

Sex-related variations in Bovine LH isoforms revealed by chromatofocusing

Running title: LH isoforms in steers and heifers by chromatofocusing

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Abstract

Luteinizing hormone (LH) is a gonadotropin found in both blood and pituitary tissue, and it plays a critical role in reproductive regulation in ruminants and other species. Distinct isoforms produced by post-translational modifications have been previously reported in the pituitary gland of ovariectomized cows and castrated bulls, treated with progesterone and estradiol, respectively. However, it remains unclear whether variations exist in the molecular forms of this hormone in intact prepubertal cattle. Anterior pituitary extracts of steers and heifers underwent chromatofocusing across a pH gradient (10–3.5), allowing classification of the eluted hormone forms into basic (pH \geq 7.5), neutral (pH 7.4–6.5), and acidic fractions (pH \leq 6.4) and on the basis of distinct isoforms (12 peaks of A–K). Radioimmunoassay was used to establish the immunological activity of LH isoforms. Overall, basic LH isoforms were present at higher concentrations than neutral or acidic forms in both steers and heifers. However, in steers the analysis by distinct isoforms of LH exhibited a slightly greater proportion of acidic isoforms J (pH 3.4–5.4) and neutral isoform G (pH 6–6.9) compared to heifers. In contrast, heifers showed slightly higher proportions of basic isoforms B (pH 9.2–9.9) and E (pH 7.8–8.7), neutral isoform F (pH 7.1–7.7), and acidic isoforms I (pH 5.7–6.4) and K (pH 3.3–4.9) than steers. Notably, the overall pituitary LH patterns observed in both groups were consistent with previously reported profiles, suggesting that these patterns are relatively stable despite physiological variation. Nevertheless, the subtle differences identified between sexes suggest that sex may influence the synthesis of LH isoforms at the pituitary level.

Keywords: Luteinizing hormone; Isoforms; Steers; Heifers; Pituitary gland.

Study contribution

The main contribution of this study is the demonstration that the overall patterns of hormone distribution in the pituitary of intact prepubertal cattle align with previous reports, indicating resilience to physiological variations. Nonetheless, subtle sex-related differences were identified: steers showed a slightly higher proportion of acidic and neutral forms, whereas heifers displayed a greater abundance of specific isoforms, including basic (B and E), neutral (F), and acidic (K and I) fractions. These findings indicate that sex may influence both the synthesis and post-translational modification of this hormone, offering new insights into its regulatory mechanics in ruminant reproduction.

Introduction

In domestic animals, reproductive function is primarily controlled by pituitary-derived gonadotropins, namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones are essential for the control of ovarian activity, and variations in their circulating levels are closely associated with reproductive status.⁽¹⁾ Although the fundamental role of gonadotropins is conserved across species, intrinsic differences in reproductive rates are observed among different species and breeds. These variations may be explained at least in part by species-specific differences and similarities in gonadotropin structure, regulation, and physiological responsiveness.

The endocrine system relies on hormones to orchestrate body functions. In the case of protein hormones, it is now well established that variants can arise due to gene mutations.^(2–4) Additionally, isoforms may result from alternative splicing and/or post-translational modifications.⁽⁵⁾ These hormone variants and isoforms often exhibit distinct structural, functional, and immunochemical properties both within individuals and across animal populations and species. One such hormone is LH, a heterodimeric glycoprotein,^{(6–}

⁸⁾ that plays a critical role in regulating sexual and reproductive functions. Beyond its well-known role, it is now widely recognized that at least three LH variants arise from gene mutations,^(9, 10) with some of these variants potentially contributing to pathological conditions.⁽¹¹⁾

In addition to these genetic variants, LH also exists in multiple isoforms, which are less frequently acknowledged. These isoforms—classified as basic, neutral, or acidic—are generated by the addition of distinct oligosaccharide side chains to the LH molecule.^(6, 8, 12, 13) LH isoforms have been identified in pituitary extracts,^(14–17) and serum samples,^(16, 18, 19) across various mammalian species. Each isoform exhibits distinct physicochemical and immunochemical properties, as well as unique biological effects.^(20–24) For instance, in female cattle, acidic LH isoforms become more abundant in the pituitary gland,^(14, 15) and circulation,⁽¹⁸⁾ as estradiol levels rise, whereas basic LH isoforms predominate when progesterone levels increase.⁽¹⁷⁾

Similarly, in castrated male rats, sheep, and cattle, an increase in pituitary basic LH isoforms has been reported,^(14, 25–28) while testosterone replacement leads to a shift toward a more acidic isoform profile.^(28–31) Given these findings, it is plausible that LH isoforms may contribute to the regulation of reproductive function. Based on this premise, the present study aimed to investigate potential sex-related differences in LH synthesis before the onset of puberty by analyzing and comparing the molecular heterogeneity of this hormone in the pituitary tissue of intact prepubertal females and males.

Materials and methods

Ethical statement

The study did not require review by the Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México (FMVZ-UNAM) Ethics Committee since the material was collected from a state slaughterhouse.

Animal selection and postmortem evaluation

Animals selected for pituitary gland collection met specific morphological and anatomic criteria. Heifers were identified by a finer body conformation and less developed musculature. Postmortem examination confirmed the presence of a uterus and ovaries with developing follicles and minimal corpora lutea. In contrast, steers exhibited more developed musculature, particularly in the cervical and shoulder regions, and postmortem examination confirmed the presence of seminal vesicles, prostate, and penis.

Pituitary gland collection

The pituitary glands were collected from steers (n = 4) and heifers (n = 4) were collected at a local abattoir formerly known as “Ferreria slaughterhouse” located in the City of México, México. Each pituitary gland was collected after animal sacrifice within 60 min. Each gland was meticulously dissected and washed with physiological saline, promptly transported in containers kept at 4°C to the laboratory, and then stored at -70°C.

Pituitary homogenate

Following pituitary dissection, the anterior lobe was homogenized and centrifuged under conditions previously described.^(16, 17) The resulting supernatant was fractionated into 2 mL aliquots and subsequently lyophilized.

LH chromatofocusing

Prior to chromatofocusing, each anterior pituitary lobe extract was resuspended in 5 mL of 0.01 M ammonium bicarbonate buffer (pH 7.5) and dialyzed for 48 h against the same buffer using a dialysis membrane with a molecular weight cut-off (MWCO) of 12–14 kDa. The dialyzed extract was lyophilized. Chromatofocusing was carried out using 50 mg of protein from each extract following a previously adapted procedure.⁽¹⁸⁾ Briefly, protein samples were loaded onto a PBE-118 ion-exchange column, pre-equilibrated with 0.025 M triethylamine-HCl buffer (pH 11.0) and eluted through a continuous pH gradient from 10.5 to 3.5 using Pharmalyte and Polybuffer 74 solutions. Fractions were collected and neutralized according to their pH. The pH and absorbance at 280 nm were recorded throughout the procedure. All neutralized fractions were stored and subsequently analyzed by radioimmunoassay.

Radioimmunoassay for pituitary samples

The immunoreactive concentration of LH in each fraction collected during the chromatofocusing analysis of anterior pituitary extracts was determined in a heterologous radioimmunoassay (RIA) for bovine LH, performed with a 120-hour incubation at 4°C.⁽¹⁷⁾ The RIA buffer was composed of 0.05 M phosphate at pH 7.4, supplemented with 0.14 M sodium chloride and 0.01 % bovine serum albumin (BSA).

The assay employed ovine LH (oLH), as the reference standard (NIDDK-oLH-I-2, National Institute of Diabetes and Digestive and Kidney Diseases). The radiolabeled tracer was generated by iodination of oLH with [¹²⁵I] sodium via the IODO-GEN method.⁽³²⁾ The standard curve range and the primary antibody, anti-NIDDK-oLH 26 raised in rabbit, were used at a working dilution of 1:80 000 in normal rabbit serum (diluted 1:1 600), following a previously described protocol.⁽¹⁷⁾ Bound and free hormone fractions were

separated following a previously validated protocol.^(16, 17) Briefly, 1 mL of 0.05 M phosphate-buffered saline (PBS, pH 7.4) supplemented with 10 µL/mL of *Staphylococcus aureus* protein A (Pansorbin®) was added to each reaction tube, followed by centrifugation at 1 500 × *g* for 15 min at 4°C. Samples were analyzed at dilutions of 1:20, 1:40, 1:80, and 1:400, under the same conditions as the standard curve. The assay sensitivity was 0.25 ng per tube and intra- and inter-assay coefficients of variation were 2 and 3.5 %, respectively.

Evaluation of LH isoform activity

Following chromatofocusing and RIA analysis, the LH-like immunoactivity of the isolated isoforms was evaluated by comparing their displacement to that of the reference standard NIDDK-oLH-I-2 using the same radioimmunoassay protocol. Each protein peak was dialyzed (12–14 kDa MWCO membranes) for 48 h at 4°C, with deionized water replaced every 8 h. After dialysis, the proteins were lyophilized, reconstituted in PBS, and quantified using the Bradford method⁽³³⁾ with BSA (Albumin concentrate 20 %, Immunochemical Products Ltd.) as the standard.

Parallelism between the standard and each LH isoform was assessed by comparing their dose-response curves. The relative concentration of each isoform was estimated by determining the effective concentration required to displace 50 % of the radiolabeled hormone (EC_{50}), calculated using logit–log linear regression.⁽³⁴⁾ The similarity of slopes and correlation coefficients between the standard and each isoform curve was used as a criterion for parallelism (**Table 1**). All assays were performed in quintuplicate. A representative parallelism test for major LH isoforms is shown in **Figure 1**.

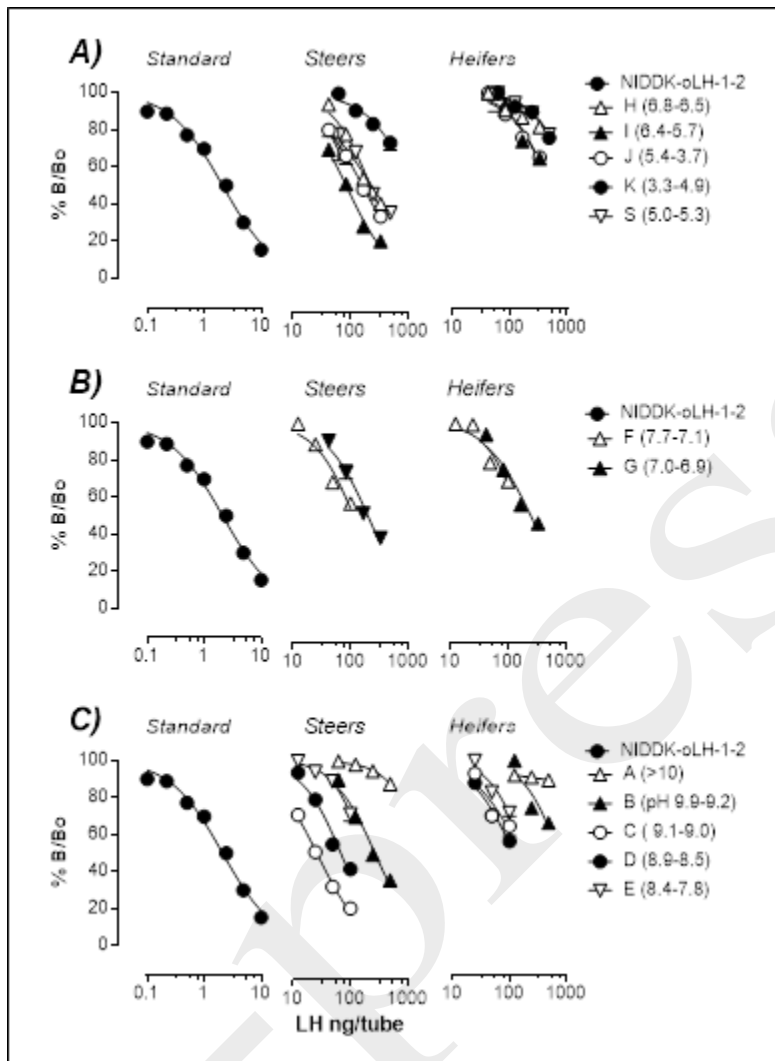


Figure 1. Dose-response curves for LH isoforms in pituitary extracts from steers and heifers. (A) Reference standard (NIDDK-oLH-1-2) and acidic isoforms (H–K). (B) Standard and neutral isoforms (F, G). (C) Standard and basic isoforms (A–E). Each curve depicts the percentage of tracer binding (% B/Bo) as a function of the logarithmic concentration (ng/tube).

Statistical analysis

For statistical analyses, LH isoforms were categorized according to their elution pH range and specific elution pH. The resulting proportions were transformed using the arcsine square root transformation to stabilize variances and then analyzed by one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's post hoc test was applied to identify pairwise differences between groups. All statistical procedures were conducted using SAS software (Statistical Analysis System, Cary, NC, USA). A p -value of less than 0.05 was considered statistically significant. Two-tailed tests, unless otherwise indicated. For the analysis of LH isoform profiles, the methodology was updated to incorporate a modeling approach that is more consistent with the discrete nature of the molecular isoforms.

1. Preprocessing and normalization

Prior to the analysis, LH concentrations were normalized using a two-step approach to correct for inherent inter-individual variability while preserving population-level patterns:

- Individual standardization. Z-score standardization was applied within each animal to eliminate differences in individual basal levels.
- Global rescaling. Subsequently, the data were re-scaled using the global population mean and standard deviation to maintain biological interpretability.

2. Modeling and selection

A generalized linear model (GLM) with up to eighth-order polynomial terms was initially explored. This model, however, showed limited explanatory power ($R^2 = 0.195$) and lacked biological interpretability, as the data pattern was more

consistent with discrete molecular peaks than with a continuous polynomial function. We then explored a Gaussian mixture model (GMM) since LH occurs as multiple, discretely charged isoforms with distinct isoelectric points. In the GMM, each Gaussian component was assumed to represent a putative LH isoform eluting at a specific pH.

To identify the most influential components, we utilized a greedy forward selection algorithm. This algorithm systematically evaluated combinations of all possible isoforms (A–S) to determine the optimal model configuration. Under the optimal configuration, the GMM consistently demonstrated a superior fit compared to the GLM and the original Analysis of Variance (ANOVA)-based methodology. Information criteria (Bayesian Information Criterion, BIC = 12 709.2; Akaike Information Criterion, AIC = 12 671.8) favored a model with four principal isoforms: C, E, B, and I.

3. Statistical comparison

Finally, to evaluate whether sex influences the expression patterns of the isoforms, LH levels between prepubertal males and females were compared within the four significant isoforms using Student's t-test. Additionally, the global fit of a model that included sex as a predictor was evaluated to verify its impact on overall LH secretion. The $P < 0.05$ was considered statistically significant. To evaluate the immunoreactive activity of each LH isoform, dose–response curves were constructed using Prism 6.0 software (GraphPad Software, Inc., USA). This software applies the Hill equation:

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{(\log EC_{50} - X) \cdot h}}$$

Where X is the logarithm of the concentration, Y is the response (which increases as X increases), *Top* and *Bottom* represent the asymptotic maximum and minimum response values, $\log EC_{50}$ is the agonist concentration at which 50 % of the maximal response is observed, and h is the Hill coefficient, indicating the slope of the curve.

The EC_{50} and Hill slope values obtained from the fitted curves were compared using the F-test included in Prism 6.0. This test evaluates whether two isoforms differ significantly in their dose–response characteristics. A $P > 0.05$ indicates no significant difference between the parameters compared.⁽²³⁾

Results

The elution profiles obtained from pituitary glands of heifers and steers are shown in **Figure 2**. A total of twelve LH fractions were identified in both sexes, each fraction presumably corresponding to a distinct LH isoform. These isoforms were designated A through K, along with an additional salt-eluted peak labeled as S. Five fractions eluted between pH 7.8 to 10 (basic isoforms; A–E), three between pH 6.5 to 7.7 (neutral isoforms; F–H), and three between pH 6.4 to 3.3 (acidic isoforms; I–K). The final fraction, S, was eluted with 1 M NaCl and is referred to as the salt peak.

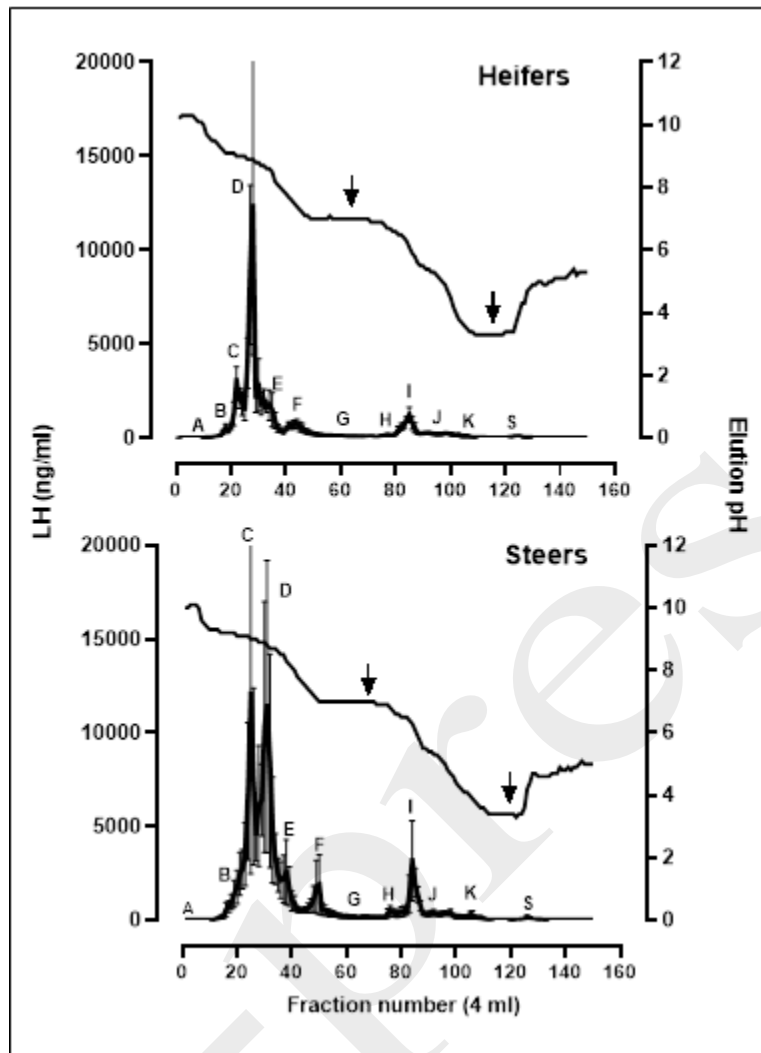


Figure 2. Elution pattern of LH-like immunoreactivity from anterior pituitary extract using chromatofocusing. The graph displays mean LH ($n = 4$) \pm standard error of the mean (SEM) obtained across 160 collected fractions (4 mL each) during chromatographic separation. A total of 50 mg of protein was loaded onto a PBE-118 ion-exchange column pre-equilibrated with 0.025 M triethylamine–HCl buffer (pH 11.0). Proteins were resolved along a continuous pH gradient ranging from 10.5 to 3.5. Arrows denote the transition points between different buffer systems used during the gradient. Elution peaks were sequentially labeled from A to K, corresponding to decreasing isoelectric points. Residual

proteins retained on the column after the pH gradient were eluted with 1 M NaCl and are indicated as the salt-associated fraction (S).

Conversely, the combined proportion of neutral and acidic LH isoforms was significantly greater in heifers (neutral: 12.9 ± 1.61 %, acidic: 13.8 ± 2.2 %) compared to steers (neutral: 9.5 ± 1.68 %, acidic: 12.6 ± 2.06 %). Despite these discrete sex-related differences, basic isoforms consistently represented the predominant LH population in both groups. The initial analysis using a GLM was rejected due to limited explanatory power ($R^2 = 0.195$) and lack of biological interpretability. The adopted GMM, which aligns with the hypothesis of discrete molecular isoforms, provided a superior fit, achieving an $R^2 = 0.283$ with optimal selection criteria (BIC = 12 709.2, AIC = 12 671.8).

The GMM identified four principal isoforms that dominated the prepubertal LH profile: C (pH 9.0–9.1), E (pH 7.8–8.4), B (pH 9.2–9.9), and I (pH 5.7–6.4). Here, the term principal refers to their contribution to the GMM fit (explained variance) rather than to immunoactivity parameters (EC_{50} /Hill). Collectively, these four components accounted for over 95 % of the variance explained by the model.

Sex-related differences in isoform abundance.

To assess the influence of sex, the levels of LH were compared between prepubertal males and females specifically within the four significant isoforms identified by the GMM. Isoform B (pH 9.2–9.9) showed a significant difference between sexes, with heifers exhibiting considerably higher levels (2.8 ± 0.9 %) compared to steers (0.2 ± 0.1 %) ($t_{47} = 3.04$, $p = 0.004$, Cohen's $d = 0.88$). The remaining principal isoforms (C, E, and I) showed no significant difference between sexes ($p > 0.19$ for all comparisons). Although a specific difference was detected in isoform B, the inclusion of sex as a global predictor

did not improve the overall model fit (increase in BIC; $\Delta\text{BIC} = +17.8$), suggesting that sex differences are localized to specific isoforms rather than reflecting a generalized effect on LH secretion.

Although the distribution of LH isoforms by pH range did not differ significantly between sexes, specific differences emerged when individual isoform subtypes were compared (**Table 1**). For example, among the basic LH isoforms, fractions C and D represented the most abundant LH isoforms in both sexes. Notably, isoform D was more prevalent in steers ($46.5 \pm 8.3\%$) compared to heifers ($31.2 \pm 7.7\%$). Several isoforms showed significant sex-related variation ($P < 0.05$), including B, E, F, G, I, J, and K. Isoforms B, E, F, I, and K were more abundant in heifers, whereas G and J were predominant in steers. Notably, isoform B in heifers showed a marked difference, reaching $2.8 \pm 0.9\%$ in heifers versus only $0.2 \pm 0.1\%$ in steers.

In terms of immunological activity, isoform D exhibited the lowest EC_{50} values in both sexes (70.8 ng/tube in steers and 119.6 ng/tube in heifers), indicating a relatively high immunoactivity or stronger receptor affinity. In contrast, isoform B in heifers displayed the highest EC_{50} value (736 ng/tube), indicative of reduced immunoreactivity. The Hill slopes of the dose-response curves ranged from -0.83 to -1.56, with only minor variations across isoforms and sexes, supporting consistent parallelism with the reference standard (**Figure 1**). These results underscore sex-specific differences in both the distribution and immunological potency of pituitary LH isoforms.

Table 1. Comparative analysis of LH isoform abundance and immunoreactivity in steers and heifers

Hormone	pH elution	LH ¹		Immunoreactive concentration of LH ²			
		LH (%)		EC ₅₀ (ng/tube)		Slope	
		Steers	Heifers	Steers	Heifers	Steers	Heifers
NIDDK-oLH	----	----	----	2.1	2.1	- 0.94	- 0.94
A	≥ 10	0.01 ±0.01	0.05 ±0.03	ND	ND	-1.43	ND
B	9.2–9.9	0.2 ±0.1 ^a	2.8 ±0.9 ^b	27.4	736.0	-1.07	-1.36
C	9.0–9.1	23.6 ±9.7	17.2 ±6.2	27.4	160.3	-1.07	-1.02
D	8.5–8.9	46.5 ±8.3	31.2 ±7.7	70.8	119.6	-1.25	-1.12
E	7.8–8.4	5.4 ±1.3 ^a	18.4 ±1.3 ^b	183.0	183.0	-1.56	-1.44
F	7.1–7.7	4.4 ±0.7 ^a	8.7 ±1.5 ^b	117.3	167.2	-1.26	-1.44
G	6.9–7.0	4.7 ±0.7 ^a	2.9 ±0.2 ^b	208.4	260.9	-1.20	-1.10
H	6.5–6.8	1.6 ±0.3	1.3 ±0.4	227.9	ND	-1.28	ND
I	6.4–6.7	6.9 ±0.8 ^a	10.2 ±1.9 ^b	87.9	488.2	-1.11	-1.29
J	3.4–5.4	6.0 ±1.5 ^a	4.5 ±1.9 ^b	169.7	542.2	-0.99	-1.14
K	3.3–4.9	0.8 ±0.3 ^a	2.7 ±0.9 ^b	ND	ND	-1.10	-1.30
S	5.0–5.3	0.01 ±0.01	0.07 ±0.01	245.4	ND	-0.83	-1.31

¹ Data represent the percentage of LH recovery for each isoform (mean ± SEM, n = 4).

Means within the same row that do not share a letter differ significantly (P < 0.05). ² LH

concentrations were measured using a heterologous radioimmunoassay for ovine LH (NIDDK-oLH-I-2), radiolabeled with Na¹²⁵I via the IODO-GEN technique.⁽³²⁾

The immunoreactive concentration was estimated as the hormone dose required to displace 50 % of tracer binding (B/Bo). Parallelism between isoform curves and the standard was assessed based on slope comparison. ND = not detectable.

Discussion

Post-translational modifications of the mature, bioactive LH lead to the generation of a family of isoforms that may be subject to differential physiological regulation. This structural diversity opens the possibility that each isoform could play distinct roles in reproductive and sexual functions.⁽³⁵⁾ In several species, it has been shown that the availability of sex steroids modulates the pattern of LH isoforms in males and females.^(14–16, 18, 26, 28–30)

In this context, the GMM analysis provided a novel and robust framework for characterizing the LH profile. The GMM, which identified four principal isoforms (C, E, B, and I), confirmed that while the overall elution patterns were similar between steers and heifers, significant differences were observed in the relative proportions of specific isoforms. Specifically, our GMM analysis demonstrated that the primary sex-related difference is localized to isoform B (pH 9.2–9.9), which was significantly more abundant in heifers. This finding supports the hypothesis that the relevance of LH during prepubertal development resides not in the overall concentration of the hormone, but rather in the specific composition and abundance of its glycoforms.

The fact that the inclusion of sex as a global predictor did not improve the GMM's overall fit ($\Delta\text{BIC} = +17.8$) further supports this localized regulatory mechanism. These sex-related differences suggest that the relative abundance and functional diversity of LH isoforms may be modulated by distinct regulatory mechanisms governing gonadotropin synthesis or post-translational modifications. Furthermore, the predominance of acidic LH isoforms observed in heifers aligns with previous reports in ovariectomized cows, regardless of progesterone treatment,⁽¹⁷⁾ supporting the idea that the pituitary LH isoform profile remains relatively stable and is not markedly affected by short-term physiological changes.^(26, 28, 29)

The biochemical basis underlying interspecies differences in LH isoforms remains incompletely understood. However, variations in the structure and composition of oligosaccharide chains, particularly at their terminal residues, appear to play a crucial role. For instance, in rat LH, sialic acid is the predominant terminal sugar.⁽³⁶⁾ In human LH, approximately 53 % of the oligosaccharides are sialylated, while 42 % are sulfated. In contrast, bovine and ovine LH molecules exclusively possess sulfated oligosaccharide chains, characterized by the presence of N-acetylgalactosamine-4-sulfate (GalNAc-4-SO₄) as the terminal residue,⁽³⁷⁾ with a distribution of approximately 78 % monosulfated branches and 22 % desulfated residues.⁽³⁸⁾ These structural differences in glycosylation profiles support the hypothesis that species-specific oligosaccharide composition contributes to the diversity of LH isoforms and the pronounced charge heterogeneity commonly observed among glycoprotein hormones.^(6, 39)

The analysis of pituitary extracts from steers and heifers identified at least eleven immunoreactive fractions, with seven of them presenting apparent isoelectric points between pH 7.0 and ≥ 10 . This elution profile aligns with previous findings in rats,⁽²⁶⁾

rams,^(28, 29) and cattle.^(14, 17, 27) Likewise, the isoforms detected within the acidic pH range (pH \leq 6.7 to 4.1) resembled those described in sheep,^(28, 29) and cattle.^(15, 17) These parallels indicate that the observed LH isoform profiles in both sexes are consistent with those reported in ruminants and other species, reinforcing the value of chromatofocusing as a robust method for characterizing the charge-based heterogeneity of LH isoforms. The LH-specific radioimmunoassay showed consistent parallelism across the various pituitary fractions obtained from steers and heifers through chromatofocusing. This was supported by the similarity in the slopes of the dose–response curves when compared with the NIDDK-oLH-I-2 standard, confirming that each fraction contained immunoreactive LH.

A lower concentration of immunoreactive LH was required to reach the EC₅₀ in protein fractions eluted at basic pH values (8.1 to 9.1), compared to those recovered in the acidic range (pH 4.5 to 7.4). This variation in immunoreactivity may reflect, at least in part, the characteristics of the antibody employed, which was raised against LH purified at pH 9.5, according to United States Department of Agriculture (USDA) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) protocols. Additionally, differences in glycosylation among LH isoforms may contribute to this pattern. Isoforms eluting at higher pH values are likely to exhibit lower sialic acid content and less complex oligosaccharide chains, favoring increased antibody binding. Conversely, isoforms recovered at more acidic pH may contain heavily glycosylated or sulfated structures that hinder epitope accessibility, thereby requiring greater hormone concentrations to achieve similar displacement in the radioimmunoassay.

Conclusion

Our results confirm the presence of multiple LH isoforms in pituitary extracts from steers and heifers, with sex-related differences in their distribution and immunoreactivity. In particular, the GMM statistical analysis revealed that, in heifers, the phase of prepubertal sexual differentiation is characterized by the prevalent presence of LH acidic isoforms — particularly I and K— accompanied by the specific enrichment of the more basic isoform B, rather than by generalized differences in total LH secretion. These findings underscore the importance of investigating LH heterogeneity in comparative endocrinological studies to understand the subtle regulatory mechanisms of reproduction.

Data availability

All data and material are available from the corresponding author upon request.

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Conflicts of interest

The authors declare that they have no competing interests.

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Writing-review and editing: G. Gutiérrez-Ospina, E. González-Padilla, G. Perera-Marín.

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