Changes in plasma cortisol concentrations during the ovulatory cycle of the mare

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SUMMARY

Daily blood samples from four mares were assayed for cortisol through a total of eight ovulatory cycles. Mean cortisol concentrations on days 14, 13, 10, 9 and 8 before ovulation (dioestrus) were greater than on days 2 to 2 (260±28 nmol/l) and the lowest on day 2 (142±14 nmol/l). A single episode on a day in late dioestrus characterized the maximum cortisol value per cycle for five of eight cycles. Extraction of plasma samples with petroleum ether or chromatography before assay, to eliminate interference from progesterone and its metabolites, did not alter the pattern of high dioestrus and low oestrous cortisol concentrations. Maximum follicular diameter at ovulation was negatively correlated with mean cortisol concentration for that cycle. These results indicate that in the mare the adrenals secrete cortisol more actively during dioestrus than during oestrus and suggest that a decline in cortisol values at oestrus may favour full follicular growth and ovulation.

INTRODUCTION

Adrenal glucocorticoids have been shown to influence reproductive processes in many species (reviewed by Andrews, 1976). In earlier studies in this laboratory, administration of the synthetic glucocorticoid dexamethasone reduced the incidence of sexual behaviour in ovariectomized mares (Asa, Goldfoot, Garcia & Ginther, 1980a), and suppressed oestrus, follicular growth, luteinizing hormone (LH) concentrations in plasma and ovulation in intact mares during the oestrous cycle (Asa & Ginther, 1982).

Endogenous glucocorticoids have not been found to fluctuate during the menstrual cycles of women (Moore, Kawagoe, Davajan, Nakamura & Mishell, 1978) or rhesus monkeys (Leshner, Toivola & Terasawa, 1978), but are higher on the day of pro-oestrus in the mouse (Nichols & Chevins, 1981) and rat (Buckingham, Döhler & Wilson, 1978; Phillips & Poolsanguan, 1978). In spite of demonstrations that dexamethasone profoundly affects reproductive events in the mare, measurements of the natural glucocorticoids relative to the phases of the ovulatory cycle are lacking. The present study was performed to assess possible changes in adrenal hormones, as reflected by plasma concentrations of cortisol, the primary glucocorticoid in the horse (Zolovick, Upson & Eleftheriou, 1966), throughout the ovulatory cycle.

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MATERIALS AND METHODS

The eight pony mares with intact ovaries and three vasectomized pony stallions ranged in age from 3 to 18 years and weighed between 160 and 250 kg. Between tests mares were together at pasture and stallions were stabled separately. The study period extended from 18 July to 2 September, encompassing two ovulations per mare. Because cortisol concentrations in the mare follow a circadian rhythm (Zolovick et al. 1966), all procedures were performed each day in the early afternoon. To minimize the influence of handling on cortisol levels, blood was taken before rectal palpation and behavioural testing.

Blood was collected daily by means of jugular venepuncture into heparinized tubes, the plasma was separated by centrifugation and stored at −15°C until assayed. Cortisol concentrations were measured by radioimmunoassay using a specific antibody (New England Nuclear, Boston, Massachusetts, U.S.A.) raised against cortisol-21-succinyl-bovine serum albumin. Except for the antibody and a modification in incubation time, the general assay protocol was identical to the procedures previously published (Robinson, Scheffler, Eisele & Goy, 1975; Bielert, Czaja, Eisele, Scheffler, Robinson & Goy, 1976; Sholl, Robinson & Wolf, 1979). The antiserum was appropriately diluted to give 50% binding of the [3H]cortisol (New England Nuclear). The range of the standard curves was 0.1–2.8 pmol with blank values of <0.02 pmol being obtained in the assays. The mean intra- and interassay coefficients of variation were 11.6 and 15.8% respectively.

Cross-reactivities of the antibody with other similar steroids were all less than 25% as published by New England Nuclear and confirmed in our laboratory. Progesterone, in particular, was found to cross-react only 5%, but to test further for possible interference by this steroid in these assays, an additional experiment was carried out. Since progesterone, but not cortisol, is selectively extracted in petroleum ether (Johansson, 1969a, b), the assay results of 39 plasma samples from two ponies with and without prior extraction with petroleum ether were compared. Cortisol recoveries in our laboratory, using straight ethanol extraction, is routinely 95% or better. However, recovery estimates after double extraction (petroleum ether followed by ethanol) were somewhat lower, 87.1 ± 0.7 (S.E.M.)%.

To prevent interference by corticosterone, progesterone and other progestins, 16 of the above samples were also analysed after chromatographic separation on Sephadex LH-20 columns using a hexane:benzene:methanol solvent system. Progesterone and other neutral steroids were eluted in the first 9–12 ml with corticosterone coming off in the 8–14 ml fractions. Cortisol was collected in the 15–21 ml fractions and then assayed. Cortisol recovery estimates after extraction and column chromatography were 89.4 ± 3.35%.

To monitor follicular growth and ovulation, ovaries were palpated rectally every third day during dioestrus and daily during oestrus. Follicle size to the nearest 0.5 cm was estimated by comparison with simulated follicles of known diameter. The smallest follicle diameter recorded was 15 mm. Ovulation was detected by the absence of a large follicle that had been present on the previous day.

Behavioural oestrus was determined by daily 20-min observations of mares in harem groups. Two harem groups were randomly assembled each day, with four mares plus one vasectomized stallion per group. As in our previous studies (Asa et al. 1980a, b), mares which showed two urinations plus ≥20 s of tail raise near a stallion per 20-min test were considered to be in full oestrus. Behaviour was recorded by means of System 7 SSR event recording keyboard (Semeiotic Systems Corp., Madison, Wisconsin, U.S.A.).

Analysis of variance for repeated measures was used to evaluate daily cortisol levels before and after ovulation. When significant differences were found individual means were compared by Duncan's multiple range test (Bruning & Kintz, 1977). Values of cortisol during dioestrus and oestrus which resulted from assays after petroleum ether extraction or chromatography were compared by Student's t-test. Pearson Product Moment correlation...
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(Bruning & Kintz, 1977) was used to compare the day of maximum cortisol value to cycle length and to day of onset of behavioural oestrus and to compare maximum follicle diameter for each cycle to mean cortisol concentration for that cycle. The highest cortisol value for each ovulatory cycle was compared to the mean for that cycle by z-score; i.e., a z-score of greater than 1.96 (equivalent to 1.96 standard deviations from the mean) is significant at $P<0.05$, while a z-score of greater than 2.58 is significant at $P<0.01$ (McCall, 1975).

RESULTS

Each of the four mares ovulated twice during the course of the study. The first ovulation of one mare occurred on the third day of sampling, providing too few data points for inclusion in the analysis. Consequently, unless otherwise indicated, the results were based on samples surrounding seven ovulations.

As illustrated in Fig. 1, the highest mean value of the daily cortisol concentrations occurred at day $-10$ and the lowest mean value at day $-2$ before ovulation. Mean levels were greater ($P<0.05$) during mid- to late-dioestrus than during oestrus. In an analysis by

![Graph of plasma cortisol concentrations during the ovulatory cycles of four mares.](image)

**Fig. 1.** Mean ($\pm$S.E.M.) plasma cortisol concentrations (nmol/l) during the ovulatory cycles of four mares. Points without common superscripts are different ($P<0.05$) (analysis of variance for repeated measures). $n$ represents the number of mares.

days before ovulation (Fig. 1), mean cortisol concentrations on days $-14$, $-13$, $-10$, $-9$ and $-8$ before ovulation were greater than on days $-5$ to $-1$ ($n = 7$ cycles). Analysis by days after ovulation ($n = 4$ cycles) revealed an increase ($P<0.05$) at day 9 after ovulation which was above the nadir at day $-2$ before ovulation.

Assay values were lower after petroleum ether extraction or chromatography. Before these procedures, the mean ($\pm$S.E.M.) cortisol concentration for dioestrous days $-14$, $-13$, $-10$, $-9$ and $-8$ for the two ponies was $201\pm 16$ nmol/l and for oestrous days $-5$ to $-1$ it was $146\pm 14$ nmol/l. After extraction or chromatography, the mean value for dioestrus was $192\pm 18$ nmol/l and for oestrus it was $137\pm 14$ nmol/l. The lower values were likely to be the
consequence of the extra manipulative steps which were not adjusted for recovery. However, the values for dioestrus were still greater (P<0.05) than those for oestrus.

The maximum cortisol value per cycle for each mare occurred as a single spike/day between days −7 and −14 before ovulation. For each of five of the seven cycles, the maximum cortisol value was significantly greater than the mean for the cycle (P<0.05; z-score >1.96). The maximum for the sixth cycle approached significance (z-score = 1.87). For the remaining cycle, cortisol was raised, though not significantly, on days −10, −12 and −14 with z-scores between 1.3 and 1.56. None of the highest values occurred on the same calendar date. For all cycles the second highest cortisol value was never significantly greater than the cycle mean. The number of days before ovulation on which the maximum value occurred was not correlated to cycle length (r = −0.45; t = 0.87) nor with onset of behavioural oestrus (r = −0.5; t = 1.29). However, the maximum follicle diameter for each cycle was negatively correlated with the mean cortisol concentration for that cycle (r = −0.7; P<0.05).

DISCUSSION

During the ovariary cycle, mean plasma cortisol values were higher during mid- to late-dioestrus than during oestrus, with the lowest mean value occurring 2 days before ovulation. This pattern of cortisol secretion appears to be negatively and positively correlated with the patterns reported for oestradiol and progesterone respectively (Noden, Oxender & Hafs, 1975) and it contrasts with the pattern of cortisol secretion in the rat (Buckingham et al. 1978; Phillips & Poolsanguan, 1978) and the mouse (Nichols & Chevins, 1981), in which cortisol is low during dioestrus and highest on the afternoon of prooestrus and positively correlated with oestradiol. An explanation for the cortisol pattern seen in mice and rats is provided by the ability of oestrogens to stimulate glucocorticoid production, as demonstrated in numerous studies (reviewed by Brien, 1981). A third pattern is that reported for the menstrual cycles of women (Moore, Kawagoe, Davajan, Nakamura & Mishell, 1978) and rhesus monkeys (Lesner et al. 1978), in which no significant changes in mean daily cortisol were found. The absence of increases in cortisol during the preovulatory phase of the menstrual cycle is postulated to be due to a threshold for the stimulatory effect of oestrogen (Moore, Kawagoe, Davajan, Mishell & Nakamura, 1978). This threshold is apparently exceeded during pregnancy but not during the ovariary cycle.

The pattern of cortisol secretion shown by the mare has not been reported for other species. The similarity of the curve of mean cortisol to that found for progesterone (Noden et al. 1975) suggested possible cross-reactivity with progestins in our assay. However, despite finding lower cortisol values either after petroleum ether extraction or by chromatographic separation of cortisol from progestins, the same pattern was evident. Thus, the higher assay values of cortisol seen during dioestrus were not due to interference by progesterone or other progestins.

To our knowledge, oestrogens have not been shown to inhibit nor progesterone to stimulate production of glucocorticoids. It is known, however, that glucocorticoids can suppress oestrogens (Cortes-Gallegos, Gallegos, Bedolla Tovar, Cervantes & Parra, 1975; Cunningham, Caperton & Goldzieher, 1975; Hsueh & Erickson, 1978). Although oestrogens were not measured during dexamethasone administration to ovariary mares in our laboratory, lowered oestrogen levels would have been consistent with the resulting decreases in incidence of oestrous behaviour, LH concentrations, maximum follicle size and ovulation (Asa & Ginther, 1982). In the light of this evidence, the observation in the present study that mean cortisol concentrations for an ovariary cycle were negatively correlated to maximum follicle diameter suggests a possible inhibitory influence of cortisol on follicle growth.
Another apparently unique aspect of adrenal cortical involvement in the mare ovulatory cycle was the identification of a single cortisol spike near the end of dioestrus in six out of seven cycles. The spike seemed to coincide in time with the expected peak of prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$), which is related to corpus luteum regression (Douglas & Ginther, 1976). Unlike some other species, the mare does not rely on a local transport of uterine PGF$_{2\alpha}$ directly to the ovaries through the uterine vein and adjacent ovarian artery, but apparently secretes PGF$_{2\alpha}$ into the systemic circulation (Ginther, 1979). In the mare administration of PGF$_{2\alpha}$ results in transient increases in LH, progesterone, and oestradiol (Noden, Oxender & Hafs, 1978). Because PGF$_{2\alpha}$ also has been found to stimulate secretion of adrenal cortical steroids in some cases (Flack, Jessup & Ramwell, 1969; Spat, Antoni, Balla, Bonta & Siklos, 1977), the late dioestrous spike of cortisol may be a direct consequence of increased systemic PGF$_{2\alpha}$ at that time. If so, the subsequent decline in cortisol may also follow the fall in PGF$_{2\alpha}$.

These results suggest that the adrenal cortex, perhaps influenced by uterine PGF$_{2\alpha}$, may have an inhibitory effect on ovulatory processes. Perhaps a decline of cortisol in late dioestrus is necessary for the resurgence of follicular growth and accompanying events which culminate in ovulation.

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