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Origin of First Trimester 17-Hydroxyprogesterone Levels as Determined in Pregnancies by Donor Oocyte Fertilization

Abstract

The study presented herein measured 17-hydroxyprogesterone (17-OHP) levels in women with ovarian failure who conceived by transfer of embryos which resulted from donor oocyte fertilization. A significant increase in 17-OHP during the first trimester was seen compared to baseline nonpregnant levels. The 17-OHP levels increased from a baseline average of 47.7 ± 9.7 ng/dl to a first-trimester average of 175.8 ± 80.6 ng/dl in the donor oocyte recipients vs. 63.0 ± 38.0 ng/dl baseline to 295.0 ± 83.9 ng/dl first-trimester in the control group. Initially these data may appear to contradict previous findings demonstrating a lack of 17-OHP secretion by the first-trimester placenta. However, by comparing the first-trimester progesterone (P) levels of normal pregnant women, and also measuring 17-OHP in patients with natural menopause and surgical menopause given exogenous P we concluded the following about the origin of first-trimester sera 17-OHP levels: hydroxylation of P to 17-OHP by the ovaries, some secretion by the first trimester placenta; and also increased adrenal conversion of P to 17-OHP. Contributing to the total serum 17-OHP level is the fact that there is cross-reactivity with P to 17-OHP.

Key Words

Donor oocyte

First trimester

Placenta

17-Hydroxyprogesterone

Introduction

In a study published more than 20 years ago, data were presented indicating that the placenta has, at best, a limited capacity for 17-hydroxylation. The study by Yoshimi et al. [1] has led to the widely held belief that the corpus luteum appears to be the primary source of progesterone (P) during pregnancy until at least 5-8 weeks of gestation, at which time the placenta assumes that role [2, 3]. In fact, since serum 17-hydroxyprogesterone (17-OHP) is thought to be exclusively produced by the corpus luteum, 17-OHP

can be used as an indication of corpus luteum function during early pregnancy [3].

The achievement of pregnancy using donor oocytes in women with ovarian failure (i.e. absence of corpora lutea) now allows us to test the theory that 17-OHP during the early first trimester is almost exclusively a product of the corpus luteum of pregnancy. In the present study, 17-OHP levels in women with ovarian failure before and after conception following donor oocyte fertilization were measured and compared to 17-OHP in a group of controls who had normal pregnancies. Also measured for compari-

son were the 17-OHP levels in a second control group of non-pregnant post-menopausal women, including some with surgical absence of the ovaries.

Materials and Methods

Study Subjects

The study group was comprised of 10 women who were diagnosed with ovarian failure, but were able to achieve a pregnancy by fertilization with donor oocytes and intrauterine transfer of the embryos.

The oocytes were donated by other patients undergoing in vitro fertilization - embryo transfer (IVF-ET) in exchange for sharing donor costs. All donors were treated with the long leuprolide acetate (LA), human menopausal gonadotropin (hMG) protocol [4]. LA was started at 1 mg subcutaneously in the mid-luteal phase and continued for at least 10 days before hMG was introduced (300 IU given i.m.); the LA was reduced to 0.5 mg when the hMG was started. After 6 days administration of LA to the donor, the recipient was started on 17 β -estradiol orally at 4 mg/day, gradually increasing the dosage to 8 mg/day. Progesterone 50 mg i.m. was also introduced at the time of the donor oocyte retrieval. Both patients were switched to oral micronized P after the first positive beta human chorionic gonadotropin (hCG) level. The range of P supplementation was 400-600 mg/day. The oocytes were shared equally between donors and recipients. Of the total 10 recipients evaluated, patients 3 and 5 had successful twin deliveries, patients 4 and 6 had missed abortions while the remainder delivered singletons at term.

Control Subjects

One of the two control groups consisted of 21 patients registered in the infertility center, who conceived without the use of ovulation-inducing drugs. Each patient conceived within ± 5 days of the recipient. All showed detectable beta hCG levels 16-18 days from ovulation and on the same day as the recipient. These controls received P supplementation during the luteal phase and throughout the first trimester. The dosage ranged from 400 to 600 mg micronized P/day according to patient need, as previously described [5].

A second control group included 6 patients. These were nonpregnant women in natural or surgical menopause who were given oral micronized P 400 mg/day. The ovaries in 2 of these patients had been surgically removed.

Variables Measured

For the pregnant women, sera 17-OHP levels were measured before pregnancy (baseline) and several times during the first 11 weeks of the first trimester. All baseline 17-OHP levels were obtained in the morning, and for the 21 spontaneous pregnancies baseline levels were measured in the follicular phase. The first-trimester 17-OHP level for each patient was defined as the average of the first trimester 17-OHP levels available for the patient. For women in the second control group, sera 17-OHP levels were obtained prior to therapy and then 7 days after P therapy.

Assays for 17-OHP and P

The serum samples of 17-OHP levels were run in duplicate using a double antibody radioimmunoassay (RIA; Pantex, Santa Monica, Calif., USA). The normal range of the assay was 11 to 80 ng/dl. To

Table 1. Comparison of mean 17-OHP levels between pregnant donor oocyte recipients (n = 8) and pregnant control group (n = 21)

	Baseline ^a ng/dl	First trimester ^b ng/dl
Donor oocyte recipients	47.7 \pm 9.7	175.8 \pm 80.6 ^c
Pregnant controls	63.0 \pm 38.0	295.0 \pm 83.9 ^d

^a p > 0.05, unpaired t test.

^b p < 0.01, unpaired t test.

^c p < 0.05, paired t test.

^d p < 0.001, paired t test.

determine the percentage of cross-reactivity between P and 17-OHP, purified progesterone standards of known concentration (Amersham Corporation, Arlington Heights, Ill., USA) were assayed by double antibody RIA (Pantex, Santa Monica, Calif., USA). Corrected 17-OHP levels are presented. Progesterone was measured by competitive binding enhanced luminescence (Amersham Corporation).

Statistical Analysis

A paired t-test was performed to compare the mean baseline 17-OHP level to the mean first trimester 17-OHP level within each group. Between group comparisons were tested using a t-test for independent samples. A 0.05 level of significance was used.

Results

The mean (± 1 SD) baseline and first-trimester 17-OHP levels for the recipients and the pregnant control group are presented in table 1. The baseline levels were not statistically different for both groups; however, the first-trimester levels are higher for the control group. The increase in 17-OHP comparing baseline to first-trimester levels was significant in both recipients (p < 0.05) and controls (p < 0.001).

Only 17 of 146 (11.6%) sera 17-OHP levels taken from the pregnant controls were below 200 ng/dl and three of these were contributed by 2 women who aborted. Only 2 recipient patients failed to reach a minimum 17-OHP level of 150 ng/dl but they had levels over 100 ng/dl. Twenty of the 58 serum 17-OHP levels (34.5%) obtained during the first trimester in the recipients were higher than 200 ng/dl.

Table 2 indicates the peak level of 17-OHP reached by each patient and at what week the maximum was achieved. Patient 3 with twins had a 17-OHP level which was 85 ng/dl 4 days after transfer, and continued to rise above 200 ng/dl 36 days after transfer. Her next 4 levels

Table 2. 17-OHP levels in pregnant women with donor oocytes

Patient No.	Baseline 17-OHP	Peak 17-OHP level, ng/dl	Serum P, ng/ml	Corrected 17-OHP	First trimester samples	Weeks from transfer for highest level
1	39	275	75.6	124	2	11
2	39	483	90.0	293	4	2
3	<30	239	45.0	149	13	9
4	64	153	41.0	73	3	4
5	48	213	81.0	51	6	8
6	44	235	81.5	72	2	1
7	<30	115	50.5	14	3	9
8	42	146	40.9	65	6	3
9	<30	280	75.6	139	8	4
10	13	346	92.0	162	13	5

(with the last one 58 days from transfer) remained above 200 ng/dl. Another patient with a singleton pregnancy had the initial 17-OHP level measured 4 days following embryo transfer; levels increased to 198 ng/dl at 17 days from transfer and rose to 224 ng/dl at 31 days. Her 17-OHP not only remained above 200 ng/dl for her last 6 levels but rose above 300 ng/dl in 4 of them, with the highest level recorded at 346 ng/dl. She delivered at term. Two patients, No. 4 and 6, had missed abortions and their maximal 17-OHP levels were low.

The data on cross-reactivity between 17-OHP and P are seen in table 3. We found a similar degree of cross-reactivity of 2.3% claimed by the manufacturers at a wide range of circulating P levels. For the purpose of analyzing data in this study, an average of 2.0% cross-reactivity was utilized.

The mean (± 1 SD) serum P levels during the first trimester were 44.6 ± 17.2 ng/dl for the recipients versus 47.3 ± 21.4 ng/dl for the controls. Correcting for cross-reactivity, the mean first-trimester serum 17-OHP for recipients should be reduced to 86.8 ng/dl which is not significant when compared to the baseline (47.7 ng/dl). However, in all cases, the 17-OHP levels were higher during pregnancy than were the baseline measurements. The highest 17-OHP level recorded in the pregnant recipients was 483 ng/dl in patient 2 at 2 weeks from transfer. Even after correcting for cross-reactivity and baseline contributions, there is still an increase in 17-OHP level of 254 ng/dl. No consistent trend was noted in individual patients corrected 17-OHP levels throughout the first trimester.

A comparison of the mean baseline, first trimester and corrected first-trimester 17-OHP levels between the donor oocyte pregnancies and the normal pregnancies is presented in figure 1.

Table 3. Cross-reactivity with P in the Pantex 17-OHP assay

P standard value ng/dl	17-OHP assayed value, ng/dl	Cross-reactivity, %
20.4	42	2.1
50.3	87	1.7

The mean baseline 17-OHP level in 6 nonpregnant women with ovarian failure and the presence of at least one ovary was 48.6 ng/dl and the mean level after 7 days of P treatment was 203.7 ng/dl. The mean serum P level was 49.4 ng/dl, indicating that 56.3 ng/dl was not explained. The mean baseline 17-OHP level in the 2 women with surgical menopause was 61.5 ng/dl and after 1 week of P therapy, this level increased to 154 ng/dl. The level increased to 180 ng/dl in one woman after 10 days. Both serum P levels averaged 46.2 after 1 week so that the entire rise for these two women in serum 17-OHP can be explained by their baseline level plus cross-reactivity with P.

Discussion

Previous data have suggested that the placenta either has no capacity for making 17-OHP [6] or may, perhaps, have a limited capacity for 17-hydroxylation [7]. The demonstration that both the serum P and 17-OHP levels increased from the luteal phase until a peak at 6-8 weeks with a subsequent rise again in P but a steady decline in 17-OHP levels provides further support for the concept

that (a) the corpus luteum and not the placenta is primarily responsible for P secretion during the early first trimester and (b) 17-OHP is made almost exclusively by the corpus luteum except for the small amount contributed by the adrenal gland [1] although placental contribution of 17-OHP cannot be excluded.

Although the data presented herein demonstrated a statistically higher mean 17-OHP level in normal pregnancies than those achieved in ovarian failure patients using donor oocytes, the data also showed a significant rise during the first trimester in the recipients compared to their baseline nonpregnant levels. This raises the question as to how the rise in 17-OHP can occur in the absence of corpora lutea in these patients.

The data also demonstrate that a significant part of the increase in serum 17-OHP in donor oocyte recipients during the first trimester is related to cross-reactivity with the higher serum P levels caused by exogenous hormone supplementation. However, patients 1, 2, 3, 9 and 10 did attain at least one first-trimester level that was greater than could be explained by cross-reactivity.

The late rise in 17-OHP in patients 1 and 3 might be explained by placental production at 9 and 11 weeks from conception, close to or which is already into the second trimester. Indeed in the second trimester Albrecht and Pepe [8] have shown that the baboon placenta is capable of 17-OHP formation. The increase seen in case 3 at 9 weeks may be attributed to the twin pregnancy, where placental mass is obviously larger and therefore, local production of 17-OHP at this time may be more evident. This is also supported by the recent study of Devroy et al. [9] who found a similar increase in P levels in patients with pregnancies in the absence of ovaries. There are several alternate hypotheses to explain the rise in the other 3 cases. It was recently shown that despite lack of change in plasma CRH and ACTH levels, there is an increase in circulating control levels, an index of adrenal activation in the first trimester [10]. In order to enable the cortisol to increase, a stimulation of 17-OHP by the adrenal might be necessary.

The postmenopausal ovary may be capable of hydroxylating exogenous P to 17-OHP in some instances. We provided evidence that in postmenopausal women, P administration caused an increase in 17-OHP which may be from the ovary itself, or from substrate conversion by the adrenal glands. Perhaps if more cases of surgical menopause were used, similar rises in some cases might have been seen despite the absence of the ovaries. Another theory is that spontaneous ovulation or at least the formation of a corpus luteum may have occurred.

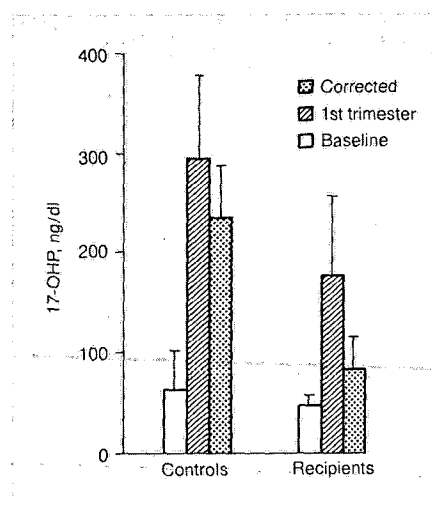


Fig. 1. A comparison of the mean baseline, first-trimester and corrected first-trimester 17-OHP levels between donor-oocyte pregnancies (RECIPIENTS) and normal pregnancies (CONTROLS). 17-OHP levels were corrected for cross-reactivity with progesterone (P).

Stimulation of folliculogenesis has been reported following the use of higher doses of exogenous estrogen [11] and, in fact, there is a published report of one pregnancy following spontaneous ovulation which occurred after the use of high-dose estrogen supplementation for frozen-embryo transfer [12].

Thus, according to our findings, it still seems reasonable to suggest that serum 17-OHP levels may be used as markers of corpus luteum function during the first trimester. One study demonstrated that despite supplementation of P during the first trimester, serum P levels were approximately the same in aborters vs. nonaborters; however, the 17-OHP levels were much lower in the aborters [13]. At the time of that publication, the amount of contribution merely from cross-reactivity from P was not fully realized so that corpus luteum function was even lower in these spontaneous aborters than previously appreciated.

The data presented herein demonstrate that the first-trimester serum 17-OHP levels represent a combination not only of the normal baseline adrenal contribution, and the contribution of the corpus luteum of pregnancy, but also it is falsely increased by cross-reactivity of the RIA for 17-OHP and P. Whether the postmenopausal ovary might also hydroxylate P to 17-OHP can only be deter-

mined by measuring 17-OHP after administering exogenous P to a postmenopausal woman with bilateral adrenalectomy. However, 17-hydroxylation seems more likely from the postmenopausal ovary rather than adrenal since in the 2 women with surgical removal of the ovaries no unaccountable serum 17-OHP was demonstrated.

The early first trimester rise in 17-OHP in some patients seems to challenge the concept that the first tri-

mester placenta is incapable of making 17-OHP. Other explanations, e.g., increased adrenal conversion of P to 17-OHP should be considered as well.

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