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Comparative Analysis of Reproductive Cycles in Female Persian Gazelle (*Gazella subgutturosa subgutturosa*) (Central Asia) and Sand Gazelle (*Gazella subgutturosa marica*) (Arabian Peninsula)

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This study was conducted at the Bukhara breeding centre (Ouzbekistan, Central Asia) on Persian gazelles (*Gazella subgutturosa subgutturosa*) and at Thumammah (Saudi Arabia) on sand gazelles (*Gazella subgutturosa marica*). Plasma prolactin (PRL) and progesterone were determined in groups of females. Because these females were under two different photoperiods (20°N in Saudi Arabia and 40°N in Uzbekistan), some Persian females were treated with melatonin implants. Both groups of females living in natural environment in Uzbekistan and in Saudi Arabia exhibited an estrus (autumn and early winter) and an anestrus season (spring and summer). Both groups of females exhibited estrous and anestrous seasons. The estrous season was shorter in females from Uzbekistan. During the anestrous season, progesterone concentrations remained at basal levels in Persian gazelles (Uzbekistan) while in sand gazelles (Saudi Arabia) hormonal concentrations were higher than in Persian gazelles until August and decreased to similar concentrations in September–October. Moreover, the pattern of PRL significantly differed in the two groups. Persian gazelles exhibited a clear seasonal pattern with significantly high levels of PRL in June whereas in the sand gazelle PRL did not rise significantly in summer and fluctuated widely. Melatonin treatment significantly depressed PRL concentrations but did not affect the length of the estrous season in Persian gazelles, suggesting that the reproductive cycle was entrained by an endogenous rhythm.

INTRODUCTION

The reproductive strategies in ungulates is strongly dependent upon environmental factors (Baharav, 1983). Numerous species from the genus *Gazella* breed twice a year and sometimes throughout the year. Some mountain gazelles in Israel (*Gazella gazella*) and also some wild populations of Farasan gazelle (*G. g. farasani*), as well as gazelles introduced in the reserve of Haawtah (Saudi Arabia), exhibited continuous breeding (Baharav, 1983; Habibi, 1991). Populations of mountain gazelles have also two fawning peaks, in spring and autumn (Shalmon, 1987; Dunham, 1997) and mountain gazelles living in captivity seasonally have two birth seasons (Baharav, 1983; Habibi, 1991). Baharav (1983) showed that mountain and Dorcas gazelles can breed throughout the year when food and water are abundant.

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Another species, *Gazella subgutturosa* has two subspecies, the Persian gazelle (*G. s. subgutturosa*), which is present in Central Asia (Gepner *et al.*, 1961), and the sand gazelle (*G. s. marica*), which lives in Saudi Arabia (Habibi, 1992). Only one spring fawning period has been reported in Persian gazelle (*Gazella subgutturosa subgutturosa*) (Djevnerov, 1984; Kutcheruk, 1995; Pereladova *et al.*, 1998). Only one birth in autumn has been recorded for the sand gazelles (*G. subgutturosa marica*) living in captivity in Saudi Arabia (N. Brown, personal data).

It is well known that photoperiod regulates and adjusts sexual cycles in animals that exhibit estrous and anestrous seasons (Marshall, 1937; see also Thimonier and Sempéré, 1989). Photoperiod can control the reproductive cycles of cervids when the animals live in temperate environments. The influence of photoperiod on the sexual cycles is stronger when animals are living under boreal latitudes (latitude more than 30°; Bubenik, 1986; Loudon and Brinklow, 1991). At these latitudes, the timing of birth coincides with maximum of food abundance. Whereas tropical and subtropical deer living between 20° north and 20° south breed all year round and there is no calving season (Loudon and Curlewis, 1988). These equatorial deer exhibited no obvious changes when they were kept at 52° north (Loudon and Brinklow, 1991). Moreover, equatorial animals are not sensitive to melatonin implants (Loudon and Curlewis, 1988).

Some experiments have shown that in the ewe, the end of the breeding season in early spring could result from refractoriness to long and "short days" mimicked by melatonin implants (Robinson and Karsch, 1984; Malpaux *et al.*, 1989) or conversely, the cessation of the breeding season in cervids would result from increasing day length in spring mainly when the day length is more than 12 h (Sempéré *et al.*, 1995).

Persian and sand gazelles live in arid environments with different photoperiods (Pereladova *et al.*, 1998; Habibi, 1991). In Central Asia, Persian gazelles live up to 40 to 43°N latitudes (Gepner *et al.*, 1961) whereas in Saudi Arabia, sand gazelles are located to 2°N. What is the influence of photoperiod on the sexual cycles of these animals?

The purpose of the study was to compare the sexual cycles of female Persian and sand gazelles classified as the same gazelle species but living at two different latitudes. The role of photoperiod on the sexual cycles was also investigated.

MATERIALS AND METHODS

Centres of Studies

The Bukhara Breeding Centre (Uzbekistan) (40°N, 65°E). The Bukhara Breeding Centre was established in 1978 by the Ministry of Ecology of Uzbekistan. The centre comprises a fenced territory of 5126 ha, pens, and buildings. Persian gazelles (*G. subgutturosa subgutturosa*) were collected in different areas of Uzbekistan and set free in the centre in 1978 (Pereladova *et al.*, 1998). Our animals were caught for hand rearing in the centre (see Pereladova *et al.*, 1998) and kept in captivity. The fawns were fed with cow and occasionally goat milk and received some additional fresh lucern in small parks (20 m^2). When they were 6 months old, the animals were moved into larger pens (500 m^2 to half hectare) and fed *ad libitum* with dried and fresh alfalfa and lucern, dry pellets, hay, and water. Particular care was taken to minimise the stress of capture, similar to the process utilised for roe deer (Sempéré *et al.*, 1992). Animals were gently moved through a corridor and then to a darkened shed directly into trap wood boxes. They were blinded with a mask and then taken in the arms by the technician for weighing and sampling. The animals were always calm and never agitated. After sampling, the animals were brought to the pen by the technician, the mask was removed, and the animals were laid on the ground and released.

KKWRC (King Khalid Wildlife Research Centre), Thumamah (Saudi Arabia, 24°N, 46°E). This centre is located about 70 km north of Riyadh and was managed for a large collection of gazelles. Sand gazelles (*G. subgutturosa marica*), also called the rheem in Saudi Arabia, were collected from the few hundred free-ranging animals surviving in Saudi Arabia and held in a 560 ha enclosure (Rietkerk *et al.*, 1991). They were transferred to an enclosure of 150 ha, and some animals were kept in small pens (0.5 ha) for research purposes. All animals were fed *ad libitum* with dried and fresh lucern, dry pellets, hay, and water.

To reduce the sampling stress, and because animals were completely dependent on artificial food supplies

(Mubarak *et al.*, 1991), the animals were enclosed in small capture pens (10 m^2) where the food was given daily a few hours before handling. For sampling, two people entered the pens and rapidly after manual restraint, females were blindfolded and bled by jugular venipuncture within 30 s of the beginning of manipulations as previously described for Arabian oryx (Sempéré *et al.*, 1996).

All females studied (Bukhara and Thumamah) were 2–3 years old and were separated from the males when they were 6 months old.

Experiment 1

In both experimental centres, blood was collected weekly or within 10 days (mean interval of 10.11 ± 0.61 days in Bukhara and 9.6 ± 0.64 days, NS) in Thumamah.

Bukhara. Eight tamed adult females were kept in two pens, without males. Blood was taken over 15 months, from September 1995 to December 1996. In summer, blood samples were collected by jugular venipuncture, kept in heparinized tubes for 1 h in a cooled box, and centrifuged for 15 min at 1500*g*, and the plasma was stored at −20° until assayed.

Thumamah. For this experiment, seven tamed adult females were isolated in small individual pens (20 m^2) and blood was collected from September 1992 to December 1993. Plasma was stored, following the same process as in Uzbekistan.

Experiment 2 (Bukhara)

Another group of four adult Persian gazelles living in a park, received sc (trocar 2 mm diameter) two implants (Melovine TM CAMCO) each containing 18 mg melatonin (Sempéré *et al.*, 1995), from 21 December and monthly until June. Animals were sampled every 2 weeks.

In ungulates, ovulation can be considered when plasma progesterone concentrations are above the 1 ng ml^{-1} and persist for at least 6 days (Gaillard *et al.*, 1992).

Hormone Assays

It has been shown that ovariectomy abolishes progesterone secretion in roe deer (Sempéré, 1977), indicating that the progesterone is of luteal secretion but in some cervids, progesterone from adrenal gland has been demonstrated (Sempéré, 1992). Plasma progesterone concentrations were measured by radioimmunoassay (RIA) using an antibody raised in rabbits against progesterone 11α-hemisuccinate-BSA conjugate, (Sempéré *et al.*, 1989) and [^{3}H]progesterone (3.55 TBq / mMol; CEA-Saclay). The sensitivity was 6.25 pg / tube (0.06 ng / ml). At the 50% displacement level for 50 pg progesterone / tube, cross-reactivities were 1.7% for 20α-hydroxyprogesterone and <0.3% for 17α-estradiol, cortisol, androstenedione, androstenediol, cortisone, 20α-hydroxyprogesterone, and testosterone. The mean recovery from extracts of plasma was 97.7 ± 0.01 (SE). Interassay variation was 12% and intraassay variation was 6.2%. The sensitivity was 6.25 pg / tube. The mean recovery from extracts was 96.7 ± 0.01 (±SE). Interassay variation was 10% and intraassay variation 6%.

Plasma prolactin concentrations were assayed using an heterologous system (Sempéré *et al.*, 1995). Assay sensitivity was 0.3 ng ml^{-1} and interassay coefficient of variation was 13.1%. Intraassay variation, calculated from 12 reference plasma samples, was 4.8%.

Statistical Analysis

Comparisons between hormone concentrations from animals of Uzbekistan and Saudi Arabia were performed using the two-way repeated measures analysis of variance (ANOVA 2). Comparisons between the different periods in the same group of animals were made using the post-ANOVA PLSD Fisher test. Hormone concentrations are presented as means and standard errors (SEM).

RESULTS

Experiment 1

Progesterone Profiles

The two groups exhibited estrous and anestrous seasons ($P < 0.001$) with different annual variations (Fig. 1).

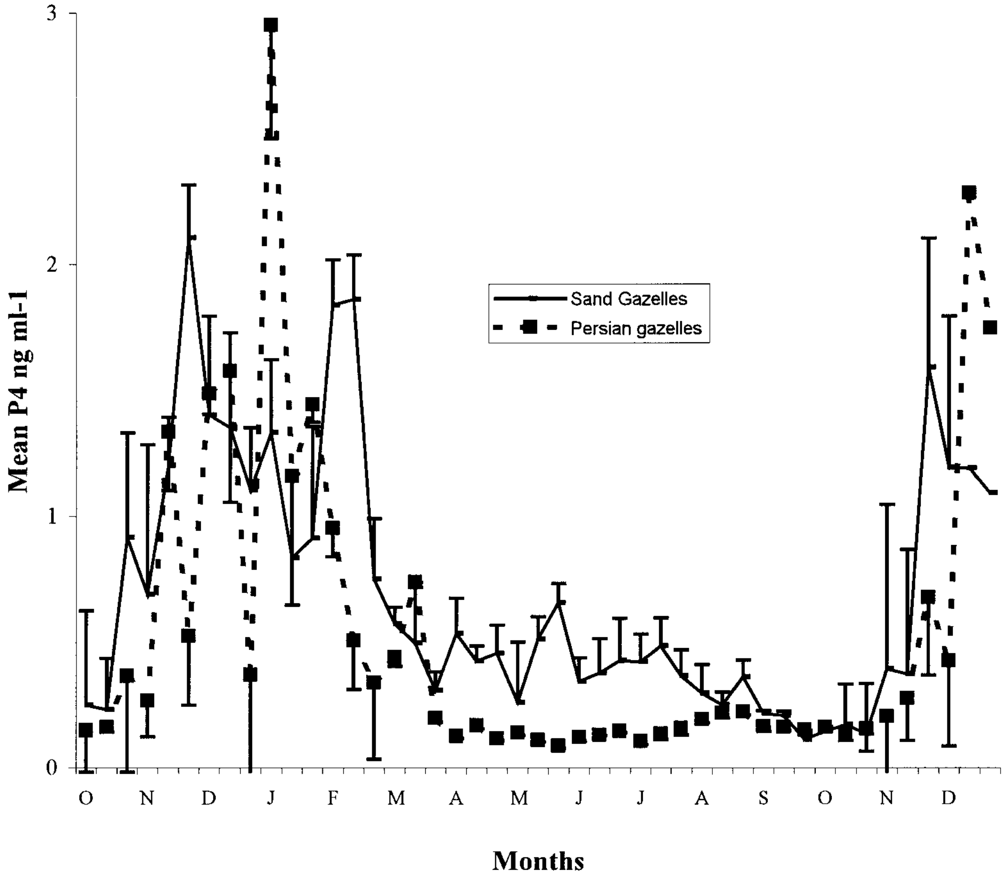

FIG. 1. Plasma progesterone profiles in sand gazelle (*Gazella subgutturosa marica*) living in Saudi Arabia and in Persian gazelle (*Gazella subgutturosa subgutturosa*) living in Uzbekistan.

Estrous season (from October to March). In both groups of females, progesterone concentrations rose between 9 and 25 November indicating that the estrous season began in November. However, plasma progesterone concentrations in November were greater in sand gazelles (Saudi Arabia) than in Persian gazelles (Uzbekistan (Fig. 1). During the estrous season there were large variations in plasma progesterone concentrations. However, during the second half of the estrous season (23 December–24 February) a significant hormonal decrease was observed in Persian gazelles ($F = 3.284$, $P < 0.0077$) while in sand gazelles it remained high during this period. In Persian gazelles, from February progesterone concentrations were always lower than 1 ng ml^{-1} and dropped to minimum concentrations on 25 March (0.20 ± 0.04 ng ml^{-1}). From individual profiles, it appeared that some sand gazelle exhibited high progesterone concentrations (>1 ng ml^{-1}) from early November to late February. Therefore, the length of the estrous season was significantly shorter in Persian gazelles than in sand gazelles (80.86 ± 5.77 days vs 116.44 ± 8.24 days: $P < 0.005$, $F = 10.440$).

Anestrous season (from April to November). The pattern of plasma progesterone concentrations differed during the anestrous season in the two groups of females (Fig. 1).

- Persian gazelles

Plasma concentrations always remained at basal levels in Persian gazelles both during the first period of anestrous (0.15 ± 0.04 ng ml^{-1} in April–August) and during the end of the anestrous period in September–October (0.16 ± 0.05 ng ml^{-1}).

- Sand gazelles

From February to July. Plasma progesterone concentrations always remained significantly higher in sand

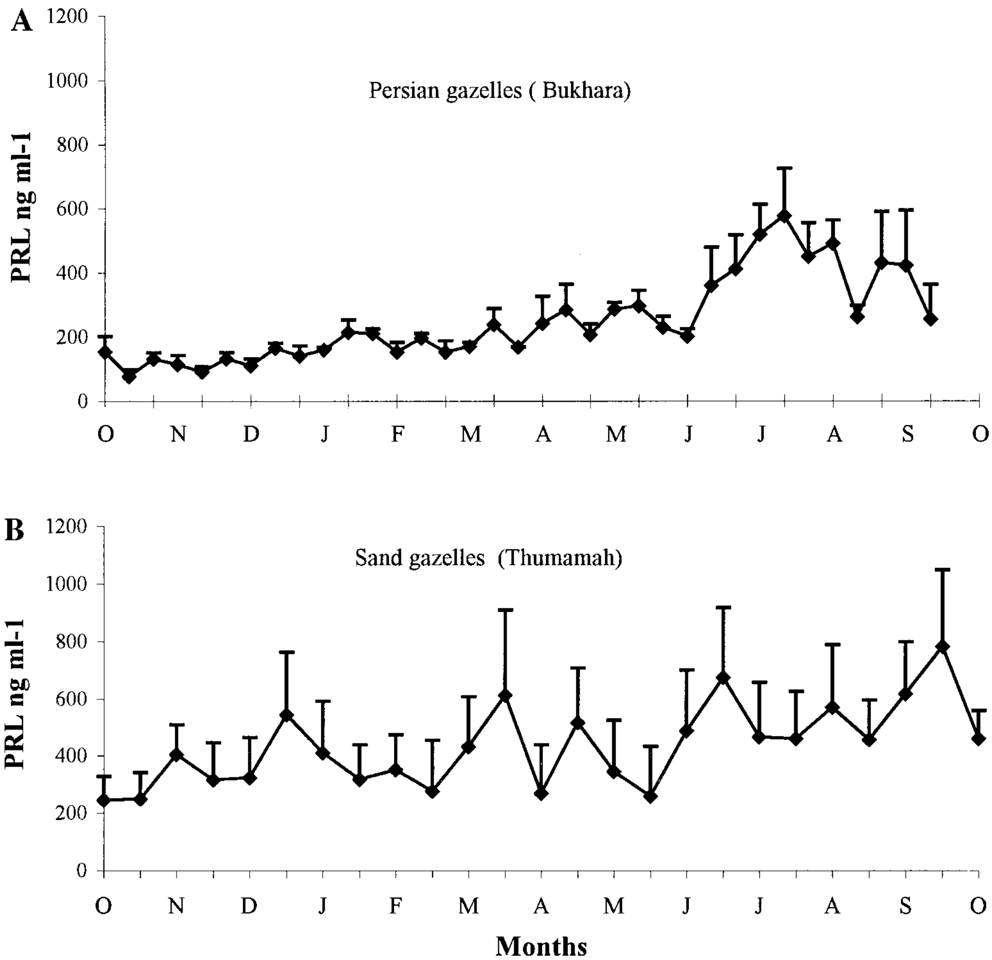

FIG. 2. Mean plasma prolactin concentrations in Persian gazelle (A) and sand gazelle (B).

gazelles than in Persian gazelles (except for Day 23 March: $P = 0.6582$).

From July to October. In sand gazelles, plasma progesterone concentrations were significantly higher from July to August (0.38 ± 0.05 ng ml^{-1}; $N = 78$) than from September to October (0.19 ± 0.4 ng ml^{-1}, $n = 48$) ($P < 0.001$). In September–October, plasma progesterone concentrations were similar in both populations.

Prolactin

Plasma concentrations of prolactin (PRL) exhibited different patterns in sand (Fig. 2B) (Thumamah, 2°N) and in Persian gazelles (Bukhara, 4°N) (Fig. 2). In sand gazelles, plasma PRL fluctuated widely throughout the year (October to September) but not significantly (Table 1) whereas in Persian gazelles (Bukhara) (Fig. 2A), the lowest concentrations were in autumn and winter (Period 1) while significantly higher levels were observed during summer (Period 2) ($P < 0.001$) (Table 1). Plasma PRL concentrations were also significantly lower ($P < 0.01$) during the first period (autumn–winter) in Persian gazelles than in sand gazelles whereas it did not differ between the two groups during Period 2 (summer) (Table 1).

Experiment 2

Persian females treated with melatonin implants for 6 months from the winter solstice (21 December) exhibited lower PRL concentrations than control females in winter and in spring (December to June) (126.29 ± 9.6 ng / ml in controls vs 85.88 ± 9.0 in treated females:

TABLE 1
Comparative Analysis of Plasma PRL Concentrations during the Short Photoperiod (Period 1) and the Long Photoperiod (Period 2) in Both Groups of Gazelles

	Period 1 (October–March)		Period 2 (April–September)		
	Mean ($ng\ ml^{-1}$)	SE	Mean ($ng\ ml^{-1}$)	SE	*P*
Thumamah (24°N,31°E)	374.1	30.4	489.1	47.0	ns
Bukhara (40°N,65°E)	151.5	7.1	325.8	24.8	<0.01
P	<0.01		ns		

$P < 0.001$) (Fig. 3). Furthermore, PRL in treated females rose significantly in summer (December–June $85.88 \pm 6.05\ ng\ ml^{-1}$ vs $133.93 \pm 13.05\ ng\ ml^{-1}$ July–September, $P < 0.002$) while their plasma concentrations remained significantly lower in summer than in control females ($P < 0.001$). Both groups of females (treated and control females) exhibited the same pattern of plasma progesterone and ended their estrous season at the same time by the end of February (Fig. 3).

DISCUSSION

Gazelles could more or less regularly exhibit two calving seasons a year; females could have a new estrous cycle in early spring with the second breeding season (postpartum estrus) occurring some days after parturition and not inhibited by lactation (Loggers, 1992; Baharav, 1983). Mountain gazelles living in High Galilee (arid conditions) give birth only in spring when in the same species living in Low Galilee at a distance of 80 km, where drinking water is available all year round, births occur year round (Baharav, 1983). Dorcas gazelles in western Lybia have two peaks of rutting behaviour in April and November (Essaghaier, 1981). Nevertheless, in all cases, the most important breeding season takes place in autumn and birth mainly occurs in spring when food availability is

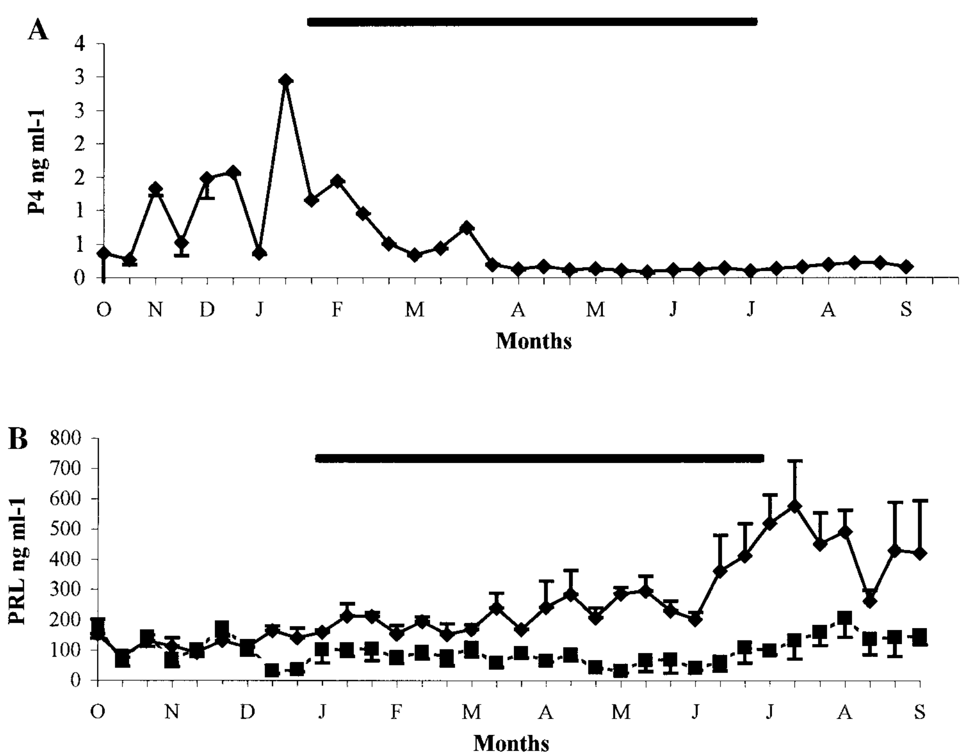

FIG. 3. Comparative profiles of plasma progesterone and prolactin (PRL) in Persian gazelles treated with melatonin (broken lines) and control females (full lines). Arrows indicate the day when melatonin implants were received.

maximum. These data suggest that these gazelles could have a long estrus season or could exhibit estrous cycles year round. In such cases, the length of estrous season and / or the fertility of females could be affected by a biotic factors such as food and water.

The present results show that both *G. subgutturosa* subspecies (sand gazelle from Saudi Arabia and Persian gazelles from Uzbekistan) display polyestrous cycles during the estrous season, from late autumn to late winter. The length of the estrous season was shorter in Persian (Uzbekistan: 40°N) than in sand gazelles (Saudi Arabia: 2°N). Under such conditions, and regarding the length of pregnancy (150 days, Marmosinskaya, 1990, 1996; Pereladova *et al.*, 1998), Persian females could be fertilized by mid-November and give birth by mid-April. Parturition would thus occur after the end of the normal estrous season and during the anestrous season. A second estrous period or a single estrous cycle during the anestrous season has never been observed in Persian gazelles (cf. Figs. 1 and 2) (Gepner *et al.*, 1961; Djevnerov, 1984; Kutcheruk, 1995). This suggests that the females cannot have a post partum estrous in spring, because they were already in anestrus from April (present data).

The estrous season in sand gazelles is longer than in Persian gazelles and the patterns of the anestrous season differed. In Persian gazelles, plasma progesterone concentrations remained basal during all of the anestrus season while sand gazelles have some ovarian activity until the next summer (Fig. 1) because two females of the present group exhibited estrus cycles until May (female N°359) and June (female N°108). In that case, it may be that for some pregnant females that can have such long estrous season (from November to May-June), a postpartum estrous is possible. However, this possibility would depend not only on the ovarian capacity but, also on the body condition of the female. Therefore, it seems that under such photoperiods, some sand gazelle females, living in captivity and receiving supplemented food (N. Brown, personal data), could have an exceptional second birth period during the year.

All these results indicate that the reproductive pattern in females depends not only on the species (i.e., Persian gazelle which always exhibit one cycle a year) but also the body condition of females for the species that can give birth twice a year (Loggers, 1992; Baharav, 1983). Prolactin is under photoperiod control (Bubenik, 1986). In general, concentrations are maximum during the long photoperiod in summer and minimal during the winter solstice (December). It appears that Persian gazelles have a clear seasonal PRL pattern whereas sand gazelles do not. This difference in hormonal pattern of PRL between the two subspecies could be related to the difference in these photoperiod regimes.

The length of estrus and the pattern of the anestrus in sand gazelle and Persian gazelle could have resulted from the inhibitory action of the long photoperiod as in temperate deer (Sempéré *et al.*, 1995). Such inhibition is less pronounced or does not exist when the animals live under or around 20° of latitude (Loudon and Brinklow, 1991). Persian females from Uzbekistan (40°N) were exposed to a photoperiod which ranged from short days (8L:16D) in winter to long days in summer (16L:8D), whereas sand gazelles in Saudi Arabia (2°N) live under a photoperiod regime less pronounced (2–3 h difference between solstices). Therefore, females living in Saudi Arabia (2°N) may be less influenced by photoperiod. In fact, the low sensitivity to the photoperiod was reflected in the pattern of PRL in sand gazelle females (Saudi Arabia). The lack of significant annual variation and the large difference in PRL concentrations between animals (reflected by the large standard errors) would indicate that in Saudi Arabia, the animals were not truly led by the photoperiod which acts as a proximate factor for the beginning of the breeding season in animals exposed to greater photoperiod variations (more than 20° of latitude).

In deer, melatonin treatment prevents anestrus (Adam *et al.*, 1985; Sempéré *et al.*, 1995). Malpaux *et al.* (1989) demonstrated that increased photoperiod in spring was not necessary to terminate the breeding season in ewes, suggesting that the end of the estrous season in sheep was entrained by an endogenous rhythm. In the present study, melatonin, which inhibited PRL secretion, had no effect on the end of the estrous season of Persian gazelles. These results strongly suggest that the end of the estrous season in Persian gazelles is led by an endogenous rhythm as in sheep.

These physiological processes of the reproductive cycle might be related to the adaptations of the species to arid environments. These two species are very well adapted to high temperatures, and dry summers have a positive effect on breeding success (Pereladova *et al.*, 1998). On the other hand mortality was very high in Persian gazelles during the cold winter (continental climate in Uzbekistan). In Saudi Arabia, winters are not very cold (Ancrenaz and Flammand, 1995) and sand gazelles are less affected and can survive. Moreover, rainfall which is higher in Central Asia (150 mm) than in Saudi Arabia (50 mm) (Dunham, 1991) can affect heat loss and because of problems of thermoregulation can induce a high mortality in Persian gazelles (Pereladova *et al.*, 1998). Females spend a lot of energy during lactation and much more for ungulates with twins (Mauget *et al.*, 1997). Therefore, the climate in Central Asia (very hot summer and cold winter) demands a lot of energy for Persian females which have newborn twins to feed (Pereladova *et al.*, 1998). Such females must be in good condition if they breed twice a year.

If photoperiod is a proximate factor in controlling the timing of estrus for numerous species of mammals, other environmental factors (rainfall, temperatures) also determine genetic selection. For example, Eld's deer (*Cervus eldi thamin*), which is a tropical species, initiate the estrous season in spring and give birth in autumn when the food is abundant because of the summer Monsoon (Monfort *et al.*, 1991; Asher *et al.*, 1994).

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