Comparison of an enzyme-linked immunosorbent assay vs radioimmunoassay for measuring serum progesterone at low levels

J. H. CHECK - C. LAUER - L. UBELACKER - R. KUHN

Summary: Reports have suggested a correlation between low serum progesterone (P) levels prior to human chorionic gonadotropin (hCG) administration and increased pregnancy rates in patients undergoing in vitro fertilization (IVF) patients. We have published two opposite conclusions, dependent upon the methodology used. Pregnancy rates were higher when P by radioimmunoassay (RIA) was < 1 ng/mL, but no increase in pregnancy rates were found when P was measured by the same company's non-isotopic assay. To test if the lack of correlation was attributable to the P method, sera from IVF patients were assayed by two methods, RIA and enzyme linked immunosorbent assay (ELISA). There was 81.8% agreement between methods. Further studies are needed to determine the importance of low P; however, if non-isotopic methods are used, the IVF center should carefully determine the accuracy of their assay in the low range.

Key words: Non-isotopic; Coefficient of variation; Isotopic.

INTRODUCTION

Many clinical laboratories, motivated by the automation and lack of radioactive waste offered by newer technologies, are replacing radioimmunoassay (RIA) for the measurement of hormone levels. While these methods were designed to correlate with RIA, modifications made to accommodate automation, such as specimen size and incubation times, have sometimes compromised the dynamic range of the assays. Although this might not be a problem for general use of the assays, it could prove problematic in specific applications. Serum progesterone (P) measurements are a good example.

Measurement of follicular phase sera levels of P is widely utilized in the treatment of infertility including women undergoing in vitro fertilization (IVF). Several publications have suggested a correlation between low serum P levels in IVF patients prior to the administration of human chorionic gonadotropin (hCG) and increased pregnancy rates ([1, 2]). A recent study suggested that the adverse effect of this subtle rise of P is on the endome-

Received 3-11-1995 from the
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School at Camden
Cooper Hospital/University Medical Center
Department of Obstetrics and Gynecology
Division of Reproductive Endocrinology & Infertility
Camden, New Jersey, USA
Revised manuscript accepted for publication 20-1-1996.

trium and not the follicle ([3]). The levels cited require accurate measurement of P levels less than 1 ng/mL, and in all these incidences RIA was used. These data have recently been challenged based on P levels measured by non-isotopic methods. Our own group has arrived at two opposite conclusions based on the methodology used for P measurements ([4, 5]).

Utilizing RIA, we found pregnancy rates were higher when the patient's serum P prior to hCG was less than 1 ng/mL ([4]). However, when serum P levels were measured with the same company's non-isotopic enhanced luminescent assay, no such correlation was found ([5]). It was our hypothesis that the lack of correlation in our second study could have been due to the method utilized for measurement of P, but the lack of commercial availability of the specific kits involved precluded further study. Therefore, we decided to compare a readily available non-isotopic P methodology with the RIA procedure used by other investigators. We were particularly concerned with correlation at the low end of the assay ($P \leq 1$ ng/mL) and whether on not patient classification as good or poor responders (more or less likely to achieve pregnancy) would be the same, regardless of P method used.

The study presented herein evaluated an enzyme-linked immunosorbent assay (ELISA) method for measuring serum P to see if certain modifications would allow accuracy in determining the serum P levels in the low range by comparing to levels obtained with a well established RIA method.

MATERIALS AND METHODS

Serum samples obtained from 22 IVF patients immediately prior to hCG injection were divided and assayed for P by two methods. The patients agreed to have their medical records released for research purposes as long as confidentiality was assured in compliance with the guidelines of the Institutional Review Board.

The samples were assayed according to manufacturer's directions using the Coat-A-Count RIA (Diagnostic Products Corporation, Los Angeles, CA), a solid phase, competitive binding technique. The kit supplied standards at levels of 0, 0.1, 0.5, 2.0, 10.0, 20.0 and 40.0 ng/mL. The manufacturer's claims of intra-assay precision of 6.4% at 1.1 ng/mL, inter-assay precision of 10.0% at 1.3 ng/mL, and sensitivity of 0.03 ng/mL were confirmed.

The split specimens were assayed on the ES 300 (Boehringer Mannheim Diagnostics, Indianapolis, IN), a fully automated system utilizing an ELISA. Early releases of the P assay for this system had featured a low standard of 0.25 ng/mL and the low end correlation with RIA was unacceptable. Boehringer Mannheim modified the assay by replacing the 0.25 standard with a 0 standard and enhancing the peroxidase-labelled P constituent to give higher absorbance readings. At the time of this study, lot specific standards ranged from 0 to 33 ng/mL. Within run the coefficient of variance (CV) was 12.87% at 0.85 ng/mL, run to run CV was 10.72% at 0.96 ng/mL and sensitivity was 0.16 ng/mL.

The method correlation coefficient utilizing only the low end results was found to be 0.915.

RESULTS

The patients were classified as poor or good responders based on their sera P levels according to the criteria of Silverberg *et al.* ([2]) and Schoolcraft *et al.* ([1]) ($P \leq 0.5$ ng/mL) and according to the criteria established by Check *et al.*, $P \leq 1.0$ ng/mL ([4]) (Table 1).

Using the low range of 0.5 ng/mL, there would have been 22 poor responders by the RIA method and 18 by ELISA, while four were classified as poor if the RIA method was used but good with the ELISA results (81.8% agreement). Using 1 ng/mL as the cutoff, there were 5 poor and 13 good responders regardless of method. The classification of the remaining four was method dependent. The percent agreement was again 81.8.

Table 1. – *Serum progesterone levels and response classification.*

Patient	RIA ng/mL	ELISA ng/mL	0.5 ng/mL cut-off	1.0 ng/mL cut-off
1	0.80	0.70	Poor/Poor	Good/Good
2	0.60	0.60	Poor/Poor	Good/Good
3	0.50	0.50	Poor/Poor	Good/Good
4	1.10	0.80	Poor/Poor	Poor/Good[a]
5	2.00	1.50	Poor/Poor	Poor/Poor
6	0.60	0.30	Poor/Good[a]	Good/Good
7	0.50	0.80	Poor/Poor	Good/Good
8	0.70	0.50	Poor/Poor	Good/Good
9	1.30	1.00	Poor/Poor	Poor/Poor
10	1.00	0.90	Poor/Poor	Poor/Good
11	1.80	1.30	Poor/Poor	Poor/Poor
12	1.00	0.80	Poor/Poor	Poor/Good
13	0.60	0.40	Poor/Good[a]	Good/Good
14	0.90	0.70	Poor/Poor	Good/Good
15	0.70	0.70	Poor/Poor	Good/Good
16	0.70	0.50	Poor/Poor	Good/Good
17	1.10	0.90	Poor/Poor	Poor/Good[a]
18	0.80	0.30	Poor/Good	Good/Good
19	1.70	1.20	Poor/Poor	Poor/Poor
20	0.80	0.70	Poor/Poor	Good/Good
21	0.50	0.40	Poor/Good[a]	Good/Good
22	3.10	2.60	Poor/Poor	Poor/Poor

([a]) Indicate disparate classifications.

DISCUSSION

Hormonal assay methods available worldwide vary greatly, with some of the biggest differences being between isotopic and non-isotopic methods. In vitro fertilization centers routinely establish intervention ranges based upon hormonal results generated by specific assay methods and/or manufacturers. If a new assay method is introduced, routine method comparison protocols may not be sufficient to assess the adequacy of the new method, especially if the intervention range is at the low end of the assay's reportable limits. It may be desiderable to assess any new assay introduced in terms of clinical correlation as well as statistical correlation.

This study will not necessarily validate the intervention range. Obviously, further studies are needed to corroborate or refute the importance of a low serum P pre-hCG in IVF programs and this study, based on just 22 patients, would have done little to allay that controversy. Therefore, no attempt was made to assess pregnancy rates. The concerns were, rather, could the previously reported disparate outcomes be due to differences in the P assay used and was there a non-isotopic P assay available which could replace the less desirable RIA?

While the cut-off level chosen yields obviously disparate classifications, this study shows that if IVF patients were classified as good or poor responders solely on the basis of their serum P prior to hCG, they would be classified the same almost 82% of the time, despite the method used. Considering that this classification is based on P levels obtained at the very low end of the curve, the agreement is very good. For physicians and/or laboratories who need to measure accurately serum P levels less than 1 ng/mL, the Boehringer Mannheim ES300 offers an acceptable alternative to RIA.

REFERENCES

1) Schoolcraft W., Sinton E., Schlenker T. Huynh D., Hamilton F., Meldrum D. R.: " Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate ". *Fertil. Steril.,* 1991, *55,* 563.
2) Silverberg K. M., Burns W. N., Olive D. L., Riehl R. M., Schenken R. S.: " Serum progesterone levels predict success of in vitro fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins ". *J. Clin. Endocrinol. Metab.,* 1991, *73,* 797.
3) Check J. H., Hourani C., Choe J. K., Callan C. Adelson H. G.: " Pregnancy rate in donors versus recipients according to the serum progesterone level at the time of human chorionic gonadotropin in a shared oocyte program ". *Fertil. Steril.,* 1994, *61,* 262.

4) Check J. H., Lurie D., Askari H. A., Hoover L., Lauer C.: " The range of subtle rise in serum progesterone levels following controlled ovarian hyperstimulation associated with lower in vitro fertilization pregnancy rates is determined by the source of manufacturer ". *Eur. J. Obst. Gyn. Reprod. Biol.,* 1993, *52,* 205.

5) Check J. H., Lurie D., Hoover L., Stumpo L., Summers D.: " Association of higher pregnancy rates with low serum progesterone levels (by radioimmunoassay) at the time of human chorionic gonadotropin not corroborated when using a non isotopic immunoassay ". *J. Assist. Reprod. Genetics,* 1993, *10,* 384.

Address reprint requests to:
J. H. CHECK
7447 Old York Road
Melrose Park, PA 19027 (USA)