Effect of duration of infusion of stress-like concentrations of cortisol on follicular development and the preovulatory surge of LH in sheep

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Abstract

Stress-like levels of cortisol suppress follicular growth and development and block or delay the preovulatory surge of LH when cortisol is continuously administered during the late luteal and early follicular phases of the ovine oestrous cycle. We postulated that cortisol infusion of shorter duration would have a similar effect. To test this hypothesis the oestrous cycles of mature ewes were synchronized using progestin-treated vaginal pessaries. Ewes were randomly assigned to one of four treatment groups. Animals received cortisol (0.1 mg/kg/h; n=8) or vehicle alone (n=8) beginning 5 days before, and continuing for 5 days after, pessary removal (PR). Additional groups received cortisol only during the 5 days period before (n=7), or the 5 days period after (n=8), PR. Continuous delivery of cortisol established stable serum concentrations of cortisol of 72.0±2.5 ng/ml within 6 h of initiation of infusion. Serum concentrations of oestradiol increased progressively during the period after PR in control animals receiving vehicle alone and the preovulatory surge of LH was evident in all control animals (eight of eight) 55.5±5.0 h after PR. In contrast, follicular development and the preovulatory surge of LH were evident during the period of cortisol infusion in only one of eight animals receiving stress-like levels of cortisol over the entire 10-day infusion period. Similarly, neither follicular development nor surge-like secretion of LH were evident during the infusion period in animals (zero of eight) receiving cortisol during the 5-day period after PR. This cortisol-dependent suppression of ovarian activity in sheep receiving stress-like levels of cortisol during the 5 days after PR was temporary and follicular development, the ovulatory surge of LH, and subsequent luteal function were evident in six of eight ewes after cessation of cortisol delivery. Similarly, follicular development and the preovulatory surge of LH were noted within 5 days after PR in four of seven ewes receiving cortisol only during the 5-day period prior to PR. Collectively,
these data indicate that stress-like levels of cortisol reduce fertility of sheep by suppressing follicular development and the preovulatory surge of LH. Additionally, cortisol delivery during the follicular phase has a more profound suppressive effect on follicular development than cortisol administration during the luteal phase. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Cortisol; Sheep; LH surge; Follicular development; Oestradiol; Stress

1. Introduction

Stressful stimuli reduce fertility in primates (Norman et al., 1994; Xiao et al., 1998) and domestic species (Welsh and Johnson, 1981; Wilson et al., 1998). Indeed, climatic extremes (Doney et al., 1973), transportation (Ehnert and Moberg, 1991; Smart et al., 1994) or laparoscopy (Martin et al., 1981) suppress or delay expression of behavioural oestrus and ovulation in sheep. In addition to reducing fertility, these stressors also stimulate the activity of the hypothalamic-pituitary-adrenal axis, and a marked increase in serum concentration of cortisol is commonly associated with management-related stressors (Martin et al., 1981; Ehnert and Moberg, 1991; Komesaroff et al., 1998).

Although the causal link between stress and infertility has not been precisely defined, several studies indicate that glucocorticoids may contribute to the anti-gonadal effect of stress (Dobson and Smith, 1995; Chrousos et al., 1998). Indeed, intra-hypothalamic or systemic administration of glucocorticoid blocks follicular development and ovulation in rodents (Smith et al., 1971; Baldwin and Sawyer, 1974). Exogenous glucocorticoid has a similar effect in primates (Cunningham et al., 1978; Hayashi and Moberg, 1990) and some domestic species (Barb et al., 1982; Stoebel and Moberg, 1982). Moreover, recent studies in our laboratory indicate that follicular development and ovulation are blocked or delayed in sheep receiving stress-like concentrations of cortisol during the late luteal and early follicular phases of the oestrous cycle (Daley et al., 1999a). The study presented here examines oestradiol production and LH secretion in sheep receiving stress-like levels of cortisol during the luteal and/or follicular phases of the oestrous cycle in sheep. We hypothesized that stress-like concentrations of cortisol would block or delay follicular maturation and suppress the preovulatory surge of LH.

2. Materials and methods

2.1. Animals

Thirty-one mature Rambouillet ewes of proven fertility were used to assess the effects of chronic stress-like concentrations of cortisol on follicular development and ovulation. Ewes were housed in an open-sided barn under natural lighting and were allowed free access to water and alfalfa pellets. This study was conducted during November, a period of high reproductive activity for sheep at this latitude (38°N). All experimental procedures involving the use of animals were conducted in accordance with National Institutes of Health guidelines and were reviewed and approved by the Animal Use and Care Committee for the University of California, Davis.
2.2. Cannulation

Intravenous cannulae (Intramedic PE 190, Clay Adams, Parsippany, NJ) were placed in the right and left jugular veins and used for hormone infusion and blood collection, respectively. All cannulae were passed through a protective plastic tubing sheath along a halter and lead rope to the exterior of the animal holding area. Animals were freely mobile at the end of a 1 m lead. The cannulae were inserted 2 days prior to initiation of treatment to permit acclimation to the conditions of experimentation.

2.3. Hormone delivery

Cannulae for the delivery of cortisol or vehicle (50% ethanol-saline) were connected to syringes placed in Harvard infusion pumps (Model 2265, Harvard Bioscience, South Natick, MA). Cortisol (0.1 mg/kg/h; Sigma Chemical Co., St. Louis, MO) or a comparable volume of vehicle was delivered by continuous infusion at a rate of 1.0–1.2 ml/h.

2.4. Experimental design

Prior to the initiation of infusion ewes received two injections of prostaglandin F₂α (Lutalyse, Upjohn Co., Kalamazoo, MI) given 10 days apart to synchronize oestrous activity. Vaginal pessaries (Chrono-gest, Intervet International, Boxmeer, Holland) impregnated with a synthetic progestin (40 mg flugestone acetate) were inserted 10 days after the second prostaglandin injection. Luteolysis was simulated by pessary removal (PR) 10 days after insertion.

Animals were assigned at random to one of four treatment groups (Fig. 1). Animals in Groups 1 (n=8) and 2 (n=8) received vehicle or cortisol, respectively, beginning 5 days before and continuing for 5 days after pessary removal (PR). Additional animals (Group 3; n=8) received vehicle before PR and cortisol beginning at PR and continuing for 5 days thereafter. A final group (Group 4; n=7) received cortisol during the 5-day period before PR and vehicle thereafter.
before, and continuing for 5 days after, PR. Animals in Group 3 \((n=8)\) received vehicle prior to PR, with infusion of cortisol initiated at PR and continued for 5 days thereafter. Conversely, animals in Group 4 \((n=7)\) received cortisol during the 5-day period before PR and vehicle thereafter. Blood samples were collected daily prior to PR and at 3 h intervals for 5 days thereafter. Additional blood samples were collected at 3 h intervals for 3 days after cessation of cortisol infusion from animals in Group 3. Luteal function (serum concentration of progesterone) was also monitored in blood samples collected at 2 days intervals during the post-infusion period. Serum was isolated by centrifugation within 12 h of sample collection. Serum samples were rapidly frozen and stored at \(-20^\circ\)C for later endocrine analysis.

2.5. Hormone analysis

Serum concentrations of LH, cortisol, progesterone, and oestradiol were determined using previously validated radio-immunoassay procedures (Adams et al., 1975, 1988; Sakurai et al., 1992; Daley et al., 1999b). The LH (NIAMDD-oLH-26) reference standard was a gift from the National Hormone and Pituitary Program (Baltimore, MD). In all cases, intra-and inter-assay coefficients of variation were <10%. The minimum sensitivity of the LH, oestradiol, progesterone, and cortisol assays was 0.1 ng/ml, 0.5 pg/ml, 0.1 ng/ml, and 1 ng/ml, respectively.

2.6. Statistical analysis

Statistical significance of treatments was determined by ANOVA. Where significant treatment effects were noted, mean comparisons were made using Duncan’s Multiple Range Test. Data are presented in the text as mean±SEM. \(\chi^2\) analysis was used to determine the significance of differences between treatments in number of ewes displaying surge-like secretion of LH (Gill, 1978). Preovulatory surge-like secretion of LH was defined as an abrupt, but short-lived increase in the serum concentration of LH (peak LH concentration >10 ng/ml) that was preceded by increased serum concentration of oestradiol (peak oestradiol >4 pg/ml) and followed by sustained increase in progesterone lasting 10–14 days (peak progesterone >1 ng/ml).

3. Results

3.1. Cortisol

Continuous infusion of cortisol at a rate of 0.1 mg/kg/h elevated serum concentrations of cortisol to 72.0±2.5 ng/ml within 6 h of the initiation of infusion (Fig. 2). Serum concentrations of cortisol were maintained at this level for the remainder of the infusion period and returned to the basal level (7.7±1.0 ng/ml) 3 h after the termination of cortisol infusion. In contrast, serum concentrations of cortisol in control animals receiving vehicle alone were 11.6±2.6 ng/ml.
3.2. Follicular development and the preovulatory surge of LH

At PR serum concentrations of oestradiol were below the limit of detection (<0.5 pg/ml) in all treatment groups. Serum concentrations of oestradiol increased progressively during the period after PR in animals (Group 1) receiving vehicle alone during the 10-day infusion period. Maximal serum levels of oestradiol (6–8 pg/ml) were noted 48–60 h after PR and a preovulatory surge of LH was evident in all control animals (eight of eight), with peak LH noted 55.5±5.0 h after PR (Table 1).

In contrast, serum concentrations of oestradiol were maintained at basal (<0.5 pg/ml) levels throughout the infusion period in sheep receiving stress-like levels of cortisol

Table 1
The effect of continuous infusion of stress-like levels of cortisol during the simulated luteal and/or early follicular phases of the oestrous cycle on the appearance and magnitude of the preovulatory surge of LH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Animals displaying ovulatory surge of LH</th>
<th>Time of surge (h)</th>
<th>Amplitude of surge (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>8 a</td>
<td>55.0±5.0</td>
<td>23.2±2.3</td>
</tr>
<tr>
<td>Cortisol–luteal and follicular phases</td>
<td>8</td>
<td>1 b</td>
<td>54</td>
<td>26.5</td>
</tr>
<tr>
<td>Cortisol–follicular phase</td>
<td>8</td>
<td>0 b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cortisol–luteal phase</td>
<td>7</td>
<td>4 c</td>
<td>63.8±12.6</td>
<td>21.3±3.3</td>
</tr>
</tbody>
</table>

a Animals received vehicle or stress-like levels of cortisol (0.1 mg/kg/h) beginning 5 days before and continuing for 5 days after pessary removal (PR). Additional animals received cortisol beginning at the time of PR and continuing for 5 days thereafter. A final group received cortisol during the 5-day period before PR and vehicle thereafter.

b Sheep displaying a preovulatory surge of LH during the 5-day period after PR; values within the column that do not share a common letter are significantly different (P<0.05).

c Interval from PR to the peak of the LH surge.

d Serum concentration of LH at the height of the surge.
Fig. 3. Serum concentrations of progesterone in sheep receiving vehicle ((○); n=8) or stress-like levels of cortisol ((●); n=6) during the 5-day period after pessary removal (PR). Two animals from the cortisol-treated group (Group 3) did not show evidence of luteal function (serum progesterone maintained concentrations below 0.2 ng/ml) during the post-treatment period. Data from these animals have not been included in the graph presented here.

beginning 5 days before and continuing for 5 days after PR (Group 2). Additionally, surge-like secretion of LH was evident in only one of eight animals receiving stress-like concentrations of cortisol throughout the 10-day infusion period.

Similarly, serum concentrations of oestradiol were maintained at basal levels throughout the infusion period in sheep receiving stress-like levels of cortisol beginning at PR and continuing for 5 days thereafter (Group 3). Although surge-like secretion of LH was not evident during the infusion period in sheep (zero of eight) receiving stress-like levels of cortisol during the 5-day period beginning at PR, surge-like secretion of LH was evident in six of eight sheep within 3 days (6–8 days after PR) of cessation of cortisol infusion. In addition, the development and subsequent regression of the corpus luteum in animals receiving stress-like levels of cortisol during the period after PR was delayed, relative to luteal activity in control animals (Fig. 3).

In contrast to the suppression noted in sheep receiving cortisol after PR, the preovulatory surge of LH was evident within 5 days after PR in four of seven ewes receiving cortisol only during the 5-day period prior to simulated luteolysis (Group 4).

4. Discussion

The results presented here demonstrate that administration of stress-like levels of cortisol, beginning during a simulated luteal phase and continuing through the early follicular phase of the ovine oestrous cycle, suppresses oestradiol secretion and prevents the preovulatory surge of LH. Oestradiol is derived almost entirely from developing ovulatory follicles (Bjersing et al., 1972; Carson et al., 1981) and, therefore, serum concentrations of oestradiol are often used as a rough measure of follicle maturation. Indeed, recent studies indicate that only ovine follicles larger than 3.5 mm in diameter acquire the capacity to synthesize oestradiol (Huet et al., 1997). The suppression of oestradiol secretion during cortisol
infusion indicates that stress-like levels of cortisol may arrest follicular development prior to that stage of maturation. Alternatively, cortisol may selectively impair oestrogen synthesis without compromising other aspects of follicular development.

Similarly, administration of stress-like levels of cortisol beginning at the onset of the follicular phase also suppressed follicular maturation and the ovulatory surge of LH. Conversely, cortisol delivery only during the simulated luteal phase compromised oestradiol secretion and the preovulatory surge of LH during the subsequent follicular phase in some, but not all, ewes so treated. This anti-ovulatory effect of cortisol is consistent with the response to exogenous glucocorticoid noted in women (Cunningham et al., 1978), rodents (Baldwin and Sawyer, 1974), and other domestic species (Barb et al., 1982; Stoebel and Moberg, 1982). However, these results differ from a previous report that indicated that daily administration of a synthetic glucocorticoid (dexamethasone) did not compromise follicular development or ovulation in sheep (Phillips and Clarke, 1990). The physiologic basis for this discrepancy between our studies and this prior work is not clear, however, difference in the type of glucocorticoid, or dose and mode of administration, may contribute to these divergent observations.

In the work presented here we have attempted to approximate the increase in serum levels of cortisol noted in sheep during exposure to repetitive or persistent stress by infusion of the natural glucocorticoid. Continuous delivery of cortisol at a rate of 0.1 mg/kg/h established serum concentrations of cortisol comparable to those noted in sheep subject to moderate stressors, such as repeated laparoscopy, isolation and restraint, or bacterial infection (Martin et al., 1981; Minton, 1994; Battaglia et al., 1998). The use of the natural glucocorticoid at physiological concentrations strengthens our conclusion that stress-like concentrations of cortisol impair fertility in sheep. However, it should be noted that stress-induced increase in cortisol secretion is generally short-lived, suggesting that cortisol alone may not account in full for the infertility associated with stress.

Cortisol-dependent suppression of follicle maturation may reflect direct actions of the glucocorticoid at ovarian sites or, alternatively, action at hypothalamic or hypophyseal sites to limit secretion of the gonadotrophins. Recent studies indicate that cortisol-dependent arrest of follicular development in sheep is reversed by episodic administration of GnRH (Daley et al., 1999a). This suggests that stress-like levels of cortisol restrain pulsatile GnRH release during the follicular phase. Although, stress-like levels of cortisol do not affect the frequency or amplitude of episodic release of LH in ovariectomized sheep (Breen et al., 1999), LH pulse frequency is markedly reduced in sheep treated concurrently with cortisol and low concentrations of oestradiol (Breen et al., 1999; Daley et al., 1999b). This suggests that stress-like levels of cortisol may synergize with the low concentration of oestradiol characteristic of the early follicular phase to suppress GnRH pulse frequency and, thereby, limit gonadotrophin secretion.

We previously reported that continuous delivery of cortisol during the late luteal and follicular phases of the oestrous cycle blocked follicular maturation and the ovulatory surge of LH in about 50% of sheep so treated and delayed these processes in the remainder (Daley et al., 1999a). We note here that stress-like levels of cortisol during the follicular phase blocked follicular development and ovulation in 100% of sheep so treated. The increased inhibitory response noted in the current study may reflect the higher rate of cortisol delivery (0.1 versus 0.08 mg/kg/h) used in the study reported here. This resulted in increased serum
concentrations of cortisol (~75 versus ~65 ng/ml) and indicates that the suppressive effect of cortisol increases with increasing serum concentration over the physiological range.

In addition, we note here that cortisol-dependent suppression of follicle development and the LH surge is expressed even when cortisol delivery is initiated at PR. This suggests that stress-like levels of cortisol act relatively rapidly to suppress follicular maturation. However, the suppressive effect of cortisol appears to be temporary since four of seven sheep showed normal follicular development and ovulation within 5 days of PR when cortisol delivery was halted at PR. Similarly, luteal function was evident within 10 days of cessation of cortisol infusion in six of eight sheep receiving cortisol during the 5-day period after PR. These observations also indicate that the cortisol-dependent block to ovulation is temporary and follicular development, ovulation and subsequent luteal activity in most sheep are reinstated shortly after release from the inhibitory effects of stress-like levels of cortisol.

Taken together, these data are consistent with our working hypothesis that the infertility induced by stress or stress-like concentrations of cortisol is due, at least in part, to cortisol-dependent increase in the negative feedback potency of oestradiol. During the follicular phase of the oestrous cycle the augmented negative feedback potency of oestradiol would be expected to suppress the frequency and/or amplitude of episodic release of GnRH and decrease gonadotrophin secretion below the threshold required to maintain the normal progression of follicular development.

References


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