Temporal concentrations of cortisol and LH in virgin ewes acutely exposed to rams during the transition into the breeding season

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Temporal concentrations of cortisol and LH in virgin ewes acutely exposed to rams during the transition into the breeding season

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Abstract

The objectives of this study were to determine if exposing seasonally anovular ewes to rams would alter patterns of cortisol concentrations, and if these changes are associated with changes in characteristics of LH concentrations. Seasonally anestrous ewes were assigned to be exposed to rams (RE; n = 11) or wethers (NE; n = 12). Blood samples were collected at 15-min intervals beginning 120 min before introduction of males (time = 0 min), and continued for 360 min after male exposure. Characteristics of cortisol and LH concentrations included: mean and baseline concentrations, pulse amplitude, duration, frequency, and time to first pulse. Mean and baseline cortisol concentrations, and cortisol pulse amplitude, frequency, and time to first pulse after male exposure did not differ between RE and NE ewes. Cortisol pulse duration was longer ($P < 0.05$) in RE ewes than in NE ewes. Mean LH and LH pulse amplitude, duration, and time to first pulse after male exposure did not differ between RE and NE ewes. Baseline LH concentrations and LH pulse frequency were greater ($P < 0.05$) in RE than in NE ewes. In RE ewes, but not NE ewes, LH pulse frequency tended to increase ($P = 0.06$) as pulse frequency of cortisol decreased. In conclusion, exposing ewes to mature rams during the transition into the breeding season increased LH pulse frequency which hastened ovulatory activity. However, the results do not support the hypothesis that changes in cortisol concentrations plays a significant role in the 'ram effect'.

1. Introduction

The biostimulatory effect of rams on ewes is known to cause a relatively rapid increase in LH pulse frequency that accelerates resumption of seasonal ovulatory activity (Martin et al., 1980; Poindron et al., 1980). The biostimulatory effect of males has been reported to involve changes in adrenal cortical glucocorticoids in rodents (Nichols and Chevins, 1981; Marchlewska-Koj and Zacharczuk-Kakietek, 1990), humans (Wyart et al., 2007) and cattle (Tauck et al., 2007, 2010). Activation of the hypothalamic–pituitary–adrenal axis and cortisol secretion negatively impact reproductive functions (Smith and Dobson, 2002; Dobson et al., 2012). In this regard, Tauck et al. (2010) reported that cortisol pulse frequency decreased and pulse amplitude increased in postpartum, anovular, suckled cows exposed to bulls. Alterations

in hypothalamic–pituitary–adrenal axis activity was suggested to play a role in the physiologic mechanism of the biostimulatory effect of bulls to accelerate resumption of ovulatory activity in the bovine. Whether activation of the hypothalamic–pituitary–adrenal axis is involved with the biostimulatory effect in sheep is not known. Therefore, it was of interest to determine if changes in temporal cortisol concentration patterns were associated with the biostimulatory effect of rams on ewes during the transition into the breeding season. The objective of this experiment was to determine if temporal patterns of cortisol concentrations are altered in 18-mo-old virgin Targhee ewes exposed to rams during the transition into the breeding season. The null hypotheses were that (1) exposing seasonally anovular ewes to rams would not alter patterns of cortisol or LH concentrations, and (2) that temporal characteristics of cortisol are unrelated to that of temporal characteristics of LH concentrations.

2. Materials and methods

2.1. Animals and treatments

Thirty-five 18-mo-old virgin Targhee ewes that had been isolated from males since weaning during the previous yr, were used in this study. Additionally, three sexually experienced, epididymectomized rams and three wethers that had been castrated before secondary sex characteristics developed were used in this experiment. This experiment was conducted at the Montana State University Fort Ellis Research and Teaching Facility, near Bozeman, Montana. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee.

Jugular venous blood samples were collected from each ewe 10 and 15 d before exposure to males and assayed for progesterone (P4). All ewes used in this study had concentrations of P4 less than 1.0 ng/mL on these 2 d and were considered to be anovular. Additionally, one sample from the intensive sampling day was also assayed for P4 to assess whether ovulation had occurred in any of the ewes during the intervening time before exposure to males. Ewes were stratified by BW and assigned randomly to be exposed to rams (RE; $n = 17$) or exposed to wethers (NE; $n = 18$). Ewes within exposure type were then assigned randomly to an intensive sampling day; 1 (RE-1; $n = 5$, NE-1; $n = 6$), 2 (RE-2; $n = 6$, NE-2; $n = 6$), or 3 (RE-3; $n = 6$, NE-3; $n = 6$). Intensive sampling days were 3 d apart starting on August 18, 2009.

2.2. Pre-treatment handling

Each ewe received an indwelling jugular catheter 3 d before exposure to males. Catheters were 5¼″ 16 gauge extended use catheters (Jorgenson Laboratories, Loveland, CO). Catheters were flushed twice daily with heparinized saline (10 IU/mL in 0.9% sterile NaCl solution) until the night before ewes were exposed to either a ram or wether. Ewes in both exposure types were adapted to the sampling conditions, including sampling pens and human contact for 8 h/d during the 3 d before exposing them to males.

2.3. Blood sampling for LH and cortisol

At 08:30 (−2 h), RE and NE ewes were placed randomly into 1.5 m × 2 m pens (3 ewes/pen). Blood samples (~10 mL) were collected at 15-min intervals for 2 h. When the 0 min sample was obtained, rams or wethers were placed into pens holding ewes (1 ram or wether/3 ewes). Blood collection continued at 15-min intervals for 6 h. An equal volume of sterile saline solution (0.9%) was used to flush the catheters of each ewe after each blood sample was collected.

Blood samples were promptly cooled and stored overnight at 4 °C then centrifuged at 1850 × *g* for 30 min. Sera was harvested and stored at −20 °C until assayed for LH and cortisol.

2.4. Luteinizing hormone, cortisol and P4 assays

Concentrations of LH in serum samples were determined in duplicate by a double antibody RIA (Niswender et al., 1969). The primary antibody was NIDDK anti-oLH-1 AFP 192279RB and bLH AFP 11743B was used for the iodination and standards. Both assay reagents were obtained from the National Hormone and Peptide Program (NHPP) and Dr. A. Parlow (University of San Francisco, San Francisco, CA). Intra- and inter-assay CV were 9.6 and 16.3%, respective.

Cortisol concentrations in serum samples were assayed in duplicate using solid-phase RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA) validated for bovine serum in our laboratory (Berardinelli et al., 1992). Percent recoveries for sheep serum were determined by adding cortisol standard (7.81 ng/mL) to a sheep serum pool and assaying this pool at three different volumes. Percent recoveries ranged from 94 to 100%. Sensitivity of the assay using sheep serum was 1.95 ng/mL. The intra- and inter-assay CV were less than 10% in serum pools that contained 91 and 21.5 ng/mL.

Progesterone concentrations were determined in serum samples in duplicate by a solid-phase RIA kit (Siemens Healthcare Diagnostics, Los Angeles, CA) validated in our laboratory for bovine serum (Custer et al., 1990). The intra- and inter-assay CV were less than 5% in a serum pool of sheep that contained 2.4 ng/mL.

2.5. Characteristics of temporal patterns of LH and cortisol concentrations

Characteristics of temporal patterns of LH and cortisol included: (1) mean concentration, (2) baseline concentration, (3) pulse amplitude, (4) pulse frequency, (5) pulse duration, (6) time to first pulse. For each hormone in each ewe, during the pre-exposure sampling period and the exposure period, a plot of hormone concentration over time was generated. Baseline concentrations were identified and the mean baseline concentration was the mean of these concentrations. Concentrations of LH or cortisol that were >2 SD above the mean baseline concentration were considered as concentrations within a pulse of each hormone. Pulse amplitude (ng/mL) was calculated by subtracting the mean baseline concentration and the highest

concentration in each pulse. Pulse duration (min) was defined as the time interval between an increase from the baseline concentration to a peak concentration, then the return to a baseline concentration after that peak. Pulse frequency (peaks/h) was number of peaks during the blood sampling period divided by the time of that period. Time to first pulse was the time after exposure began until the zenith of first peak of each hormone.

2.6. Statistical analyses

Characteristics of temporal concentration patterns for LH and cortisol, including mean and baseline hormone concentration, as well as, pulse amplitude, frequency and duration and time to first pulse were analyzed two-way ANOVA for a completely randomized design using PROC GLM in SAS (SAS Inst., Inc., Cary, NC). The model included exposure type, intensive sampling day, and the interactions between these factors. Animal was the experimental unit. Means were separated using Bonferroni's method in SAS.

Relationships between temporal patterns of cortisol and LH concentrations were determined by regressing characteristics LH concentrations on characteristics of cortisol concentrations within exposure type using the PROC REGRESS procedure of SAS. Mean concentrations of LH were regressed on mean concentrations of cortisol, and baseline concentrations of LH were regressed on baseline concentrations of cortisol, and so on for all other characteristics (i.e., cortisol concentration pattern characteristic was the independent variable and LH concentration pattern characteristic was the dependent variable).

3. Results

Based on progesterone concentrations on the intensive sampling day, five RE and six NE ewes had resumed luteal activity and were excluded from analyses. Additionally, one ewe from the RE treatment was excluded from analyses because several characteristics of LH were statistically identified as outliers. Initial analyses revealed no effect of intensive sampling day or its interactions on any dependent variable so data for each sampling day were pooled.

Mean and baseline concentrations, pulse frequency and amplitude of cortisol pulses did not differ between RE and NE ewes (Table 1). Cortisol pulse duration was longer ($P = 0.02$) in RE than in NE ewes (Table 1). Mean LH concentration, pulse amplitude and duration of LH did not differ between RE and NE ewes. However, baseline LH concentrations were greater ($P = 0.03$) in RE than in NE ewes. Additionally, LH pulse frequency was greater ($P = 0.02$) in RE than in NE ewes (Table 2). Time until the first cortisol or LH pulse after introduction of males did not differ between RE and NE ewes (Table 2).

There were no linear relationships for mean and baseline concentrations, pulse amplitude and pulse frequency of LH regressed on mean and baseline concentrations, pulse amplitude and pulse frequency of cortisol within exposure type. However, there was a tendency ($P = 0.06$) for a negative, linear relationship between LH pulse frequency and cortisol pulse frequency in RE ewes, but not in NE ewes (Table 3).

Table 1
Characteristics of temporal patterns of cortisol concentrations patterns in virgin Targhee ewes collected at 15-min intervals for 6 h after exposure to rams (RE) or wethers (NE) during the transition into the breeding season.

Item	Exposure type		SEM[a]	*P*-value
	RE	NE		
n	11[b]	12[b]		
Mean, ng/mL	15.34	11.56	5.53	0.12
Baseline, ng/mL	4.68	4.62	0.023	0.95
Frequency, pulse/h	0.72	0.75	0.19	0.71
Amplitude, ng/mL	20.67	16.92	80.50	0.24
Duration, min	73.81[c]	62.26[d]	11.07	0.04
Time to 1st pulse, min	46.36	58.75	38.15	0.44

[a] Pooled standard error of mean.
[b] Number represents ewe from each treatment that were pooled from 'sampling days 1 and 2' for RE, $n = 5$ and 6, respectively, and for NE, $n = 6$ and 6, respectively.
[c,d]Values within rows differ.

Table 2
Characteristics of temporal patterns of LH concentrations patterns in virgin Targhee ewes collected at 15-min intervals for 6 h after exposure to rams (RE) or wethers (NE) during the transition into the breeding season.

Item	Exposure type		SEM[a]	*P*-value
	RE	NE		
n	11[b]	12[b]		
Mean, ng/mL	1.51	0.78	1.02	0.11
Baseline, ng/mL	0.82[c]	0.46[d]	0.75	0.03
Frequency, pulse/h	1.02[c]	0.85[d]	0.17	0.02
Amplitude, ng/mL	2.01	1.12	1.39	0.14
Duration, min	52.12	51.73	13.05	0.94
Time to 1st pulse, min	33.31	48.19	1.92	0.188

[a] Pooled standard error of mean.
[b] Number represents ewe from each treatment that were pooled from 'sampling days 1 and 2' for RE, $n = 5$ and 6, respectively, and for NE, $n = 6$ and 6, respectively.
[c,d]Values within rows differ.

Table 3
Linear regression of LH pulse frequency on cortisol pulse frequency within ewes exposed to rams (RE) and ewes exposed to wethers (NE) during the transition into the breeding season.

Variable	RE	*P*-value	NE	*P*-value
n	11[a]		12[a]	
LH pulse frequency on cortisol pulse frequency				
Intercept	1.59	0.0002	1.43	0.004
Slope (pulses/h)/(pulses/h)	−0.78	0.057	−0.809	0.101

[a] Number represents ewe from each treatment that were pooled from 'sampling days 1 and 2' for RE, $n = 5$ and 6, respectively, and for NE, $n = 6$ and 6, respectively.

4. Discussion

The rationale for this experiment was that changes in adrenal cortisol in anovular females are associated with the biostimulatory effect of males in other species, as reported by Tauck et al. (2010). Specifically, the purpose of this experiment was to determine if temporal concentrations of cortisol are altered in ewes during acute exposure to rams during the transition into the breeding season, and if

alterations in temporal cortisol concentrations were related to temporal concentrations of LH.

Exposing ewes to rams during the transition into the breeding season in the present study caused an increase in cortisol pulse duration; however, other characteristics of cortisol concentrations patterns were not altered by exposure to rams compared to exposing ewes to wethers. This change in concentration pattern without a change in the overall magnitude of the signal may be useful for understanding how cortisol may be able to modulate reproduction in ways other than the stereotypical inhibitory effects. Whether there is a direct or indirect effect of this change on LH pulse duration or frequency, this particular concentration pattern appeared to be associated with hastened resumption of luteal activity in ewes exposed to ram during the transition into the breeding season.

In the present study we found that cortisol pulse duration was longer in ewes exposed to rams than in ewes exposed to wethers. This result is consistent with the findings of Tauck et al. (2010) who reported that cortisol pulse duration tended to increase in postpartum, anovular, suckled cows exposed to bulls compared with that in cows not exposed to bulls. Tauck et al. (2010) also reported lower cortisol pulse frequency in postpartum, anovular, suckled cows exposed to bulls than in cows not exposed to bulls. However, results from the current study did not show decreased cortisol pulse frequency in ewes exposed to rams compared to ewes exposed to wethers. The discrepancy between the result report by Tauck et al. (2010) and the results of the present study might reflect difference between physiological states, i.e., transition into the breeding in ewes is related to photoperiodic regulation; whereas, in cows, the transition is regulated by postpartum factors, such as cow–calf bonding and lactation.

Exposing ewes to rams during the transition into the breeding season increased LH pulse frequency and baseline LH concentration relative to ewes exposed to wethers. These results are consistent with findings of Martin et al. (1980) and Poindron et al. (1980) in that seasonally anestrous ewes exposed to rams exhibited increased LH pulse frequency and a greater baseline concentration of LH. This is evidence of a change in the signal that LH conveys to the ovary (i.e., an increase in stimulation). Luteinizing hormone pulse frequency is increased during the transition into the breeding season allowing for follicular maturation and ovulation (for review, see, Karsch et al., 1980). In ewes exposed to rams, there was a tendency for a negative, linear relationship between LH pulse frequency and cortisol pulse frequency. The inference of this statistical result is that as the number of cortisol pulses increase the number of LH pulses decrease in ewes exposed to rams. However, no such relationship was observed in ewes exposed to wethers. The physiological explanation for this observation not obvious. One might argue that this is simply a stress response associated with exposing ewes to mature, intact rams.

In conclusion, the only temporal change in cortisol concentration patterns in response to exposing ewes to rams during the transition into the breeding season was a duration of cortisol pulses. It is not clear from the results of this study if this change caused significant changes in temporal concentration patterns of LH patterns that ultimately lead to resumption of ovulatory and luteal activity, or if this changes in cortisol pulsatility was merely a coincident effect associated with a stress response to a ram and does not in itself mediate changes in LH concentration patterns. Thus, results of this experiment do not support the hypothesis that the hypothalamic–pituitary–adrenal axis plays a significant role in the 'ram effect' in sheep during the transition into the breeding season.

Conflict of interest

None of the authors have any conflict of interest to declare.

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