Radioimmunoassay of FSH in the plasma of post-partum dairy cows

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Summary. A method for measurement of bovine plasma FSH has been established with an interassay coefficient of variation of 15·5% over the workable range of the assay. Compared to values at the end of pregnancy (42–122 ng/ml), FSH concentrations were greater (P < 0·01) between 0 and 20 days post partum in 3 cows.

Introduction

For optimal breeding efficiency a dairy cow must calve every 12 months and pregnancy must therefore be achieved by 80 days after calving. Cystic ovarian disease, especially during the period 28 to 100 days post partum, accounts for a large proportion (37·5%) of delayed calving intervals (Morrow, Roberts, McEntee & Gray, 1966), and has been attributed to abnormal hormone levels (Nadaraja & Hansel, 1976). When the present study of the endocrine changes in normal cows post partum was begun, no method for measurement of bovine plasma FSH was available, although post-partum patterns of LH and progesterone had been reported (Erb, Surve, Callahan, Randel & Garverick, 1971; Schams, Hoffman, Fischer, Marz & Karg, 1972; Tribble et al., 1973).

The object of the present study was to develop a method for the measurement of plasma FSH in cows and to compare the changes in plasma FSH concentration with those of LH and progesterone in dairy cows during the post-partum period.

Materials and Methods

Radioimmunoassay

A heterologous system was chosen for FSH measurement. The buffer used throughout was 0·1 m-phosphate-buffered saline (PBS), pH 7·0, containing 0·01% sodium merthiolate. The antibody, to rat FSH (S6: kindly donated by NIAMDD), was used at an initial dilution of 1:2000 in PBS containing 0·05 M-ethylene diamine tetracetic acid, disodium salt. Highly purified bovine FSH (LER-1695-2), kindly donated by Dr L. E. Reichert, was iodinated for 30 sec by the chloramine T method of Greenwood, Hunter & Glover (1963), and used immediately, diluted in PBS. The reference standard NIH-FSH-B1, donated by NIAMDD, was diluted to the required concentrations in PBS containing 1% egg albumin. Aliquots of plasma (0·2 ml) and known amounts of NIH-FSH-B1 in 0·2 ml PBS were incubated at 4°C for 6 days with 0·1 ml antiserum (1:2000) and 0·1 ml 125I-labelled bovine FSH (containing approximately 100 000 c.p.m.). Antibody-bound and unbound 125I-labelled FSH were then separated by incubation for 2 days at 4°C with 0·1 ml anti-rabbit γ-globulin serum (Wellcome Reagents Ltd; diluted to 1:30 in 1:400 normal rabbit serum). After dilution with 1 ml distilled water and centrifugation at 6000 g and 4°C, the supernatant was decanted and the precipitate counted in a Tracerlab Gamma/guard 180 counter. Results were expressed in ng equivalents of NIH-FSH-B1/ml plasma.

The reliability of the method was assessed over 10 assays by measuring known amounts of NIH-FSH-B1 (10–50 ng) added to plasma obtained from a cow on Day 7 of the oestrous cycle. The FSH content of two bovine plasma pools was also assessed in each assay. Parallelism was tested against a crude homogenate of bovine pituitaries and against plasma samples obtained from a cow in oestrus.
Cross-reactions were tested against LH (NIH-B8), TSH (NIH-B6), prolactin (NIH-B3) and growth hormone (NIH-B17) in amounts up to 1000 ng. No comparison with any other method of FSH measurement was made.

Progesterone was measured by the method of Kanchev, Dobson, Ward & Fitzpatrick (1976) without modification. The antibody used (RD/4.10) was raised in this laboratory against progesterone-11-succinyl-bovine serum albumin and only had significant cross-reactions with 11α- (64%) and 11β-hydroxyprogesterone (72%). The sensitivity of the method was 20 pg with inter- and intra-assay coefficients of variation of 17 and 8.7% respectively. LH was measured by the unmodified method of Dobson, Cooper & Furr (1975). The antibody to bovine LH used was raised in a horse by Dr R.B. Snook and the only significant cross-reaction was with TSH (90%). Purified bovine LH (LER 1072/2) was used as the 125I-labelled preparation. The sensitivity of the method was 0.2 ng with inter- and intra-assay coefficients of variation of 10.6 and 8.3% respectively. The results are expressed as ng equivalents of NIH-LH-B8/ml plasma.

**Animals**

Six Friesian cows, 2–5 years old, were bled by jugular venepuncture daily for 100 days starting 14 days before the expected date of calving. Plasma was obtained immediately by centrifugation and stored at −15°C. The cows were part of a commercial dairy herd and were observed at least twice a day for oestrus and milked twice daily. Each calf was removed from its mother 1 day after birth.

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**Text-fig. 1.** Inhibition curves for bovine pituitary preparations and plasma in the FSH assay system. The radioactivity is expressed as % of 125I-labelled FSH bound to antibody in the buffer control tubes. ●, NIH-FSH-B1; △, NIH-LH-B8; ○, NIH-TSH-B6; □, NIH-P-B3; ■, NIH-GH-B17; *, crude pituitary extract; ▲, serum.
Results

Over 10 FSH assays the average binding (± s.d.) of 125I-labelled FSH was 13·2 ± 3·6% (13 200 c.p.m.) in the absence of unlabelled hormone, decreasing to 7·7 ± 1·6% (7700 c.p.m.) with the addition of 50 ng FSH. The non-specific binding was 4·2 ± 2·0% (4200 c.p.m.). The minimal detectable amount of standard (defined as the first point in one standard curve with two standard deviations difference from the zero point) was 4 ng. The results in Text-fig. 1 show that there was no significant cross-reaction with any other bovine pituitary hormone and good parallelism was exhibited with the crude pituitary homogenate and with plasma. The cross-reaction of 5% against TSH is equivalent to the contamination with FSH quoted by NIAMDD. The two plasma pools assessed in each assay gave mean values (± s.d.) of 108·1 ± 15·7 ng/ml and 124·0 ± 17·0 ng/ml. Table 1 gives the results of known amounts of FSH added to bovine plasma. The interassay variation was 15·5% between 15 and 37·5 ng. This was considered to be the workable range of the assay and, if necessary, plasma samples were diluted to be within these values.

Table 1. Recovery (mean ± s.d.) of known amounts of FSH added to 0·2 ml bovine plasma over 10 assays

<table>
<thead>
<tr>
<th>FSH added (ng)</th>
<th>No. of duplicates</th>
<th>FSH recovered (ng)</th>
<th>Interassay coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>8</td>
<td>10·2 ± 2·6</td>
<td>25·5</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>15·2 ± 2·5</td>
<td>16·5</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
<td>26·0 ± 3·6</td>
<td>13·8</td>
</tr>
<tr>
<td>37·5</td>
<td>9</td>
<td>36·1 ± 5·9</td>
<td>16·3</td>
</tr>
<tr>
<td>50</td>
<td>9</td>
<td>46·1 ± 15·6</td>
<td>33·8</td>
</tr>
</tbody>
</table>

Oestrus was detected in 5 of the 6 cows at least once during the sampling period and all 6 were successfully inseminated by 120 days post partum. A characteristic example of the hormone patterns obtained is shown in Text-fig. 2 and the concentrations of LH and FSH are summarized for all 6 cows in Table 2. The pattern for plasma progesterone concentrations confirmed that reported by others (Erb et al., 1971; Schams et al., 1972; Tribble et al., 1973). In 5 cows plasma progesterone increased slightly (< 2 ng/ml) for 5–8 days before the resumption of normal cyclic activity. The mean LH concentration was higher between 21 and 48 days after calving than between 0 and 20 days due to the occurrence of intermittent peaks of LH ranging from 4 to 14 ng/ml. Compared to values at the end of pregnancy the mean FSH concentrations was significantly greater between 0 and 20 days post partum in 3 of the 6 cows (see Table 2). FSH values were generally lower after the resumption of cyclic activity.

Text-fig. 2. Plasma concentrations of FSH (▲), progesterone (●) and LH (vertical bars) during the post-partum period of a dairy cow. O = oestrus.
Table 2. Mean (± s.d.) plasma LH and FSH concentrations during the post-partum period (Day 0 = day of calving) in 6 dairy cows

<table>
<thead>
<tr>
<th></th>
<th>Cow</th>
<th>41</th>
<th>26</th>
<th>25</th>
<th>22</th>
<th>20</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>0-20 days</td>
<td>1.9 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>1.9 ± 0.1</td>
<td>2.3 ± 0.6</td>
<td>0.6 ± 0.3</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>21-48 days</td>
<td>2.7 ± 0.7</td>
<td>1.6 ± 0.3</td>
<td>2.5 ± 0.5</td>
<td>3.0 ± 1.0</td>
<td>2.7 ± 3.1</td>
<td>4.0 ± 1.7</td>
</tr>
<tr>
<td>FSH</td>
<td>Last 14 days of pregnancy</td>
<td>65 ± 24</td>
<td>42 ± 16</td>
<td>111 ± 31</td>
<td>65 ± 15</td>
<td>42 ± 5</td>
<td>122 ± 41</td>
</tr>
<tr>
<td></td>
<td>0-20 days</td>
<td>105 ± 46*</td>
<td>67 ± 21*</td>
<td>124 ± 40</td>
<td>67 ± 14</td>
<td>55 ± 11*</td>
<td>139 ± 35</td>
</tr>
<tr>
<td></td>
<td>21-48 days</td>
<td>149 ± 25</td>
<td>48 ± 19</td>
<td>88 ± 29</td>
<td>73 ± 16</td>
<td>56 ± 7</td>
<td>117 ± 30</td>
</tr>
<tr>
<td></td>
<td>During 1st progesterone increase</td>
<td>141 ± 24</td>
<td>51 ± 19</td>
<td>74 ± 21</td>
<td>—</td>
<td>45 ± 3</td>
<td>101 ± 45</td>
</tr>
<tr>
<td></td>
<td>During 1st cycle</td>
<td>118 ± 27</td>
<td>47 ± 16</td>
<td>65 ± 22</td>
<td>74 ± 9</td>
<td>33 ± 7</td>
<td>109 ± 37</td>
</tr>
<tr>
<td></td>
<td>During 2nd cycle</td>
<td>47 ± 21</td>
<td>57 ± 16</td>
<td>26 ± 5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Values significantly higher (P < 0.01; Student's t test for unpaired data) than those during the last 14 days of pregnancy.

Discussion

The low cross-reactions with other pituitary hormones, good recovery results and reproducibility of control samples demonstrate that this heterologous radioimmunoassay system for FSH is adequate for measuring this hormone in bovine peripheral plasma. During the development of this system, descriptions of other radioimmunoassays for measurement of bovine plasma FSH have been published (Akbar, Reichert, Dunn, Kaltenbach & Niswender, 1974; Derivaux, Ectors, Hendrick & Franchimont, 1974; Schams & Schallenberger, 1976). These assays all have reliability criteria similar to those of the present assay, but the assay described here is believed to be preferable because all the reagents, especially the antibody, are readily available through the generosity of NIAMDD.

The plasma FSH concentrations of cows during the post-partum period have not previously been reported. Pituitary FSH content decreases after calving to Day 20 and then increases until the time of first oestrus, whereas LH changes inversely (Labhsetwar, Collins, Tyler & Casida, 1964; Saiduddin, Reisen, Tyler & Casida, 1968). The present results accord with these pituitary assessments if it is assumed that an increase in plasma concentration is reflected by decreased pituitary content.

The results suggest that re-establishment of ovarian cycles after calving depends on initial ovarian stimulation by FSH and this secretion of FSH is later regulated by progesterone from the corpus luteum. It is probably disturbance in achieving this equilibrium which is responsible for ovarian cystic disease: a larger proportion of cows have ovarian cysts before the first oestrus than thereafter (Morrow et al., 1966). The difference in LH and FSH patterns reported here provide further evidence that the controlling mechanisms for the two gonadotrophins are independent.

I thank the Animal Health Trust for a Wooldridge Fellowship; the A.R.C. for further financial support; Professor R. J. Fitzpatrick for encouragement and facilities; Sandie Midmer for technical assistance; NIAMDD, Bethesda, U.S.A., for radioimmunoassay materials; Dr R. B. Snook for the LH antibody; and Dr L. E. Reichert for the purified gonadotrophins.

References


Received 17 March 1977