Pituitary–ovarian axis during lactational amenorrhoea. II. Longitudinal assessment of serum FSH polymorphism before and after recovery of menstrual cycles

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BACKGROUND: The association of normal serum levels of immunoassayable gonadotrophins with anovulation during lactational amenorrhoea (LA) has not been fully explained. METHODS: Serum FSH polymorphism was analysed in 10 women during LA between days 60 and 70 post-partum and again, in the mid-follicular phase (MFP), after resuming menstrual cyclicity. FSH microheterogeneity was characterized according to charge, using preparative isoelectric focusing, and according to the inner structure of carbohydrate chains, using lectin chromatography. RESULTS: A significantly higher proportion of FSH charge isoforms isolated below pH 4.10 and a lower proportion of FSH isoforms bearing highly branched oligosaccharides were observed during LA when compared to MFP. Further analysis with higher resolution showed that FSH charge isoforms, isolated in the lower pH range in LA, corresponded to FSH molecules bearing highly branched and biantennary oligosaccharides. FSH isoforms bearing hybrid-type oligosaccharides were only present during LA. The circulating FSH isoform mix was significantly less bioactive in LA than in MFP. LA is characterized by a more acidic mix of FSH isoforms, containing hormone bearing less processed oligosaccharides, with decreased biopotency in comparison with the follicular phase. CONCLUSIONS: This FSH microheterogeneity may be one of the critical factors contributing to incomplete follicular development and anovulation during LA.

Key words: FSH polymorphism/follicular growth/breastfeeding/anovulation/amenorrhoea

Introduction

Limited growth of ovarian follicles, low levels of estradiol in serum and anovulation are characteristic features associated with lactational amenorrhoea (LA). Several studies have suggested that suckling-induced alteration of the GnRH pulsatile secretion pattern and increased hypothalamic sensitivity to the negative feedback of estradiol are the main mechanisms involved in the suppression of ovarian cyclicity during lactation (Perheentupa et al., 2000; McNeilly, 2001). LH secretion characterized by low frequency and variable interpulse intervals has been observed during this period (Tay et al., 1992). In addition, it has been reported that this hormone exhibits diminished biopotency during lactational amenorrhoea (Seron-Ferre et al., 1995).

During breastfeeding, FSH serum levels remain at a level comparable to that observed during the follicular phase of normal ovulatory cycles (Glasier et al., 1983; Shaaban et al., 1987). Therefore, anovulation associated with breastfeeding has not been ascribed to alterations in FSH secretion. However, we have found that lactational amenorrhoea is characterized by a profound dissociation between follicular growth and follicular endocrine activity in spite of normal immunoactive FSH serum levels, suggesting that FSH bioactivity might be altered (Velasquez et al., 2006).

FSH is a polymorphic hormone whose heterogeneity arises from differences in the carbohydrate composition and structure of the peptide-bound oligosaccharides. The terminal sugar residue of the carbohydrate chain—sialic acid—determines the metabolic clearance rate of the FSH molecule and, therefore, its in vivo biopotency (Wide and Wide, 1984; Wide, 1986). It has been reported that follicles in culture respond to the addition of more acidic FSH isoforms (isolated at a pH range 3.6–4.6), with a slower growth rate and delayed and reduced estradiol production (Ulloa-Aguirre et al., 2003). However, FSH glycosylation variants differ not only in charge, which in this particular gonadotrophin is mainly determined by sialic acid content, but also in the inner structure of the carbohydrate chains. Differences in both sialic acid content and inner structure of the glycans modulate the full biological expression of...
study to establish the molecular characteristics of circulating FSH in terms of charge and inner structure of peptide-bound oligosaccharides, in a group of women during amenorrhoea associated with full nursing, and after resumption of their cyclic ovarian function.

**Materials and methods**

**Subjects**

Ten normal healthy nursing women participated in this study. Women were instructed not to give their baby any liquid or solid food or water and to use their breast as the only source of water and nutrients, except for the administration of vitamin drops, for 6 months. All subjects were instructed not to give their baby any liquid or solid food or water during menstruation and to use their breast as the only source of water and nutrients, except for the administration of vitamin drops, for 6 months.

**Study protocol**

From day 60 to day 90 post-partum, vaginal ultrasound examination of the ovaries and concomitant blood sampling were performed at various intervals in order to assess follicular growth and hormonal profile. At this time all women were on exclusive breastfeeding and were lactational amenorrhoeic (LA). When menstrual cycles resumed, additional vaginal ultrasound examination and blood sampling were performed on the 2nd and 3rd post-partum menstrual cycles. All samples were collected ≥2 h after the preceding breastfeeding episode. Venous blood was allowed to clot and then centrifuged to harvest the serum, which was stored frozen at −20°C until assayed. The study protocol was approved by the Ethics Review Committee of ICMER and all women enrolled gave informed written consent.

**Endocrinological assessment**

Microheterogeneity of circulating FSH was assessed in a sample obtained during LA between days 60 and 70 post-partum and during follicular phase between days 7 and 10 after onset of menses of the 3rd post-partum cycle. Previous studies showed that in this population usually all women ovulate in the second cycle post-menorrhoea (Diaz et al., 1992). The shortest cycle studied lasted 25 days and the longest 34 days; therefore, assuming a luteal phase of 13 days, samples obtained on days 7–10 of the cycle were most likely collected 4.5–12.5 days before ovulation and will be referred to as mid-follicular phase (MFP). Serum LH, prolactin, estradiol levels and immunoassayable and bioassayable FSH levels were determined in the same samples in which FSH microheterogeneity was assessed in order to document the functional level of the pituitary–ovarian axis. For the same purpose, ultrasonographic assessment of the population of antil follicles >3 mm was also done on the days of blood sampling. Additional endocrine and ultrasonographic data are presented in Velasquez et al. (2006).

**Determination of serum FSH polymorphism**

Figure 1 shows the general outline of the analytical procedure.

**Preparative isoelectric focusing (IEF)**

The varying distribution and content of negatively charged sialic acid allows distinction of FSH isoforms on the basis of their isoelectric point (Ulloa-Aguirre et al., 1984, 1992c; Wide, 1989). Serum samples obtained from women during LA (n = 10) and MFP (n = 10) were individually analysed. Each sample was dialysed in cellulose membrane tubing (mol. wt threshold 12,000; Sigma Chemical Co., St Louis, MO, USA) against 0.01 mol/l NaCl at 4°C for 2 h.

Preparative IEF was carried out using a Rotorof system (Rotofer Preparative Cell; Bio Rad Laboratories Inc., CA, USA) as previously described by Bedecarras et al. (1998). Before applying the sample, a pre-focusing step was included to establish the pH gradient and avoid protein exposure to extreme pH. This step was carried out by filling the focusing chamber with 35 ml of deionized water, ampholytes (pH range 3–10, 3% and pH range 2.5–5, 0.75%; Pharmalyte, Sigma) and 3-(3-cholamidopropyl)dimethyl-ammonio-1-propane sulphonate, CHAPS, 1% (Sigma). After this procedure, 4–6 ml of serum containing 1500–2100 ng immunoassayable FSH (I-FSH; LER-907) were loaded into the cell (the final volume of the chamber always being 50 ml) and focusing was carried out at 12 W constant power (Electrophoresis Constant Power Supply, ECPS 2000/300; Pharmacia Fine Chemicals AB, Uppsala, Sweden) for 4 h, maintaining the chamber refrigerated (Refrigerated Circulator; FORMA Scientific Inc., OH, USA). When focusing was completed, 20 fractions (2 ml each) were harvested and their pH was determined. Five hundred microlitres of 5 mol/l NaCl were added to each tube (final concentration 1 mol/l NaCl) to avoid ampholytes and detergent interaction with sample proteins. Each individual fraction was exhaustively dialysed to completely eliminate ampholytes and detergent. Dialysis was carried out against 1 mol/l...
Preparative Isoelectrofocusing (IEF) → pH

Fraction dialysis and concentration → FSH RIA

Distribution profile of FSH charge isoforms (Fig 2: panel A)

Combination and concentration of fractions → FSH bioassay

Con-A chromatography

UB

WB

FB

Dialysis and concentration

Lectin-based profile of FSH isoforms (Fig 3; Fig 4, panels B, C and D)

Figure 1. General outline of the analytical procedure followed to isolate FSH glycosylation variants in lactational amenorrhoea (LA) and mid-follicular phase (MFP) according to their charge and to the inner structure of their carbohydrate chains. Oligosaccharide structures of unbound (UB), weakly bound (WB) and firmly bound (FB) FSH isoforms isolated by Concanavalin A chromatography are shown: UB = triantennary and bisecting oligosaccharides; WB = biantennary and truncated oligosaccharides; FB = hybrid-type and high mannose oligosaccharides; Asn = asparagine; □ = N-acetyl glucosamine; ▪ = mannose; ○ = galactose; ▲ = Stalic acid; ▼ = fucose.

NaCl for 24 h at 4°C in a relationship of 1 fraction per litre 1 mol/l NaCl, replacing this solution by a fresh one every 8 h. During the following 12 h the concentrated saline solution was changed to physiological solution, refreshing it every 3 h. This procedure assured the complete absence of interfering substances in future hormone determinations and further characterization steps. After dialysis, each fraction was concentrated using Centriprep-10 membrane (threshold ten thousand; Amicon, Beverly, MA, USA) and the content of FSH was determined by double-antibody radioimmunoassay (RIA). Remaining material in IEF fractions containing FSH were combined, concentrated and stored at −20°C until measurement of bioactive FSH (B-FSH) and Concanavalin A (Con A) chromatography. The range of recovered FSH was 500–1160 ng I-FSH, LER-907 (48–64%).

In order to analyse FSH charge isoforms with higher resolution, two pools (7 ml each, containing 3000 ng I-FSH, LER-907) were prepared by combining serum aliquots of equal volume from seven subjects during LA and MFP (subjects 4–10; Table II in Velasquez et al., 2006). Samples from these subjects were pooled in order to have an appropriate hormone level. This analysis was carried out to establish the distribution profile of FSH isoforms isolated at different pH ranges, according to the inner structure of oligosaccharides. LA and MFP pools were individually applied to the Rotofor cell. The IEF was carried out as described above with a different combination of ampholytes that rendered a 20 fraction pH gradient with a narrow pH range (2.5–6.5). A mix of ampholytes (Pharmalyte; Sigma) with pH range 2.5–5 (3%), 4.2–4.9 (1%) and 4–6.5 (1%) was used. After determination of pH, each IEF fraction was dialysed as described above and the content of I-FSH determined. Fractions containing FSH within the following pH ranges: 3.00–3.79 (more acidic), 3.80–4.09 (mid-acidic) and 4.10–4.99 (less acidic) were combined and concentrated. Due to the low proportion of FSH charge isoforms recovered within the less acidic region of pH gradient from LA pooled sera, they were not submitted to further analysis. Neither were enough FSH charge isoforms recovered in the more acidic region of the pH gradient from MFP to continue their analysis. Consequently, only four preparations were suitable for analysis: LA more and mid-acidic FSH isoforms, and MFP mid- and less acidic FSH isoforms. These preparations were individually applied to four Con A columns.

Concanavalin A chromatography

The technique previously described by Cheng et al. (1984) and modified by Creus et al. (1996) was employed. Con A columns (0.6×15 cm) containing 3 ml of the immobilized lectin gel (Concanavalin A conjugated to Sepharose 4B; Sigma) were equilibrated with equilibrium buffer (0.050 mol/l Tris–HCl; pH 7.4, 0.5 mol/l NaCl, 0.001 mol/l MgCl2, 0.001 mol/l CaCl2, 0.001 mol/l MnCl2). An aliquot of combined and concentrated FSH charge isoforms obtained after IEF (containing 300–600 ng I-FSH, LER-907), from subjects 1 to 7 during LA and MFP, were individually applied to Con A columns. The above-mentioned four preparations of FSH charge isoforms obtained after IEF of LA and MFP pooled sera were also individually applied to Con A columns.

All samples were loaded onto the columns and allowed to interact with the lectin for 30 min. Unbound material was eluted with 7 ml of equilibrium buffer (unbound FSH isoforms, UB). Then, 0.5 ml of equilibrium buffer containing 0.010 mol/l methyl-α-D-glucopyranoside (glucoside) was added and left for 30 min. Seven millilitres of the same buffer were added and the weakly bound material was collected (weakly bound FSH isoforms, WB). Finally, 0.5 ml of equilibrium buffer containing 0.1 mol/l methyl-α-D-mannopyranoside (mannside) was added to the column and incubated for 30 min. An additional 7 ml was added, and the firmly bound material was collected (firmly bound FSH isoforms, FB). Eluates containing UB, WB and FB FSH isoforms were dialysed against 0.01 mol/l NaCl for 24 h and concentrated (Centriprep membranes, Amicon). The whole procedure was carried out at 4°C. The range of recovered FSH was 120–400 ng LER-907 (76–87%). Concentrates were stored frozen at −20°C until I-FSH was determined.

Hormone measurements

Immunoactive FSH, LH, prolactin and estradiol levels in serum samples were determined by immunoassays as previously described (Velasquez et al., 2006). The external quality control program (progba@cemic.edu.ar) indicates that our E2 assay consistently reads values that are 30% below the mean of the other laboratories enrolled in that QC programme. This should be taken into account when considering the absolute values presented here.

The FSH content of samples applied to the Rotofor cell, isoelectric focusing fractions and lectin affinity chromatography eluates was determined by double-antibody RIA using reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA (NIDDK). The reference preparation LER-907 (1 mg LER-907 = 53 IU 2nd International Reference Preparation, HMG) was used to construct the standard curve. The polyclonal human antibody anti-hFSH-6 was used as antiserum. Purified hFSH (hFSH-I-SIAFP-1) was iodinated by the chloramine-T method as described by Greenwood et al. (1963). The iodinated hormone was...
Hormonal profile and ultrasonographic findings

Bioactive FSH (B-FSH) was determined by an in vitro rat Sertoli cell aromatase bioassay as described by Padmanabhan et al. (1987) and modified by Creus et al. (2001). LER-907 was used to construct the standard curve. Estradiol production was measured by RIA (Escobar et al., 1976). The sensitivity of the FSH bioassay was 9.5 ng/ml. Intra- and inter-assay CV were <9 and <12% respectively. The sensitivity of the assay was 4.6 ng (LER-907)/tube.

Breastfeeding episodes were markedly more frequent during lactational amenorrhoea (12 ± 1 per 24 h) than at the time of follicular phase sampling (5 ± 1 per 24 h), when their number had decreased to almost 50% (P < 0.01). Three women had begun weaning, and one had already weaned.

Results

Statistical analysis

Results are expressed as mean ± SEM. The Shapiro–Wilk test was applied to assess the assumption of normality for all variables. Before statistical analysis, the percentage of FSH isoforms was transformed to arcsine [p]1/2, where 0 < p < 1; prolactin, FSH and estradiol values were transformed to decimal logarithm. Comparisons between two groups were assessed using paired Student’s t-test. Comparisons among more than two groups were carried out using paired two-factor analysis of variance, followed by appropriate multiple comparisons tests. P < 0.05 was considered statistically significant.

Results

Breastfeeding pattern at the time of sampling

Breastfeeding episodes were markedly more frequent during lactational amenorrhoea (12 ± 1 per 24 h) than at the time of follicular phase sampling (5 ± 1 per 24 h), when their number had decreased to almost 50% (P < 0.01). Three women had begun weaning, and one had already weaned.

Serum hormonal profile and ultrasonographic examination

Table I shows mean number of ovarian follicles with a diameter >3 mm, the diameter of the largest follicle and mean serum FSH, LH, estradiol and prolactin levels observed in each condition. The number of follicles >3 mm was significantly higher during LA than MFP (P < 0.05). The mean diameter of the largest follicle tended to be lower in MFP (range 4.6–11.0) than that observed in LA (range 7.5–13.5); however, this difference was not statistically significant. No significant difference was observed in FSH, LH and estradiol serum levels when LA was compared to MFP. As expected, higher prolactin serum levels were found during LA when compared to MFP (P < 0.01).

|$P$ < 0.05 when LA and MFP.

*This assay mean has a slight positive bias as results below the detection limit of the assay (30%) were expressed as the assay detection limit.

LA = lactational amenorrhoea; MFP = mid-follicular phase.

### Distribution and bioactivity of FSH charge isoforms after preparative IEF (individual analysis)

Figure 2A shows the distribution profile of FSH charge isoforms isolated after preparative IEF during LA and MFP. Immuno-FSH was detected in nine fractions. The pH value of the fractions containing the highest amount of FSH in each individual was significantly lower in LA than in MFP (4.08 versus 4.29; $P = 0.05$).

A higher proportion of FSH charge isoforms isolated below pH 4.10 was observed during LA compared to MFP: 60.4 ± 1.9 versus 40.8 ± 2.1%; $P < 0.01$ (Figure 2B). The proportion of FSH charge isoforms isolated above pH 4.50 increased 2-fold during MFP when compared to LA (13.4 ± 1.8 versus 6.2 ± 1.7%; $P < 0.05$).

After IEF, FSH containing fractions isolated from each subject were combined and concentrated maintaining separately individuals and LA from MFP. Thus, seven samples from LA and seven from MFP were tested in the Sertoli cell bioassay. FSH in vitro bioactivity was: LA = 51.3 ± 1.7 versus MFP = 66.0 ± 5.2 ng/ml LER-907 ($P < 0.01$). Therefore, a statistically significant reduction of 22.6% in FSH bioactivity was detected during LA in comparison with MFP.

### Distribution profile of FSH isoforms after Con A chromatography (individual analysis)

Con A columns were used to isolate three groups of FSH isoforms according to the inner structure of their carbohydrate chains (Narasimhan et al., 1979): (i) unbound (UB); FSH isoforms bearing triantennary and bisecting oligosaccharides; (ii) weakly bound (WB): FSH isoforms bearing biantennary and truncated oligosaccharides; and (iii) firmly bound (FB): FSH isoforms bearing high mannose and hybrid-type oligosaccharides. Three groups of isoforms were isolated in samples obtained during LA. UB and WB FSH isoforms, corresponding to those bearing complex type oligosaccharides, were present in similar proportions (49.2 ± 2.0 and 40.0 ± 2.0% respectively). A small proportion of FSH was recovered in the FB fraction that corresponds to isoforms bearing high mannose and hybrid-type oligosaccharides (11.3 ± 1.9%) (Figure 3A).

A different profile was observed in samples obtained at MFP. Only UB and WB isoforms were isolated by the lectin. No FSH was detected in the FB fraction (Figure 3B). Thus, the

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<th>Table I. Hormonal profile and ultrasonographic findings</th>
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<td>No. of follicles</td>
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<td>LA 5.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>MFP 3.4 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
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Data are expressed as mean ± SEM (n = 10).

<sup>a</sup> P < 0.05 between LA and MFP.
predominant FSH isoforms observed at MFP were those bearing triantennary and bisecting oligosaccharides (UB versus WB: 65.9 ± 3.0 versus 34.0 ± 3.0%, *P* < 0.001). The proportion of UB FSH isoforms was significantly higher in MFP than in LA (*P* < 0.01).

**Further characterization of FSH (pooled sera)**

Figure 4A shows the distribution profile of FSH charge isoforms at more restricted pH intervals after IEF carried out with higher resolution, in a narrow pH range gradient (2.5–6.5). Under these experimental conditions the proportions of more acidic FSH charge isoforms (pH < 3.20–3.79) were 33.1 and 25.2%, mid-acidic FSH charge isoforms (pH range 3.80–4.09) were 37.7 and 22.5% whereas less acidic FSH charge isoforms (pH 4.10–4.99) were 29.2 and 52.2%, in LA and MFP respectively. The more acidic and mid-acidic FSH charge isoforms isolated from LA pooled sera, and the mid-acidic, and less acidic FSH charge isoforms isolated from MFP pooled sera, were analysed by Con A chromatography (Figure 4B–D). More acidic and less acidic FSH isolated at both extremes of the pH gradient from LA and MFP respectively showed the presence of FSH isoforms bearing complex type oligosaccharides (UB and WB) (Figure 4B and D). However, most of the LA more acidic FSH isoforms corresponded to those bearing triantennary and bisecting oligosaccharides (UB: 80.6%); whereas similar proportions of UB and WB FSH isoforms were present in MFP less acidic FSH isoforms (49.2 and 50.7% respectively). Figure 4C shows the distribution profile obtained when mid-acidic FSH isoforms from LA and MFP were analysed.

**Discussion**

Lack of ovulation during lactational infertility is not easy to explain since it frequently occurs in the face of normal circulating levels of gonadotrophins. Therefore, one has to assume that during lactation something makes the ovary less responsive to gonadotrophins and/or that hormones reach the ovary in a biologically less potent form.

The results obtained in the present study confirm previous reports showing similar levels of FSH and LH at 9 weeks...
In contrast, in MFP, not only did this proportion decrease significantly but ~10% of the circulating hormone was isolated at an even higher pH value, where no immunoactive FSH was present during LA. These results suggest that resumption of ovarian cyclicity is associated with the secretion of less acidic FSH glycosylation variants.

Sialic acid, the terminal residue of the carbohydrate chain, determines the metabolic clearance rate of the hormone and therefore its in vivo biopotency (Morell et al., 1971). Heavily sialylated molecules circulate for longer periods than those less sialylated (Wide, 1986; Ulloa-Aguirre et al., 1992b) but they exhibit low in vitro bioactivity due to their reduced receptor affinity (Zambrano et al., 1999). The predominance of more acidic FSH charge isoforms observed during LA was associated with a relative decrease in biopotency confirmed in the Sertoli cell bioassay. Consequently, during LA, FSH molecules may have longer half-life but are less able to induce a full biological response in the ovarian follicle. Interestingly, these low bioactive glycosylation variants may play an important role in the pre-pubertal period and at the early follicular phase of the menstrual cycle since the presence of a mix of acidic FSH isoforms has been observed in these two physiological conditions (Zambrano et al., 1995; Phillips et al., 1997; Anobile et al., 1998). Based on the above-mentioned facts, it can be proposed that a more acidic mix of FSH isoforms circulating during LA is unable to induce full follicular growth and endocrine activity, preventing ovarian cyclicity under this physiological condition.

A significant increase in the relative abundance of less acidic, more bioactive FSH isoforms may provide a more intense gonadotrophic signal in the follicle, thus contributing to the restoration of ovarian cyclicity. We speculate that a higher proportion of less sialylated FSH isoforms is secreted as the effectiveness of breast suckling to inhibit the gonadal axis wanes. These glycosylation variants may in turn enable FSH to stimulate granulosa cell proliferation, induce mRNA expression and tissue-type plasminogen activator enzyme activity, as well as maximal cyclic AMP and estradiol production (Timossi et al., 1998, 2000; Barrios-De-Tomasi et al., 2002). More biopotent, short-lived FSH isoforms observed at MFP would ensure effective stimulation of the final stages of follicular growth, leading to complete follicular maturation and ovulation.

Differences in FSH microheterogeneity during LA and MFP also involved variations in the relative abundance of isoforms bearing fully processed oligosaccharides. The distribution profile observed at MFP confirmed previous results showing that only FSH isoforms bearing complex type oligosaccharides circulate in the follicular phase of normal menstrual cycles, when a fully functional gonad is present (Creus et al., 1996). During LA, there is a relative decrease of circulating FSH isoforms bearing highly branched carbohydrate chains, concomitantly with the appearance of a small proportion of isoforms bearing incomplete hybrid-type oligosaccharides. The presence of three groups of isoforms, bearing oligosaccharides with diverse degrees of branching and completion of branch synthesis, differs from that observed in post-menopausal women (Creus et al., 1996).

postpartum and during MFP of recovered menstrual cycles (Glasier et al., 1983; Kremer et al., 1991; Nunley et al., 1991). Our study shows that this also holds true when the same women are compared under both conditions, suggesting that the amount of FSH in the circulation is not a limiting factor for adequate follicular development. A likely explanation is that the mix of FSH isoforms being secreted is not endowed with full biological activity, but differences in the molecular structure of FSH secreted in these two periods had not been previously explored.

The molecular microheterogeneity of circulating FSH during LA and MFP was evaluated according to the charge of FSH molecules, reflecting their sialic acid content, and the inner structure of the carbohydrate chains attached to the protein core. The results obtained show that FSH polymorphism is different during LA and MFP.

The distribution profile of FSH charge isoforms isolated from sera obtained during LA showed a high proportion of acidic FSH molecules (recovered below a pH value of 4.10),

![Graph A: Distribution profile of circulating FSH isoforms after Concanavalin A chromatography in seven women: (A) during lactational amenorrhoea, (B) in the mid-follicular phase of the third cycle after resumption of menstrual cyclicity. Results are expressed as percentage of total recovered immunoactive FSH. UB = triantennary and bisecting oligosaccharides; WB = biantennary and truncated oligosaccharides; FB = hybrid-type and high mannose oligosaccharides.

![Graph B: Distribution profile of FSH isoforms circulating during lactational amenorrhoea and mid-follicular phase.](https://example.com/graph.png)
Interestingly, a similar pattern was described in normal men and in the male rat (Rulli et al., 1996, 1999; Fernandez-Vera et al., 1997). Analysis of pooled sera obtained during LA showed that a high proportion of more acidic FSH isoforms bears complex type oligosaccharides. This is an expected result since these molecules have a greater chance of incorporating sialic acid residues. In contrast, during MFP, the relative abundance of isoforms bearing highly branched carbohydrate chains is significantly increased, but they have not incorporated the terminal sugar residue which would decrease their biopotency.

In summary, the present results demonstrate very clearly that circulating FSH isoforms during lactational amenorrhoea differ significantly in their carbohydrate moiety from those found in the same women after recovery of ovarian cyclicity. During LA, an incomplete processing of oligosaccharides renders the circulating mix of FSH isoforms less biopotent. These findings support the hypothesis that one mechanism through which breast suckling prevents ovulation during lactation is by modulating the oligosaccharide structure of secreted FSH isoforms with compromised ability for inducing full follicular maturation. It can be postulated that specific carbohydrate structures present in the hormone may activate different signal transduction systems in antral follicles during these two physiological conditions.

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