Circulating FSH isoform patterns during recurrent increases in FSH throughout the oestrous cycle of heifers

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Blood samples were collected from heifers (n = 6; 450 ± 7.7 kg) 2–4 times a day during the first and second follicular waves, and during the gonadotrophin surge to determine whether changes in circulating FSH isoforms occur during cyclic quantitative changes in FSH throughout the oestrous cycle. Serum was assayed for FSH, LH, oestradiol and progesterone. Selected samples collected during the first (samples 1–3) and second (samples 4–6) postovulatory recurrent FSH increase and during the subsequent gonadotrophin surge (samples 7 and 8) were analysed for FSH isoforms by chromatofocusing. No change (P > 0.05) in isoform profile occurred during the first or second recurrent FSH increase, when oestradiol concentrations were 0.6 ± 0.07 and 0.6 ± 0.09 pg ml⁻¹ and progesterone concentrations were 0.03 ± 0.01 and 2.4 ± 0.19 ng ml⁻¹, respectively. The percentage of FSH eluting in the pH range 7.4–7.0 increased (P < 0.05) from 14.2 ± 2.2 during the luteal phase (samples 1–6) to 20.2 ± 2.3 (sample 7) and 31.4 ± 3.4% (sample 8) during the preovulatory gonadotrophin surge, while oestradiol concentrations were higher (P < 0.05; 4.9 ± 0.39 pg ml⁻¹) than in the luteal phase of the cycle. In summary, FSH isoform patterns did not change during the cyclic quantitative changes in FSH associated with emergence of the first or second follicular wave. However, during the gonadotrophin surge, in association with increased oestradiol concentrations, an increase in the amount of less acidic isoforms of FSH was observed. Therefore, qualitative changes in FSH are not important in the physiological regulation of follicle turnover during the luteal phase of the oestrous cycle of heifers.

Introduction

Recurrent cyclic changes in concentrations of FSH during the oestrous cycle are associated with sequential follicular wave emergence in cattle (Adams et al., 1992; Sunderland et al., 1994). FSH exists as a series of isoforms which have been identified in urine, serum and pituitary extracts of several mammals (Harlin et al., 1986; Padmanabhan et al., 1992; Kojima et al., 1995). It is now accepted that the observed molecular heterogeneity of these hormones arises through differential post-translational modification of core carbohydrate structures, leading to hormones with identical amino acid sequences but with distinct carbohydrate moieties (Chappel, 1995; Ulloa-Aguirre et al., 1995; Cooke et al., 1996). These carbohydrates differ in their relative amounts of terminal sialic acid and sulfate, yielding isoforms with different pl values; that is, those containing more sialic acid are described as being more acidic than those with less terminal sialylation. The overall biological activity of an isoform is influenced by the identity of its carbohydrate moieties, as illustrated by the fact that glycosylation has been shown to affect receptor binding ability, half-life in vivo and the ability of a hormone to elicit second-messenger responses in target tissues (Morell et al., 1971; Liu et al., 1984; Calvo et al., 1986). There is substantial evidence for a role of the gonadal steroids, especially oestradiol, in modulating FSH heterogeneity. A change in FSH isoform profile has been observed during the oestrous cycle in hamsters (Cameron and Chappel, 1985) and rats (Ulloa-Aguirre et al., 1988) and during the human menstrual cycle (Padmanabhan et al., 1988). In these species, a shift from the more acidic (displaying less in vitro bioactivity) to the less acidic (displaying more in vitro bioactivity) isoforms of FSH has been observed during the follicular phase of the cycle when preovulatory concentrations of oestradiol are present.

To date, few studies have been carried out to characterize the patterns of circulating gonadotrophin isoforms in farm animals during different physiological states (Cooke et al., 1996). Many of the data generated are based on the patterns of isoforms present in the anterior pituitary rather than in the circulation (Chappel and Ramaley, 1985; Ulloa-Aguirre et al., 1988; Stumpf et al., 1992). However, analysis of circulating isoforms of FSH, rather than those found in the pituitary (some of which may represent intermediates in the FSH biosynthetic pathway) will help determine if they play a biological role in relation to FSH action on follicular growth and development.

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Thus, the aim of this study was to characterize the pattern of circulating FSH isomers at strategic stages of the oestrous cycle in heifers related to recurrent quantitative changes in FSH and follicular wave development. The specific hypothesis was that a shift in FSH isomers from the more acidic to the less acidic forms would occur during the follicular phase (in the presence of high concentrations of oestradiol) compared with other stages of the cycle. In addition, it was hypothesized that no change in isomer profile would occur between the early luteal phase, when progesterone concentrations are low (< 1.0 ng ml⁻¹), and the luteal phase of the cycle, when progesterone concentrations are higher (> 2 ng ml⁻¹).

Materials and Methods

Animals

Eight crossbred beef heifers (450 ± 7.7 kg body weight) were administered a prostaglandin F₂α analogue (PGF₂α 15 mg luprostiol; Frosolvin: Intervet Ireland Ltd, Finglas, Dublin) during the luteal phase to initiate luteolysis. Heifers were observed for oestrous behaviour four times a day for 3 days from 12 h after PGF₂α injection. They were housed in slatted accommodation, had access to grass silage and water ad libitum, and were supplemented with 1.4 kg concentrate feed per heifer per day throughout the experimental period. Subsequently, PGF₂α was injected on the second day of dominance of the second dominant follicle to induce luteolysis and ovulation of the second dominant follicle in all heifers. A follicle was defined as dominant when (i) it was ≥ 8.5 mm in diameter, (ii) it was ≥ 2 mm greater in diameter than any of the subordinate follicles in its cohort, and (iii) the subordinate follicles had become static or regressed based on daily ultrasound scanning of the ovaries (Müh et al., 1994). All animal experimentation was performed in compliance with regulations set down by the BioMedical Centre, University College Dublin, and the Cruelty to Animals Act (Ireland), 1897.

Scanning of ovaries and collection of blood samples

From 2 days after the onset of oestrus, ovaries of heifers were scanned daily using an ultrasound scanner (Concept 500, Dynamic Imaging, Livingston) and a 7.5 MHz transrectal linear probe. The size, number and location of all follicles ≥ 5 mm were recorded as described by Savio et al. (1988). From the onset of heat (day 0), 60 ml blood samples were collected every 6 h for 48 h; sampling frequency was then reduced to twice a day until day 4 and once a day on day 5 and day 6. Twice a day, samples were collected from day 7 until 24 h after the second prostaglandin injection; thereafter, samples were collected every 6 h until the second dominant follicle ovulated. Blood samples were maintained at room temperature for 1 h, at 4°C for 18–24 h and centrifuged at 1600 g for 20 min. Serum was separated and divided into two aliquants of approximately 20 ml and 5 ml. All serum samples were stored at −20°C until assay. Serum samples were assayed for FSH, LH, oestradiol and progesterone (using the 5 ml aliquant), while the larger samples were used for chromatofocusing of FSH isomers.

Hormone assays

Previously validated radioimmunoassays were used to quantify oestradiol (Prendiville et al., 1995), progesterone (Ronayne and Hynes, 1990) and FSH (using USDA bFSH B1 as the standard preparation; Crowe et al., 1997). Mean interassay coefficients of variation (CV; n = 3) for serum samples containing 1.4, 2.9 and 6.2 pg oestradiol ml⁻¹ were 17.2, 14.3 and 14.7%, respectively, while the intra-assay CV (n = 6) for the same samples were 11.3, 5.5 and 7.1%. The sensitivity of the oestradiol assay was 0.2 pg ml⁻¹. In the progesterone assay, the mean interassay CV (n = 4) for serum samples containing 0.2, 0.8 and 2.7 ng progesterone ml⁻¹ were 10.9, 7.0 and 6.4%, respectively, while mean intra-assay CV (n = 6) for the same samples were 9.1, 7.1 and 4.3%. The sensitivity of the progesterone assay was 0.1 ng ml⁻¹. The mean interassay CV (n = 4) for serum samples containing 10.7, 18.0 and 34.1 ng FSH ml⁻¹ were 16.7, 15.4 and 6.4%, respectively, while intra-assay CV (n = 6) for the same samples were 10.5, 8.9 and 9.6%. The assay sensitivity was 2.0 ng USDA bFSH B1 ml⁻¹. Fractions from chromatofocusing columns were assayed for FSH using a modified version of the assay described by Crowe et al. (in press). The sample volume was increased from 200 µl to 400 µl for both sample and standard. Assays were carried out at 4°C and standard curves were prepared using 0.025 ml imidazole 1⁻¹ HCl, pH 7.4 as buffer. This increased the sensitivity of the assay from 2 ng USDA bFSH B1 ml⁻¹ to 0.5 ng ml⁻¹. Intra-assay CV (n = 4) for serum samples containing 7.8, 16.6 and 22.2 ng USDA bFSH B1 ml⁻¹ were 9.5, 10.5 and 14.1%, respectively. Intra-assay CV (n = 6) for the same samples were 11.9, 10.0 and 12.5%.

Serum LH concentrations were determined by radioimmunoassay using a modification of the method described by Matteri et al. (1987). Briefly, 200 µl aliquots of serum or standard (NIH bLH B9, NIDDK, MD), 100 µl of monoclonal antibody (518B7 anti LH-B; 1:150 000 dilution; supplied by J. Roser, University of California, Davis, CA) and 100 µl bLH (USDA bLH-I-1 AFP-0000, 1²¹⁵) radioligand (approximately 10 000 c.p.m. per tube; iodinated using a mild chloramine-T procedure; Crowe et al., 1997) were added to 11 mm x 64 mm polyethylene tubes on day 1. Tubes were vortex-mixed and incubated at room temperature for 24 h. On day 2, 50 µl donkey anti-mouse second antibody (SAC-CELL, A-SAC 4, IDS, Boldon, Tyne and Wear) was added to each tube, vortexed and incubated at room temperature for 30 min. 250 µl of distilled water was added and tubes were centrifuged for 5 min at 1600 g. The supernatant was aspirated and amount of radioactivity of iodinated LH in the precipitate was determined using a gamma counter. Cross-reactivity at 50% binding with bFSH (USDA bFSH I2) was 1.05%. Mean interassay CV (n = 2) for samples containing 4.9 and 9.9 ng LH ml⁻¹ were 1.9 and 4.3%, respectively. Intra-assay CV (n = 6) for the same samples were 8.9 and 4.1%, respectively. The sensitivity of the LH assay was 0.1 ng ml⁻¹.

Chromatofocusing of serum samples

Serum samples were selected for chromatofocusing analysis so that FSH isomer profiles could be determined during the quantitative changes in FSH that occur throughout the cycle. Eight samples were analysed from each animal: samples 1–3.
correspond to the first FSH increase, samples 4–6 to the second FSH increase, while samples 7 and 8 were collected during the gonadotrophin surge (Fig. 1). Chromatofocusing was carried out as described by Padmanabhan et al. (1988). Briefly, 16 ml serum samples were dialysed (molecular mass cut-off of dialysis tubing, 12 000–14 000 kDa) against 5 l of 0.025 mol imidazole l⁻¹ HCl, pH 7.4, overnight, at 4°C. Samples of dialysed serum (16 ml) were applied to chromatofocusing columns (bed height, 20 cm; internal diameter, 1.0 cm; PBE 94; Pharmacia, Uppsala) previously equilibrated in 0.025 mol imidazole l⁻¹ HCl, pH 7.4, eluted with a 1:10 dilution of Polybuffer 74 (Pharmacia, Uppsala) HCl, pH 3.6 at a flow-rate of 30 ml h⁻¹, and approximately 95 fractions of 2 ml were collected, while at 4°C. This generated an elution gradient between pH 7.4 and pH 3.6. Once the eluent decreased to < pH 3.8, the elution buffer was changed to 0.01 mol NaCl l⁻¹ and a further 10–15 fractions were collected. The pH of all fractions was measured and fractions were assayed for immunoreactive FSH the next day. After measurement of immunoreactive FSH in each eluent fraction, the amounts of FSH eluting in each of the pH ranges 7.4–7.0, 6.9–6.0, 5.9–5.0, 4.9–3.8 and in the salt peak were calculated as percentages of the total FSH recovered from the column.

Statistical analyses

One heifer developed cystic follicles during the induced oestrous cycle and another that failed to ovulate the second dominant follicle were excluded from the analyses. Changes in concentrations of FSH, oestradiol and progesterone were evaluated by comparing mean hormone concentrations at different time points using paired t test analysis. Mean LH concentrations were calculated and used to confirm the timing of the gonadotrophin surges, but did not undergo statistical evaluation. Changes in size of dominant follicles were evaluated by ANOVA of follicle diameter on different days of the cycle. The mean percentage of FSH eluting in each pH range of samples 1–8 from all heifers was calculated, and compared among samples using a general linear model ANOVA. Fisher's LSD test was used to determine specific differences between means after a significant F test (Snedecor and Cochran, 1989).

Results

The first increase in FSH concentrations after the LH/FSH preovulatory surge occurred between day 0.5 and day 1.2 (day 0 = oestrus) when concentrations (mean ± SEM) increased (P = 0.002) from 9.8 ± 1.2 ng FSH ml⁻¹ (day 0.5 ± 0.1) to a maximum of 24.1 ± 3.3 ng FSH ml⁻¹ (day 1.2 ± 0.1; Fig. 2), and then decreased (P < 0.001) to 11.9 ± 1.2 ng FSH ml⁻¹. This increase in FSH concentration was coincident with decreasing concentrations of oestradiol and basal concentrations.
of progesterone. Between day 2 and day 5, after the emergence of the first follicular wave and in the presence of a growing dominant follicle, oestradiol concentrations increased ($P < 0.001$) from nadir values (mean ± SEM) of $0.33 ± 0.07$ pg oestradiol ml$^{-1}$ to a maximum of $1.83 ± 0.41$ pg oestradiol ml$^{-1}$, and declined ($P = 0.001$) again to nadir concentrations by day 6. Between day 3 and day 7, the first dominant follicle increased in diameter (mean ± SEM; $P < 0.001$) from $8.0 ± 0.5$ mm to $15.5 ± 1.0$ mm and remained at this diameter ($P > 0.2$) until day 16, when it decreased ($P < 0.05$) to $11.6 ± 1.8$ mm. The second dominant follicle became dominant on day $13.5 ± 0.4$ at a diameter of $10.6 ± 0.3$ mm and ovulated in all animals. A sustained basal increase ($P < 0.001$) in progesterone concentrations was observed between day 26 and day 14.0 (mean days), and then returned to basal concentrations by day 15, in response to PGE$_2$ injection.

During the first FSH increase, oestradiol concentrations were higher ($P = 0.01$) in sample 1 compared with sample 3; however, there was no difference ($P > 0.05$) in oestradiol concentration between sample 2 and sample 3 (Table 1). Progesterone concentrations were not different ($P > 0.05$) in samples during the first FSH increase. Concentrations of oestradiol and progesterone did not differ ($P > 0.05$) in samples collected during the second FSH increase (samples 4 to 6; Table 1) or the preovulatory LH/FSH surge (samples 7 and 8; Table 1). However, oestradiol concentrations during the preovulatory LH/FSH surge (samples 7 and 8; Table 1) were higher ($P < 0.001$) than during the rest of the cycle (samples 1–6; Table 1) and progesterone concentrations in samples collected during the second FSH increase (samples 4–6; Table 1) were greater ($P < 0.001$) than in those collected during the first FSH increase or during the LH/FSH surge.

The chromatofocusing profiles of samples 1 and 8 from a representative heifer are shown (Fig. 3). There was no difference ($P ≥ 0.7$) in the FSH isoform profile in heifers during the first or second recurrent FSH increase (Table 2), despite the differences in circulating steroid concentrations. However, the FSH isoform profile was different in samples collected during the preovulatory rise, where a greater proportion of FSH eluted in the less acidic region of the pH gradient in sample 8 compared with samples 1–7. In samples 1–7, approximately $50\%$ of FSH eluted in the salt peak, indicating that up to $50\%$ of FSH in circulation at these times has a pI value less than 3.8 (pH 3.8 is the lower limit of the gradient). The percentage of FSH eluting in this region for sample 8 was decreased ($P < 0.001$) more than twofold. Between 10% and 20% of FSH eluted in the upper limit of the pH gradient in samples 1–7, indicating the presence of FSH isoforms with a pI value ≥ 7.4. An increase ($P < 0.05$) in the percentage of FSH eluting in the pH region 7.4–7.0 was observed in samples from the gonadotrophin surge (approximately 30% for sample 8), compared with samples during the first and second recurrent FSH increase (1–6). The percentage of FSH eluting in this less acidic region (pH 7.4–7.0) in sample 7 was higher ($P < 0.05$) than in samples 2 and 4. In addition, in sample 8, the percentage of FSH eluting in the pH region 6.9–6.0 increased fourfold ($P < 0.001$), while the proportion of FSH eluting in the salt peak decreased twofold ($P < 0.001$) compared with samples 1–7.

### Table 1. FSH, oestradiol and progesterone concentrations (mean ± SEM) in serum samples collected during the first and second recurrent FSH increase and during the gonadotrophin surge of the oestrous cycle of cattle, which were subsequently analysed for FSH isoforms

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Stage of cycle</th>
<th>FSH (ng ml$^{-1}$)</th>
<th>Oestradiol (pg ml$^{-1}$)</th>
<th>Progesterone (ng ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>First FSH rise</td>
<td>$9.8 ± 1.2^a$</td>
<td>$1.05 ± 0.23^d$</td>
<td>$0.05 ± 0.03^d$</td>
</tr>
<tr>
<td>2</td>
<td>First FSH rise</td>
<td>$24.1 ± 3.3^b$</td>
<td>$0.54 ± 0.12^c$</td>
<td>$0.02 ± 0.01^d$</td>
</tr>
<tr>
<td>3</td>
<td>First FSH rise</td>
<td>$12.0 ± 1.2^a$</td>
<td>$0.39 ± 0.05^c$</td>
<td>$0.01 ± 0.01^d$</td>
</tr>
<tr>
<td>Mean 1–3</td>
<td>First FSH rise</td>
<td>$0.6 ± 0.07^a$</td>
<td>$0.03 ± 0.01^a$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Second FSH rise</td>
<td>$14.7 ± 1.5^a$</td>
<td>$0.68 ± 0.15^d$</td>
<td>$2.03 ± 0.31^d$</td>
</tr>
<tr>
<td>5</td>
<td>Second FSH rise</td>
<td>$28.8 ± 2.6^b$</td>
<td>$0.67 ± 0.18^d$</td>
<td>$2.42 ± 0.38^d$</td>
</tr>
<tr>
<td>6</td>
<td>Second FSH rise</td>
<td>$13.7 ± 1.6^a$</td>
<td>$0.49 ± 0.17^d$</td>
<td>$2.41 ± 0.38^d$</td>
</tr>
<tr>
<td>Mean 4–6</td>
<td>Second FSH rise</td>
<td>$0.6 ± 0.09^a$</td>
<td>$2.29 ± 0.19^y$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Gonadotrophin surge</td>
<td>$9.5 ± 2.0^a$</td>
<td>$5.01 ± 0.58^d$</td>
<td>$0.06 ± 0.03^d$</td>
</tr>
<tr>
<td>8</td>
<td>Gonadotrophin surge</td>
<td>$45.0 ± 5.2^c$</td>
<td>$4.77 ± 0.57^d$</td>
<td>$0.08 ± 0.02^d$</td>
</tr>
<tr>
<td>Mean 7–8</td>
<td>Gonadotrophin surge</td>
<td>$4.9 ± 0.39^y$</td>
<td></td>
<td>$0.06 ± 0.01^a$</td>
</tr>
</tbody>
</table>

$^a,b$: Within a column, means with different superscripts are different ($P < 0.05$).
$^c,d$: Within a stage of cycle, means with different superscripts are different ($P < 0.05$).
$^y$: Between stages of cycle, means with different superscripts are different ($P < 0.05$).

### Discussion

Despite distinct alterations in circulating FSH isoforms in a variety of species, at different physiological and developmental stages, the biological significance of FSH heterogeneity remains undetermined. Many of the studies carried out to date have examined FSH isoform distribution in the pituitary, owing to the relative abundance of FSH there. Fewer experiments have examined FSH forms in the circulation (Blum and Gupta, 1985; Padmanabhan et al., 1988, 1992; Shand et al., 1991; Hassing
et al., 1993; Wide and Bakos, 1993; Wide and Naessen, 1994) of species such as rats, humans, horses and sheep, and this is the first published study to do so in cattle.

This experiment shows that, during the oestrous cycle of cattle, the profile of circulating FSH isoforms is not different during luteal phase FSH increases but is different during the gonadotrophin surge of the follicular phase compared with the luteal phase. Specifically, an increase in the proportion of more basic isoforms (pl ≥ 6.0) was observed in serum at a time coincident with increased concentrations of oestradiol. This finding is in broad agreement with many of the studies of FSH heterogeneity in other species. Ulloa-Aguirre et al. (1988) reported that during the late morning and early evening of pro-oestrus in rats, associated with increased circulating concentrations of oestradiol and GnRH secretion, an increase in the percentage of less acidic FSH with a pl of 6.2–5.5 was observed. Cameron and Chappel (1985) examined FSH heterogeneity during the oestrous cycle and after ovariectomy in female golden hamsters, and found that the predominant forms (86%) of FSH within and secreted in vitro from the anterior pituitary after ovariectomy (hence, in the absence of gonadal steroids) had pl values < 4.5. However, during dioestrus II, when oestradiol concentrations are high (Chappel et al., 1978), a large proportion of FSH (52%) in the pituitary had more basic pl values (pl 7.5–4.5). During the oestrous cycle of ewes, Phillips et al. (1994) demonstrated that changes in circulating bioactive FSH (determined using a rat Sertoli cell bioassay) occur several times during the cycle; the bioactive:immuno-active (B:I) ratio of FSH increased in the late follicular phase, suggesting a greater proportion of more bioactive (basic) isoforms associated with increased oestradiol concentrations.

There are conflicting data in studies carried out during the human menstrual cycle. Padmanabhan et al. (1987) showed an increase in B:I ratio of FSH at the time of the gonadotrophin surge, while Jia et al. (1986) and Reddi et al. (1990) observed no change in B:I ratio during the different phases of the menstrual cycle. The discrepancy in these results may be explained by methodological differences, specifically, the use of different radioimmunoassays and in vitro bioassays. However, significant changes in B:I ratio within any one study suggest changes in the distribution of FSH isoforms.

It is unclear whether the shift in FSH isoforms observed in the present study was mediated directly through modulation of FSH secretion at the pituitary by oestradiol or through an oestradiol-mediated alteration in GnRH secretion. Several studies have reported an oestradiol-induced change in isoform profile in favour of more basic forms of the hormone in women (Padmanabhan 1988; Wide and Naessen, 1994) and men (Matikainen et al., 1994). However, direct administration of oestradiol to ovarioctomized heifers (Kojima et al., 1995) failed to alter pituitary FSH isoforms compared with ovarioctomized and cyclic control animals. In the present study, no change in FSH isoforms was observed during the first recurrent FSH increase, despite significant changes in oestradiol concentrations in the samples from this phase of the cycle. These results indicate that, in cattle, oestradiol alone does not appear to alter pituitary FSH isoform distribution. Stumpf et al. (1992) failed to show an alteration in pituitary FSH isoforms in heifers throughout sexual maturation, a time of presumed increase in GnRH secretion, in contrast to studies in other species (Chappel et al., 1983; Wide 1989). Many studies using GnRH antagonists (Kessel et al., 1988; Mortola et al., 1989; Matikainen et al., 1992), GnRH agonist (Kessel et al., 1988) and direct GnRH administration (Wide and Albertsson-Wikland, 1990; Phillips and Wide 1994) support a role for GnRH in control of FSH heterogeneity.

The lack of change in serum FSH isoforms during sequential FSH increases associated with different stages of the first and second waves of follicle growth during the oestrous cycle of heifers suggests that heterogeneity in bovine FSH is not biologically significant in the regulation of follicular wave dynamics. Although there is evidence of differential biological to immunological potency and circulatory clearance rates of FSH isoforms, the current data show that changes in isoform pattern in blood do not play a meaningful physiological role in relation to the FSH-induced emergence of a new follicular wave; neither do changes in this pattern seem to be critical in relation to the selection or subsequent atresia of the dominant follicle. Despite the differential functional capacities of different FSH isoforms demonstrated in vitro, the current data in heifers in vivo do support the contention that there is a putative change in FSH function during the preovulatory period, as well as the mainly circumstantial evidence that different FSH

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**Fig. 3.** Chromatofocusing profiles of FSH (A) in two serum samples from a representative heifer. Sample 1 (a) was collected during the first recurrent FSH increase, while sample 8 (b) was collected during the gonadotrophin surge, of the oestrous cycle. Samples were loaded onto chromatofocusing columns and eluted with a pH gradient, as illustrated by the decreasing pH profile (D). Once the lower end of the pH gradient was reached, 1 mol NaCl l⁻¹ was added (arrow) to elute any FSH remaining on the column. All fractions were assayed for FSH by radioimmunoassay.
isoforms may play a role during the dynamic periovulatory period. These results highlight the fact that future research into FSH heterogeneity should focus on the demonstration of altered function of different FSH isoforms in vivo.

During the first and second recurrent increases in serum FSH concentrations of the oestrous cycle of heifers, the pattern of circulating FSH isoforms did not change. However, before and during the gonadotrophin surge, an increase in the percentage of less acidic isoforms in circulation was observed. In conclusion: (i) changes in concentration of progesterone are not involved in the regulation of FSH isoforms in cattle; (ii) oestadiol alone does not appear to be involved in the regulation of FSH heterogeneity; however, it may be involved in conjunction with other gonadal or hypothalamic factors (for example, GnRH) in regulating circulating isoforms of FSH; and (iii) FSH pleiomorphism is not important in the physiological regulation of follicle turnover throughout the cycle, but may play an as yet undetermined role in the events around the time of ovulation.

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<table>
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<tr>
<th>Sample number</th>
<th>Stage of cycle</th>
<th>pH 7.4–7.0</th>
<th>pH 6.9–6.0</th>
<th>pH 5.9–5.0</th>
<th>pH 4.9–3.8</th>
<th>Salt peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>First FSH rise</td>
<td>14.2 ± 2.0b</td>
<td>6.2 ± 1.2a</td>
<td>10.5 ± 1.6</td>
<td>19.7 ± 1.7</td>
<td>49.4 ± 4.3b</td>
</tr>
<tr>
<td>2</td>
<td>First FSH rise</td>
<td>13.2 ± 2.1a</td>
<td>5.2 ± 1.6a</td>
<td>7.6 ± 1.8</td>
<td>24.2 ± 1.9</td>
<td>49.8 ± 3.2b</td>
</tr>
<tr>
<td>3</td>
<td>First FSH rise</td>
<td>15.4 ± 2.7b</td>
<td>5.6 ± 1.1</td>
<td>9.9 ± 1.5</td>
<td>17.8 ± 2.8</td>
<td>51.3 ± 3.0b</td>
</tr>
<tr>
<td>4</td>
<td>Second FSH rise</td>
<td>14.1 ± 1.2a</td>
<td>4.7 ± 0.8a</td>
<td>6.8 ± 2.0</td>
<td>17.2 ± 2.1</td>
<td>57.2 ± 3.7a</td>
</tr>
<tr>
<td>5</td>
<td>Second FSH rise</td>
<td>14.2 ± 2.9b</td>
<td>4.9 ± 1.2b</td>
<td>6.3 ± 1.6</td>
<td>20.8 ± 3.0</td>
<td>53.7 ± 3.8b</td>
</tr>
<tr>
<td>6</td>
<td>Second FSH rise</td>
<td>14.2 ± 2.2b</td>
<td>3.7 ± 0.7</td>
<td>6.0 ± 0.7</td>
<td>18.4 ± 2.4</td>
<td>57.6 ± 2.5a</td>
</tr>
<tr>
<td>7</td>
<td>Gonadotrophin surge</td>
<td>20.2 ± 2.3b</td>
<td>4.4 ± 0.5b</td>
<td>8.0 ± 0.7</td>
<td>20.7 ± 4.4</td>
<td>46.0 ± 4.2b</td>
</tr>
<tr>
<td>8</td>
<td>Gonadotrophin surge</td>
<td>31.4 ± 3.4a</td>
<td>15.3 ± 1.4b</td>
<td>10.6 ± 0.7</td>
<td>19.5 ± 2.0</td>
<td>23.1 ± 1.5c</td>
</tr>
</tbody>
</table>

abc: Means within a column with differing superscripts are different (P < 0.05).


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