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Serum Progesterone and 17-Hydroxyprogesterone in the Diagnosis of Ectopic Pregnancies and the Value of Progesterone Replacement in Intrauterine Pregnancies when Serum Progesterone Levels Are Low

Key Words

Progesterone
Pregnancy
17-Hydroxyprogesterone

Abstract

The corpus luteum function was evaluated in patients with surgically confirmed ectopic pregnancy (EP) in a multicenter study. In addition, the minimal threshold of serum progesterone (P) concentration required for salvaging intrauterine pregnancies (IUP) was also examined. Results show that single P or 17-OHP measurements are not diagnostic for EP, since mean P levels in EP were similar to those with spontaneous abortion though significantly lower than those in controls. 17-OHP levels in EP overlapped in 50% with IUP, and the mean levels were significantly lower only at 6-7 weeks. The 17-OHP levels when compared to hCG supports the view that corpus luteum defect is primary. In IUP, P levels < 8 ng/ml still were associated with viable (60%) pregnancy; thus no minimal threshold could be established.

Introduction

Matthews et al. [1] published data suggesting that the serum progesterone (P) level could be useful in identifying patients with ectopic pregnancies (EP). They suggested that a level of 15 ng/ml of serum P distinguished viable from nonviable pregnancies, and all EPs fell below this level. Yeko et al. [2] supported the findings of Matthews et al. [1] and even suggested that in any patient with a suspected EP a serum P < 15 ng/ml should prompt a dilation and evacuation followed by laparoscopy if no prod-

ucts of conception were found, in lieu of the more standard approach of serial ultrasound and serum β -hCG levels [2]. The possibility exists, however, that an insufficient number of cases were evaluated to be certain that a viable pregnancy was not possible below a P level of 15 ng/ml. Furthermore, no attempts were made to treat with P, and, in fact, a recent study demonstrated a 70% viable pregnancy rate in women aggressively treated with P who initially presented with serum P levels under this critical level during their pregnancy [3]. 17-OHP is recognized as a marker of the corpus luteum (CL) in the nonpregnant

and pregnant states since placental hydroxylation capacity is very limited in early pregnancy. Thus, this steroid may serve as a good marker in assessing CL function in early pregnancy. Previously low P and 17-OHP levels were found in EP which is likely to be caused by a primary luteal phase defect [4-6]. This was, however, based on a single measurement.

A study was thus performed to evaluate a larger series of pregnancies to determine if there is truly a critically low level of P that is consistent with EP and not consistent with a viable intrauterine pregnancy (IUP). These patients would be aggressively treated with P to determine whether this therapy was ineffective below a certain new critical P level. The influence of fertility drugs and/or luteal phase support with P in causing serum steroid levels to be above the critical discriminatory P level in women with EP would be evaluated as well. Finally, 17-OHP and hCG levels were examined in EP patients in an attempt to correlate CL function with that of the placenta.

Materials and Methods

The studies consisted of combined data from three different institutions: the State University of New York (SUNY) Health Science Center at Syracuse N.J., USA; the University of Medicine and Dentistry of New Jersey (UMDNJ) at Camden, N.J., USA, and the Yale New Haven Hospital, N.H., USA.

From 1986 to 1988, serum samples at SUNY were obtained from patients suspected of having either EP or abnormal IUP. The specimens were stored at -20°C until assayed. Each patient's gestational age was established at initial presentation by last menstrual period. The pregnancy outcomes were evaluated retrospectively and divided into three groups: group 1 ($n = 72$), normal IUP defined as intrauterine fetal heart activity on ultrasound; group 2 ($n = 31$), surgically confirmed EP; group 3 ($n = 91$), spontaneous abortion (SAB) verified histologically. Patients on P supplement therapy or ovulation-inducing drugs (OVID) were eliminated from the study. The remaining 194 patients constituted the study population. The stored serum P concentrations were assayed at the end of the study period.

The UMDNJ study evaluated the serum P and 17-OHP levels of two groups of previously infertile women with surgically confirmed EP ($n = 66$) and those who had IUP ($n = 33$). The data were examined retrospectively from 1984 to 1988.

Patients with EP were divided into those taking OVID ($n = 34$) and those who did not ($n = 32$). A further subdivision of these two groups was made on the basis of whether or not the women were taking P supplements at the time that the P determinations were made (24 patients with no supplemental P and 42 patients with supplemental P). Of the 24 patients who were not on P, 15 patients had taken no fertility drugs. Furthermore, a total of 33 patients with viable IUP who had serum P < 12 ng/ml and 15 patients with serum P < 8 ng/ml were evaluated to determine if aggressive P therapy could alter the pregnancy outcome.

Table 1. Levels of P (ng/ml) in patients with IUP, SAB and EP

	IUP	SAB	EP
Mean \pm SD	22.7 \pm 9.6	5.8 \pm 7.3*	7.9 \pm 7.8*
Range	8.1-42.2	0.2-35	0.6-33.4
Patients	72	91	31

* $p < 0.001$ vs. control.

The Yale study group consisted of 35 patients with surgically confirmed diagnoses of EP at 6-11 weeks from the last menstrual period from 1983 to 1985. Plasma measurements of hCG and 17-OHP were performed on frozen stored samples, to aid in clinical management. The mean number of samples per patient in the study group was 2.3 (range 1-13). In 15 patients, multiple blood samples were obtained prior to surgery. A total of 79 samples were available for analysis. The control IUP group for hCG and 17-OHP consisted of 27 women for the former and 21 women for the latter hormone who were matched for gestational age (6-11 weeks from the last menstrual period). No P replacements were given to any of the patients.

Assays

P levels were measured by RIA 4.5 and 5.5% intra- and interassay variability (Diagnostic Product Corporation, Los Angeles, Calif., USA). 17-OHP was measured by using double antibody RIA with a 2% cross-reactivity with progesterone (Pantex, Santa Monica, Calif., USA). In the Yale study prior to RIA of 17-OHP, an extraction with petroleum ether and chromatography on a Sephadex LH-20 (Pharmacia, Piscataway, N.J., USA) was carried out. hCG was measured by standard RIA.

Statistical analyses were made using one-way ANOVA and Student's t test for unpaired observations.

Results

Table 1 derived from SUNY data demonstrated the following: group 1 contained 72 patients with normal IUP. Their gestational ages ranged from 4 to 12 weeks at initial presentation. The patient's P levels varied from 8.1 to 42.2 ng/ml (mean \pm SD: 22.7 \pm 9.6). In group 2, EP ($n = 31$), serum P levels ranged from 0.6 to 33.4 ng/ml (mean \pm SD: 7.9 \pm 7.8). In group 3, SAB ($n = 91$), serum P levels ranged from 0.2 to 35 ng/ml (mean \pm SD: 5.8 \pm 7.3).

The mean level of serum P in patients with normal IUP was significantly higher than in patients with EP or SAB ($p < 0.001$). However, between patients with EP and SAB the mean level of serum P was not statistically different. All patients with normal IUP, 35.5% of patients with EP and 22% of patients with SAB had serum P levels above 8 ng/ml.

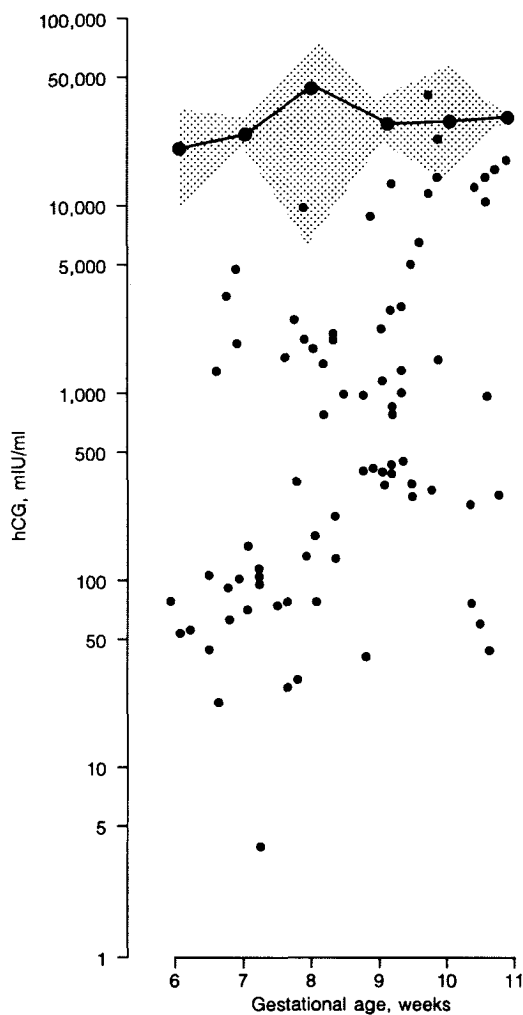


Fig. 1. Distribution of hCG levels in EP, between 6 and 11 weeks of gestation. The shaded area represents the 95% confidence interval of hCG for normal IUP.

The mean serum P levels of the UMDNJ patients with EP who had taken OVID (22.2 ± 17.2 ng/ml) was significantly lower than that of patients with normal IUP who had taken OVID (59.9 ± 34.3 ng/ml, $p < 0.005$). Among the patients with EP who were taking OVID, the mean serum P level was higher in those taking P supplements (34.8 ± 21.3) than in those not receiving exogenous P (15.9 ± 11.2); the difference between these values was not statistically significant.

The mean serum P levels among the patients following natural ovulation cycles were also significantly lower for

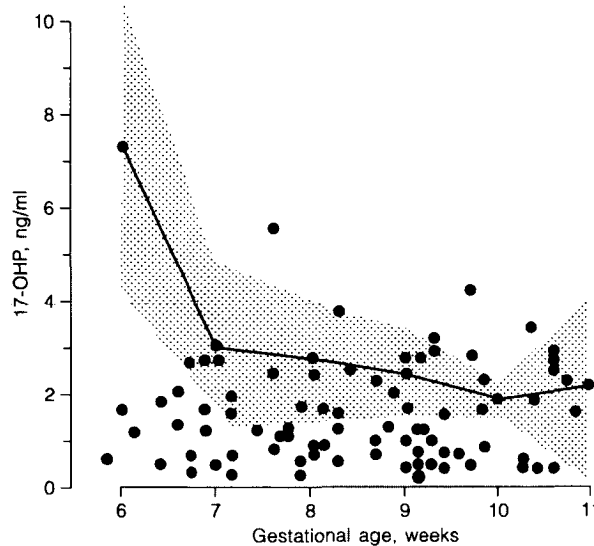


Fig. 2. Distribution of 17-OHP levels in EP, between 6 and 11 weeks gestation. The shaded area represents the 95% confidence interval of 17-OHP for normal IUP.

patients with EP (14.9 ± 8.5 ng/ml; $p < 0.05$) than for viable IUP (30.1 ± 15.8 ng/ml). In the subgroup of patients with EP and natural ovarian cycles, P supplementation resulted in higher serum P levels (21.5 ± 9.2) than those observed for patients who did not receive exogenous P (11.0 ± 5.2).

In patients with EP, 17-OHP levels were lower than in those who had normal IUP. These differences were statistically significant for serum levels obtained at the 5th gestational week in patients taking OVID and for weeks 3–5 after ovulation in patients not taking OVID. Twenty-two out of 33 patients (66%) with serum P below 12 ng/ml developed normal pregnancies with aggressive supplemental P therapy. Using the 8 ng/ml cutoff level, 9/15 (60%) had successful viability with P therapy.

In the Yale study, the distribution of hCG levels in EP is shown in figure 1. The shaded area represents the 95% confidence interval for hCG in normal IUP. Mean plasma levels of hCG in the EP group were significantly lower than those in the IUP group ($p < 0.05$), at all times between 6 and 11 weeks of gestation. The mean (\pm SEM) value of hCG for the entire EP group under study was $3,051 \pm 685$ mIU/ml, and the median value was 397 mIU/ml.

Plasma levels of 17-OHP are illustrated in figure 2 showing that mean plasma levels of 17-OHP in the EP

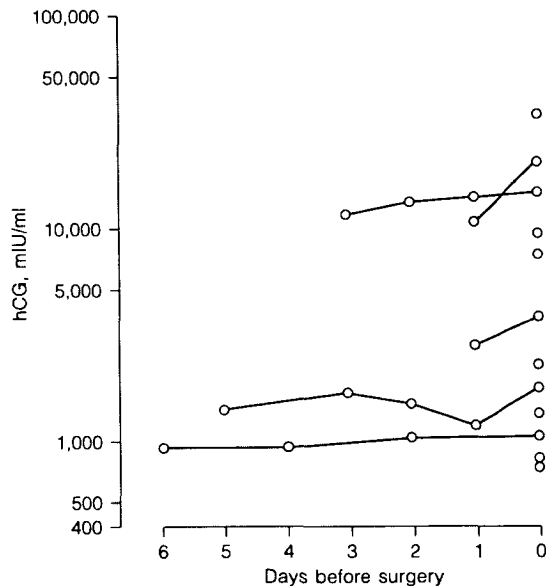
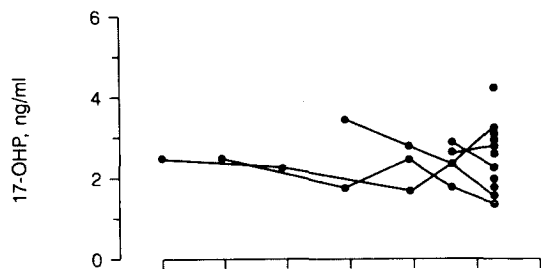


Fig. 3. Levels of hCG (above median) in EP patients with normal 17-OHP, up to day of surgery (day zero).

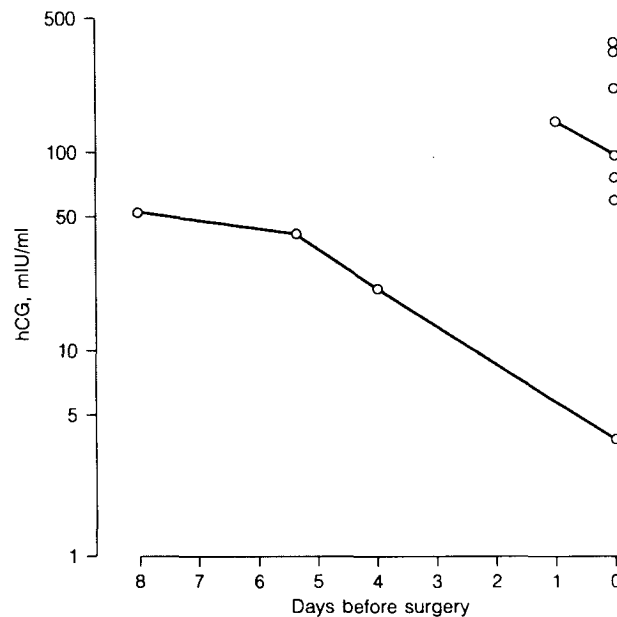
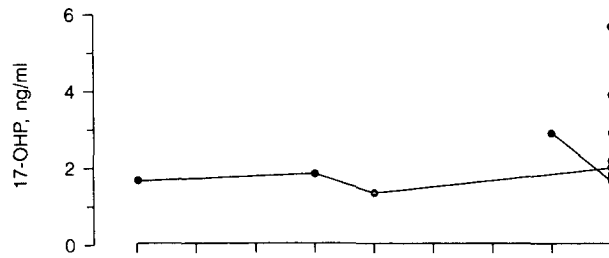


Fig. 4. Levels of hCG (below median) in EP patients with normal 17-OHP, up to day of surgery (day zero).

group were significantly lower at 6 and 7 weeks gestation but not later, compared to those in the IUP group ($p < 0.05$).

Fifteen out of 35 EP patients had 17-OHP levels which were within the 95% confidence interval for normal IUP between 6 and 9 gestational weeks. Another 4 patients actually had 17-OHP levels above the upper limit of the 95% confidence interval. In these 19 EP patients with normal 17-OHP levels, 12 patients had hCG values above the median for the entire EP population (fig. 3). The remaining 7 patients with normal 17-OHP had hCG levels below the median for the entire EP population (fig. 4). In this latter group, 1 patient was followed for 8 days prior to surgery and showed normal 17-OHP values despite

hCG ranging from 4 to 50 mIU/ml (fig. 4). The remaining 16 patients had a wide range of hCG values (26–13,797 mIU/ml), but 17-OHP levels were below the lower limit of the 95% confidence interval for normal IUP (fig. 5).

Discussion

The larger series provided in the SUNY data suggests that in untreated patients a cutoff of serum P of 8 ng/ml would better prevent the possibility of iatrogenically caused embryonal demise by premature surgical intervention through dilation and evacuation. The UMDNJ data

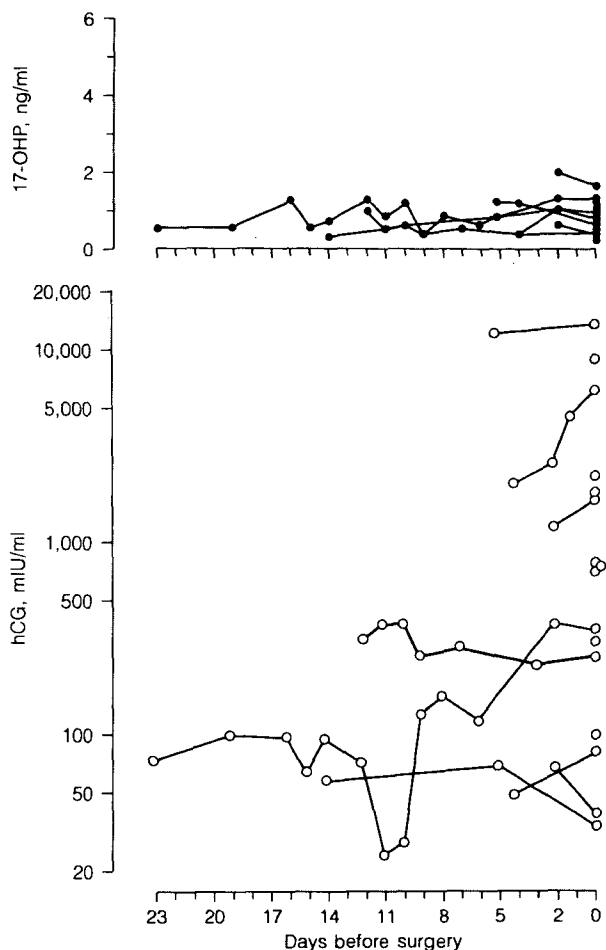


Fig. 5. Levels of hCG in EP patients with subnormal 17-OHP, up to day of surgery (day zero).

demonstrated, however, that even at that lower cutoff, aggressive P therapy could still salvage 60% of IUPs.

The use of fertility drugs or supplemental luteal phase and first trimester P therapy decreases the confidence that a serum P level above the discriminatory zone reduces the chances of an EP. Using the lower 8 ng/ml cutoff as opposed to the 15 ng/ml level of Matthews et al. [1] and Yeko et al. [2] increases the false negatives for EP, but lowers the risk of terminating a potentially viable fetus. Nevertheless, since aggressive P therapy was almost as effective in salvaging viable pregnancies with serum P < 8 ng/ml (60%) as in IUPs with serum P < 15 ng/ml (70%), the data suggest that women interested in the possibility of salvaging an IUP should be evaluated by more

conventional serial β -hCG levels and ultrasound. At a minimum, a low serum P level is a helpful ancillary test to the other clinical studies to aid the physician in determining the presence or absence of an EP.

The P levels in the luteal phase are approximately in the 15–20 ng/ml range. It is well established that low (< 8 ng/ml) P levels at that stage do not preclude implantation, but have been shown to cause a high rate of miscarriage. The question arises whether there is a critical level below which the pregnancy is doomed to fail irrespective of P replacement. The second question which remains to be answered is when is it too late in the pregnancy to start P administration.

The other aspect of the study examined CL function in patients with EP without P supplementation with serial hormone measurements. It shows that low levels of hCG can still support adequate CL function, though in 50% of all cases the levels of 17-OHP were significantly lower than gestational-age-matched controls. However, this difference is only transient and is evidenced at 6–7 weeks, but not later, in two different centers studied. This would negate the usefulness of this steroid measurement in predicting EP at a later gestational age. The use of 17-OHP for diagnosing EP would also require a precise knowledge of the menstrual age at 6–7 weeks which in many patients is difficult to ascertain and also ultrasound at that stage is already clearly diagnostic.

The serial measurements of 17-OHP support the view that, in those cases where 17-OHP levels are low, we are dealing with a primary CL defect rather than the consequence of a decreased hCG secretion. Indeed, the rescue of the CL is accomplished with hCG levels that are very low, i.e. 10–30 mIU, seen within 5 days after implantation. Thus, such an explanation is not likely since the low CL function seen in EP patients all have levels of hCG which are higher than those present in the luteal phase. The refractoriness of the CL to hCG may be operative here since hCG injection given at the end of the luteal phase can delay menses only for a limited amount of time [7]. Also, most measurements were done when the CL function was already declining, since peak 17-OHP levels in the first trimester are found at 5 weeks of gestation. This raises the possibility that the ectopic trophoblast is lacking or has low levels of a putative CL stimulator which makes further CL support difficult, i.e. shortening its life span.

The question of altered steroid levels in EP was examined by us previously, finding that the levels of P and estradiol (though they increase) are significantly lower than those in controls. Such observations were made by

others as well [8]. The reasons for such occurrences are not easily explained since the bioactivity of hCG is not the cause of the CL defect since that activity was well preserved in patients with EP who were matched with those having an IUP with similar hCG levels [5].

In conclusion, P and 17-OHP alone used as a single measurement are not adequate for diagnosing EP. Thus,

early ultrasonic evaluation and serial hCG measurements are required for precise diagnosis. Also, in spite of very low P levels, many pregnancies can be salvaged by aggressive P replacement once IUP is diagnosed. Finally, primary defect of the CL is likely to cause the low 17-OHP in EP, despite the presence of adequate hCG levels.

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