Hormonal characterization of the reproductive cycle and pregnancy in the female Mohor gazelle (*Gazella dama mhorri*)

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The oestrous cycles of seven captive Mohor Gazelles (*Gazella dama mhorri*) were investigated. Hormone profiles obtained from faecal samples collected each day from cyclic females indicated that the mean duration of the oestrous cycle was 18.62 ± 0.26 days (range 16–22 days; $n = 37$ oestrous cycles). No inter-individual differences in the concentration of faecal progesterone metabolites excreted were observed, but mean faecal oestrogen excretion during both the luteal and inter-luteal phases of the oestrous cycle varied among females ($P < 0.001$ and $P = 0.070$, respectively). Oestrous cycles were synchronized using controlled internal drug release (CIDR) devices, before natural mating with an intact male. Concentrations of faecal progesterone metabolites remained

approximately constant for the first 10 weeks of gestation (mean $\pm$ SEM = 4048 ± 407 ng g⁻¹ faeces), before increasing to a mean of $23\,556 \pm 1176$ ng g⁻¹ faeces. Two of seven female gazelles conceived immediately after removal of the CIDR device, a similar proportion to that conceived at the postpartum oestrus under natural conditions. Life history data for these individuals indicated that the mean time to conception in female gazelles is positively correlated with peak values in the ratio of excreted oestrogen:progesterone during the inter-luteal period of their oestrous cycles ($R^2 = 0.58$; $P < 0.05$). This finding indicates that interactions between steroid production and metabolism may influence the likelihood of conception occurring in this species.

Introduction

Derived from a founder population consisting of the last 17 surviving individuals remaining in the Western Sahara (Alados *et al.*, 1988), the Mohor gazelle (*Gazella dama mhorri*) has been bred naturally in captivity since 1970 (Holt *et al.*, 1996a), and has been successfully reintroduced into protected wild habitats (Cano *et al.*, 1993; Abáigar *et al.*, 1997). The resultant population represents a useful model for investigating approaches for conservation of endangered ungulates. The success of this captive breeding programme means that subpopulations held in different geographical locations, that is, various zoos, are likely to suffer genetic drift and further inbreeding. Artificial insemination (AI) and the establishment of a genetic resource bank have been proposed as a way of minimizing these inbreeding effects, and might also prove useful in maximizing the genetic diversity of stock currently being reintroduced (Holt *et al.*, 1996b; Wildt *et al.*, 1997). However, to date, no studies of female reproductive physiology have been undertaken in this species and this severely limits the development of AI protocols. Therefore, the objective of this study was to characterize endocrine attributes of the oestrous cycle and pregnancy. Non-invasive faecal hormone analysis was used to avoid stressing the animals.

Effective breeding programmes for the conservation and management of endangered species require reliable information about the ovarian activity of any given female, particularly in situations in which the application of assisted reproductive techniques is considered appropriate (Hodges, 1995). However, by definition, access to adequate numbers of endangered individuals is limited, and basic physiological data are difficult to obtain. One solution to this problem is to study a more common, but closely related species, in the belief that reasonably accurate extrapolations can be made. This approach may provide useful background information, but inherent and species-specific factors may impose unexpected limitations on the applicability of reproductive technologies. Although such limitations may, for example, restrict the success rates of procedures such as artificial insemination and embryo transfer, determining whether they are related to genetic or environmental factors may provide valuable insights about causes of population declines and the likelihood of successful recoveries. Furthermore, such analyses may help guide management decisions concerning optimal conservation strategies. The aim of this study was to address some of these issues.

In an earlier AI study, oestrous synchronization was attempted by the insertion of intravaginal controlled internal drug release (CIDR) devices that were impregnated with progesterone. This is a standard technique, widely

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Table 1. Reproductive life history parameters of seven female Mohor gazelles (*Gazella dama mhorri*) subjected to oestrous synchronization

Animal	Age at first conception (years)	Number of full term pregnancies before CIDR insertion	Percentage of pregnancies in which ≥ 1 calf survived > 30 days	Age at CIDR insertion (years)
420	1.45	7	57	7.20
425	1.25	7	100	7.06
519	1.05	6	50	5.72
542	1.45	4	100	5.17
577	1.35	3	66	4.19
672	0.95	3	0	3.81
687	0.75	3	66	3.36

CIDR: controlled internal drug release device.

used in livestock breeding, where the objective is to minimize limitations on fertility due to inappropriate timing of insemination or poor semen quality (Holt *et al.*, 1996a). Subjective assessment of the reproductive tract at the time of laparoscopic insemination indicated that the synchronization protocol had been effective, but no endocrine investigations were undertaken to confirm these observations. Pregnancy was diagnosed by ultrasonography in four of 13 females (30.77%) on day 52 after AI. Of these, only one female carried her calves to term, possibly because the stress of intervention for ultrasonography may have caused fetal loss in the other female gazelles.

Evidence from other ungulate species indicates that conception rates after oestrous synchronization by the insertion of a CIDR device and AI are comparable to natural fertility rates and are usually in excess of 65% (see, for example Asher *et al.*, 1988; Jabbour *et al.*, 1993). One objective of the present study was to compare conception and pregnancy rates obtained after experimental manipulation with the natural fertility of this species, using data obtained from breeding records. Conception rates observed in CIDR-synchronized female gazelles that were allowed to mate naturally with intact males were also recorded in an attempt to identify the cause of any impaired reproductive function. The opportunity to undertake this investigation on seven female Mohor gazelles is rare for an endangered species, and allowed significant information about the physiology of this species to be documented.

Materials and Methods

Animals and management

The Mohor gazelles (*Gazella dama mhorri*) used in this study were selected from the breeding herd maintained and managed by the Estación Experimental de Zonas Áridas (EEZA), Consejo Superior de Investigaciones Científicas, Almería, Spain. Normal day-to-day management procedures, as described by Alados *et al.* (1988), were observed for the duration of the study. Conception dates for each pregnancy were considered as 200 days before the day of parturition, the duration of gestation in this species (Furley, 1986).

Reproductive life history data for each female gazelle studied were collated from the EEZA records for this species. Factors considered relevant to this analysis are summarized (Table 1). All females were within their normal reproductive lifespan, which on average is about 11 years in this species; however, in exceptional cases, births have been reported in 14-year-old female gazelles (Cano, 1991).

Determination of oestrous cycles

Seven multiparous female gazelles (> 2 years of age) were penned in groups in the presence of a vasectomized male, for longitudinal oestrous cycle monitoring over approximately 3 months. One female (Studbook number 542) was pregnant at the onset of sample collection, but gave birth on day 9 of the study. Faecal samples were collected early in the morning, each day. Individuals were identified by their ear tags, and approximately 5 g of freshly deposited faeces was collected and stored at -20°C until analysed.

Sexual interactions between the vasectomized male and the female gazelles were recorded each day for 1 h before nightfall so that faecal hormone concentrations could be correlated with courtship behaviour. The behaviours recorded were limited to:

(i) Male follows female, who runs away (that is, is unreceptive); (ii) male follows female, who does not run away (that is, is receptive); (iii) male mounts female without an erect penis; and (iv) male mounts female with an erect penis.

These results and the identity of the female to which the behaviour was directed were stored in a field computer (Psion Organiser II, Psion Computers, London) with automatic allocation of date and time.

Oestrous synchronization and pregnancy monitoring

Oestrous synchronization was performed in the same seven female gazelles. Each female was fitted with a single progesterone-containing intravaginal CIDR type-G device (9% progesterone; CHIH Plastic Moulding, Hamilton) for 12 days, as described by Holt *et al.* (1996a). CIDR devices were inserted in July 1996, approximately 1 month after the

end of the period of behavioural and oestrous cycle monitoring, when the species is known to be naturally fertile. The females were distributed between three social groups at the time of CIDR device removal, each in the presence of a male of proven fertility. For management reasons it was not possible to collect faeces when the CIDR devices were in place. From day 2 after CIDR device removal, until between 4 and 6 weeks after parturition, faecal samples were collected once a week, and stored as described above. Faecal samples were also collected (where possible) on the day of parturition.

Faecal progesterone analysis

Faecal samples were collected and analysed for their progesterone content by ELISA, according to the method described by Shaw *et al.* (1995), using an antiserum raised in rabbits against 4-pregnen-20 α -ol-3-one (20 α -hydroxyprogesterone, donated by M. J. Peddie, Department of Physics and Pharmacy, University of Southampton). This antibody shows a degree of crossreactivity with other faecal progesterone metabolites (principally 5 α -pregnan-3 α ,20 α -diol (1.4%), 5 α -pregnan-3 β ,20 α -diol (11.1%) and 5 β -pregnan-20 α -ol-3-one (47.2%); Garnier *et al.*, 1998). Standard curves were prepared using 20 α -hydroxyprogesterone (Sigma P6288; Sigma Chemical Co., Poole), and ranged between 0.0625 and 16.0 ng ml⁻¹. Comparison of the regression lines for a random subset of the standard curves ($n = 15$) and the displacement curves for serial dilutions of faecal extracts ($n = 2$) showed no evidence of a departure from parallelism ($t_{138df} = 4.243$; ns). Samples collected during the oestrous cycle were diluted 1:50 (v/v) in assay buffer, and samples collected during gestation were diluted 1:300 or 1:500 (v/v) in assay buffer. Precision of extraction, calculated as the coefficient of variation of 20 α -progesterone concentrations for repeated extraction of the same sample, was $14.23 \pm 0.03\%$. The intra-assay coefficients of variation were 17.7% for high (3.37 ± 0.51 ng ml⁻¹) and 5.2% for low (0.28 ± 0.10 ng ml⁻¹) concentration quality control samples. Interassay variation was 18.4% and the average sensitivity of the assay was < 0.0625 ng ml⁻¹.

Faecal oestradiol analysis

The oestrogen-containing potassium hydroxide fraction of the faecal extract was diluted 1:20 (v/v) with assay buffer and analysed using a tritiated radioimmunoassay as described by Hodges *et al.* (1983). The antiserum raised in rabbits (No. 35, 7.6.83, a gift from I. Swanston, MRC Reproductive Biology Unit, Edinburgh) crossreacted 100% with oestradiol, 27% with oestradiol-17 α , 8% with oestrone and $< 0.1\%$ with testosterone, progesterone and androstenedione. The inter-assay coefficients of variation for pooled quality control samples containing 167.75 ± 9.68 and 24.38 ± 1.77 pg oestradiol ml⁻¹ were 5.77% and 7.28%, respectively. The mean intra-assay coefficient of variation was 8.83%, and the limit of sensitivity of the assay at 90%

binding was < 6.25 pg ml⁻¹. The precision of the extraction procedure was $10.39 \pm 1.55\%$. Parallelism between serial dilutions of faecal extracts ($n = 3$) and the oestradiol standard curves ($n = 17$) was observed ($t_{120df} = 1.190$; ns).

Data analysis

Faecal progesterone and oestradiol concentrations were expressed as ng g⁻¹ faeces. Data collected after oestrous synchronization were aligned according to the week during which conception occurred for each female. Owing to variability in the duration of each oestrous cycle, the progesterone data generated when the females were housed in the presence of a vasectomized male were not aligned to any specific time point, but were investigated independently, using the entire data set for each female.

Different phases of the oestrous cycle were defined as follows:

Inter-luteal phase. The mean ($\pm$ SD) concentration of faecal progesterone during the inter-luteal phase was calculated separately for each oestrous cycle, and individual samples were checked to confirm that if included, or excluded, they fell within, or without, the mean (± 2 SD) concentration for that oestrous cycle. The term inter-luteal phase has been used as the analysis of progesterone excretion precludes identification of the period during which recruited ovarian follicles are maturing.

Luteal phase. The onset of the luteal phase was determined as the first of two successive faecal samples of increasing progesterone concentration, which was at least 2 SD greater than the mean concentration of progesterone in the preceding inter-luteal phase. The end of the luteal phase was assigned as the sample preceding the first sample to fall within the criteria established above for inclusion in the subsequent inter-luteal phase.

The duration of the oestrous cycle was calculated as the time between the onset of two successive luteal phases, and was calculated separately within and between individuals and tested statistically by ANOVA to investigate differences between individuals. Similarly, the durations of the luteal and inter-luteal periods of the oestrous cycle were examined, and these were correlated by linear regression analysis to investigate the source of variation in the duration of these phases of the oestrous cycle. This information was used to determine the oestrogen content of all samples collected during the inter-luteal phase, but alternate samples during the luteal phase of the oestrous cycle were analysed. The oestrogen:progesterone ratio was calculated for the days on which both hormones were analysed, as this ratio has proved useful for detecting the time of ovulation in humans (Lenton *et al.*, 1989).

For each animal, mean ($\pm$ SEM) faecal concentration of progesterones, oestrogens, and the faecal oestrogen:progesterone ratio were calculated separately for each luteal and inter-luteal period of the oestrous cycle, as defined above. These

data were examined by ANOVA, using animal as a block structure to identify whether there were inter-individual differences in hormone excretion profiles. Differences between minimal and maximal concentrations excreted during each phase of the oestrous cycle were also calculated and analysed using the same method.

The faecal progestagen concentration during the luteal phase was compared with the concentration of progestagen excreted during the first 10 weeks of gestation (paired Student's *t* test); linear regression analysis was used to investigate the relationships between hormone concentrations and reproductive life history characteristics. All statistical analyses were performed using Microsoft Excel 97. Statistical differences were considered significant at $P < 0.05$, unless stated otherwise.

Results

Characteristics of the oestrous cycle

Faecal progestagen excretion during each oestrous cycle fluctuated regularly with a mean ($\pm$ SEM) frequency of 18.62 ± 0.26 days. A range of 16–22 days was recorded for the duration of the oestrous cycle ($n = 37$ cycles). The duration of the luteal phase was between 10 and 17 days (mean $\pm$ SEM = 13.45 ± 0.27 days), whereas the duration of the inter-luteal phase was between 2 and 9 days (mean $\pm$ SEM = 5.06 ± 0.24 days). The modal values for the luteal and inter-luteal phases of the oestrous cycle were 13 and 4 days, respectively. The duration of the luteal and inter-luteal phases of the oestrous cycle were inversely related ($R^2 = 0.14$; $P = 0.023$), and differed significantly between individuals (luteal phase: $P = 0.039$; inter-luteal phase: $P = 0.032$).

Behavioural data showed that the lowest faecal progestagen concentrations corresponded to peaks in courtship behaviour between the male and the females. Therefore, it was assumed that the cyclic variability in progesterone metabolite excretion genuinely reflected changes in the peripheral progesterone concentrations associated with ovarian activity. Concentrations of progestagen metabolites during the inter-luteal phase of the oestrous cycle were 295.40 ± 18.83 ng g⁻¹ faeces (mean range = 79.50–762.10 ng g⁻¹ faeces). These concentrations were achieved 0–2 days after the onset of behavioural interest from the male, which indicated that the female was in oestrus (Fig. 1). When mean luteal phase and inter-luteal phase progestagen concentrations were calculated separately for each oestrous cycle within each female, there were no differences among females in the concentration of progestagens excreted.

Concentrations of faecal progestagen during the luteal phase were 2065 ± 116 ng g⁻¹ faeces (mean range = 1017–4212 ng g⁻¹ faeces), and were lower than the concentration of progesterone metabolites excreted during the first 10 weeks of gestation ($P < 0.001$). Nevertheless, the range of faecal progestagen concentrations in the two physiological states showed considerable overlap when the absolute

concentration of individual samples was considered (range during the luteal phase = 55–17 530 ng g⁻¹ faeces; range during first 10 weeks of gestation = 87–13 884 ng g⁻¹ faeces).

Hormone profiles during pregnancy

Faecal progestagen concentrations remained approximately constant during the first 10 weeks of gestation (mean $\pm$ SEM = 4048 ± 407 ng g⁻¹ faeces), before increasing to a mean of $23\,557 \pm 1176$ ng g⁻¹ faeces. The concentration of progestagens excreted did not decrease significantly before the week of parturition and baseline values were reached within 7 days after parturition. A composite profile of progestagen excretion during pregnancy was generated using the combined data from all seven female gazelles investigated (Fig. 2).

Sources of individual variation in relation to conception

Three female gazelles conceived within 11 days of removing the CIDR device (see Table 2). The faecal progestagen concentrations in the initial samples collected from females (Nos 542 and 672) were low (399 and 426 ng g⁻¹ faeces, respectively), indicating that the increased plasma progesterone concentrations expected as a result of CIDR device insertion had decreased 2 days after CIDR device removal. The third female (No. 420) had a high faecal progestagen concentration at this time (2772 ng g⁻¹ faeces). Conception in the remaining four individuals occurred between week 3 and week 17 after CIDR device removal. All but one (No. 519) of these females had low progestagen concentrations in their first faecal sample.

The faecal progestagen concentrations of the females that conceived at the ovulation after removal of the CIDR device were not significantly different during gestation from those that returned to oestrus between CIDR device removal and conception. In females that failed to conceive immediately after CIDR device removal, the faecal progestagen concentrations recorded between CIDR device removal and the establishment of pregnancy (as determined from their date of parturition) were similar to concentrations during the luteal phase, and lower than those observed during the first trimester of gestation.

The seven gazelles used in this study represented a range of ages and had experienced different numbers of pregnancies before the onset of this investigation (Table 1). Life history data showed that one female (No. 672) suffered from 100% calf mortality in the first month after parturition. The mean time to the next conception for this female (6 days) indicated that this species does not experience a period of postpartum anoestrus and anovulation. Overall, 26.9% of gestations resulted in no calves surviving beyond 30 days after parturition.

For the group as a whole, the mean time between parturition and conception after the death of all calves within the first month of life was 38.31 ± 10.24 days (range from 1 to 101 days). When at least one calf survived for at least 1 month, the interval between parturition and conception was

Fig. 1. Excretion of faecal steroid hormone metabolites and associated mating behaviour during the oestrous cycle of a representative Mohor gazelle (*Gazella dama mhorr*). Faecal progesterone concentration (ng g⁻¹ faeces): pink squares; faecal oestrogen:progesterone ratio: blue squares.

on average 75.72 ± 15.83 (range from 3 to 411 days). Thus, lactational anoestrus was not observed in this species. There was no significant relationship between the time from CIDR device removal to conception, and the natural interval to conception on the basis of the reproductive life history data for each individual (Table 2). The seven female gazelles used in this study had experienced collectively 33 pregnancies (26 inter-gestational periods) before the onset of this experiment, and had produced 49 calves. Conception occurred within one oestrous cycle after parturition in 31.5% of the cases in which at least one calf survived the first month of life, and in 28.6% of cases in which all calves died within 1 month of birth.

Significant differences in oestrogen excretion were observed between females during both the luteal and inter-luteal phases of the oestrous cycle (Table 3). The oestrogen:progesterone ratio did not differ between the females, but its peak value during the inter-luteal phase was positively correlated with the mean time to conception for each individual as determined from their life history data ($R^2 = 0.582$; $P < 0.05$; Fig. 3).

Discussion

Traditional methods for monitoring reproductive physiology in domestic species, such as the measurement of steroid hormones circulating in the plasma, are inappropriate for endangered species because of the handling required for

sample collection, and the impracticalities associated with repeated sampling from intractable individuals (Lasley and Shideler, 1993). These factors limit the ability to collect basic physiological data needed to understand and manipulate reproduction. As a result, alternative methods for monitoring reproduction in both captive and free-roaming species have been developed. Intensive observations of behavioural activities associated with breeding, and monitoring of steroid hormone metabolites excreted, usually urine or faeces (for a review, see Heistermann *et al.*, 1995), but also including saliva (Digiano *et al.*, 1992; Czekala and Callison, 1996; Thorne *et al.*, 1998), have proved invaluable tools for conservation biologists managing populations of endangered species. The management constraints associated with studying endangered animals impose sampling limitations that would be unacceptable in similar studies of domestic livestock.

CIDR devices have been used to synchronize oestrus in a number of non-domesticated ungulates (Haigh and Bowen, 1991; Argo and Loudon, 1992; Jabbour *et al.*, 1993; Monfort *et al.*, 1993). In the present study, conception rates resulting in parturition 200 days after CIDR device removal were low (28.6%) and were similar to conception rates obtained after oestrous synchronization and AI (30.8% as diagnosed by ultrasonography) in a previous study by Holt *et al.* (1996a). Similar results were reported after the use of CIDR devices alone to synchronize oestrus before AI in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*; Asher

Fig. 2. Composite (mean $\pm$ SEM; $n = 7$) faecal progesterone concentrations before conception (red triangles), during gestation (blue diamonds) and after parturition (red squares) in the Mohor gazelle (*Gazella dama mhorri*).

et al., 1993). However, improved conception rates ($> 70\%$ in fallow deer: Asher *et al.*, 1992; Morrow *et al.*, 1995; 40–60% in red deer, Asher *et al.*, 1993) were achieved after manipulation of the oestrous synchronization or AI protocols.

The unexpectedly low rates of conception after oestrous synchronization and natural insemination in the Mohor gazelle, led us to consider whether our original expectation, that AI would produce higher conception rates, was unrealistically optimistic. If so, the underlying causes of low conception rates may be related to poor reproductive function, perhaps caused by inbreeding depression or other factors associated with populations originating from a small number of founders (Crnokrak and Roff, 1999).

The possibility that the protocol used to synchronize oestrus in the Mohor gazelle was inadequate was considered. In another study (Holt *et al.*, 1996a), laparoscopic examination of the uterine horns at the time of AI indicated that the CIDR devices are effective in synchronizing the reproductive cycles of female gazelles. This result was provisionally confirmed within the present study, in which five of seven female gazelles showed low concentrations of faecal progesterone on day 2 after CIDR device removal, within the range of the inter-luteal phase progesterone concentrations recorded in the presence of the vasectomized male. This finding indicates that 71.4% of

female Mohor gazelles should be in oestrus within 2 days of CIDR device removal when using this protocol. As insemination timing and procedure can be eliminated as limiting factors in this study, conception rates of $> 30\%$ may, therefore, be expected. Findings from the present study indicate that the underlying cause of conception failure is a consequence of the female physiology, and possibly that ovulation has not occurred after CIDR device removal.

Analysis of oestrous cycle data for the Mohor gazelle indicated considerable variation in the duration of the cycle both within and between females. These results also showed that the luteal phase in this species could exceed the time that the CIDR devices were in place in the vagina. Thus, depending on the stage of the oestrous cycle at the time of CIDR device insertion, the synchronization protocol used in this study may have failed to override the endogenous luteal phase of the two females in which high faecal progesterone concentrations were recorded 2 days after CIDR device removal. Therefore, modifications of the oestrous synchronization protocol are required to increase its effectiveness, which may include the use of higher concentrations of progesterone or the insertion of the CIDR device for a longer period, or the application of exogenous hormones to enhance follicular maturation or induce luteolysis. In a similar study in scimitar-horned oryx (*Oryx*

Table 2. Comparison of time to conception after synchronization (with natural insemination) and time to conception in non-synchronized female Mohor gazelles (*Gazella dama mhorri*)

Animal	Number of days from CIDR removal to conception*	Mean number of days to conception under natural conditions* (range)
420	11	64.67 (8–222)
425	24	37.17 (17–91)
519	123	51.40 (12–90)
542	3	137.33 (112–172)
577	44	116.0 (104–128)
672	2	6.0 (1–11)
687	101	90.5 (75–106)

*Based on an assumed gestation period of 200 days.
CIDR: controlled internal drug release device.

Table 3. Faecal steroid metabolite concentrations during the oestrous cycle (separated into luteal and inter-luteal phases) of seven female Mohor gazelles (*Gazella dama mhorri*)

Animal	Faecal progestagen (ng g ⁻¹)		Faecal oestrogen (ng g ⁻¹)		Faecal oestrogen:progestagen ratio	
	Luteal phase	Inter-luteal phase	Luteal phase	Inter-luteal phase	Luteal phase	Inter-luteal phase
420	2244.56 ± 268.05	331.45 ± 52.58	5.65 ± 0.45	7.15 ± 0.60	0.0036 ± 0.0004	0.0263 ± 0.0043
425	2613.14 ± 348.17	413.36 ± 82.87	6.33 ± 0.33	7.38 ± 0.65	0.0040 ± 0.0005	0.0240 ± 0.0036
519	2309.72 ± 256.92	279.75 ± 21.10	5.70 ± 0.09	6.07 ± 0.58	0.0042 ± 0.0040	0.0230 ± 0.0015
542	1635.40 ± 208.88	291.09 ± 44.03	6.45 ± 0.23	6.95 ± 0.65	0.0059 ± 0.0008	0.0391 ± 0.0147
577	2035.37 ± 309.80	229.22 ± 19.23	5.54 ± 0.43	5.16 ± 0.42	0.0079 ± 0.0036	0.0276 ± 0.0032
672	2116.70 ± 382.33	239.69 ± 29.99	4.11 ± 0.19	5.49 ± 0.43	0.0049 ± 0.0010	0.0277 ± 0.0032
687	1414.78 ± 169.32	261.13 ± 25.81	5.94 ± 0.41	6.41 ± 0.59	0.0060 ± 0.0006	0.0288 ± 0.0019
P value	ns	ns	< 0.001	0.070	ns	ns

Values are mean ± SEM.
ns: not significant.

dammah), Morrow *et al.* (2000) successfully synchronized oestrus with two prostaglandin injections, and found that this approach was more effective than additional progestagen administration.

Recent evidence that population density can alter the effect of the environment on reproductive outcome in ungulates (Kruuk *et al.*, 1999) implies that care should be taken when extrapolating life history data collated from small and captive populations in an attempt to predict population- or species-level responses. Nevertheless, to understand fully the processes that occur in this experimental system, breeding records for the animals used in this study were investigated. For each female, the interval between CIDR device removal and conception was not related directly to the natural fertility of the females investigated, when compared with their reproductive life history characteristics. There was no common trend in the time from parturition to conception in each female under natural conditions, although this period tended to reduce with increasing age and successive pregnancies in all females. A comprehensive investigation of the breeding records for the entire Mohor gazelle population housed at the EEZA would be necessary to gain a greater

Fig. 3. Correlation between maximum inter-luteal phase oestrogen:progestagen ratio and mean time to conception for seven female Mohor gazelles (*Gazella dama mhorri*). Data are taken from life-history characteristics and represent 26 inter-gestational periods.

understanding of inherited aspects of fertility for this species; this is beyond the scope of the present study. However, a collective analysis of the life history data for the seven female gazelles manipulated in this investigation

revealed that an overall conception rate of only 30.8% at the first postpartum oestrus was achieved when no reproductive manipulation was used. It may, therefore, be unrealistic to expect higher conception rates after reproductive manipulation, particularly given the collective evidence that natural conception rates according to breeding records (this study), conception rates after oestrous synchronization with natural insemination (this study) and conception rates after oestrous synchronization and AI (Holt *et al.*, 1996a) are equitable (30.8%, 28.6% and 30.8%, respectively).

The frequency of faecal sample collection (once per week) was not sufficient for characterizing oestrous cycles of females that failed to conceive after oestrous synchronization. Therefore, the possibility that conception had occurred, but was followed by early pregnancy loss could not be investigated directly. Nevertheless, statistical comparisons of faecal progesterone concentrations during the luteal phase of the oestrous cycle and the first trimester of pregnancy indicate that pregnant female gazelles excrete significantly higher progesterone concentrations than cyclic female gazelles. This finding may, with further analysis and assessment of the expected limits of accuracy for an unknown individual, form the basis of a practical early pregnancy diagnostic test for this species. There was no evidence to indicate that the use of a CIDR device causes mid-term fetal loss and, therefore, should be contra-indicated in this species. Females that failed to conceive after oestrous synchronization did not show a consistent trend towards faecal progesterone concentrations exceeding those of the luteal phase of the oestrous cycle. However, it is possible that any true effect may be masked by the overlapping range of faecal metabolite concentrations in the two reproductive states and by the infrequent sampling during the pregnancy investigation. Increasing the frequency of sample collection during this period should address this issue in more detail.

The duration of the oestrous cycle was variable within and among female gazelles, but was comparable to that of other Bovidae of similar body size, for example impala, *Aepyceros melampus* (Fairall, 1970); blackbuck, *Antilope cervicapra* (Holt *et al.*, 1988); Pronghorn, *Antilocapra americana* (Pojar and Miller, 1984); goats, *Capra hircus* (Rogers *et al.*, 1969), *Capra ibex* (Stuwe and Grodinsky, 1987), *Capricornis crispus* (Ito, 1971); sheep, *Ovis aries* (Grubb and Jewell, 1973; Yenikoye *et al.*, 1981; Yenikoye, 1984), *Ovis canadensis* (Turner and Hansen, 1980) and Kob, *Kobus* (= *Adenota*) *kob* (Morrison, 1971; Morrison and Buechner, 1971). Despite variability within and among female gazelles, there was no evidence to indicate that the animals used in this study were capable of adjusting the duration of their oestrous cycle to dominate matings over other females in the herd. Behavioural and physiological studies of other ungulates, for example the scimitar-horned Oryx (Morrow *et al.*, 1999) and sable antelope, *Hippotragus niger* (Thompson, 1995) indicate that these species adopt such a social strategy. The Mohor gazelle shows a hierarchical social structure based on the age of the other

females in the group; therefore, it might be expected that dominant (older) females could alter their ovarian function to attract increased attention from the resident male.

The oestrogen:progesterone ratio was useful for interpreting the data generated during this study. This ratio provides a means of accounting for potential differences in steroid metabolism and excretion, and has proved more valuable for the prediction of ovulation than the analysis of circulating oestradiol or progesterone in isolation in humans (Lenton *et al.*, 1989). It remains to be determined how peaks in the oestrogen:progesterone ratio relate to ovulation in this species. However, the significant relationship between the maximum inter-luteal phase oestrogen:progesterone ratio and the mean time to conception recorded for these gazelles, indicates that there may be a relationship between steroid metabolism and excretion and successful conception, and is worthy of further investigation. Relative concentrations of these two steroids have been used for monitoring the success of reproductive manipulation in a number of human studies (Maclin *et al.*, 1990; Kamada *et al.*, 1992; Csemiczky *et al.*, 1996; Xia and Younglai, 2000), and studying oocyte quality in cattle (Mihm *et al.*, 1999; Oussaid *et al.*, 2000).

In conclusion, the likelihood of improving the success of reproductive manipulations of the Mohor gazelle may be constrained by inherent limitations on steroid production or metabolism. This information should be taken into account when considering the economics of undertaking a programme of assisted breeding. Similarly, this information is also relevant when predicting the population dynamics of reintroduced populations breeding under natural conditions, but experiencing additional environmental pressures when compared with captivity.

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