1)

Chi-square values were significant at  $P \leq 0.01$  at 4 to 7 weeks; NS - Non-significant chi-square value; Numbers in brackets are the number of female camels

Table 2. Serum progesterone levels (ng/mL) in camels (treatment-masked) after mating

Camel N <sup>o</sup>	Progesterone concentration on days post- mating (ng/mL)							
	0	7	15	30	45			
359	0.13	0.27	2.4	1.9	1.75			
63 *	0.12	1.5	0.75	0.18 (M)	0.34 (7)	6.0 (15)	1.6 (30)	1.8 (45)
69	ND	0.34	2.2	2.2	2.3			
77	ND	0.28	0.25	0.18	0.10			
439	0.17	0.34	1.8	0.45	0.25			
342	0.16	1.08	0.62	0.35	0.13			
Mean ± S.E.	0.11 ± 0.03	0.59 ± 0.19	1.69 ± 0.77	1.11 ± 0.36	1.02 ± 0.39			

ND - Not detectable i.e. <0.10 ng/mL; \* J 63 was re-mated, words and figures in bracket indicate blood collection on the day of mating and 7, 15, 30 and 45 days after mating

Periodic examination of the ovaries of female camels in the masked group revealed follicular growth. Table 1 shows that by the end of the second and third weeks follicles of measurable size ( $\geq 0.5$  cm diameter) could be observed in 14.3% and 100% camels respectively. One (14.3%) camel had a follicle of ovulating size (diameter  $\geq 0.9$  cm) by the end of the third week. During the fourth week ovaries of 28.6% of the camels were observed to have follicles of ovulating size, while one camel each week (14.3%) was observed to have a follicle of ovulating size during the fifth, sixth and seventh weeks of treatment. In one camel (431), the follicle did not grow in size beyond 0.65 cm in diameter up to the seventh week, which was considered not to be of ovulating size therefore, the camel was not mated. In the remaining six camels, the follicular growth was sufficient to attain ovulating size (0.9 cm or more in diameter) and therefore, they were mated with virile studs.

The follicle size ranged from 0.94 to 3.23 cm diameter when the females were mated, and ovulation took place in all except one camel. The follicle size in this camel was 0.91 cm diameter at the time of mating. Ovulation occurred in 100% (5/5) of matings when follicle size ranged from 1.01 cm to 3.72 cm diameter. Whereas 50% (1/2) of the matings resulted in ovulation when follicle size was  $< 1.0$  cm.

In the control camels, none showed follicle growth up to the fourth week (Table 1). A small follicle ( $\geq 0.5$  cm in diam.) was observed in only one camel in the fifth week. Subsequent examinations in the sixth and seventh weeks revealed that the diameter of the follicle in the same camel remained  $< 0.6$  cm. The minimum size of follicles suitable for ovulation in response to mating in camels is reported to be 0.9 cm (SKIDMORE et al., 1996a). Therefore no control camel was mated.

Table 2 shows the serum progesterone profile (ng/mL) in six camels that were mated. Serum progesterone levels (ng/mL) in one camel remained low ( $< 0.3$  ng/mL) up to 45 days of mating. Ovulation did not occur post-mating in this animal. In two camels (359 and 69) serum progesterone concentration increased to 2.4 and 2.2 ng/mL respectively on day 15 and remained high ( $> 1.0$  ng/mL) up to 45 days of mating. They were diagnosed as pregnant by ultrasound scanning. In camels no. 439 and 342 the serum progesterone levels increased ( $> 1.0$  ng/mL) initially but declined subsequently. The ovulation did take place in these camels as a result of copulation but pregnancy could not be established. In camel no. 63, the progesterone level increased ( $> 1.0$  ng/mL) and then declined (0.18 ng/mL) on day 30. This camel was remated and progesterone levels were found to be  $> 1.0$  ng/mL at 15, 30 and 45 days of post mating. This camel was diagnosed pregnant by ultrasound scanning at 20, 30 and 40 days after mating.

The ultrasonography on 20, 30 and 40 days post mating revealed 50% (3/6) of camels to be pregnant and the pregnancy continued to term.

## Discussion

Seasonal reproductive activity is regulated by an endogenous rhythm. This rhythm is synchronised with the geophysical year by environmental stimuli, the most important of which is the photoperiod. Unlike temperature, availability of food, rainfall or other environmental cues, photoperiod provides information about the season and remains constant from year to year (GERLACH and AURICH, 2000). Photoperiod regulation of pineal-hypophyseal-gonadal function has been elucidated in small mammals and appropriate manipulations are regularly undertaken to enhance growth, puberty and bring the animal into cyclicity during the non-breeding season (MALPAUX et al., 1993). The dromedary camel also exhibits a reproductive rhythm during the short photoperiod and goes into anestrus during the long photoperiod (MUSA et al., 1993). Only a few scientific studies have addressed the effects of the photoperiod on the reproductive rhythm of camels (VYAS et al., 1997; AGARWAL and KHANNA, 1998).

The results of the present experiment indicated that protecting eyes from exposure to solar light by applying masks over the eyes 4 to 7 weeks prior to the breeding season stimulated ovarian activity in 85.7% of female camels which were otherwise sexually quiescent. The reproductive performance of camels following application of masks over their eyes was somewhat poorer than during the normal breeding season, where the conception rate has been reported to be 70.2% (SAHANI et al., 1997) as against 50% obtained in the present study. However, the conception rate is fairly comparable or better than that reported after augmentation of reproductive activity by hormonal therapy during the non-breeding season (ELIAS et al., 1985; AGARWAL et al., 1997).

Photoperiodism has been found to be one of the important environmental factors affecting reproductive responses in the several seasonally breeding species (REITER, 1980). The secretion of the hormone melatonin is inhibited by light so that it provides an endocrine index of timing of the scotophase i.e. darkness (ROLLAG and NISWENDER, 1976; REITER, 1980; PELTIER et al., 1998). The secretion of melatonin in camel is also reported to be in the scotophase (VYAS et al., 1997). The main effect of melatonin is control of the frequency of the LHRH pulsatile discharge from the hypothalamus which in turn controls the pulsatile secretion of LH and this effect could be sufficient by itself to explain the regulation of seasonality of reproduction in sheep (MALPAUX et al., 1990). In sheep, which are known to be short day breeders similar to camels, the photoperiod was found to be the main environmental factor responsible for the seasonality of reproduction. The timing of reproductive activity could be driven by modifying only the photoperiodic component of the environment and the reversal of the photoperiodic cycle was reported to cause the breeding season to phase shift by six months (MALPAUX et al., 1993). The response of the camels as a result of the longer dark phase, but with small intermittent light phases, indicates that a long night with a small flash of light in the middle may

be perceived as a long night in camels. Although melatonin profiles during various reproductive phases and events in camels are not known, it is presumed that like sheep, which are also short day breeders, melatonin may also be the primary controlling factor in the onset of ovarian activity in camels (AGARWAL and KHANNA, 1998).

The results of the present study reaffirm the earlier findings (SKIDMORE et al., 1996a), that chances of ovulation of follicles measuring 0.9-1.9 cm diameter are more than those measuring <0.9 cm diameter.

The present findings indicate that serum progesterone levels in the pregnant camels remain higher than 1.0 ng/mL. These results are in agreement with previous findings in the dromedary camel (MARIE and ANOUASSI, 1986; AGARWAL et al., 1987; SKIDMORE et al., 1996b).

The results of the present experiment indicate that protecting eyes from exposure to sunlight by applying masks over the eyes during the months of October and November stimulated ovarian activity in 85.7% of female camels, which were otherwise sexually quiescent. Therefore masks over the face and eyes may be used as a photoperiodic control measure in female camel to augment follicular activity by one and half to two months ahead of the breeding season.

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**VYAS, S., R. SINGH, G. N. PUROHIT, P. K. PAREEK, M. S. SAHANI: Ultrazvučna procjena reakcije jajnika nakon primjene mjera svjetlosnoga režima u deva (*Camelus dromedarius*). Vet. arhiv 78, 39-48, 2008.**

**SAŽETAK**

Istraživanje je provedeno u svrhu ultrazvučnoga praćenja utjecaja svjetlosnoga razdoblja kontroliranog pomoću maske na očima na tijek i dinamiku folikulogeneze u deva i određivanja razine progesterona za praćenje cikličnih promjena na jajnicima. Promatrano je 14 deva u dobi od sedam do 11 godina koje su bile podijeljene u pokusnu (n = 7) i kontrolnu skupinu (n = 7). Devama u kontrolnoj skupini svakodnevno je stavljana maska na oči tijekom 6 sati u razdoblju od 7 tjedana izvan rasplodne sezone. Deve su ultrazvučno pregledavane jednom tjedno za vrijeme čitavoga trajanja istraživanja. One su bile parene s plodnim mužjakom kad je uočen folikul na jajniku ( $\geq 0,9$  cm promjera, ovulacijski folikul). Kad je uočen ovulacijski folikul, jajnici su bili pregledavani ultrazvukom svakih 12 sati u razdoblju od 48 sati radi potvrđivanja ovulacije, a potom nakon 20, 30 i 40 dana radi potvrđivanja gravidnosti. Uzorci krvi bili su uzeti 0., 7., 15., 30. i 45. dana nakon

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parenja, a plazma je bila analizirana komercijalnim RIA kompletom za određivanje koncentracije progesterona. Prije početka istraživanja i tijekom prvog tjedna istraživanja nijedna deva nije imala folikule na jajnicima. Do trećega tjedna istraživanja, uočeni su folikuli u svih deva pokusne skupine, ali su većinom bili maloga promjera (0,5 - 0,89 cm, 6/7), osim u jedne deve u koje je folikul bio ovulacijske veličine (>0,9 cm). Folikuli koji su po veličini odgovarali ovulacijskim folikulima ustanovljeni su u 28,6% deva tijekom 4. tjedna, 14,3% tijekom 5. tjedna, 14,3% tijekom 6. i 14,3% deva tijekom 7. tjedna istraživanja. Koncipiralo je 50% deva (3/6). Razina progesterona u krvi porasla je nakon ovulacije i bila je veća od 1 ng/ml u svih gravidnih deva. U kontrolnoj skupini samo je jedna deva imala folikul (0,6 cm) na jajniku tijekom 5. tjedna, ali on nije dosegnuo ovulacijsku veličinu. Rezultati istraživanja pokazuju da zaštita očiju od svjetlosti mjesec do dva prije sezone parenja potiče razvoj folikula u deva i može dovesti do gravidnosti ako se deve tada pare.

**Ključne riječi:** jajnik, fotoperiod, progesteron, ultrazvuk, *Camelus dromedarius*

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