

CAMDEN, NEW JERSEY

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

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INTRODUCTION

There have been several studies suggesting that lower serum progesterone (P) levels at the time of human chorionic gonadotropin (hCG) are predictive of higher pregnancy rates (PRs) with in vitro fertilization (IVF), even when down-regulation of gonadotropin is accomplished by gonadotropin releasing hormone agonists (GnRH_a). Two studies using a leuprolide acetate (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 radioimmunoassay (RIA) from Diagnostic Products Corporation (DPC), Los Angeles, CA] at the time of injection of hCG (1,2); another study using the same COH regimen, but a different RIA kit (Amersham-Amerlex, Arlington Heights, IL), found the best

PRs when serum P was ≤ 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 nonisotopic immunoassay methods. The study presented here was designed to determine if we could corroborate our previous data with RIA in which a serum P of ≤ 1 ng/ml at the time of hCG was predictive of higher PRs with IVF, only this time using a nonisotopic 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 (Amer-sham Corp., Arlington Heights, IL), a competitive technique, based on enhanced luminescence. In-house reproducibility studies found the intraassay coefficient of variation (CV) in the 0.6- to 1.2-ng/ml range to be 12.25%, with an interassay CV of 11.78%. In comparison, the manufacturers' CVs of other RIA procedures were as follows: Coat-a-Count (DPC), interassay CV 6.4% and intraassay CV 10.7%; and Amerlex (Amersham), interassay CV 11.9% and intraassay CV 9.0%.

The COH regimen was a modification of the luteal-phase LA-hMG regimen originally reported by Meldrum *et al.* (3) and has been described previously (4). The hormonal replacement regimen for recipients has also been described previously (5). Cycles using a different COH regimen were not included.

The following data were recorded for each retrieval: date of retrieval, number of oocytes retrieved, percentage fertilization, number of embryos transferred, pregnancy outcome, P level on day of hCG injection, patient's age, infertility fac-

tor, and number of embryos frozen. Data available for each cryopreserved cycle included date of transfer, number of embryos thawed, number of embryos frozen, and 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: Group 1, $P \leq 1$; Group 2, $P < 1.1$ to 1.5; Group 3, $P = 1.6$ to 2.0; and Group 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

There were 224 fresh and 74 frozen embryo transfers (FETs) evaluated. The PRs for fresh and frozen cycles presented according to serum P ranges are shown in Table I; pregnancy refers to ultrasound evidence of pregnancy, and ongoing/delivered refers to patients who completed at least their first trimester with a viable fetus on ultrasound. The estimated PRs for the groups in which the serum P was >1.5 ng/ml are based on a small sample size. Therefore, the statistical analysis used could compare only the PRs between Group 1 and Group 2 and between Group 1 and the combination of Groups 2 and 3 (if serum P between 1 and 2 ng/ml). Cycles in which the serum P levels were >2.0 ng/ml were not included in the statistical analysis. There was no difference in PRs according to serum P levels in either fresh transfers or FETs.

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 $P \leq 0.5$ ng/ml was 10.3% and that for P of 0.6 to 0.8 ng/ml was 18.5%.

The mean values of relevant IVF parameters in the fresh cycles did not differ in the four P groups with age (years) (35.2 ± 5.1 , 35.0 ± 5.1 , 33.3 ± 5.5 , 36.8 ± 5.3); neither did the mean number of em-

Table I. Pregnancy Rates for Fresh and Frozen Cycles According to Serum P Ranges

Group	Clinical pregnancies		Viable pregnancies	
	Fresh transfers (%)	Frozen transfers (%)	Fresh transfers (%)	Frozen transfers (%)
$P < 1.0$	20/132 (15.1)	7/35 (20.0)	14/132 (10.6)	7/35 (20.0)
$P < 1.1$ to ≤ 1.5	12/69 (17.4)	2/33 (6.1)	10/69 (14.5)	2/33 (6.1)
$P 1.6$ to ≤ 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
$P 1.0$ to ≤ 2.0	19/87 (21.8)	2/35 (5.7)	14/87 (16.1)	2/35 (5.7)

bryos transferred (3.8 ± 1.3 , 4.2 ± 1.1 , 4.5 ± 1.1 , 3.8 ± 1.5) or the percentage of fertilization (66.1 ± 23.9 , 63.8 ± 26.8 , 56.7 ± 26.3 , 62.2 ± 29.2). The groups differed only in mean number of oocytes retrieved, mean number of embryos frozen, and mean P levels on the day of hCG. The patients with lower serum P levels had fewer oocytes retrieved (10.1 ± 5.7 , 15.6 ± 8.1 , 16.3 ± 9.2 , 9.8 ± 3.4) and thus had fewer embryos available for freezing (2.3 ± 3.3 , 3.9 ± 4.1 , 3.5 ± 5.1 , 2.3 ± 2.0). The number of embryos transferred was the same at all levels of P.

DISCUSSION

This study failed to find any correlation of 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 and 0.5 ng/ml, which are exactly the groups that Schoolcraft *et al.* and Silverberg *et al.* found to have the highest PR when using the Diagnostic Product Corp. (DPC) RIA (1,2).

There has been at least one other study published failing to find an association between low serum P levels and high PR, using an RIA by Pantex (Santa Monica, CA) (6). However, this study had been criticized because of small numbers and the large CV of the Pantex assay (34%) (7,8). The nonisotopic immunoassay used to measure P in this study did have a slightly higher intraassay CV (12.2%) than previously utilized RIA procedures, e.g., Amersham (Amerlex) of 9.0 or DPC (Coat-a-Count) of 10.7%. The interassay CV was higher than that claimed by one RIA manufacturer; however, in-house studies found the RIA and nonisotopic methods from the same manufacturer to have equivalent interassay CVs. The interassay CV of the nonisotopic method was reevaluated at the critical level of 1.0 ng/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 the time of hCG has the potential to be more clinically useful than merely as a prognosticator. If ultrasound or serum 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 hCG sooner. Furthermore, some centers might even freeze all em-

bryos and delay the transfer. Thus, it is very important to continue studies of serum P at the time of hCG and perhaps the serum should be evaluated simultaneously with RIA and nonisotopic methodologies.

The incidence of premature luteinization with IVF is rare where the gonadotropins are first suppressed by LA (9). Thus, unless these low levels of serum P are found to be predictive of better success, there will be little reason to obtain serum 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 a subtle rise in P has any adverse effect on PRs in natural cycles or subsequent frozen embryo transfer cycles.

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