Precision and method bias of two assays for oestradiol: consequences for decisions in assisted reproduction

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Oestradiol monitoring in assisted reproduction is empirical, with no proof of benefit. Precise oestradiol estimation will be an essential pre-requisite for high quality evaluation of possible differences between combined and ultrasound-only monitoring. Objectives of the present trial were independent method comparison and bias estimation of chemiluminescent immunoassay (CLIA) versus radioimmunoassay for oestradiol. In a prospective comparison, 505 consecutive samples were split and assayed concurrently. Precision (reproducibility), relative bias and logistics were analysed and compared to manufacturers’ findings. Correlation between CLIA and radioimmunoassay was excellent. Positive bias with CLIA necessitated altering decision points for therapy. Precision (reproducibility) was superior with CLIA, making it an appropriate candidate method for future randomized trials of the effectiveness of combined oestradiol/ultrasound monitoring for assisted reproduction.

Key words: bias/immunoassay/IVF/oestradiol/precision

Introduction

Oestradiol monitoring in assisted reproduction is empirical, with no proof of benefit (Golan et al., 1994). Since information is inversely related to variance of measurement (M.N.Patel, University of Illinois, personal communication), advances in assay technology that increase precision (reproducibility) of oestradiol estimation have the potential to enhance clinical care. High quality evaluation of possible differences between ultrasound-only and combined oestradiol and ultrasound monitoring will depend on randomized design, precise estimation of oestradiol, and sufficient sample size for adequate power.

Oestradiol monitoring has a singular role in populations of automation and the avoidance of radioisotopes. One alternative to radioimmunoassay is chemiluminescent immunoassay (CLIA), based on the principle of chemical production of light detected by a luminometer. However, differing assay technologies for oestradiol produce systematic disparities (Lee et al., 1991; Rojanasakul et al., 1994). The objectives of the present trial were independent method comparison and bias estimation of CLIA and radioimmunoassay for oestradiol (National Committee for Clinical Laboratory Standards, 1995) and the evaluation of the consequences of method bias on clinical decision making in assisted reproduction. Precision (reproducibility), relative bias and logistics for determining oestradiol using CLIA versus RIA were analysed and compared to manufacturers’ findings.

Materials and methods

Determinations of serum oestradiol using CLIA and radioimmunoassay were performed at Gamma-Dynacare Medical Laboratories from July 15 through September 30, 1996. Five hundred and five consecutive serum samples from patients undergoing assisted reproduction were split and analysed daily by both methods. Serum samples were obtained each day and measured concurrently. Technologists were blinded to results of the other method when performing analyses.

The CLIA was carried out using a commercially available assay, ACS Estradiol-6 (Chiron Diagnostics, Markham, Ontario, Canada) using the automated chemiluminescent immunoassay system ACS™-180 (Chiron Diagnostics, Markham, Ontario, Canada), according to the manufacturer’s protocol. The assay principle involves oestradiol in the patient sample competing with oestradiol labelled with dimethyl-acridinium ester (the light reagent) for a limited amount of rabbit anti-oestradiol antibodies. Rabbit anti-oestradiol antibodies are captured by mouse anti-rabbit IgG coupled to paramagnetic particles. There is an inverse relationship between the amount of oestradiol in the patient sample and the amount of relative light units detected. Samples in excess of 6000 pmol/l required manual dilution. All samples were analysed in duplicate.

The radioimmunoassay was also carried out using a commercially available assay, Coat-a-Count® Estradiol (Diagnostic Products Corporation, Los Angeles, CA, USA). This assay is a solid phase coated tube technology and a 60 min incubation was used in accordance with the manufacturer’s protocol. Samples in excess of 10 000 pmol/l required manual dilution. All samples were analysed in duplicate.

Sample size and statistics

The sample size of 500 was chosen arbitrarily and this number was reached during the study period. Statistical analysis was performed using Winstar statistical software (Anderson-Bell Corp., Arvado CO, USA).

The mean of duplicates for CLIA and radioimmunoassay were compared using paired t-tests. Correlation was determined by linear
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**Table I. Comparison of assay precision**

<table>
<thead>
<tr>
<th>Oestradiol quality control</th>
<th>CLIA</th>
<th>RIA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (%)</td>
<td>8.6</td>
<td>15.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Medium (%)</td>
<td>6.0</td>
<td>10.6</td>
<td>0.01</td>
</tr>
<tr>
<td>High (%)</td>
<td>5.3</td>
<td>8.0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CLIA = chemiluminescent assay, RIA = radioimmunoassay.

Regression and calculated overall and in ranges previously described (Loumaye et al., 1997). Imprecision (non-reproducibility) was expressed as coefficient of variation (CV) [(standard deviation/mean)×100] and compared using independent t-tests. Reliability of agreement was assessed by κ. Results are expressed as mean ± SD or mean ± 95% confidence intervals (CI). In analyses, P < 0.05 was considered significant.

**Results**

**Correlation**

Overall correlation between CLIA and radioimmunoassay was excellent (r = 0.99). Figure 1 is a scatterplot of CLIA and RIA results. At lower concentrations, correlations declined (r = 0.87 in 100–500 pmol/l range and r = 0.45 in the 500–750 pmol/l range).

**Precision**

Precision was estimated as CV at three levels of quality control material (Lyphochek, Biorad Laboratories, Mississauga, Ontario, Canada). The CV was calculated for CLIA and radioimmunoassay at low, medium and high standards as shown in Table I. When results were below detectable limits, the lowest value detectable was assigned as the value. Minimal detectable oestradiol was 36 pmol/l for CLIA and 32 pmol/l for radioimmunoassay. At all three levels, CV was greater with radioimmunoassay compared to CLIA.

**Table II. Comparison of assay reliability**

<table>
<thead>
<tr>
<th>Reliability for ‘ovarian suppression’, n = 505</th>
<th>‘Suppressed’ RIA</th>
<th>‘Not suppressed’ RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Suppressed’ CLIA</td>
<td>44</td>
<td>26</td>
</tr>
<tr>
<td>‘Not suppressed’ CLIA</td>
<td>7</td>
<td>428</td>
</tr>
<tr>
<td>κ = 0.69 (95% confidence interval 0.59–0.79) indicating good agreement.</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reliability for ‘increase stimulation’, n = 505</th>
<th>‘Increase’ RIA</th>
<th>‘No increase’ RIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Increase’ CLIA</td>
<td>103</td>
<td>20</td>
</tr>
<tr>
<td>‘No increase’ CLIA</td>
<td>14</td>
<td>368</td>
</tr>
<tr>
<td>κ = 0.81 (95% confidence interval 0.75–0.87) indicating strong agreement.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CLIA = chemiluminescent assay, RIA = radioimmunoassay.

**Bias estimation and reliability of agreement after adjustment for bias**

Results were higher with CLIA than with RIA (Figure 2). In the present trial, the relationship between CLIA and radioimmunoassay was CLIA = 1.04 (radioimmunoassay) + 75 pmol/l, higher than determined by the manufacturer [CLIA = 1.03 (radioimmunoassay) + 19 pmol/l]. Positive bias with CLIA was 110 (92–132) pmol/l overall, 57 (47–67) pmol/l in the range 100–500 pmol/l and 110 (75–145) pmol/l in the range 500–750 pmol/l.

Due to positive bias the cut-point for ovarian suppression, ≈150 pmol/l using RIA (Yuzpe et al., 1994), was adjusted to ≈200 pmol/l for CLIA. The cut-point for increasing ovarian stimulation with gonadotrophins after 6 days, 250 pmol/l using radioimmunoassay, was adjusted to 300 pmol/l for CLIA.

Reliability of agreement was compared using the κ statistic for categorical variables of ‘suppressed’ versus ‘not-suppressed’ and ‘increase’ versus ‘no increase’. Results of agreement by category are shown in Table II. Kappa indicated good agreement for ‘suppression’ and strong agreement for ‘increase stimulation’.

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**Figure 1.** Scatterplot of chemiluminescent assay (CLIA) versus radioimmunoassay (RIA) data.

**Figure 2.** Plot of positive bias with chemiluminescent assay (CLIA).
Efficiency: time to perform assays

Time to perform a typical run of eight patient samples per day was 30 min using CLIA and 120 min using radioimmunoassay. This difference reflected the automated nature of CLIA and the reduced incubation time.

Discussion

Independent comparison of methods and estimation of bias is recommended to evaluate actual performance of new assays (National Committee for Clinical Laboratory Standards, 1995). Different immunoassay methods for steroids often yield highly variable results (Lee et al., 1991; Boots et al., 1998).

While other investigators have compared CLIA to radioimmunoassay (Rojanasakul et al., 1994; Dancoine et al., 1997), the present trial evaluated the consequences of method bias on clinical decisions in assisted reproduction. In this prospective comparison, CLIA was superior in precision (reproducibility) to radioimmunoassay in all ranges. Correlation between CLIA and radioimmunoassay was excellent overall, r = 0.99 (Figure 1). Results did not correlate so well in lower concentration ranges important for clinical decisions (Figure 1). Positive method bias with CLIA was higher in this independent comparison than that reported in the product monograph for CLIA. Positive bias with CLIA necessitated upward modification of clinical decision points. After adjustment for bias, reliability of agreement was ‘good’ for ovarian suppression and ‘strong’ for need to increase stimulation. Due to precision and efficiency superior to that obtained with radioimmunoassay, CLIA has been adopted as the standard oestradiol assay for assisted reproduction at Gamma-Dynacare Medical Laboratories.

In some centres ultrasound-only monitoring is standard care (Wikland et al., 1994; Tsirigotis et al., 1995; Hurst et al., 1997). Golan and co-workers performed a randomized comparison of ultrasound-only and combined oestradiol and ultrasound monitoring and concluded that both approaches were safe and effective (Golan et al., 1994). There were fewer than 60 subjects in each group and the no difference result may have been a type II error. If all cycles in the Golan trial had had cryopreservation and results for cryopreservation consistent for the entire study group, then the outcome with combined monitoring would have been superior (t = 2.12, P = 0.02).

The custom of oestradiol monitoring is based on tradition and the need for vigilance in preventing a serious iatrogenic syndrome: ovarian hyperstimulation syndrome. More evidence regarding oestradiol monitoring is needed. Precise oestradiol estimation will be an essential prerequisite for high quality evaluation of possible differences between combined and ultrasound-only monitoring. Chemiluminescent immunoassay for oestradiol is a candidate assay method for future randomized trials.

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References


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