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Endocrine correlates of sexual behavior in the Mohor gazelle (*Gazella dama mhorr*)

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Abstract

In this study, we quantitatively examined male sexual behavior in relation to fecal estrogen and progesterone concentrations in female Mohor gazelles. We investigated the hypothesis that, during natural mating, males detect cues relating to the potential for successful conception and pregnancy. Time series analysis revealed that males could detect the approach of estrus 2–3 days before female fecal estrogens and estrogen/progestagen (E/P) ratio reached their peak values. Males also paid closer attention to those females excreting higher fecal estrogen concentrations. Mounting and copulation frequencies were positively correlated with both peri-ovulatory fecal estrogen concentrations, and the frequency of pre-copulatory courtship behaviors. These data suggested that males invest their reproductive effort selectively by mating the most fertile females, assuming that estrogen is a valid index of fertility. This assumption was investigated by examining sequential phases of the reproductive cycle for evidence that oocytes and follicles produced in a more estrogenic environment would lead to the formation of the most competent corpora lutea, thereby maximizing the chance of sustaining pregnancy. Associations between sexual behavior and hormone excretion support the hypothesis that males may use this mechanism to assess female fertility.
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Introduction

Dynamic changes in the concentration of circulating reproductive hormones, mainly gonadal steroids, elicit a repertoire of sexual behaviors in ungulates and it is widely accepted that sexual behavior reflects the reproductive physiology of individual animals. One of the main purposes of sexual behavior is to reduce aggression between the sexes, facilitating copulation and fertilization. The intensity and frequency of sexual behavior patterns are influenced by internal (genetic and experimental) and external (environmental) factors (Signoret and Balthazart, 1993) and depend upon the social organization of the species under consideration.

Male gazelles are territorial, which serves a number of

functions (Walther et al., 1983). One such function is to enable a male to gather a group of females with whom he can mate. This type of social organization has been confirmed for wild Dama gazelles [*Gazella dama;* (Grettenberger and Newby, 1986)]; of which the Mohor gazelle (*G. d. mhorr*) studied in this paper is a subspecies. As most ungulates living in temperate climates reproduce in a strictly seasonal pattern, seasonality being determined by factors such as photoperiod, temperature, and food availability (Leuthold, 1977), it follows that the females associated with a single male are likely to show some degree of reproductive synchrony. In this situation, the male must constantly monitor the females in his harem, detecting the onset of behavioral receptivity or estrus. This testing behavior, often termed "*flehmen*" (Walther et al., 1983), is then followed by a ritualized series of displays if a female is found to be approaching estrus. Flehmen itself involves a characteristic series of actions by the male. Walther et al. (1983) described it as follows; "He raises his neck, usually keeping his head

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on a horizontal plane or occasionally lifting his nose above horizontal, he opens his mouth and remains motionless in this posture for several seconds . . . males may somewhat retract the upper lip. . . ." These authors speculated that the males could learn something about the female's estrous condition by performing flehmen. In this study, we monitored the female's reproductive status by measuring steroid hormone concentrations in feces and compared the data with the male's behavior to see how accurate and sensitive the male's assessments might be. The repertoire of male displays typically includes three phases (Leuthold, 1977): precopulatory behavior, copulation, and postcopulatory behavior. The initial stimulus detected by males is thought to be olfactory, relying upon the detection of specific odors emanating from the females as they approach estrus (Fraser, 1968; Lindsay, 1965).

We have previously demonstrated associations between fecal steroid excretion and the likelihood of successful conception in the Mohor gazelle (Pickard et al., 2001). One further objective of the present investigation was to test the hypothesis that male gazelles are not only capable of detecting information about the reproductive status of females per se, but that subtle information about the likelihood of conception is also available to them during estrus itself and the period preceding estrus. We propose that the male's ability to detect this information may be manifested in differences in their behavior towards individual females. To access such information, males would be required to interpret the olfactory stimuli in terms of attributes such as the likelihood of ovulation, quality of developing oocytes, and the probability of sustaining a pregnancy after conception. A plausible mechanism for conveying this information would be through estrogen itself or female pheromones produced under estrogenic control.

A theoretical argument for this possibility can be developed from items of relevant evidence in other mammals. In domestic cattle, a relative of the gazelles, the ratio of circulating estrogen to progesterone is indicative of oocyte quality (Mihm et al., 1999; Oussaid et al., 2000). Correlated, estrogen-modulated effects on scent production may therefore be available for detection by the males. Confirmation that scent marks can be modulated in such a sophisticated manner comes from recent study of scent marking behavior in meadow voles. Leonard et al. (2001) showed that males could discriminate between female scent marks on the basis of the female's estradiol titer. While considerable evidence supports the view that such sophisticated chemical communication mechanisms exist in small mammals (Bronson, 1989; Brown and MacDonald, 1985; Johnston, 1990), experimental data is lacking for larger species. Experimental studies of scent marking behavior in giant pandas (Swaisgood et al., 1999) have shown, however, that males and females can distinguish between individual conspecifics through investigation of both urine and anogenital secretions. This suggests that the larger mammals are also capable of sophisticated olfactory communication and also

shows that the phenomenon is amenable to appropriate investigation.

One implication arising from this hypothesis is that males are able to monitor reproductive status while oocyte development is in progress, i.e., during the luteal phase of the estrous cycle, and then selectively invest effort to mate with females of the highest predicted fertility. The biological relevance of such a discriminatory mechanism in gazelles would be questionable unless females exhibit reproductive synchrony in the wild. At present, detailed reproductive cycle data is not available for wild female gazelles, so the question cannot be adequately answered. However, the reproductive cycles of captive females show strong evidence of synchrony (T. Abáigar and M. Cano, unpublished observations), resulting in synchronized calving, and it is therefore likely synchrony also exists within wild harems.

In wild bovids, the dynamic interrelationships between displays of sexual behaviors and the changing concentrations of circulating steroids are difficult to study in detail. The animals are disturbed and stressed by invasive procedures such as blood sampling for endocrine analysis. Noninvasive methods for measuring fecal hormones present a practical solution to this problem and were used in this study. These methods are now widely applied to the assessment of ovarian cyclicity (Schwarzenberger et al., 1993; Shaw et al., 1995; Wasser et al., 1991), particularly in the context of assisted reproductive technologies (Holt et al., 1988; Shaw et al., 1995) and pregnancy diagnosis (Garnier et al., 1998; Pickard et al., 2001; Safar-Hermann et al., 1987; Schwarzenberger et al., 1993; Wasser et al., 1991).

The study was carried out using Mohor gazelles as a model species. This subspecies of *G. dama* is extinct in the wild and has been the subject of a captive reproduction program since 1971 at the Estación Experimental de Zonas Áridas (Consejo Superior de Investigaciones Científicas, Almería). About 250 animals live in zoos around the world and 3 populations have been reintroduced to protected areas in Morocco, Senegal, and Tunisia (Abáigar et al., 1997; Cano et al., 1993). In Almería, the animals are closely managed in social breeding groups; their behavior can therefore be observed in detail and fecal samples collected on a frequent basis.

Material and methods

Animals and fecal collections

The Mohor gazelles 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. Seven adult females (>2 years old) of proven fertility were maintained in an enclosure together with a vasectomized adult male for the duration of the study. Normal day-to-day

management procedures (Alados et al., 1988) were observed, and fecal samples collected daily, early in the morning, from January 30th until May 30th, 1996. Each female was individually identifiable by combinations of color-coded ear tags. The observer waited until fecal deposition occurred, then placed about 5 g fresh feces into a plastic bag for storage at −20°C until required for laboratory processing.

Sexual behavior observations and recording

Focal animal sampling was used to record the male's behavioral interactions with the females in the herd, for one hour before nightfall on each day that feces were collected. All interactions between the male and females were recorded continuously using a hand-held field computer (Psion Organizer II, Psion Computers, London, UK) running a program (Focal) developed at the EEZA for this purpose. Date and time were automatically recorded, and the identity of the female towards which the behavior was directed recorded by the observer. Due to a technical problem with the computer, some of the behavioral data was lost and could not be compared with the fecal analyses. Correlative analyses were undertaken in a total of 28 of 37 estrous cycles.

For convenience, sexual behavior was divided into three phases: (1) exploratory and testing, (2) courtship, and (3) copulation (Table 1). Some of the behaviors are common to both the exploratory/testing phase and to the courtship phase and therefore Phase 1 has been subdivided into two sub-phases: Phase 1.1 (exploratory and testing behaviors) and Phase 1.2 (exploratory and/or courtship behaviors). After copulation, the male typically stands behind the female for a moment, then returns to "normal activities"; this post-copulatory behavior occurs in the majority of Antilopinae (Walther et al., 1983).

Fecal steroid hormone analyses

The progestagen content of all fecal samples was determined as described in detail by (Pickard et al., 2001). Briefly, 0.2 g of each fecal sample was extracted using a combination of potassium hydroxide, methanol, and petroleum ether. The methanol fraction was assayed for its 20α-hydroxyprogesterone concentration (Shaw et al., 1995), using an antibody raised against 4-pregnene-20α-ol-3-one (Dr. M.J. Peddie, Department of Physics and Pharmacy, University of Southampton, UK). Similarly, fecal estradiol-17β concentrations were determined in the potassium hydroxide fraction of the extract, as described by Hodges et al., 1983. Estradiol-17β concentrations were determined on alternate days during the luteal phase of the estrous cycle and daily during the peri-ovulatory period of the cycle,

Table 1
Specific sexual behaviors identified in this study

Behavioral phase	Description of behavior
Phase 1	*Exploratory and testing*
Phase 1.1	Male looks at female
	Male approaches female
	Male follows female at a trot
	Male follows female at a gallop
	Male pushes female with horns intensively
Phase 1.2	Male investigates female's genitalia
	Male licks female's urine
	Flehmen
	Male smells female's feces
	Male urinates over female's urine
	Male defecates over female's urine and feces
Phase 2	*Courtship phase*
	Male stretches head and neck forward
	During driving (mating march), the male pushes the female with his neck in her hindquarters
	During driving (mating march), the male pushes the female with his horns in her hindquarters
	Male rubs preorbital glands on female's rump
	Male stands behind female
	Male gives a 'foreleg kick'*
Phase 3	*Mounting phase*
	Mounting
	Copulation; mounting with an erect penis, followed by copulation

Classification of specific sexual behaviors adopted in this study was based on the descriptions provided by Walther et al. (1983).

* This characteristic kick has been described in detail by Walther et al. (1983).

which was determined from progestagen concentrations (see Pickard et al., 2001).

Analysis of behavioral and endocrine data

Only behavioral displays involving both males and females were analyzed; female/female interactions were not considered within this study. For daily observations, the individual components of behavior within each of the described phases were summed, and the data for each of the phases were transformed into frequencies (events/unit time). This transformation was achieved by expressing the behavior of individuals as a fraction of the total number of behavioral events occurring within each hour of observation. These data were analyzed statistically after log transformation (Zar, 1984), using Statistica for Windows (Statsoft UK, Letchworth, UK). Relationships were considered statistically significant if $P < 0.05$, unless otherwise stated. Hormone concentrations corresponding to the day of the behavioral observation were also log transformed to overcome scale effects in the data. The relationships between behavior and hormone concentration were examined by time series, correlation and regression analyses. Time series analysis was used specifically to investigate the temporal cross-correlation between steroid excretion and behavior (Box and Jenkins, 1976). Unless otherwise stated, however,
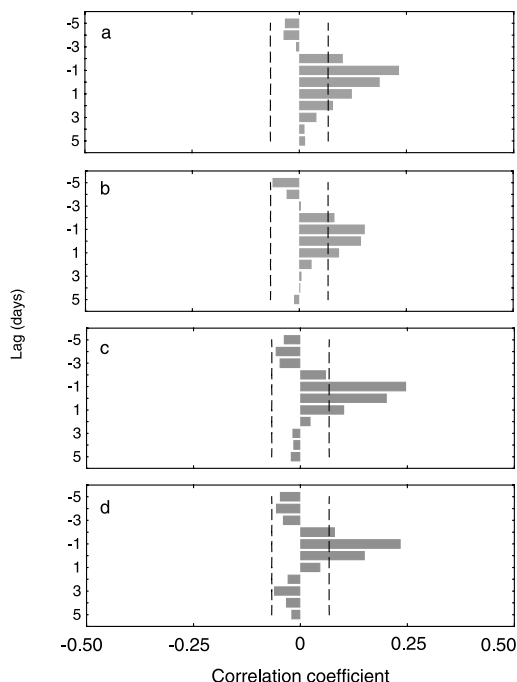
Fig. 1. a–d: Cross-correlations and lag analyses between the frequency of male behaviors and the concentration of estrogen in female feces. (a) Phase 1.1 and (b) Phase 1.2 (exploratory and testing behaviors), (c) Phase 2 (courtship behaviors), (d) Phase 3 (mounting and copulation). Horizontal bars represent the correlation coefficient of estrogen concentration vs behavior frequency when compared on the same day (day 0) or after introducing a lag of up to +/−5 days (vertical axis) between the hormone and behavior data sets. The horizontal axes represent positive or negative correlation coefficients (r). Vertical dashed lines represent the standard error of the cross-correlation coefficient; bars that project beyond the standard errors are significant ($P < 0.01$).

the analyses are presented without adjustment for time lags. The frequencies of sequential behavioral phases were also examined by correlation analyses, to see whether the male elected not to mount some females, having been through the testing and pre-copulatory phases.

Relationships between hormone concentrations within specific components of the estrous cycle were examined. For these correlations, mean estrogen and progestagen concentrations for sequential luteal phases and inter-luteal phases were calculated as described previously (Pickard et al., 2001). Probabilities of conception associated with these correlations were taken from a separate study, which commenced one month after the end of the experiment reported here. The same animals were treated with intra-vaginal progesterone-releasing CIDR devices (ART; Hamilton, New Zealand) for 12 days to synchronize estrus. After CIDR removal, they were then allowed access to an intact male. The period from CIDR removal to conception was calculated as the interval from CIDR removal to parturition −200 days (the gestation length of this species). Details of this study have been described previously (Pickard et al., 2001).

Results and Discussion

Males can discriminate the approach of estrus

During courtship the male follows the females intensely, tests their reproductive status and, if a female is approaching estrus, devotes extensive attention to that female. Sexual behavior was observed for 2–3 days of the estrous cycle (mean = 2.44 ± 0.61 days), with the majority of mountings (88.9%; N = 16) occurring 1 or 2 days before the maximum estrogen values. Time series analysis confirmed these findings; Fig. 1 (a–d) shows the results of a cross-correlation analysis aimed at identifying the lag periods between concentrations of fecal estrogen and the various behaviors. All behaviors are maximally correlated ($P < 0.01$) with fecal estrogen either on the day preceding peak fecal estrogen concentration (day −1), or the day of peak estrogen concentration itself (day 0). The 24 h delay between the fecal endocrine profile and male behavior may result from steroid recirculation within the large intestine or the rate at which digesta passes through the gut. In ruminants, gut passage rate may be related to the frequency with which water is consumed (Musimba et al., 1987). Water was provided ad libitum to the gazelles, and consumption rates were not recorded in this study.

Phase 1.1 behavior was also weakly correlated with estrogen concentrations measured 2 days before the peak value (day −2: $P = 0.046$). Moreover, while Phase 3 (mounting) behavior was strongly correlated with the maximum E/P ratio (day 0: $P < 0.002$), it was also correlated with the E/P ratio three days beforehand (day −3: $P < 0.002$). The high degree of temporal correlation between courtship behaviors and E/P ratios is more clearly illustrated in Fig. 2. Taking account of the 24 h delay between circulating and excreted hormone profiles, these results suggest that males can not only identify estrus itself, when estrogen production would be maximal, but are also able to gain
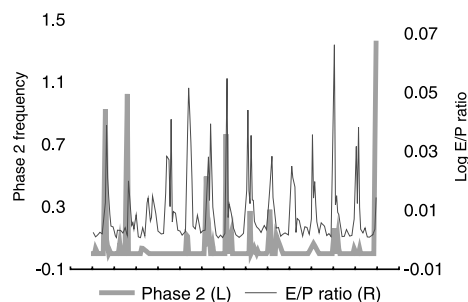

Fig. 2. Representative plot showing the frequency of Phase 2 (courtship) behaviors exhibited by the male towards one female on different days (gray shading), and her corresponding fecal E/P ratios (solid line). The horizontal axis represents consecutive days over a subset of the total duration of the experiment. (Where some E/P peaks do not show correspondingly increased behavior, this was partly due to a technical problem with data logging).
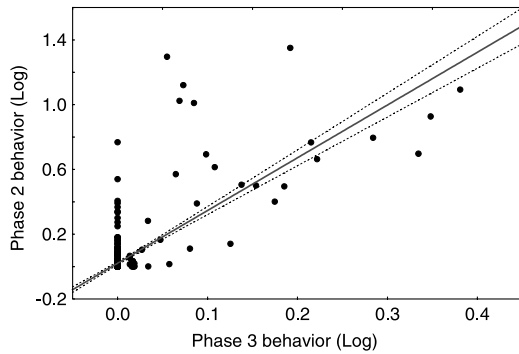
Fig. 3. Correlation between the frequency of all Phase 2 (courtship) and Phase 3 (mounting and copulation) behaviors on each day studied ($r = 0.744$; $P < 0.001$); dashed lines represent the 95% confidence limits of the regression line.

information about females when follicular maturation is at a much earlier stage.

To investigate these observations further, and to determine whether each sequential set of behaviors predicts the occurrence of the next, correlation analyses were performed between the frequencies of male behaviors in each of the phases with that in the succeeding phase. All correlations were positive, and increasingly significant with the approach of estrus ($P < 0.0001$; Phase 1.1 vs Phase 1.2; $r = 0.357$; Phase 1.2 vs Phase 2; $r = 0.653$, and Phase 2 vs Phase 3; $r = 0.744$; Fig. 3). These results support the view that males sense the approach of estrus one or two days before its onset.

We were unable to measure urinary estrogen in this study. However, as the females frequently urinate over their own fecal pellets, it is evident that males may be sensing urinary as well as fecal cues. In female ruffed lemurs (*Lemur variegatus*), both attractivity and sexual receptivity are positively correlated with urinary estrogen concentrations (Shideler et al., 1983), which may also be the case in other taxa. Data concerning the chemical nature of pheromones in ungulates is limited, and largely relates to those compounds believed to be responsible for communicating male territoriality. This information has recently been reviewed by (Rekwot et al., 2001). It has, however, been demonstrated that female Asian elephants (*Elephas maximus*) secrete a pheromone into their urine [(Z)-7-dodecen-1-yl acetate], which signals impending ovulation to the males (Rasmussen and Schulte, 1998). Nevertheless, this compound has not been isolated from the urine of other mammals.

From the observations made during this experiment, it is apparent that once a male senses that a female is approaching estrus, he commences a ritual series of behaviors that usually culminate in mounting. Observation of intense Phase 2 behavior could therefore be used by human observers as a practical substitute for estrus detection, without necessarily having to observe mounting behavior.

Males differentially discriminate the quality of estrus among females

A weak association between fecal estrogen concentrations and the corresponding frequency of Phase 2 courtship behaviors was identified ($r = 0.220$, $P = 0.053$), suggesting that the male uses these activities to provide critical information about female reproductive status. However, the frequency of mounting and copulatory (Phase 3) behavior was strongly and positively correlated with fecal estrogen concentrations ($r = 0.532$, $P = 0.004$; Fig. 4a) and E/P ratio ($r = 0.548$; $P = 0.003$; Fig. 4b). These results not only confirm the previous suggestion that males are capable of detecting peri-ovulatory increases in estrogen excretion, but suggests that they obtain information about the quality of the approaching or current estrus period. Access to such information would allow the male to pay closest attention to the females most likely to conceive successfully. In domestic cattle, E/P ratios have been used to ascertain oocyte quality (Mihm et al., 1999; Oussaid et al., 2000). Furthermore, the administration of exogenous progesterone, which
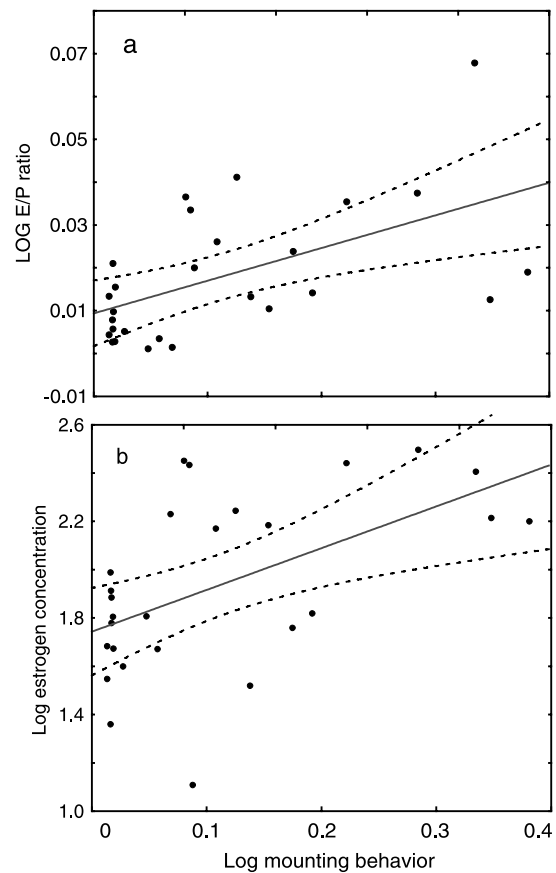

Fig. 4. a and b: Correlations between the frequency of Phase 3 (mounting and copulation) behaviors in the male Mohor gazelle and (a) fecal E/P ratio ($r = 0.548$, $P = 0.003$); (b) fecal estrogen excretion ($r = 0.532$, $P = 0.004$) in females. Data pairs where no behavioral observations were made are omitted. Dashed lines represent 95% confidence limits of the fitted regression lines.

results in raised plasma progesterone concentrations around the time of estrus, extends the duration of the estrous cycle through the continuous emergence of follicular waves (Bergfelt et al., 1991) and suppresses estrus and ovulation (Christian and Casida, 1948). Exogenous progesterone administration to heifers, designed to raise plasma progesterone concentrations to suprabasal levels rather than prolong the luteal phase of the estrous cycle, results in prolonged growth of the dominant follicle, increased estradiol secretion and reduced fertility (Duchens et al., 1995). The E/P ratio of progesterone treated heifers is reduced compared to that of control animals, suggesting that both the absolute and relative proportions of these two hormones play a key role in determining the physiological and behavioral events of estrus. In our studies of Mohor gazelles (Pickard et al., 2001), CIDR devices were used to synchronize the estrus cycles of the females, and may have inadvertently raised peri-estrus progesterone levels to suprabasal concentrations. However, it should be noted that the behavioral relationships reported here were recorded prior to the use of the CIDR devices, and that conception rates in the CIDR treated females were not different than those recorded for the same females under natural insemination (Pickard et al., 2001).

The male invests a large proportion of time in behaviors that fall into Phases 1.1, 1.2 and 2, when compared with the time invested in Phase 3 (mounting) behaviors. From these activities, the male may be able to determine the suitability of a female as a mate, and then selectively invest energy and resources in mating only those females that provide the highest probability of propagating his genes into a new generation. In the wild, males will also be occupied with activities that are not strictly necessary in captivity, such as marking and defending their territory, locating food and water for their group and predator avoidance. Thus, there would be significant energetic advantages to be gained by avoiding the effort of mating with females who are unlikely to become pregnant. The female Mohor gazelle shows no significant seasonal reproductive suppression in captivity; therefore the male could benefit by first ensuring that highly fertile females are pregnant, and then devoting time and effort into inseminating females of lower fertility. Furthermore, specific behaviors (for example urinating or defecating over a female's feces) may serve to disguise the status of a potential mate, perceived as of high fertility, thus confusing other males in the vicinity. Fecal deposition rates in male Oribi (*Ourebia ourebi*) have been shown to vary depending on the social rank of the individual and the size of the harem a dominant male is attempting to protect (Brashares and Arcese, 1999). If the supply of feces for territorial marking is limited, it may be energetically preferable to confuse potential opponents than to defend the group or individuals.

No mounting was observed during the behavioral monitoring periods in 8 of the 28 estrous cycles studied (28.6%), however it is possible that mating occurred at a time when the animals were not being observed (Shaw et al., 1995).

Previously, (Holt et al., 1988) reported that female blackbuck (*Antelope cervicapra*) experience undetected behavioral estrus, despite evidence from urinary hormone analyses to suggest that the females were, in fact, in estrus.

Hormone relationships between sequential phases of the estrous cycle

To investigate the possibility that males may be able to access information about physiological correlates of fertility, we analyzed endocrine relationships during sequential phases of the estrous cycle. Individual regression analyses were performed for each female. Firstly, we hypothesized that estrogen excretion during the luteal phase should be positively correlated with estrogen excretion during the following inter-luteal phase (a). A highly estrogenic environment during a luteal phase, when follicle recruitment and oocyte development are in progress is likely to generate a high quality oocyte with good prospects for fertilization and normal embryo development. A positive slope in (a) was interpreted as supporting this scenario, and was observed in five of the seven females studied. A neutral or negative relationship was considered to indicate the production of a lower quality oocyte or inadequate oocyte maturation. Secondly, we proposed that estrogen excretion during the interluteal phase should be positively correlated with progestagen production during the subsequent luteal phase (b). Dominant follicles, which produce large quantities of estrogen around the time of ovulation, should subsequently develop into high quality corpora lutea, capable of efficient progesterone production and supporting an ensuing pregnancy. Once again, a positive slope in (b) was observed for five of the seven females studied, and interpreted as supporting this hypothesis. The remaining two females exhibited neutral or negative correlations in (b), which were considered to demonstrate reduced fertility. Thus, within animals, matching positive slopes should be indicative of highest quality oocyte production and fertility. The outcome of these analyses for each female is summarized in Table 2, and was compared with time to conception data for each female, to see whether males were apparently capable of detecting these subtle differences.

When the seven animals were ranked by time to conception after CIDR device removal, the three which conceived within the first estrous cycle after CIDR device removal showed matching, strong positive regression coefficients in both of the analyses. The remaining four animals showed unmatched slopes and weak associations, and failed to conceive until a further one or more estrous cycles had elapsed. Evidence from other ungulates, especially cattle, suggesting that plasma progesterone concentrations can be indicative of corpus luteum competence, as well as embryo yield and quality (Gosden and Nagano, 2002; Silva et al., 2002) lends support to these interpretations.

Table 2
Relationships between (a) luteal phase fecal estrogen excretion vs subsequent inter-luteal phase fecal estrogen excretion, (b) inter-luteal phase fecal estrogen excretion vs subsequent luteal phase fecal progestagen excretion, and the time from CIDR device removal to conception in individual Mohor gazelles

Gazelle ID	(a) Slope of luteal phase estrogen concentration vs subsequent peri-ovulatory estrogen concentration*	(b) Slope of peri-ovulatory estrogen concentration vs subsequent luteal phase progestagen concentration*	Days to conception following CIDR device removal[†]
672	+	+	2
542	+	+	3
420	+	+	11
425	−	+	24
577	+	−	44
687	+	−	101
519	−	+	123

* Slopes were derived from linear regression analyses determined over between 5 and 7 estrous cycles per female. + indicates a positive regression coefficient; − indicates a negative regression coefficient.

[†] Days to conception following CIDR device removal (Pickard et al., 2001).

Hormones and olfactory cues

Our previous investigations have indicated that peri-ovulatory estrogen excretion is associated with the likelihood of successful conception in the female gazelles examined in the present experiment (Pickard et al., 2001). The data presented here indicate that either the male is also capable of detecting the fertility of the female concerned prior to ovulation, or that the intensity of his behavioral interest plays an important role in the success of conception in this species. However, the relationships between the level of hormone excretion and prospect of conception described above suggest that oocyte quality may be predetermined and therefore the first assertion, that the male selectively invests reproductive effort in mating females with a high chance of conception, is more likely to be correct.

By repeatedly copulating with a female that is signaling high fertility, the male ensures three things. Firstly, the female will be defended from non-territorial males by the continued presence of the territorial male. Secondly, should a non-territorial male successfully copulate with the female, his sperm would be diluted and potentially out-competed by that of the dominant male. Finally, repeated copulation ensures that there is fresh sperm at the site of fertilization, in anticipation of the arrival of the oocyte, in order to ensure that conceptus development is not compromised by an unnecessary delay in fertilization.

The nature of the exploratory and courtship behaviors exhibited by this species indicate the importance in communication of the olfactory cues excreted in the urine and feces. Olfactory exploration of the female's genitalia, urine and feces, including characteristic activities such as flehmen, indicate the close association between pheromone production and the excretion of reproductive steroids. The chemical nature of the pheromones responsible for olfactory communication in even common ungulate species has not been described. Attempts to purify pheromones from domestic cow urine suggest that the active compound(s) are highly volatile, polar molecules with molecular weights between 50 and 130 Daltons (Dehnhard and Claus, 1996). This is somewhat less than the biologically relevant estrogens (estrone, estradiol-17β and estriol). The striking correlation between fecal estrogen concentrations and male behavior raises the possibility that males directly detect estrogen, rather than estrogen-dependent female secretions. Although this study cannot furnish additional evidence for this, it is worth noting that such a mechanism would not be unexpected. Nevertheless, recent studies of mice lacking the estrogen receptors α- and β-($\alpha\beta$ERKO) have revealed that these receptors are essential for the normal expression of male behaviors such as mounting and ejaculation (Ogawa et al., 2000).

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