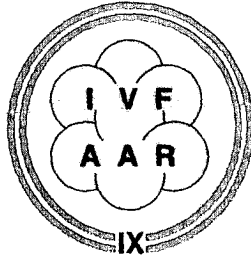


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IX

# EIA methods for measuring serum progesterone (P) at time of human chorionic gonadotropin (hCG) not predictive of subsequent pregnancy rates (PR) (1)

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## SUMMARY

The purpose of this study was to corroborate or refute by non-isotopic immunoassay previous radioimmunoassay (RIA) findings that serum progesterone (P)  $\leq 1$ ng/mL at the time of human chorionic gonadotropin (hCG) in patients undergoing in vitro fertilization (IVF) (where luteal phase leuprolide acetate (LA) was used to first suppress gonadotropins) predicts a higher pregnancy rate (PR). The clinical PR for women undergoing retrieval according to serum P at time of hCG was 15.1% with P  $\leq 1$  ng/mL; 17.4% with P 1.1-1.5; 38.9% with P 1.6-2.0; and 20.0% with P  $> 2.0$ . No significant differences were found according to serum P levels (chi-square,  $p > .05$ ), not even a trend was found for higher PRs with low serum P when using non-isotopic assays.

## INTRODUCTION

There have been several studies suggesting that lower serum P levels at the time of hCG are predictive of higher PRs with IVF, even when down-regulation of gonadotropin is accomplished by gonadotropin

releasing hormone agonists (GNRHa). Two studies using a LA-human menopausal gonadotropin (hMG) controlled ovarian hyperstimulation (COH) regimen found a higher PR when serum P was  $< 0.5$  ng/mL (as measured by a serum RIA from Diagnostic Products Corporation (DPC), Los Angeles, CA) at the time of injection of hCG (2,3); another study using the same COH regimen, but a different RIA kit (Amersham-Amerlex<sup>M</sup>, Arlington Heights, IL), found the best PRs when serum P  $\leq 1$  ng/mL (Check et al, AFS, New Orleans, LA. 1992).

Many laboratories are faced with limitations in the use of radioactivity and have replaced RIAs with non-isotopic immunoassay methods. The study presented herein was designed to determine if we could corroborate our previous data with RIA in which a serum P of  $\leq 1$  ng/mL at time of hCG was predictive of higher PRs with IVF, only this time, using a non-isotopic immunoassay by the same company.

## MATERIALS AND METHODS

All IVF cycles performed at the Cooper Institute for In Vitro Fertilization, Marlton, New Jersey, between 11/1/91 and 8/19/92 were reviewed. During this time period serum P levels were being measured by the Amerlite Progesterone Assay (Amersham Corporation, Arlington Heights, IL) a competitive technique, based on enhanced luminescence. In-house reproducibility studies found the intra-assay coefficient of variation (CV) at the 0.6-1.2 ng/mL range to be 12.25% with an inter-assay CV of 11.78%. In comparison, the manufacturers CVs of other RIA procedures were as follows: Coat-a-Count (DPC) inter-assay CV 6.4% intra CV 10.7% and Amerlex (Amersham) inter CV 11.9%, intra CV 9.0%.

The COH regimen was a modification of the luteal phase LA-hMG regimen originally reported by Meldrum et al. (4) and has been previously described (5). The hormonal replacement regimen for recipients has also been previously described (6). Cycles using a different COH regimen were not included.

The following data was recorded for each retrieval: date of retrieval, number of oocytes retrieved, percent fertilization, number of embryos transferred, pregnancy outcome, P level on day of hCG injection, patients' age, infertility factor, and number of embryos frozen. Data available for each cryopreserved cycle included date of transfer, number of embryos thawed, number of embryos frozen, and the P level from the cycle where the embryos were retrieved.

The cycles were divided into four groups based on the serum P level (ng/mL) on the day of hCG as follows: Gr 1 - P  $\leq 1$ ; Gr 2 - P  $< 1.1$  to 1.5; Gr 3 - 1.6 to 2.0, and Gr 4 P  $> 2.0$ . Pregnancy rates were computed for each COH and P group. Mean values for all IVF parameters measured were also computed.

## RESULTS AND CONCLUSIONS

There were 224 fresh and 74 frozen embryo transfers (ET) evaluated. The PRs for fresh and frozen cycles presented according to

sera P ranges are seen in Table I; pregnancy refers to ultrasound evidence of pregnancy, and ongoing/delivered refers to patients who at least completed their first trimester with a viable fetus on ultrasound. The estimated PRs for the groups in which the serum P was  $>1.5\text{ng/mL}$  are based on a small sample size. Therefore, the statistical analysis used could only compare the PR's between Gr 1 and 2 and between Gr 1 and the combination of Gr 2 and 3 (if serum P between 1 -  $2\text{ng/mL}$ ). Cycles in which the sera P levels were  $>2.0\text{ng/mL}$  were not included in the statistical analysis. There was no difference in PRs according to sera P levels in either fresh transfers or frozen ETs.

Table I - PRs For Fresh And Frozen Cycles According To Sera P Ranges

Group	Clinical pregnancies		Viable pregnancies	
	Fresh transfers (%)	Frozen transfers (%)	Fresh transfers (%)	Frozen transfers (%)
P $<1.0$ ng/mL	20/132 (15.1)	7/35 (20.0)	14/132 (10.6)	7/35 (20.0)
P $<1.1 - \leq 1.5$	12/69 (17.4)	2/33 (6.1)	10/69 (14.5)	2/33 (6.1)
P $1.6 - \leq 2.0$	7/18 (38.9)	0/2	4/18 (22.2)	0/2
P $>2.0$	1/5 (20.0)	0/3	0/5	0/3
P $1.0 - \leq 2.0$	19/87 (21.8)	2/35 (5.7)	14/87 (16.1)	2/35 (5.7)

Dividing the serum P groups into even smaller ones did not result in finding a group with better PRs with low serum P levels. The clinical PR for the group with P  $\leq 0.5\text{ng/mL}$  was 10.3% and for 0.6 to  $0.8\text{ng/mL}$  was 18.5%.

The mean values of relevant IVF parameters in the fresh cycles did not differ in the 4 P groups with age (years) ( $35.2 \pm 5.1$ ,  $35.0 \pm 5.1$ ,  $33.3 \pm 5.5$ ,  $36.8 \pm 5.3$ ) nor did mean no. embryos transferred ( $3.8 \pm 1.3$ ,  $4.2 \pm 1.1$ ,  $4.5 \pm 1.1$ ,  $3.8 \pm 1.5$ ) nor did % fertilization ( $66.1 \pm 23.9$ ,  $63.8 \pm 26.8$ ,  $56.7 \pm 26.3$ ,  $62.2 \pm 29.2$ ). The groups differed only in mean number of oocytes retrieved, mean number of embryos frozen, and mean P levels on day of hCG. The patients with lower sera P levels had fewer oocytes retrieved ( $10.1 \pm 5.7$ ,  $15.6 \pm 8.1$ ,  $16.3 \pm 9.2$ ,  $9.8 \pm 3.4$ ) and thus had fewer embryos available for freezing ( $2.3 \pm 3.3$ ,  $3.9 \pm 4.1$ ,  $3.5 \pm 5.1$ ,  $2.3 \pm 2.0$ ). The number of embryos transferred was the same at all levels of P.

This study failed to find any correlation with higher PRs and low serum P level on the day of hCG when using down-regulation of pituitary gonadotropins and hMG for COH for IVF. In fact, though not significant, the group with the lowest PR (10.3%) was the group whose serum P was between 0.3 to  $0.5\text{ng/mL}$ , which is exactly the groups that Schoolcraft et al. and Silverberg et al. found to have the highest PR when using the DPC

RIA (2,3).

There has been at least one other study published failing to find an association between low sera P levels and high PR, using an RIA by Pantex (Santa Monica, CA.) (7). However, this study had been criticized because of small numbers and the large CV of the Pantex assay (34%) (8,9). The non-isotopic immunoassay used to measure P in this study did have a slightly higher intra-assay CV (12.2%) than previously utilized RIA procedures, e.g., Amersham (Amerlex of 9.0 or DPC (Coat-a-count) of 10.7%). The inter-assay CV was higher than that claimed by one RIA manufacturer; however, in-house studies found the RIA and non-isotopic methods from the same manufacturer to have equivalent inter-assay CVs. The inter-assay CV of the non-isotopic method was re-evaluated at the critical level of 1.0ng/mL and found to be 10.5% (The published CV was 11.7% for Amerlite, 11.9 for Amerlex, and 6.4 for Coat-a-count).

The level of P at time of hCG has the potential to be more clinically useful than merely as a prognosticator. If ultrasound or sera estradiol ( $E_2$ ) criteria were close, but not exactly at the appropriate level for a given IVF center, a rising P level might suggest to the physician to give the hCG sooner. Furthermore, some centers might even freeze all embryos and delay the transfer. Thus, it is very important to continue studies of serum P at time of hCG and perhaps the sera should be evaluated simultaneously with RIA and non-isotopic methodologies.

The incidence of premature luteinization with IVF is rare where the gonadotropins are first suppressed by LA (10). Thus, unless these low levels of serum P are found to be predictive of better success, there will be little reason to obtain sera P while stimulating follicles, especially if LA suppression of gonadotropins is used. This study has raised sufficient doubt about the previous conclusions concerning the adverse effects of a subtle rise in P prior to hCG and suggest that no clinical decision should be made based on these P levels. Instead, these data strongly call for the need for a multi-center cooperative study using one methodology for measuring P when using GnRHa-gonadotropin COH regimens, to evaluate whether subtle rise in P has any adverse effect on PRs in natural cycles, or subsequent frozen ET cycles.

#### REFERENCES

1. Check JH, 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 nonisotopic immunoassay. *J Assist Reprod Genetics* 10:384-387;1993.
2. Schoolcraft W, Sinton E, Schlenker T, Huynh D, Hamilton F, Meldrum DR. Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil Steril* 55:563-566;1991.
3. Silverberg KM, Burns WH, Olive DL, Riehl RM, Schenken RS: 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* 73:797-803;1991.
4. Meldrum DR, Wiscot A, Hamilton F, Gutlay AL, Kempton W, Huynh D: Routine pituitary suppression with leuprolide before ovarian stimulation for oocyte retrieval. *Fertil Steril* 51:455-459;1989.

5. Check JH, Nowroozi K, Choe J, Dietterich C: Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization. *Fertil Steril* 56:1173-1175;1991.
6. Check JH, Nowroozi K, Choe J, Lurie D, Dietterich C: The effect of endometrial thickness and echo pattern on in vitro fertilization outcome in donor oocyte-embryo transfer cycle. *Fertil Steril* 59:72-75;1993.
7. Edelstein MC, Seltman HJ, Cox BJ, Robinson SM, Shaw RA, Muesher SJ: Progesterone levels on the day of human chorionic gonadotropin administration in cycles with gonadotropin-releasing hormone agonist suppression are not predictive of pregnancy outcome. *Fertil Steril* 54:853-857;1990.
8. Silverberg KM, Olive DL, Schenken RS: Statistical scuds, Letters-to-the-editor. *Fertil Steril* 56:153-154;1991.
9. Meldrum DR: Statistical scuds, Letters-to-the-editor. *Fertil Steril* 56:154-155;1991.
10. Check JH, Nowroozi K, Chase JS: Comparison of short versus long-term leuprolide acetate-human menopausal gonadotropin hyperstimulation in in-vitro fertilization patients. *Hum Reprod* 1:31-34;1992.

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