Comparison of day 3 FSH serum values as determined by six different immunoassays

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BACKGROUND: The accurate assessment of FSH concentration is important for evaluating ovarian function prior to IVF. However, a number of different assay techniques are currently in use, leading to inconsistencies in the hormone data being reported. To address this problem, we measured FSH concentration using a number of commercially available systems. METHODS: Day 3 serum FSH levels were measured in 215 healthy fertile women using six different immunoassays: Coatria¹²⁵I (Bio-Mérieux), ACS-180 (Bayer Diagnostics), Advia-Centaur (Bayer Diagnostics), Vitros ECi (Ortho-Clinical Diagnostics), Architect i2000 (Abbott) and Elecsys 2010 (Roche Diagnostics). RESULTS: According to the immunoassay, means ± SD of FSH concentrations were: 6.5 ± 2.2 mIU/ml for Coatria¹²⁵I, 6.8 ± 2.7 mIU/ml for Advia-Centaur, 6.7 ± 3.0 mIU/ml for Vitros ECi, 7.6 ± 3.0 mIU/ml for ACS-180, 8.2 ± 3.3 mIU/ml for Architect i2000 and 8.8 ± 3.0 mIU/ml for Elecsys 2010. CONCLUSION: Day 3 FSH values determined by six different immunoassays were significantly different (P < 0.01, paired t-test). Physicians must take care when interpreting results from different clinical laboratories.

Key words: FSH/immunoassay/menstrual cycle

Introduction

The assessment of ovarian reserve before ovarian stimulation is a major factor in the outcome of IVF. Currently, serum estradiol (E₂), FSH and inhibin B levels are examined and combined at the beginning of the menstrual cycle (day 3 or 4) to evaluate the functional status of the ovaries, providing information for appropriate ovarian stimulation treatment and prognosis for IVF outcome (Scott et al., 1989, 1990; Licciardi et al., 1995; Sharara et al., 1998).

Several studies agree about the usefulness of the specific two-site enzyme-linked immunosorbent assay (ELISA) developed by Groome and O’Brien for inhibin B determination (Groome and O’Brien, 1993). The cut-off value generally used for day 3 serum inhibin B is 45 pg/ml (Seifer et al., 1997).

Non-isotopic or isotopic immunoassays for E₂ and FSH measurements are currently used in clinical laboratories and inconsistent hormone data are frequently reported, particularly for FSH, making the results difficult to interpret. These discrepancies are due to differences in methods (resulting from FSH polymorphism, antibody specificities and preparation of the standards) and result in decision limit values being different for different immunoassays.

The aim of this study was to compare FSH values obtained on day 3 of the menstrual cycle using six different immunoassays.

Materials and methods

The study was a retrospective analytical investigation. We analysed serum from women donating oocytes in our IVF centre. These women underwent ovarian stimulation for oocyte retrieval. All were ≤35 years old, had regular menstrual cycles and were selected for the oocyte donation programme according to their day 3 hormonal status (E₂, FSH and inhibin B concentrations). Blood samples were collected in Vacutainer® (Becton-Dickinson, Plymouth, UK) glass systems without anticoagulant (serum) and were separated by centrifugation. All samples were placed in 300 µl aliquots in screw-capped Eppendorf® tubes, frozen and stored at –70°C. A serum bank of 215 sera was thus constituted. As the volume of the samples obtained was limiting, the number of samples tested by each assay was between 77 and 215. The immunoassays tested included one isotopic method (FSH Coatria¹²⁵I; Bio-Mérieux, Marcy l’Etoile, France) and five non-isotopic immunometric methods performed on automated multi-analysers: ACS-180 (Bayer Diagnostics, Tarrytown, NY, USA), Advia-Centaur (Bayer Diagnostics), Vitros ECi (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA), Architect i2000 (Abbott Laboratories, Abbott Park, IL, USA) and Elecsys 2010 (Roche Diagnostics, IND, USA). All are two-site immunoassays based on a one step method (except Architect i2000, which is a two step method). The five non-isotopic assays are visualized by direct (Architect i2000, Advia-Centaur, ACS-180) or indirect (Vitros ECi) chemiluminescence or electrochemiluminescence (Elecsys 2010). Before the clinical evaluation presented in this paper, for each method we analysed the precision (within and between runs) as
Table I. Comparison of means and SDs for day 3 serum FSH concentrations as determined by six immunoassays

<table>
<thead>
<tr>
<th>Method</th>
<th>n (mIU/ml)</th>
<th>concentration range</th>
<th>Mean (SD) (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys 2010 (Roche Diagnostics)</td>
<td>77</td>
<td>(3.2–17.4)</td>
<td>8.8± (3.0)</td>
</tr>
<tr>
<td>FSH Coatria 125I (Bio-Mérieux)</td>
<td>82</td>
<td>(3.1–15.8)</td>
<td>6.5± (2.2)</td>
</tr>
<tr>
<td>Advia-Centaur (Bayer Diagnostics)</td>
<td>99</td>
<td>(3.4–18.4)</td>
<td>6.8± (2.7)</td>
</tr>
<tr>
<td>Architect i2000 (Abbott Laboratories)</td>
<td>129</td>
<td>(3.6–21.5)</td>
<td>8.2± (3.3)</td>
</tr>
<tr>
<td>Vitros ECI (Ortho-Clinical Diagnostics)</td>
<td>133</td>
<td>(2.8–19.8)</td>
<td>6.9± (3.0)</td>
</tr>
<tr>
<td>ACS-180 (Bayer Diagnostics)</td>
<td>215</td>
<td>(3.4–20.8)</td>
<td>7.6± (3.0)</td>
</tr>
</tbody>
</table>

*P < 0.001.


Differences observed between the different methods tested may be due to the overall structure of FSH, a glycoprotein assembled from two distinct α and β subunits and which presents heterogeneities in carbohydrate and peptidestructures [different isoforms of FSH circulate (Stanton et al., 1992)] affecting immunoreactivity. The specificity of the monoclonal and/or polyclonal antibodies used in the assay may be such that some FSH isoforms are quantified by some, but not all, immunoassays. Although, some manufacturers had included in their immunoassay procedures two monoclonal (anti-βFSH and anti-αFSH) antibodies (Architect i2000 and Coatria 125I) or one monoclonal (anti-βFSH) and one polyclonal antibody (anti-αFSH; ACS-180, Advia-Centaur, Vitros ECI and Elecsys 2010), this approach was not sufficient to improve the immunoassay specificity.

The presence in the serum of various glycoproteins (LH, thyroid-stimulating hormone, HCG) structurally closely related to FSH (the same α subunit and homologies of the β subunits) could contribute to the observed discrepancies. However, it should be noted that all manufacturers have tested cross-reactivities against these molecules and found no significant cross-reactivity. A central part of a measuring system is the standardization of assay. Although all manufacturers have tested their standard curve calibration (‘secondary standard’) against the same International Standard (NIBSC, 1998), the matrix of the standard is required to be identical to the matrix of the specimen and this condition is met only rarely [note that a FSH human recombinant standard is in preparation (Rose and Gaines-Das, 1998), which could diminish the influence of imperfect test standardization].

The last component which explains differences in assay measurements could result from the mathematical relationship permitting, on the system used, the conversion of the signal into the concentration of hormone. In our study, we did not find a clear difference in serum FSH between assays using monoclonal antibodies or monoclonal and polyclonal antibodies. We also did not find differences according to the signal measured (isotope, direct or indirect chemiluminescence). It is likely that a combination of all these factors, involved in the immunoassay procedure, contributes to the divergence of
the results obtained (Büttner, 1991). This lack of agreement between FSH immunoassays is also illustrated by the French National Quality Control which clearly shows two major and distinct populations of results (République Française, 1999).

The present study, revealing statistically significant differences for day 3 FSH values according to the immunoassay used, raises issues for the interpretation of results from different clinical laboratories. FSH is widely used by physicians (in association with E2 and inhibin B) as a criterion for inclusion in assisted reproductive technology programmes. The discrepancies observed indicate that it is advisable to refer patients to selected laboratories using analytical methods for which they have defined reference values and decision limits for this clinical situation. We hope that, as soon as possible, manufacturers will harmonize their routine immunoassay systems. These efforts toward standardization would be beneficial for both physicians and clinical laboratories and could avoid travelling and expense for the patients.

References

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