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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

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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