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Increasing Concentrations of 17β-Estradiol Has Differential Effects on Secretion of Luteinizing Hormone and Follicle-Stimulating Hormone and Amounts of mRNA for Gonadotropin Subunits during the Follicular Phase of the Bovine Estrous Cycle

Andrea S. Cupp
University of Nebraska-Lincoln, acupp2@unl.edu

F. N. Kojima
University of Nebraska-Lincoln

M. S. Roberson
Oregon Health Sciences University, msr14@cornell.edu

T. T. Stumpf
University of Missouri, Columbia

M. F. Wolfe
Case Western Reserve University

See next page for additional authors

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Increasing Concentrations of 17β-Estradiol Has Differential Effects on Secretion of Luteinizing Hormone and Follicle-Stimulating Hormone and Amounts of mRNA for Gonadotropin Subunits during the Follicular Phase of the Bovine Estrous Cycle[1]

A.S. CUPP, F.N. KOJIMA, M.S. ROBERSON,[3] T.T. STUMPF,[4] M.W. WOLFE,[5] L.A. WERTH,[6] R.J. KITTOK, H.E. GROTJAN, and J.E. KINDER[2]

Department of Animal Science, University of Nebraska, Lincoln, Nebraska 68583–0908

ABSTRACT

The hypothesis tested was that 17β-estradiol (E_2) would increase amounts of mRNA for α, LHβ, and FSHβ subunits during the follicular phase of the estrous cycle prior to the preovulatory surge of gonadotropins in cows. On Day 16 (Day 0 = estrus) of the estrous cycle, all cows were treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Cows served as intact controls (CONT, n = 4) were ovariectomized (OVX, n = 5), or were ovariectomized and administered E_2 (OVXE, n = 6) in increasing doses starting at the time of treatment with $PGF_{2\alpha}$. Cows were bled for 6 h before and for 40 h after $PGF_{2\alpha}$ treatment to characterize pulsatile secretion of LH and FSH. Forty hours after $PGF_{2\alpha}$ treatment, pituitaries were collected for evaluation of amounts of mRNA for α, LHβ, and FSHβ subunits. Amplitude of LH pulses was greater ($p < 0.05$) in cows from the OVXE than from the CONT group. Concentrations of FSH were greater in cows from both the OVXE and OVX ($p < 0.01$) groups than from the CONT group. Amounts of mRNA for α and FSHβ subunits were greater ($p < 0.01$) in pituitaries of cows from the OVX than from the CONT or OVXE groups. Amounts of mRNA for LHβ subunit in pituitaries of cows from the OVX group tended to be greater ($p < 0.08$) than from the CONT group. Cows in the OVXE group tended ($p < 0.08$) to have greater amounts of mRNA for FSHβ subunit than did CONT cows. Amounts of mRNA for α and LHβ subunits in cows from the OVXE and CONT groups did not differ ($p > 0.10$). Pituitary weight and content of LH and FSH were not different ($p > 0.10$) among cows of the different groups. Ovariectomy resulted in enhanced secretion of gonadotropins and increased amounts of mRNA for gonadotropin subunits above values detected in CONT cows. Ovariectomized cows administered E_2 in follicular-phase patterns had amounts of mRNA for α and LHβ similar to those in CONT cows even though secretion of LH was enhanced in the OVXE group. We reject our hypothesis and conclude that E_2 has a divergent role in regulation of gonadotropins. Release of LH is enhanced by E_2, but E_2 reduced mRNA for gonadotropin subunits (tended to reduce mRNA for FSHβ subunit) in ovariectomized cows to amounts detected in intact cows during the follicular phase of the bovine estrous cycle.

INTRODUCTION

During the follicular phase of the estrous cycle in cows, increased 17β-estradiol (E_2) enhanced secretion of LH [1, 2]. Estradiol modulates LH secretion in the ewe by influencing secretion of LHRH [3] and up-regulating receptors for LHRH at the pituitary [4, 5]. Enhanced secretion of LHRH in rodents results in a similar up-regulation of receptors for LHRH [6, 7], and enhanced secretion of LH is a consequence of the increased population of receptors for LHRH.

Stimulation of gonadotrophs by LHRH contributes to the production of mRNA for gonadotropin subunits (ewe [8]; rat [9, 10]). Pulsatile secretion of LHRH is required to maintain greater amounts of mRNA for LHβ (ewe [11]; rat [12]) and FSHβ subunits (ewe [11]). Increases in the pulse frequency of LHRH (rat [13]) or continuous infusion of LHRH (ewe [11]) results in preferential synthesis of α subunit mRNA. Therefore, alterations in pattern of LHRH secretion from the hypothalamus results in changes in amounts of mRNA for gonadotropin subunits.

Modulation of pulse frequency of LHRH occurs during different physiological states. Pulse frequency of LHRH is increased during sexual maturation in rats [14], during the follicular phase of the menstrual cycle in primates [15–17], and prior to the preovulatory surge of gonadotropins in ewes [18, 19]. Administration of progesterone, E_2, or both steroids in combination is hypothesized to regulate the pattern of secretion of LHRH or up-regulate receptors for LHRH to cause increased production of mRNA for the LH or FSH subunit [20, 21].

In the ewe, amounts of mRNA for both α and LHβ subunits increase around estrus, whereas amounts of mRNA for FSHβ subunit are minimal [21]. In contrast, in experiments where E_2 was administered chronically to ewes, suppression of mRNA for α, LHβ [22], and FSHβ subunits occurred [23]. Experiments have not been conducted in cows to determine the effect of a physiological increase in E_2 during the follicular phase of the estrous cycle on the amount of mRNA for gonadotropin subunits. In the present experiment we tested the hypothesis that elevated concentrations of E_2 (similar to follicular phase increases) that result in

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[2]Correspondence: James E. Kinder, A224J Animal Sciences, University of Nebraska, Lincoln, NE 68583–0908. FAX: (402) 472–6362.

[3]Current address: Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

[4]Current address: Department of Animal Science, University of Missouri, Columbia, MO 65211.

[5]Current address: Department of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

[6]Current address: Department of Veterinary and Biomedical Sciences, University of Nebraska, Lincoln, NE 68583–0905.

increased secretion of LH during the follicular phase of the bovine estrous cycle prior to the preovulatory surge of gonadotropins would increase amounts of mRNA for α and LHβ subunits. In addition, we evaluated the influence of this change in E_2 on the amount of mRNA for FSHβ subunit.

MATERIALS AND METHODS

Experimental Protocol

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln. Fifteen cows of composite breeding (1/4 Hereford, 1/4 Angus, 1/4 Red Poll, 1/4 Pinzgauer) were synchronized to a common day of the estrous cycle with two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse® Sterile Solution; The Upjohn Co., Kalamazoo, MI). On Day 16 (Day 0 = estrus) of the estrous cycle, all cows were treated with $PGF_{2\alpha}$. Cows of one group served as controls (CONT, n = 4), and cows in the other two groups were either ovariectomized (OVX, n = 5) or ovariectomized and administered increasing concentrations of E_2 (OVXE, n = 6) via vaginal implants starting at the time of treatment with $PGF_{2\alpha}$. Ovariectomies were performed via high lumbar laparotomy (0 h). After ovariectomy, cows in the OVXE group received intravaginal implants (polydimethylsiloxane intravaginal implants: 3.35 mm i.d. × 4.65 mm o.d. × 13.5 cm; Dow-Corning, Midland, MI) containing E_2 (Sigma Chemical Co., St. Louis, MO). The number of implants was increased (one per time period) at 0, 10, 15, 20, and 30 h to mimic the elevation of E_2 that occurs during the follicular phase of the bovine estrous cycle (CONT).

Before $PGF_{2\alpha}$ treatment, indwelling jugular catheters were inserted, and blood samples were collected at 12-min intervals for 6 h to determine initial concentrations of hormones in circulation. After treatment with $PGF_{2\alpha}$ and initiation of treatments with E_2 in cows from the OVX and OVXE groups, cows were bled every 12-min for four 6-h periods with 4 hourly samples between each serial blood collection. The last 6-h period of blood collection ended 40 h after administration of $PGF_{2\alpha}$ and initiation of treatments with E_2. Blood samples were collected from cows in the control group after the same protocol of blood collection as that for ovariectomized cows. These blood samples were collected to characterize changes in concentration and pulsatile secretion of LH and FSH. Additional blood samples were collected every 5 h to determine plasma concentrations of E_2 and progesterone in circulation.

After the last 6-h period of serial blood collection, pituitaries were collected from all cows within 15 min after exsanguination. Anterior pituitaries were hemisected, transferred to separate vials, frozen in liquid nitrogen, and stored at −70°C until amounts of mRNA for α, LHβ, and FSHβ subunits were determined.

RIAs

Blood samples for quantification of LH were allowed to clot at room temperature and were stored at 4°C for 24 h. Serum was collected after centrifugation at 1520 × *g* for 15 min and stored at −20°C until assayed for LH. Concentrations of LH in serum samples collected at 12-min intervals and in pituitary extracts were determined by RIA [24, 25]. Intra- and interassay coefficients of variation for serum LH assays were 2.5 and 10.5%, respectively. Intra- and interassay coefficients of variation for pituitary extracts were 3.0 and 13.2%, respectively. Sensitivities of the LH assays were approximately 28 pg/ml in serum and 30 pg/ml in pituitary homogenates.

Concentrations of FSH were analyzed in all serum samples and pituitary extracts by RIA [26, 27]. Intra- and interassay coefficients of variation for FSH assays in serum were 2.1 and 11.2% and in pituitaries were 2.4 and 12.7%, respectively. Sensitivity of the FSH assays were 44 pg/ml in serum and 74 pg/ml in pituitary homogenates.

Blood samples taken every 5 h were collected in heparinized tubes and centrifuged at 1520 × *g* for 20 min, and plasma was stored at −20°C until assayed for progesterone [28] and E_2 [29]. Concentrations of progesterone were determined in a single assay, and the intraassay coefficient of variation was 5%. Intra- and interassay coefficients of variation for E_2 were 2.4 and 17.5%, respectively. Sensitivity of the progesterone assay was 600 pg/ml, and sensitivities of the E_2 assays were 0.5 pg/ml.

Pituitary Extracts

To determine pituitary content of gonadotropins, hemipituitaries were homogenized in 150 mM NaCl buffered with 50 mM Tris, pH 7.4, containing 0.5% (vol/vol) Triton X-100, 5 mM Na_2 EDTA, 1 mM phenylmethylsulphonyl fluoride, 0.5 mg/L leupeptin, and 200 U/ml aprotinin (1.0 ml/100 mg wet tissue weight) for 30 sec in a polytron homogenizer (Brinkman Instruments, Westbury, NY). Pituitary extracts were clarified by centrifugation at 100 000 × *g* for 1 h, aliquoted into 0.5-ml portions (equivalent to 50 mg tissue), and stored at −70°C until assayed for LH [24, 25] and FSH [25, 26]. Pituitary extracts were also used to quantify differences in isoforms of LH and FSH among treatments; these data are presented in a companion paper [30].

Northern and Slot Blot Analysis of Total Cellular RNA (tcRNA)

Frozen hemi-pituitaries were weighed, and tcRNA was isolated by homogenization in buffer and centrifugation through a cesium chloride gradient [22]. Precipitates of tcRNA were maintained under ethanol at −70°C until Northern (α and LHβ subunits) and slot (FSHβ subunit) blots were prepared and hybridized to appropriate subunit probes. Procedures to determine amounts of mRNA for α and LHβ subunits in pituitaries of cows using Northern blots were described previously [29].

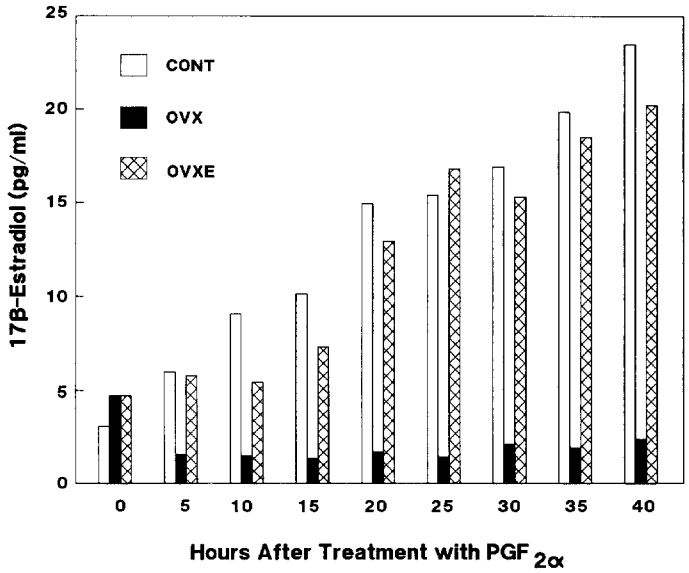


FIG. 1. Mean concentrations (in pg/ml) of E_2 in blood samples for each group collected every 5 h starting with $PGF_{2\alpha}$ treatment and/or ovariectomy and ending at pituitary collection. Pooled SEM = 0.50 pg/ml.

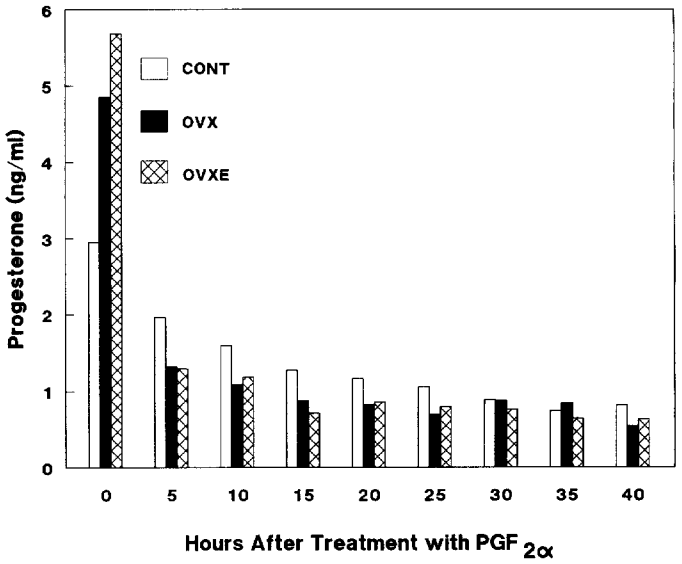


FIG. 2. Mean concentrations (in ng/ml) of progesterone in blood samples for each group collected every 5 h starting with $PGF_{2\alpha}$ treatment and/or OVX and ending at pituitary collection. Pooled SEM = 0.19 ng/ml.

Briefly, Northern blots were prepared by separating 5 μg of tcRNA from individual hemi-pituitaries in 1.5% agarose gels containing formaldehyde, where 18s and 28s bands were visualized for efficiency of RNA loading per well. Within each gel, samples of tcRNA from cows in each treatment group were represented. Total cellular RNA isolated from cerebellum and steer pituitaries were used as negative and positive controls, respectively. Size-separated tcRNA was subsequently transferred to nitrocellulose membranes (Beth Research Laboratory, Gaithersburg, MD) by capillary action and baked at 80°C for 2 h. Plasmids containing cDNA fragments encoding bovine α- [31] and LHβ [32] subunits (generously provided by Dr. Richard Maurer) were used to derive riboprobes that were labeled with α^{32}P-CTP (Amersham Corp., Arlington Heights, IL).

Slot blots to determine amounts of mRNA for FSHβ subunit were prepared with 5 μg of tcRNA and diethylpyrocarbonate distilled water to 10 μl total volume. RNA was denatured by addition of 20 μl 100% formamide, 7 μl 37% formaldehyde, and 2 μl 20-strength saline sodium citrate (SSC; single-strength SSC is 0.15 M NaCl and 0.015 M sodium citrate), heated to 68°C for 15 min, and then placed on ice [33]. Before RNA was loaded onto slot blots, wells were washed twice with 10-strength SSC (250 μl/wash for each well). Two volumes of 20-strength SSC containing bromophenol blue dye were added to RNA samples and applied to nylon filters (Zeta probe, Bio-Rad Labs., Richmond, CA) in individual wells on slot blots. The nylon filters were rinsed four times with 10-strength SSC, dried and baked for 30 min at 80°C. The cDNA probe for FSHβ subunit [34] was prepared by random-primer labeling [35] utilizing (α^{32}P-dCTP, Amersham Corp.). Heterologous oligonucleotide radioactive probes to rat α-tubulin (NEN DuPont, Boston, MA; nucleotides +958 to +987 of rat cDNA) were prepared by 3′ end labeling (3′ end labeling kit, NEN DuPont) with ^{35}S-dATP (Amersham Corp.) according to manufacturers' instructions.

Total cellular RNA bound to nylon and nitrocellulose filters were hybridized to α and LHβ subunits and α-tubulin probes for 46 h at 60°C. All treatments were represented on one slot blot that was hybridized to FSHβ subunit at 45°C for 24 h. Specific activity of probes was 1.5–2.0 × 10^9 dpm/μg. The concentration of radioactive probes was approximately 10^6 total cpm/ml hybridization buffer (5 ml/blot). After hybridization, filters were washed at room temperature (3 washes; 20 min/wash) in double-strength SSC, 0.1% SDS and then in 0.2-strength SSC, 0.1% SDS at 60°C.

Specific radiolabeled bands from Northern and slot blots were visualized and quantified by use of an Ambis Radioanalytic Imaging System. Quantification of the radiolabeled probe hybridizing to mRNA bound to nitrocellulose or nylon was expressed as net counts per minute detected within the discrete area of the radiolabeled band.

Statistical Analysis

Values for each characteristic of LH and FSH secretion were analyzed for the five 6-h periods of serial blood collection. Mean concentrations of LH and FSH (ng/ml), frequency of LH and FSH pulses (pulses/6 h), and amplitude of LH and FSH pulses (ng/ml) were determined through the use of algorithms (Pulsar Software, Univ. of Illinois, Urbana, IL, modified for the IBM-PC by J.R. Gitzen and V.D. Ramirez., Univ. of Illinois, Urbana, IL, [G-values for LH were G1—5.00, G2—2.80, G3—2.30, G4—10.00, G5—10.00; G-values for FSH were: G1—3.20, G2—2.80, G3—2.30, G4—10.00, G5—10.00]).

TABLE 1. Concentrations of LH prior to and during treatment period.

	Mean LH (ng/ml)		LH pulse amplitude (ng/ml)		LH Pulse frequency/12 h	
Group	Pretreatment	Treatment	Pretreatment	Treatment	Pretreatment	Treatment
CONT	0.87[a]	1.74[c]	1.11[a]	1.65[a,c]	1.75[a]	2.74[a,c]
OVX	0.89[a]	2.30[b,c]	1.00[a]	2.65[b]	1.40[a]	4.05[b]
OVXE	0.75[a]	2.70[b]	1.41[a]	3.07[b]	1.00[a]	3.62[b,c]
SEM	0.20	0.32	0.47	0.38	0.54	0.65
	P > 0.10	P < 0.05	P > 0.10	P < 0.01	P > 0.10	P < 0.05

[a,b,c]All values with different superscripts within columns are different at P-values listed and within rows at $p < 0.05$.

Data regarding the effect of treatment on characteristics of pulsatile secretion of LH and FSH and plasma samples of E_2 and progesterone were fitted to a linear model containing the fixed effect of blood collection period and the random effect of cow. Variance components were estimated by means of Restricted Maximum Likelihood algorithms of PROC MIXED in SAS [36, 37]. Repeated measures were accounted for by assuming an autocorrelation error structure for residual error that estimates the correlation among repeated measurements on an individual cow. Therefore, all parameters for LH and FSH were calculated over time (four 6-h blood collection periods after $PGF_{2\alpha}$) during the treatment period to determine differences between treatments. The plasma samples collected for E_2 and progesterone (every 5 h) were also analyzed over time to determine differences among groups.

The means of the characteristics for LH and FSH secretion from the combined blood collection periods after $PGF_{2\alpha}$ were compared to the initial blood collection (since this blood collection occurred before administration of treatments). In addition, comparisons for steroid hormone concentration (E_2 and progesterone) were made between the one sample collected before treatments and all collections after treatment administration. Amount of mRNA for gonadotropin subunits and α-tubulin were compared according to PROC MIXED procedures with contrast statements to determine differences among treatments [36, 37].

RESULTS

Stage of Estrous Cycle

There were no differences in characteristics of hormones in serum during the initial blood collection period prior to administration of $PGF_{2\alpha}$ and/or ovariectomy. No cows in the CONT group exhibited a surge of LH during the blood collection periods, and large ovulatory follicles with no evidence of functional CL were present when ovaries were evaluated after collection of pituitary tissue. Therefore, we conclude that all CONT cows were in the follicular phase of the estrous cycle throughout the experimental period.

Concentrations of Steroid Hormones

Data for mean concentrations of E_2 are depicted in Figure 1 for each group during the period of blood collection. Concentrations of E_2 were similar in all cows prior to $PGF_{2\alpha}$ treatment. Cows in the OVXE group had concentrations of E_2 in circulation similar to those of CONT cows ($p > 0.10$; Fig. 1). Cows in both the OVXE and CONT groups had greater ($p > 0.05$) concentrations of E_2 than did OVX cows. Concentrations of E_2 remained low in cows from the OVX group (less than level of sensitivity of the assay) throughout the experiment. Mean concentrations of progesterone for each group are depicted in Figure 2. Concentrations of progesterone were similar in cows of all groups ($p > 0.10$).

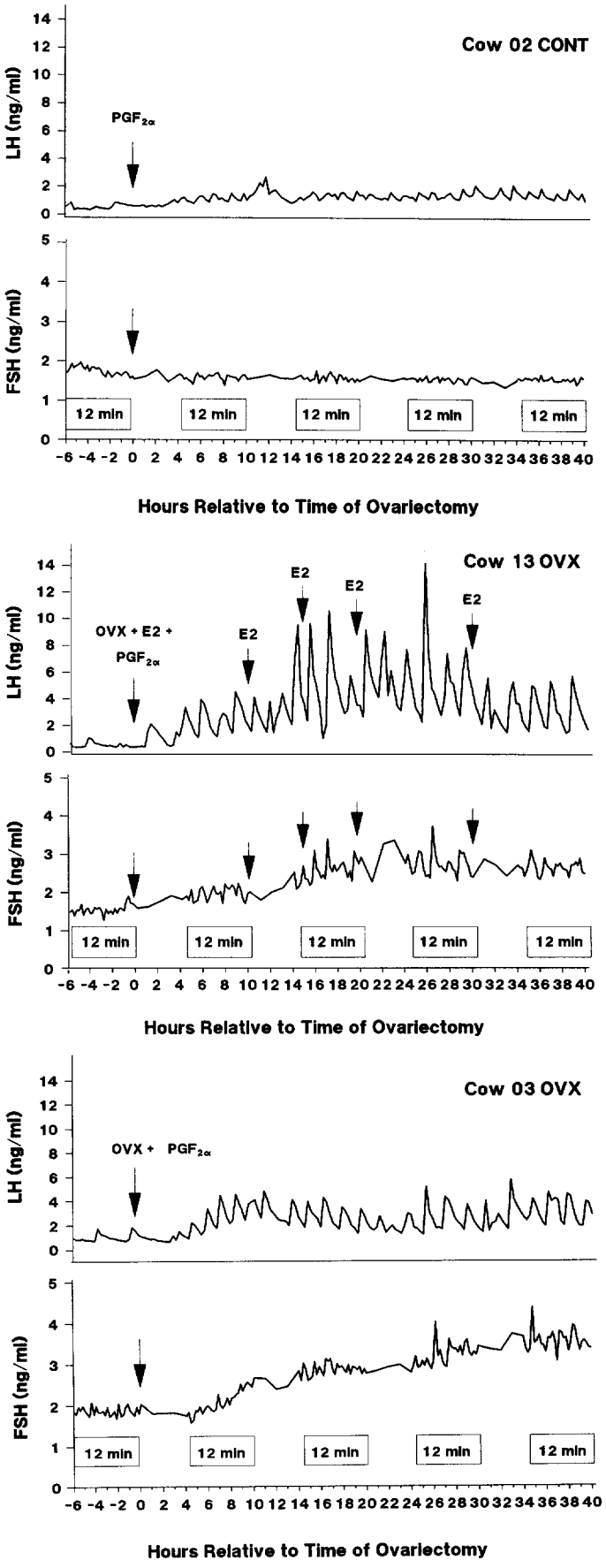
Pattern of LH Secretion

Mean concentrations of LH were greater ($p < 0.05$) in cows in the OVXE than in the CONT group when compared over all blood collection periods after $PGF_{2\alpha}$ treatment (Table 1). Mean concentrations of LH were similar among cows in the OVX and CONT groups for each of the four 6-h periods of serial blood collection after $PGF_{2\alpha}$ administration ($p > 0.10$). Frequency of LH pulses were greater ($p < 0.05$) in cows in the OVX group than in the CONT group during the combined blood collection periods after $PGF_{2\alpha}$ treatment (Table 1). Amplitude of LH pulses was greater ($p < 0.01$) in cows in the OVXE group than in the CONT group during the blood collection periods after $PGF_{2\alpha}$ was administered (Table 1). The profiles of pulsatile LH secretion during each of the five periods of serial blood collection are depicted for representative cows from each treatment in Figure 3.

Pattern of FSH Secretion

Mean concentrations of FSH were greater in cows in the OVX and OVXE ($p < 0.01$) groups than in the CONT cows during the blood collections after $PGF_{2\alpha}$ treatment (Table 2). However, FSH remained similar ($p > 0.10$) in cows in the OVX and OVXE groups during the same time period (Table 2). During the 6-h period of blood sampling prior to pituitary collection, cows in the OVX group had greater concentrations of FSH in circulation than did the cows of either the OVXE ($p < 0.01$) or CONT group ($p < 0.001$; OVX = 3.38; OVXE = 2.73; CONT = 1.67 ng/ml). In addition, cows in the OVXE group had greater concentrations of FSH than did cows in the CONT group ($p < 0.001$).

The patterns of secretion of FSH in cows from the ovariectomized groups were different from the pattern in the



CONT group (Fig. 3). During the four blood collection periods after initiation of treatment, cows in the OVX and OVXE groups had a greater ($p < 0.01$) amplitude of FSH pulses than did cows from the CONT group (0.57, 0.58, and 0.05 ± 0.14 ng/ml, respectively). Cows in the OVXE group had a greater frequency of FSH pulses than did cows in either the CONT or OVX groups (1.40, 0.12, 0.97 ± 0.33 pulses/6 h, respectively). Only two cows in the CONT group had pulses of FSH during the four serial blood collections after initiation of treatment, and in both cows pulses of FSH were low in amplitude (0.32, 0.43 ng/ml). Only two cows (one OVX and one OVXE) had pulses of FSH during the serial blood collection prior to treatment.

Gonadotropin Content and Relative Amounts of mRNA in Pituitaries

Hemisected pituitary weights (CONT = 929.0, OVXE = 860.3, OVX = 899.4 ± 78.2 mg) and concentrations of LH (CONT = 3.3, OVXE = 3.2, OVX = 2.7 ± 0.4 μg/mg tissue) and FSH (CONT = 0.088, OVXE = 0.099, OVX = 0.098 ± 0.009 μg/mg of tissue) were not different ($p > 0.10$) among cows in the different treatment groups. Concentrations of tcRNA isolated from pituitaries were not affected by treatment ($p > 0.10$) and averaged 5 ± 1 μg tcRNA/mg in pituitary tissue. Absorbance ratios of A260/A280 and A260/A230 were similar ($p > 0.05$) among pituitary tissues from cows in different groups and averaged 1.62 ± 0.03 and 2.47 ± 0.06, respectively. Total cellular RNA isolated from cow cerebellum did not specifically hybridize to either α, LHβ, or FSHβ subunit probes.

Relative amounts of α-tubulin detected in tcRNA were similar ($p > 0.10$) among pituitary tissues of cows from all groups (Fig. 4d), indicating that effects of E_2 were specific in modulating amounts of mRNA for the gonadotropin subunits. If there were nonspecific actions of estradiol in increasing amounts of mRNA in the pituitary, amount of mRNA for α-tubulin would have been influenced by treatment with E_2. There were greater amounts of mRNA for α-tubulin in cerebellum compared to pituitary tissue ($p < 0.05$).

Amounts of mRNA for α subunit were greater in cows in the OVX than in the CONT group (630 ± 55 and 406 ± 62 counts/5 μg tcRNA; $p < 0.01$; Fig. 4a). Amounts of mRNA for LHβ subunit tended to be greater in pituitaries of cows in the OVX than in the CONT or OVXE groups (225 ± 27 and 150 ± 30 or 178 ± 25 counts/5 μg tcRNA, respectively; $p < 0.08$; Fig. 4b). Cows in the OVXE group had amounts of mRNA for α and LHβ subunits similar to amounts in CONT cows ($p > 0.10$). Amounts of mRNA for FSHβ subunit were greater in cows of the OVX than in those of the CONT (p

FIG. 3. Profiles of LH and FSH secretion in representative cows from each treatment group for each of the five periods of serial blood collection, which followed the protocol described in *Material and Methods*. Top) CONT, middle) OVXE, bottom) OVX.

TABLE 2. Concentrations of FSH prior to and during treatment period.

	Mean FSH (ng/ml)		FSH pulse amplitude (ng/ml)		FSH pulse frequency/12 h	
Group	Pretreatment	Treatment	Pretreatment	Treatment	Pretreatment	Treatment
CONT	1.75[a]	1.77[a]	0.00[a]	0.05[a]	0.00[a]	0.12[a]
OVX	1.90[a]	2.79[b]	0.11[a]	0.56[b]	0.40[a]	0.97[a]*
OVXE	2.01[a]	2.55[b]	0.12[a]	0.57[b]	0.16[a]	1.39[b]
SEM	0.22	0.24	0.22	0.15	0.54	0.33
	P > 0.10	P < 0.01	P > 0.10	P < 0.01	P > 0.10	P < 0.01

[a,b]All values with different superscripts within columns are different at P-values listed and within rows at $p < 0.05$.
*Tends to be different than CONT; ($p < 0.08$).

< 0.001) or OVXE ($p < 0.01$) groups (13043 ± 1525 and 2833 ± 1705 or 7079 ± 1392 count/5 μg tcRNA, respectively, Fig. 4c). Cows in the OVXE group tended ($p < 0.08$) to have greater amounts of FSHβ subunit mRNA than did cows from the CONT group.

DISCUSSION

In the present experiment, ovariectomy and treatment with E_2 resulted in enhanced secretion of LH and FSH. Cows of the OVX group tended to have enhanced amounts of mRNA for all gonadotropin subunits (α, LHβ and FSHβ) compared to cows of the CONT group. However, amounts of mRNA for α and LHβ subunits were similar in cows of the OVXE and CONT groups. Although E_2 enhanced secretion of LH, it did not enhance amounts of mRNA for LH subunits during the follicular phase of the bovine estrous cycle as we had hypothesized. Therefore, we reject our hypothesis that increasing concentrations of E_2 during the follicular phase of the bovine estrous cycle enhance amounts of mRNA for the LH subunits coincident with the increase in secretion of LH that occurs during this part of the estrous cycle.

Many experiments have been conducted to determine the effect of E_2 on steady-state or relative amounts of mRNA for gonadotropin subunits, with mixed results. Chronic administration of E_2 reduced amounts of mRNA for gonadotropin subunits in vivo [22, 23, 38, 39]. During periods of the estrous cycle when concentrations of E_2 are greater in circulation (such as the follicular phase and around estrus), amounts of mRNA for α and LHβ subunits were increased whereas mRNA for FSHβ subunit was minimal [21].

In the present experiment, when E_2 was administered to ovariectomized cows in a pattern that mimicked that of the follicular phase of the bovine estrous cycle, amounts of mRNA for all gonadotropin subunits were suppressed compared to amounts in ovariectomized cows not treated with E_2. This demonstrates the negative effect of physiological concentrations of E_2 on amounts of mRNA for α, LHβ, and FSHβ subunits. Our results in cattle concur with those from experiments in other species (rat [40]; ewe [22, 41]) where chronically ovariectomized animals were administered either a constant high or low dose of E_2 for relatively long periods of time.

Secretion of LH (mean concentration and pulse amplitude of LH) was enhanced in ovariectomized cows treated with increasing concentrations of E_2 compared to CONT cows. Because the pattern of circulating concentrations of steroids was similar in cows of the OVXE and CONT groups, ovarian factors may exist that regulate LH secretion other than ovarian steroids. Possible ovarian factors that could elicit this effect are unknown; thus, it would be pure conjecture to speculate on their identity.

Secretion of FSH differs from that of LH because it is coordinately regulated by ovarian peptides—inhibin and

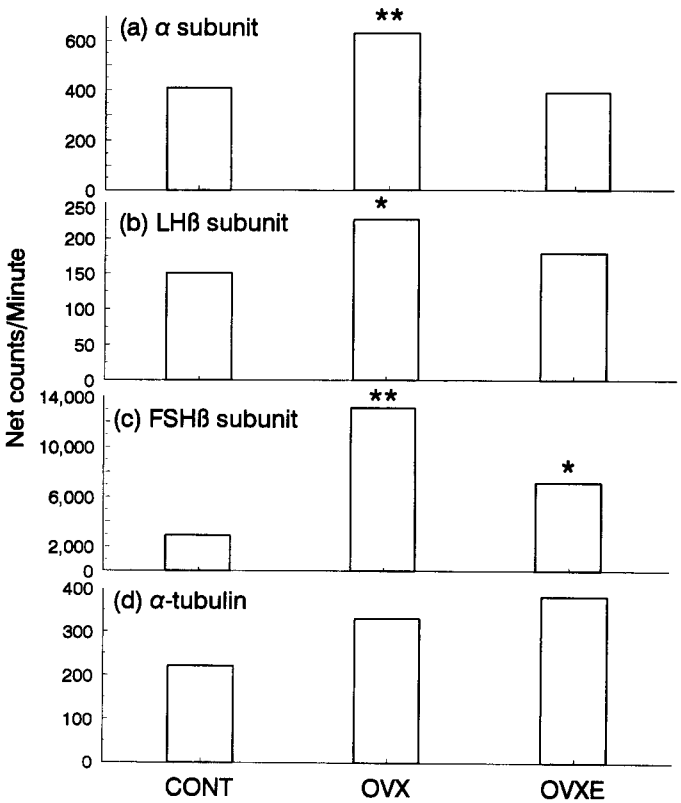


FIG. 4. Mean concentrations of (a) α subunit, (b) LHβ subunit, (c) FSHβ subunit, and (d) α-tubulin subunit mRNA expressed in net counts of radioactivity per minute for each group at pituitary collection 40 h after $PGF_{2\alpha}$ treatment. SEM for α subunit mRNA = 57 counts/min. SEM for LHβ mRNA = 27 counts/min. SEM for FSHβ mRNA = 1525 counts/min. SEM for α-tubulin mRNA = 69 counts/min. **Differences from CONT ($p < 0.01$). *Differences from CONT ($p < 0.08$).

activin—as well as LHRH [42]. In sheep, suppression of secretion of FSH is under control of both E_2 and inhibin. In combination, both of these hormones can function synergistically to suppress FSH secretion in ewes [43, 44]. It has also been suggested that inhibin and E_2 function at different times during the estrous cycle to regulate secretion of FSH in ewes [23]. Concentrations of plasma FSH during the follicular phase of the ovine estrous cycle could be regulated primarily by E_2; however, during the 24-h period following the preovulatory surge of LH, E_2 may be relatively unimportant with regard to modulation of FSH secretion [45].

Interestingly, cows in both the OVX and OVXE groups had profiles of FSH secretion with distinct pulses compared to cows in the CONT group. This difference in pulsatile secretion of FSH is probably due to the absence of inhibin or other factors following ovariectomy. Previous research from our laboratory [46] has indicated that release of FSH in intact cows is not pulsatile, but the baseline of FSH secretion may be altered during specific days of the estrous cycle. According to results from the present experiment, E_2 does not affect mean concentration of FSH (because there was no difference in OVXE and OVX groups); however, it does affect the pattern of FSH secretion. Pulse frequency of FSH was enhanced by E_2 in the absence of other ovarian factors without increasing mean concentration of FSH. It is possible that E_2 acts at the gonadotroph to regulate release of FSH as well as LH.

In most animal systems, the amount of mRNA for FSHβ subunit and the secretion of FSH are tightly coupled [47]. Unlike the common α or LHβ subunit mRNA, FSHβ subunit mRNA is regulated by ovarian factors other than steroids. Progesterone and E_2 decrease steady-state amounts of FSHβ subunit mRNA coordinately with FSH secretion in ovine pituitary cultures [48]. Amounts of FSHβ subunit mRNA in the pituitary are suppressed by administration of follicular fluid or inhibin [47]. Perfusion of pituitaries with inhibin suppresses, while perfusion with activin or LHRH increases, the amount of mRNA for FSHβ subunit [49]. It has been postulated that activin and inhibin alter the amount of mRNA for FSHβ subunit by modulating production of mRNA at the pituitary. In contrast, E_2 is hypothesized to elicit actions at the hypothalamus through altering LHRH secretion, which causes suppression of mRNA for FSHβ subunit in ewes [47]. The relationship between the amount of mRNA for FSHβ subunit present in the pituitary to the amount of FSH released from the pituitary of cows has not been evaluated.

In the present experiment, amounts of mRNA for FSHβ subunit were lowest in the CONT group; however, administration of E_2 reduced amounts of mRNA in the OVXE group as well, compared to amounts in the OVX group. These results are similar to those in rats, where treatment with E_2 at the time of ovariectomy reduced mRNA for FSHβ subunit by up to 67% when compared to that of ovariectomized rats not treated with E_2 [50]. Estradiol, therefore, has an important role in modulating amounts of mRNA for FSHβ subunit, which is independent of other ovarian hormones (such as inhibin) in the cow. In the present study, E_2 had little effect on mean concentrations of FSH (OVX compared to OVXE), but it reduced amounts of mRNA for FSHβ subunit independent of other ovarian factors.

The actions of E_2 may be elicited post-transcriptionally instead of through regulation of hypothalamic factors by altering the half-life of the mRNA for gonadotropin subunits in the pituitary. Steroid hormones may synergize with ovarian peptide hormones to alter post-transcriptional processing (through stabilization or destabilization). Activin stabilizes mRNA for FSHβ subunit, and inhibin decreases stability of mRNA for FSHβ subunit [48]. Data from the present experiment indicates that E_2 can affect amount of mRNA for all gonadotropin subunits. Whether this is through transcriptional or post-transcriptional processes still remains to be determined.

Pituitary content of either LH or FSH was not different in any of the three treatment groups. This was presumably due to the relatively short duration of treatment (40 h) compared to that in previous studies. In addition, pituitaries were collected before the preovulatory surge of gonadotropins. Considering both of these factors, secretion of hormones (LH and FSH) did not deplete the pituitary to the extent that differences in stores of the gonadotropins were detected. Collection of pituitaries at earlier time periods (12 or 24 h after $PGF_{2\alpha}$ treatment) may have resulted in different amounts of mRNA for gonadotropin subunits than we observed in the present experiment with pituitary collection at 40 h after $PGF_{2\alpha}$ treatment. The purpose of collecting pituitaries at 40 h after $PGF_{2\alpha}$ was to evaluate amounts of mRNA for gonadotropin subunits at a time when the pituitary had been exposed to E_2 for maximal amounts of time yet before initiation of the preovulatory surge of gonadotropins.

We reject our hypothesis because treatment with E_2, in a pattern typical of what occurs during the follicular phase of the estrous cycle, did not result in enhanced amounts of mRNA for the gonadotropins. Instead, cows of the OVXE group had amounts of mRNA for α and LHβ subunits similar to amounts in cows of the CONT group, whereas cows of the OVX group not treated with E_2 had greater amounts of mRNA for all three subunits than cows of the CONT group. This occurred at the same time that secretion of LH and FSH was greater in cows of the OVX and OVXE groups than in cows of the CONT group. An increased amplitude of LH pulses released from the pituitary results from treating cows with E_2. This results in greater concentrations of LH in circulation. The amount of mRNA for LH subunits is decreased as a result of treatment of cows with E_2. Estradiol, therefore, enhances LH release but suppresses amount of mRNA for LH synthesis in cows. Therefore, during the follicular phase of the bovine estrous cycle when secretion of LH is enhanced, there is not a corresponding increase in amount of mRNA for the gonadotropin subunits in cows.

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