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# Effects of 17β-Estradiol on Distribution of Pituitary Isoforms of Luteinizing Hormone and Follicle-Stimulating Hormone during the Follicular Phase of the Bovine Estrous Cycle

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# Effects of 17β-Estradiol on Distribution of Pituitary Isoforms of Luteinizing Hormone and Follicle-Stimulating Hormone during the Follicular Phase of the Bovine Estrous Cycle[1]

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## ABSTRACT

The objective of this study was to examine the influence of 17β-estradiol ($E_2$) on distribution of LH and FSH isoforms during the follicular phase of the bovine estrous cycle prior to the preovulatory surges of LH and FSH. On Day 16 of the estrous cycle (Day 0 = estrus), intact controls (CONT; n = 4) were treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to induce luteal regression and initiation of the follicular phase. Other cows were also treated with $PGF_{2\alpha}$ and either ovariectomized (OVX; n = 5) or ovariectomized and given $E_2$ implants (OVXE; n = 6) to mimic the pattern of increasing $E_2$ concentrations during the follicular phase of the estrous cycle. Pituitaries were collected 40 h after treatment with $PGF_{2\alpha}$ or ovariectomy (0 h). Aliquots of pituitary extracts were chromatofocused on pH 10.5–4.0 gradients. The LH resolved into thirteen isoforms (designated A-L and S, beginning with the most basic form) while FSH resolved into nine isoforms (designated I-IX, beginning with the most basic form). The percentage of LH as isoform F (elution pH = 9.32 ± 0.01) was greater ($p < 0.05$) in the OVX group (48.5%) than in the OVXE group (45.0%). LH isoforms I (elution pH = 6.98 ± 0.01) and J (elution pH = 6.48 ± 0.01) were more abundant ($p < 0.05$) in cows from the OVXE (2.3 and 5.8%, respectively) than the OVX group (1.4 and 3.7%, respectively). Distribution of LH isoforms in cows from the three groups did not differ ($p > 0.10$). Distribution of FSH isoforms were similar ($p > 0.05$) among all groups. In summary, removal of the ovary (OVX) resulted in a slight increase in percentage of the basic LH isoform F, while removal of the ovary and administration of $E_2$ (OVXE) in a pattern that mimicked increasing concentrations of $E_2$ during the follicular phase of the estrous cycle resulted in a slight increase in the percentage of acidic LH isoforms (I and J). There was no influence of ovariectomy or treatment with $E_2$ on distribution of FSH isoforms in the pituitary. Thus, gonadotropin heterogeneity does not appear to change significantly during the follicular phase of the bovine estrous cycle.

## INTRODUCTION

The pituitary gonadotropins, LH and FSH, from several species that exhibit microheterogeneity exist as families of charged isoforms or "isohormones" [1–5]. These variant isoforms differ in their isoelectric points (pI), receptor binding, and biological potencies [6–9], and they result in part from heterogeneity in their oligosaccharides [10, 11]. All isoforms of the rat [4, 8, 9], sheep [5], cow [12], and human [13] LH possess significant bioactivity as measured by a rat interstitial cell bioassay. Generally in most species, the most basic LH isoforms have the greatest bioactivity, whereas the more acidic LH isoforms have lower bioactivity [4, 8, 9, 13, 14], with the exception of the ovine and bovine species, in which the mid-alkaline LH isoform of F has greater bioactivity than either the more alkaline or acidic LH isoforms [5, 12]. Similarly, receptor binding activity of the hamster [15] and rat [16] FSH isoforms declines as the pI decreases. However, more acidic rat FSH isoforms display a longer circulatory half-life in vivo [17].

The distribution of the LH and FSH isoforms in the pituitary is altered by gonadal steroids. Distribution of the isoforms in the pituitary varies during stage of the reproductive cycle in rodents [18–20] and primates [21], and differs as a result of castration in rodents [3, 9, 14, 15], sheep [5], and cattle [12] or as a result of steroid hormone replacement in sheep [5] and cattle [12]. Interestingly, the amounts of biologically potent basic isoforms of LH increase during the preovulatory surge of gonadotropins in the rat [18] and during the midcycle surge of gonadotropins in primates [21, 22], whereas the amounts of biologically less potent, more alkaline or acidic rat LH isoforms remain constant throughout the entire estrous cycle [18]. These results are consistent with those of earlier studies where an increase in bioactivity of LH in circulation was observed during the preovulatory surge of LH in rats [23, 24] and during the midcycle gonadotropin surge in monkeys [25–27].

It has been reported that pattern and distribution of ovine and bovine LH [28] and rat [17], hamster [19], and human FSH [29] isoforms released from the anterior pituitary in vitro generally reflect the pituitary intracellular isoforms. According to these previous studies, changes in the endocrine milieu of animals not only alter the quantity of pituitary gonadotropins but also the quality of gonadotropins.

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Thus, changes in the profile of gonadotropin isoforms could have a role in regulating reproductive function [30].

In general, the follicular phase of the bovine estrous cycle is a transition from luteal regression to the preovulatory surge of gonadotropins. The follicular phase of the estrous cycle is accompanied by increasing concentrations of 17β-estradiol ($E_2$) after a decline in concentrations of progesterone [31, 32]. During this period, mean concentrations of LH increase linearly [33, 34], with an increase in frequency [34] and amplitude of LH pulses [35]. The preovulatory surge of gonadotropins occurs 50–70 h after treatment of cows with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [36, 37] or after the initiation of sequential $E_2$ implantation in ovariectomized cows [32]. The distribution of intrapituitary gonadotropin isoforms has not been evaluated during the follicular phase of the bovine estrous cycle, when concentrations of $E_2$ and LH are dramatically increased. The advantages of utilizing the cow as a model are the relatively large anterior pituitary glands, well-characterized reproductive cycles, and experimentally manipulatable endocrine systems. Thus, the pattern of $E_2$ in circulation can be experimentally manipulated by the exogenous source of $E_2$ to mimic the endogenous pattern during the follicular phase of the bovine estrous cycle.

Therefore, we hypothesized that different patterns of gonadotropin isoforms would exist during the preovulatory surge of gonadotropins as a result of the increased concentrations of $E_2$ that occur during the follicular phase of the estrous cycle. The change in pattern of gonadotropin isoforms may provide an appropriate stimulus for ovulation. If this is the case, the acute increase in concentrations of $E_2$ during the follicular phase of the bovine estrous cycle or increasing exogenous $E_2$ in the ovariectomized animal should alter the distribution of LH and FSH isoforms in the pituitary prior to the preovulatory surge of gonadotropins. This should be different from the case in the ovariectomized animal with no gonadal steroids in circulation. In addition, if $E_2$ is the only factor regulating distribution of LH and FSH isoforms, the follicular phase intact animal and the ovariectomized animal receiving exogenous $E_2$ should have similar patterns of isoforms.

## MATERIALS AND METHODS

### *Experimental Protocol and Collection of Pituitaries*

The experimental protocol is described in detail in a companion paper [38]. Briefly, the estrous cycles of fifteen mature beef cows (2–7 yr of age) were synchronized by two injections of $PGF_{2\alpha}$ (Lutalyse® Sterile Solution; The Upjohn Co., Kalamazoo, MI) 11 days apart. On Day 16 of the following estrous cycle (Day 0 = estrus), controls (CONT; n = 4) were treated with $PGF_{2\alpha}$ (Hour 0) to induce luteal regression and initiation of the follicular phase. Other cows were also treated with $PGF_{2\alpha}$ and either ovariectomized (OVX; n = 5) or ovariectomized and treated with $E_2$ implants (OVXE; n = 6) [35] at 0, 10, 15, 20, and 30 h (ovariectomy = 0 h) to mimic the pattern of increasing $E_2$ concentrations during the follicular phase of the bovine estrous cycle. The $E_2$ (Sigma Chemical Co., St. Louis, MO) was administered via polydimethylsiloxane intravaginal implants (3.35 mm i.d. × 4.65 mm o.d. × 13.5 cm; Dow-Corning, Midland, MI) filled with $E_2$. Pituitaries were collected 40 h after injection of $PGF_{2\alpha}$ or ovariectomy. Immediately after removal, anterior and posterior lobes of each pituitary were separated, and the anterior lobe was hemisected. Pituitary tissues were then frozen in liquid nitrogen and stored at −70°C until extracted.

### *Tissue Extraction*

Frozen pituitary tissue was homogenized for 30 sec 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) with 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 chromatofocused.

### *Chromatofocusing*

Aliquots of pituitary extract were subjected to chromatofocusing on pH 10.5–4.0 gradients. A 0.5-ml aliquot (50 mg tissue equivalent) was desalted by flow dialysis against water using 6000–8000 $M_r$ cutoff membranes (Spectra/Por 1; Spectrum Medical Industries, Inc., Los Angeles, CA). Desalted extracts were supplemented to 2% (vol/vol) with Pharmalyte 8–10.5 (pH 7.0; Pharmacia/LKB Biotechnology Inc., Piscataway, NJ). Two milligrams each of cytochrome C and myoglobin were added to the sample, and the mixture was applied to a 10-ml (0.7 × 26 cm) column (Kontes, Vineland, NJ) of PBE-118 resin (Pharmacia/LKB Biotechnology Inc.) previously equilibrated in 25 mM triethylamine (pH 11.0). The pH gradient was developed (5 ml/h) with Pharmalyte 8–10.5 diluted 1:45 with distilled water and adjusted to a pH of 7.0 with 6 N HCl at 5 ml/h. Seventy-five 1.5-ml fractions were collected to obtain a stable plateau near pH 7.0. The elution buffer was then switched to Polybuffer 74-HCl (Pharmacia/LKB Biotechnology Inc.) diluted 1:8 with distilled water and adjusted to a pH of 4.0 with 6 N HCl. An additional seventy 1.5-ml fractions were collected to reach a stable lower limiting pH of 4.0. Proteins bound to the column at this lower limiting pH were eluted with 1.0 M NaCl and collected as an additional twenty 1.5-ml fractions. The fractions were neutralized by addition of 0.15 ml of 1.1 M Tris buffer (pH = 7.0). Columns were re-equilibrated between samples with at least 50 column volumes of triethylamine. All buffers were thoroughly degassed before use and contained 1% glycerol. Recovery of immunoreactive LH and FSH from the columns averaged 89% and 85%, respectively.

### *RIAs*

Concentrations of LH in pituitary extracts and chromatofocusing fractions were determined by RIA [39] as validated in our laboratory [40]. Intra- and interassay coefficients of variation were 3.0% and 13.2%, respectively, and assay sensitivity was 29.8 pg/ml. Concentrations of FSH in pituitary extracts and chromatofocusing fractions were determined by RIA [40, 41]. Intra- and interassay coefficients of variation were 2.4% and 12.7%, respectively, and assay sensitivity was 74.1 pg/ml.

### *Statistical Analysis*

The effects of treatment on pituitary hormone characteristics were analyzed by one-way analysis of variance [42] according to the General Linear Models procedure of SAS [43]. Differences in treatment means were detected by Duncan's New Multiple Range test [42]. Percentage values were subjected to arc sine transformations (arc sine of the square root of the proportion corrected to an angle between 0 and 90°) before analysis. A probability of less than 0.05 was considered statistically significant.

## RESULTS

### *Concentrations of LH, FSH, and $E_2$ in Circulation*

Concentrations of these hormones are described in detail in the companion paper [38]. Briefly, concentrations of $E_2$ in the OVX cows remained low after ovariectomy through 40 h, at the end of which pituitaries were collected. Cows from the CONT and OVXE groups had increasing concentrations of $E_2$ in circulation during the 40-h period, and the pattern of increasing $E_2$ did not differ between these groups. Therefore, the experimental protocol for administration of $E_2$ implants (OVXE) used in the present study effectively mimicked the pattern of increasing concentrations of $E_2$ in intact animals (CONT) during the follicular phase of the bovine estrous cycle [38].

Mean concentrations of LH were greater ($p < 0.05$) in cows from the OVXE group during 40 h of blood collections (see companion paper [38] for experimental protocol for blood collections) after injection of $PGF_{2\alpha}$, ovariectomy, and/or initiation of $E_2$ implants compared to LH concentrations in the CONT group. Mean concentrations of LH were similar ($p > 0.10$) among cows from the OVX and CONT groups during 40 h of blood collections. Mean concentrations of FSH were greater ($p < 0.01$) in cows from OVX and OVXE groups during 40 h of blood collections after initiation of the treatment compared to those from the CONT group, where mean concentrations of FSH remained similar ($p > 0.10$) in cows from the OVX and OVXE groups. Mean pituitary weights and pituitary contents of LH and FSH were similar ($p > 0.10$) among all groups [38].

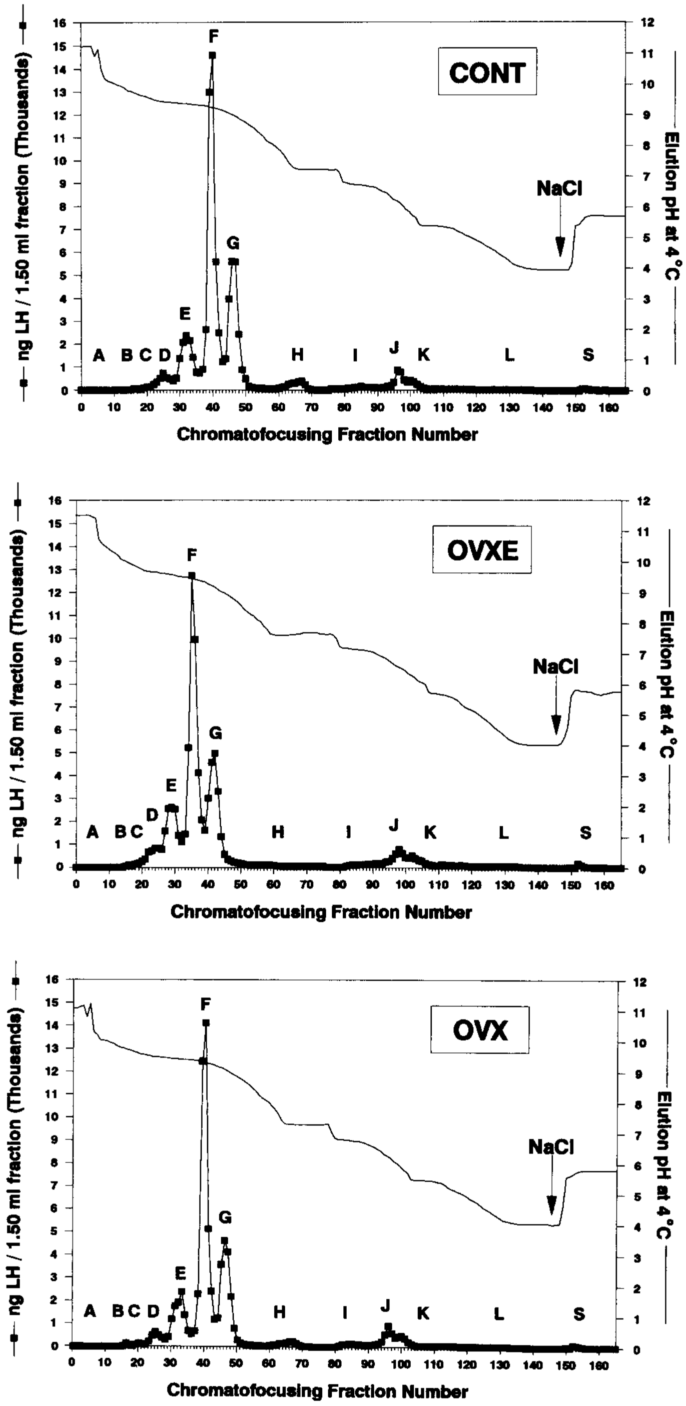


FIG. 1. Representative chromatofocusing elution profile of immunoreactive LH in pituitary. A 0.5-ml aliquot of pituitary extract (50-mg tissue equivalents) was chromatofocused on a pH 10.5–4.0 gradient. Each peak was coded with a letter beginning with the most basic form (A through L). Proteins bound to column at lower limiting pH were eluted with 1.0 M NaCl and designated peak S. Pituitaries were collected 40 h after treatment with $PGF_{2\alpha}$, ovariectomy, or initiation of $E_2$ treatment (0 h).

### *LH Isoforms*

The LH in each pituitary extract resolved into thirteen isoforms when chromatofocused over a pH 10.5–4.0 gra-

TABLE 1. Distribution of bovine LH isoforms in anterior pituitary tissue.[1]

| Isohormone | (Elution pH) | Treatments[2] | | | Pooled SEM |
|---|---|---|---|---|---|
| | | CONT | OVXE | OVX | |
| A | (11.15 ± 0.03) | <0.1 | <0.1 | <0.1 | 0.02 |
| B | (10.09 ± 0.01) | 0.3 | 0.2 | 0.3 | 0.03 |
| C | (9.68 ± 0.01) | 0.5 | 0.7 | 0.9 | 0.16 |
| D | (9.54 ± 0.01) | 2.9 | 2.6 | 3.3 | 0.40 |
| E | (9.44 ± 0.01) | 11.5 | 11.8 | 11.6 | 0.71 |
| F | (9.32 ± 0.01) | 46.8[ab] | 45.0[b] | 48.5[a] | 0.85 |
| G | (8.78 ± 0.03) | 23.1 | 22.4 | 23.2 | 0.94 |
| H | (7.51 ± 0.02) | 2.6 | 2.4 | 1.8 | 0.36 |
| I | (6.98 ± 0.01) | 1.8[ab] | 2.3[a] | 1.4[b] | 0.21 |
| J | (6.48 ± 0.01) | 5.4[ab] | 5.8[a] | 3.7[b] | 0.58 |
| K | (5.48 ± 0.02) | 3.7 | 4.9 | 4.0 | 0.65 |
| L | (4.27 ± 0.02) | 0.9 | 1.2 | 0.8 | 0.14 |
| S | (< 4.0) | 0.5 | 0.7 | 0.5 | 0.07 |

[1]Mean percentages for each isoform.

[2]CONT: control cows were treated with $PGF_{2\alpha}$ (n = 4); OVXE: ovariectomized and treated with $E_2$ (n = 6); OVX: ovariectomized (n = 5); pituitaries collected 40 h after treatment with $PGF_{2\alpha}$, ovariectomy or initiation of $E_2$ treatment (0 h).

[a,b]Means identified by different superscript letters within rows differ ($p < 0.05$).

dient (Fig. 1 and Table 1). The isoforms of LH were coded with letters A-L and S, beginning with the most basic form. Twelve isoforms eluted in the separating pH range of the column, and the thirteenth isoform was bound to the column at the lower limiting pH. The predominant LH isoforms that eluted in the basic pH range were F and G, and those forms accounted for at least 60% of the immunoreactive LH in the pituitary.

Differences existed in distribution of LH isoforms in pituitaries among cows from the OVX and OVXE groups. The percentage of LH as isoform F (elution pH = 9.32 ± 0.01) was greater ($p < 0.05$) in the OVX group (48.5%) than in the OVXE group (45.0%). LH isoforms I (elution pH = 6.98 ± 0.01) and J (elution pH = 6.48 ± 0.01) were more abundant ($p < 0.05$) in cows from the OVXE (2.3 and 5.8%, respectively) than from the OVX group (1.4 and 3.7%, respectively). Distribution of LH isoforms in cows from the CONT group did not differ ($p > 0.10$) from either the OVX or OVXE groups. The quantity of each LH isoform (data not shown) and the pituitary content of LH [38] were not different ($p > 0.10$) among groups.

### *FSH Isoforms*

The FSH in each pituitary extract resolved into nine isoforms when chromatofocused on a pH 10.5–4.0 gradient (Fig. 2 and Table 2). Isoforms of FSH were coded with Roman numerals I-IX, beginning with the most basic form. Seven isoforms eluted in the separating pH range of the column (I-VII), while VIII and IX were bound to the column at the lower limiting pH of 4.0. One isoform of FSH, I, eluted in the basic pH range (pH > 7.0), while all other isoforms of FSH eluted in the acidic pH range (pH 7.0–4.0). The distribution of FSH among its isoforms was not different ($p > 0.05$) among treatments, and the quantity of each FSH isoform (data not shown) and the pituitary content of FSH [38] were not different ($p > 0.10$) among treatments.

## DISCUSSION

The distribution of gonadotropin isoforms is thought to reflect the endocrine status of the animal. The experimental protocol used in the present study provided a means for examining the effects of $E_2$ on distribution of gonadotropin isoforms during the follicular phase of the bovine estrous cycle. Cows from the OVX group were used as a negative control (no gonadal steroids), while cows in the OVXE group were administered $E_2$ implants in a manner that mimicked the pattern of increasing concentrations of $E_2$ during the follicular phase of the bovine estrous cycle. Comparable concentrations of $E_2$ were observed in the CONT and OVXE groups. Furthermore, none of the animals initiated the preovulatory (CONT) or preovulatory-like (OVXE) LH surge before collection of pituitaries in the present study [38].

Gonadotropin isoforms were analyzed by chromatofocusing on pH 10.5–4.0 gradients. These pH gradients were extended below 7.0 to 4.0, which allowed for characterization of both LH and FSH isoforms with a single chromatographic separation for each pituitary extract [12]. The LH in extracts of bovine pituitaries resolved into thirteen isoforms when chromatofocused on pH 10.5–4.0 gradients [12]. The distribution pattern of LH isoforms observed in the present study corresponded closely to previous results obtained for sheep [5] and cattle [12, 28, 44] in the basic portion of the gradient (pH 10.5–7.0), and also corresponded to results obtained for sheep [45] and cattle [12] in the acidic portion of the gradient (pH 7.0–4.0). The predominant LH isoforms that eluted in the basic pH range were F and G, and those forms accounted for at least 60% of the immunoreactive LH in the pituitary. The present results were thus similar to those observed in the previous study using cattle [12].

The FSH in pituitary extracts resolved into nine isoforms when chromatofocused on pH 10.5–4.0 gradients. The distribution pattern of FSH isoforms observed in the present study corresponded closely to previous results obtained for cattle [12]. Seven isoforms eluted in the separating pH range of the column, and two were bound to the column at the lower limiting pH. We previously reported FSH isoform VIII in bovine pituitary extracts, which was not tightly bound to the column and eluted immediately after 1 M NaCl was applied to the column [12]; however, isoform VIII constituted less than 1% of the immunoreactive FSH in all pituitary extracts in the present study. This probably was the result of the extended plateau at the lower limiting pH of 4.0 in the present study. The most acidic isoform, IX, was tightly bound to the column and eluted as the 1 M NaCl front reached the bottom of the column; this isoform accounted for at least 23% of the immunoreactive FSH in the pituitary. Isoform IX may contain multiple components that could be resolved by an elution pH of less than 4.0 or by alternate procedures.

Analysis of isoforms of ovine FSH by chromatofocusing on pH 7.0–4.0 gradients also yields nine isoforms, in which the most basic ovine FSH isoform elutes as a flow-through peak indicating an elution pH of greater than 7.0 [46]. Similar to our previous study [12], in the present study the most basic isoform of bovine FSH, I, was observed as a distinct peak in cows of the OVX and OVXE groups. Interestingly, this peak was not observed in the intact (CONT) cows. However, neither the percentage nor quantity of FSH isoform I differed among treatments.

We hypothesized that a different composition of gonadotropin isoforms would exist in the pituitary during the period prior to the preovulatory surge of gonadotropins, which would provide an appropriate stimulus for ovulation in the bovine. The distributions of LH isoforms in the pituitaries of all groups were similar in that none of the thirteen observed isoforms constituted biologically meaningful changes in the distribution of immunoreactive LH. However, differences between cows from the OVX and OVXE groups in isoforms F, I, and J were observed. A greater percentage of LH was present as isoform F in the OVX than in the OVXE group (48.5% and 45.0%, respectively), whereas the less prevalent isoforms I and J were more abundant in cows from the OVXE than the OVX group (1.4 and 2.3%; 3.7 and 5.8%, respectively). The distribution of all LH isoforms in cows from the CONT group was not different from that in either the OVX or OVXE groups. Nonetheless, differences in the distribution of LH isoforms in the pituitary among treatments were relatively small.

It has been reported that castration significantly increased the percentage of basic isoforms in rats [47] and sheep [5, 48], and castration or ovariectomy followed by treatment with $E_2$ resulted in a significantly greater percentage of acidic isoforms of LH in sheep [5] and cattle [12]. The experimental protocol used in the present study allowed for manipulation of the pattern of $E_2$ in circulation of animals in a short-term period analogous to the hormonal milieu observed during the follicular phase of the

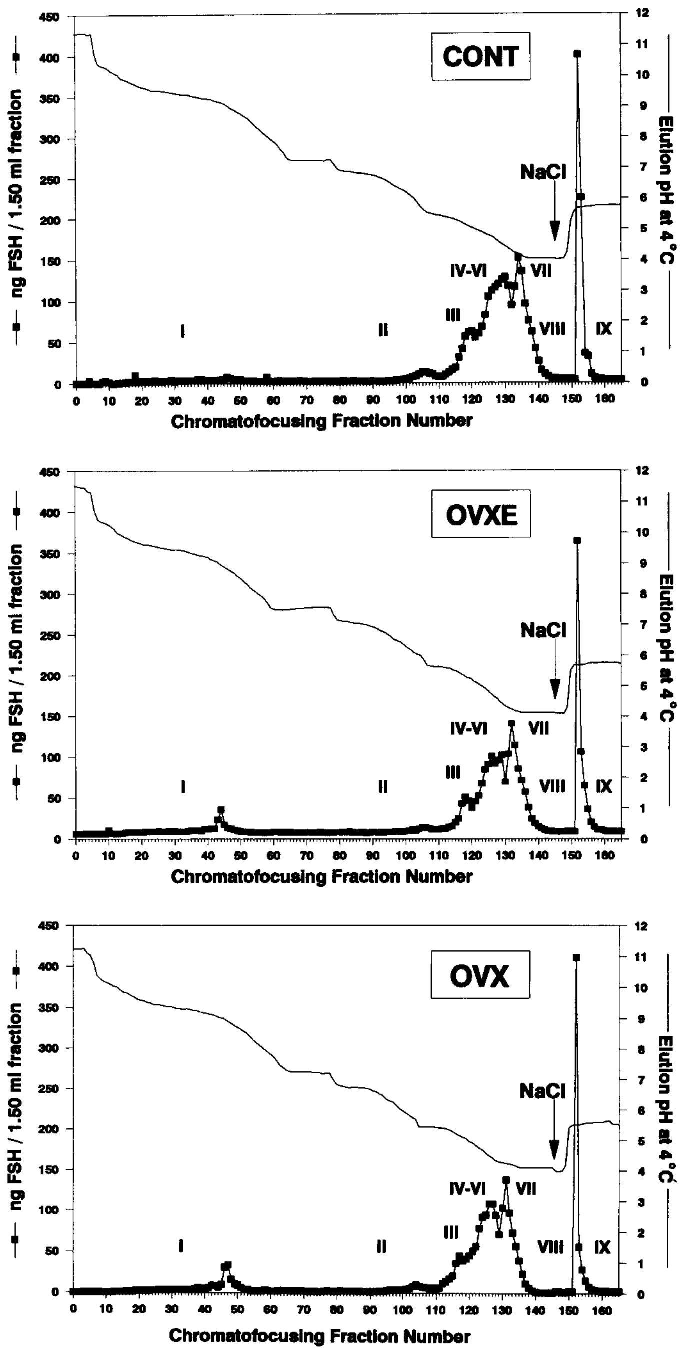


FIG. 2. Representative chromatofocusing elution profile of immunoreactive FSH in the pituitary. A 0.5-ml aliquot of pituitary extract (50-mg tissue equivalents) was chromatofocused on a pH 10.5–4.0 gradient. Each peak was coded with Roman numerals beginning with the most basic form (I through VII). Proteins bound to column at lower limiting pH were eluted with 1.0 M NaCl and designated peaks VIII and IX. Pituitaries were collected 40 h after treatment with $PGF_{2\alpha}$, ovariectomy, or initiation of $E_2$ treatment (0 h).

TABLE 2. Distribution of bovine FSH isoforms in anterior pituitary tissue.[1]

| Isohormone | (Elution pH) | Treatments[2] CONT | OVXE | OVX | Pooled SEM |
|---|---|---|---|---|---|
| I | (9.07 ± 0.003) | 9.5 | 8.6 | 8.8 | 0.88 |
| II | (6.46 ± 0.009) | 5.5 | 5.9 | 3.8 | 0.93 |
| III | (5.30 ± 0.023) | 10.2 | 8.0 | 8.6 | 0.85 |
| IV–VI | (4.66 ± 0.017) | 29.6 | 29.9 | 33.4 | 1.34 |
| VII | (4.06 ± 0.018) | 21.3 | 22.9 | 21.9 | 1.09 |
| VIII | (4.59 ± 0.026) | 0.6 | 0.9 | 0.6 | 0.17 |
| IX | (< 4.0) | 23.3 | 23.8 | 23.0 | 1.59 |

[1]Mean percentages for each isoform.
[2]CONT: control cows were treated with $PGF_{2\alpha}$ (n = 4); OVXE: ovariectomized and treated with $E_2$ (n = 6); OVX: ovariectomized (n = 5); pituitaries collected 40 h after treatment with $PGF_{2\alpha}$, ovariectomy or initiation of $E_2$ treatment (0 h).

estrous cycle in the cow. This experimental protocol resulted in slight shifts in the distribution of LH isoforms. However, the differences observed in the present study were not of the same magnitude as reported previously in rats [47], sheep [5, 48] and cattle [12]. It is likely that long-term gonadectomy has a more pronounced effect on distribution of LH isoforms. The increase in the percentage of either basic or acidic isoforms observed in previous studies [5, 12, 47, 48] might have developed over a longer period of time, whereas short-term changes in the hormonal milieu would be required to change the pattern of LH isoforms during the estrous cycle. Therefore, the experimental protocols that include long-term gonadectomy and/or steroid hormone replacement, which resulted in significant changes in the pattern of gonadotropin heterogeneity, might not represent a normal physiological state of the animal.

Furthermore, similar distributions of LH isoforms were observed throughout sexual maturation in heifers [12], and no major changes in pattern of LH isoforms occurred between follicular and luteal phases of ewes [49]. These studies with intact animals have disclosed minor changes in LH heterogeneity, at least in sheep [49] and cattle [12]. Therefore, the present and previous studies indicate that there are not significant changes in the distribution of LH isoforms in the pituitary of intact animals with functional gonads during the time when peripheral concentrations of LH exhibit markedly divergent patterns. The changes in the distribution of FSH isoforms were less pronounced than changes in LH isoforms during sexual maturation in heifers [12] and during the follicular phase of the estrous cycle just prior to the preovulatory surge of gonadotropins in cows (present study). In addition, Prewitt et al. [50] recently developed an in vivo model to determine the half-lives of purified ovine LH isoforms (B, C, D, E, and F) in circulation and assessed their abilities to stimulate testicular steroidogenesis in rams. Results were particularly interesting, because the LH isoforms examined had similar half-lives and biological potencies in vivo.

A variety of other studies indicated that changes in gonadotropin heterogeneity occur during the estrous cycle of rodents [18–20] and during the menstrual cycle of primates [21, 22]. If the pattern of gonadotropin isoforms changes significantly during normal reproductive cycles in some species (rodents and primates) but not in others (sheep and cattle), this component of endocrine regulation may not be universal across species.

Results of the present study indicated that there are relatively small changes in the pattern of isoforms in the pituitaries of sheep and cattle with functional gonads; nevertheless, during the follicular phase of the estrous cycle, meaningful changes in the distribution of gonadotropin isoforms did not occur when maximum concentrations of $E_2$ were observed. In contrast, biologically potent basic isoforms of LH increase during the preovulatory surge in rats [18] and during the midcycle surge in primates [21, 22], while biologically less potent, more alkaline or acidic rat LH isoforms remain constant throughout the entire estrous cycle [18]. These results may indicate that there are species differences in gonadotropin heterogeneity. Therefore, changes in LH and FSH heterogeneity do not appear to be a significant mechanism in regulating reproductive function in sheep and cattle.

In summary, removal of the ovary (OVX) slightly increased the percentage of the basic LH isoform F over that in the OVXE group, whereas removal of the ovary and administration of $E_2$ in a pattern that mimicked the increase in concentrations of $E_2$ during the follicular phase of the bovine estrous cycle (OVXE) resulted in a slight increase in the percentage of the acidic LH isoforms (I and J) over the percentage in the OVX group. However, distribution of LH isoforms in intact cows (CONT) during the follicular phase of the estrous cycle did not differ from that of either the OVX or OVXE groups. There was no influence of ovariectomy or treatment with $E_2$ on distribution of FSH isoforms in the pituitary. Therefore, we reject our hypothesis because a different distribution pattern of gonadotropin isoforms does not exist in the pituitary during the follicular phase of the bovine estrous cycle just prior to the preovulatory surge of gonadotropins.

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