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## **Estradiol deficiency associated with spontaneous abortion even when adequate progesterone levels are maintained**

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### **INTRODUCTION**

Studies on the prediction and prevention of spontaneous abortions in the first trimester have centered around the hormones progesterone and estradiol produced by the corpus luteum. Estradiol during the early first trimester is produced partly in the corpus luteum and partly in the placenta<sup>1</sup>. Jones and Delpha<sup>2</sup> have estimated that inadequate functioning of the corpus luteum may be responsible for 35% of all recurrent abortions. Most experts agree that if progesterone drops too low, spontaneous abortion is likely to occur<sup>3</sup>. Witt and colleagues<sup>4</sup> have shown that although progesterone is the single most reliable predictor of outcome in high and low-risk pregnancy, serum estradiol levels are also likely to be low in aborters. Low first-trimester estradiol levels in aborters have also been reported by Miyakawa and colleagues<sup>5</sup>, Hertz and co-workers<sup>6</sup> and Yuen and colleagues<sup>7</sup>.

Despite findings on low serum estradiol levels in aborters, the modern treatment of an inadequate luteal phase, to prevent spontaneous abortion, has centered on supplementing progesterone during the first trimester. Our group has previously published data showing reduced spontaneous abortion rates with progesterone supplementation in anovulatory women<sup>8,9</sup>, in habitual aborters<sup>10</sup> and those with threatened abortion and

low serum progesterone levels<sup>11</sup>.

The possibility exists that progesterone deficiency is the main hormone deficiency leading to spontaneous abortion and that low serum estradiol levels merely indicate waning corpus luteum function with subsequent low progesterone levels. The study presented here was designed to measure the average sera estradiol levels in the first trimester of pregnancy for women whose serum progesterone was carefully maintained at normal levels by the use of exogenous progesterone. We were also interested in determining if women treated with exogenous progesterone and who still aborted during the first trimester, had different estradiol levels compared to the women treated with exogenous progesterone who did not abort.

#### **MATERIALS AND METHODS**

The study group consisted exclusively of new patients in our infertility practice presenting either with:

- (1) a history of infertility related to anovulation,
- (2) a history of two or more previous abortions, or
- (3) infertility related to luteal phase deficiency (LPD) as diagnosed by a late luteal phase biopsy being out of phase by more than 2 days in two consecutive cycles.

The first 100 consecutive patients with the above diagnoses who became pregnant were prospectively enlisted in the study. A progressively increasing human chorionic gonadotropin (hCG) level up to 500 mIU/ml was required to confirm the pregnancy. Three patients were unable to return for serial hormone assays and three refused progesterone therapy; these six were eliminated, leaving a total of 94 patients in the study.

The 94 patients were classified as follows: 24 with anovulation, 28 with recurrent abortions and 42 with LPD. All were treated in the luteal phase, initially with progesterone therapy using progesterone vaginal suppositories 25 mg twice daily. If the repeat biopsy was still out of phase by more than 2 days, the patients were instructed not to conceive until their biopsy was in phase and the dose of progesterone was adjusted higher. Dosages were adjusted through the first trimester to keep the serum progesterone levels between 30 and 45 ng/ml.

Additionally, ovulation-inducing drugs were prescribed for 39 women in the cycle of conception with 26 taking human menopausal gonadotropins

(hMG) and 13 using clomiphene citrate. Human menopausal gonadotropin was used in 18 of the 24 anovulatory women and in eight of the 42 with LPD<sup>13</sup>. Clomiphene citrate was administered to the remaining six anovulatory women and in seven women with LPD but who released oocytes from immature follicles (as determined by peak serum estradiol level in the cycle not reaching 200 pg/ml)<sup>13</sup>.

A  $\beta$ -hCG level was obtained 16 days from the most mature follicle. Maturity was determined using a sonographic measurement of average follicle diameter of 18–24 mm and serum estradiol levels of at least 200 pg/ml. With the establishment of a positive pregnancy test, the progesterone dose was doubled and the patient was requested to have a serum progesterone, estradiol and  $\beta$ -hCG level measured weekly, from week 4 following conception up to the first pelvic sonographic examination performed at 7–8 weeks. Women with viable fetuses continued with weekly estradiol and progesterone monitoring until 12 weeks following conception, but the  $\beta$ -hCG level was no longer obtained. If viability was not confirmed, the ultrasound was to be repeated 1 week later, at which time the last estradiol, progesterone and  $\beta$ -hCG values would be obtained. Non-aborters had an average of 5.91 serum samples drawn, aborters an average of 4.2.

Serum estradiol measurements were performed by solid-phase radioimmunoassay (Diagnostic Products, Los Angeles, California). The serum progesterone and  $\beta$ -hCG testing was done by radioimmunoassay techniques based on enhanced luminescence (Amersham, Arlington Heights, Illinois). Ultrasound examination for follicle diameter and fetal viability was performed by one of three experienced sonographers using an ATL Ultramark 4 (Advanced Technology Laboratories, Bothell, Washington) with a 5 MHz vaginal transducer.

The mean weekly serum estradiol levels were calculated and presented by pregnancy outcome (aborters and non-aborters) and drug therapy administered in addition to progesterone (non vs. hMG or clomiphene citrate).

Since the distribution of estradiol levels in the drug-induced ovulation group had a larger variance than the distribution of estradiol levels in the non-drug-induced ovulation group during weeks 4 and 5 postconception, and the number of serum samples drawn varied from patient to patient, an ANOVA model to compare mean estradiol levels over time, by drug and pregnancy outcomes, was deemed inappropriate. Comparisons of mean estradiol levels for each week by pregnancy outcome were made within each drug therapy group using Student's *t*-test for independent groups with a probability level of 0.05.

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**Table 1** Patient characteristics by pregnancy outcome

	<i>Non-aborters (%)</i>	<i>Aborters (%)</i>
<i>Infertility factor</i>		
Anovulatory	16 (21.6)	8 (40)
Luteal phase deficiency	35 (47.3)	7 (35)
Previous abortions	23 (31)	5 (25)
<i>Age (years)</i>		
21-25	2 (2.7)	1 (5)
26-30	25 (33.8)	5 (25)
31-35	22 (29.7)	7 (35)
36-40	25 (33.8)	7 (35)
<i>Mean progesterone dosage (mg)</i>		
Luteal phase	68.4	65.5
First trimester	465	530
<i>Drug-induced ovulation therapy</i>		
None	42 (56.7)	13 (65)
Clomiphene citrate	10 (13.5)	1 (5)
Human menopausal gonadotropin	22 (29.8)	6 (30)

**RESULTS**

There were 74 women (78.8%) who successfully completed their first trimester, and 20 aborters (21.2%). A comparison of the distribution of infertility factor, age and progesterone dosage administered to aborters and non-aborters is presented in Table 1.

In the analysis, seven patients were identified whose data were atypical of the distribution of serum estradiol levels in the remaining sample, i.e. these seven patients had elevated initial serum estradiol levels (>700 pg/ml). These patients' data were not included in the calculation of mean estradiol levels for this study. In addition, it was determined that the distribution of serum estradiol levels in the drug-induced ovulation, and non-drug-induced ovulation groups were not the same. The variance of serum estradiol levels was greater in the drug-induced ovulation groups than in the non-drug-induced ovulation groups in weeks 4 and 5 following conception. These groups were therefore analyzed separately.

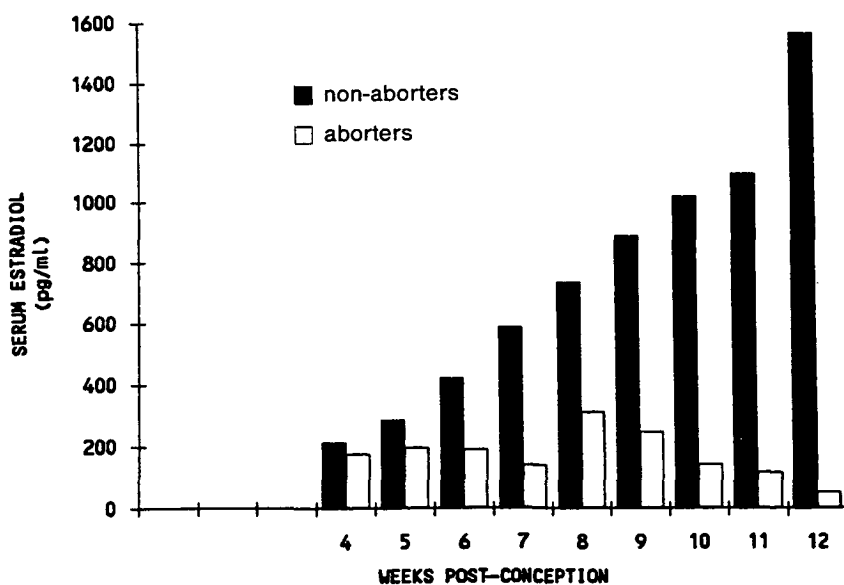
The averaged weekly serum estradiol levels for the progesterone treated group not taking ovulation drugs is presented in Table 2. Mean serum estradiol levels were significantly lower in the aborters than the non-aborters from week 5 to week 12 (Figure 1). In fact, 61.3% of the serum estradiol levels measured for aborters were at least 1 SD below the corresponding weekly mean for non-aborters.

*Low estradiol levels in first-trimester aborters*

**Table 2** Comparison of first-trimester serum estradiol levels by pregnancy outcome in patients treated with exogenous progesterone only

Weeks after conception	Mean ( $\pm$ SD) serum estradiol			
	Non-aborters	n	Aborters	n
4	212.8 $\pm$ 71.5	13	175.3 $\pm$ 89.9	10
5	287.5 $\pm$ 111.5	28	195.8 $\pm$ 157.4*	12
6	421.8 $\pm$ 216.8	34	189.1 $\pm$ 126.9*	8
7	587.8 $\pm$ 302.2	30	139.1 $\pm$ 88.4*	8
8	734.3 $\pm$ 442.9	35	311.3 $\pm$ 184.2*	7
9	888.6 $\pm$ 445.2	33	246.5 $\pm$ 130.6*	4
10	1018.9 $\pm$ 562.8	30	142.0 $\pm$ 117.0*	3
11	1096.3 $\pm$ 576.1	24	116.0 $\pm$ 98.9*	2
12	1569.6 $\pm$ 865.1	23	52.0	1

\* $p < 0.5$  (Student's *t*-test comparing mean estradiol in aborters vs. non-aborters)



**Figure 1** Mean serum estradiol levels by pregnancy outcome in patients treated with exogenous progesterone only

There was no difference in the mean serum estradiol levels in patients taking hMG as compared to patients taking clomiphene citrate. These patients were therefore combined into one drug-induced ovulation group. The average weekly serum estradiol levels for the drug-induced ovulation

**Table 3** Comparison of first trimester serum estradiol levels by pregnancy outcome in patients treated with exogenous progesterone and ovulation-inducing drugs

Weeks after conception	Mean ( $\pm$ SD) serum estradiol			
	Non-aborters	n	Aborters	n
4	348.6 $\pm$ 148.4	13	230.8 $\pm$ 54.1	4
5	480.6 $\pm$ 240.9	22	215.6 $\pm$ 78.7*	5
6	545.7 $\pm$ 227.3	24	173.9 $\pm$ 34.9*	4
7	624.6 $\pm$ 282.3	23	126	1
8	762.9 $\pm$ 393.1	21	126	1
9	835.8 $\pm$ 399.1	18		
10	885.0 $\pm$ 430.3	19		
11	1062.2 $\pm$ 438.6	13		
12	1340.8 $\pm$ 490.8	9		

\*  $p < 0.05$  (Student's *t*-test comparing mean estradiol in aborters vs. non-aborters)

group are presented in Table 3.

The aborters taking ovulation-inducing drugs all aborted by week 8 following conception so the data on which to base our conclusions is more limited. At week 4 following conception, the aborters and non-aborters had the same mean serum estradiol levels, but from week 5, the aborters had lower mean serum estradiol levels similar to the trend for the non-drug-induced ovulation group (Figure 2). A total of 73.3% of the individual serum estradiol levels measured for aborters were at least 1 SD below the corresponding weekly mean for non-aborters.

## DISCUSSION

It is hard to compare the 21.1% abortion rate of this study with the standard 15% level, since the pregnancy is not normally established in the general population as early as 4 days after the missed menses (a 500 mIU/ml  $\beta$ -hCG level was used to approximate this time). Thus, this study may have included early aborters who would not have been included in general population studies, because without early  $\beta$ -hCG measurements, and a delay in menses due to progesterone therapy, they would not have known that they were pregnant.

In the first few weeks of pregnancy, there was little difference in the serum estradiol levels between aborters and non-aborters, but the differences increased each week. The data presented here thus suggest that the failure of the corpus luteum may occur during the pregnancy, and loss of hormones, including estradiol, may account for pregnancy

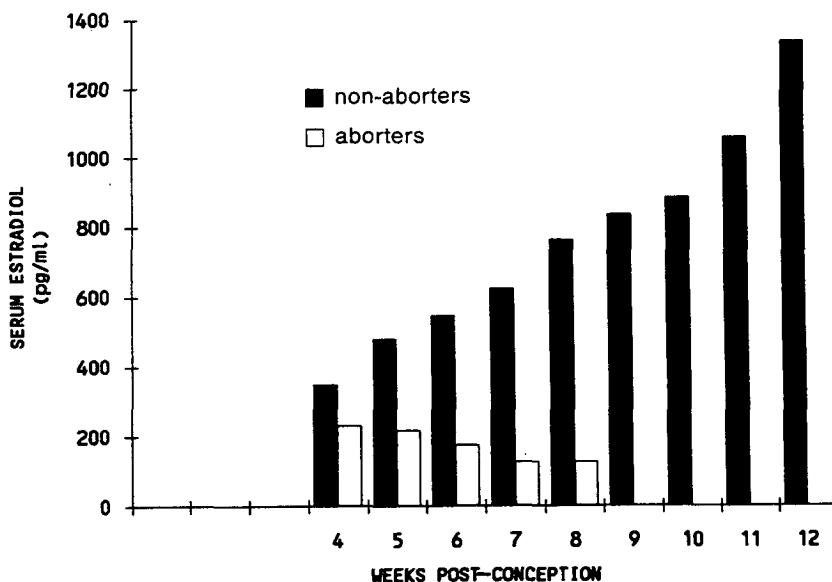


Figure 2 Mean serum estradiol levels by pregnancy outcome in patients treated with exogenous progesterone and ovulation-inducing drugs

losses, despite in-phase endometrial biopsies and maintenance of normal serum progesterone levels.

The data do not allow us to draw any definite conclusions about the efficacy of hormonal therapy in preventing spontaneous abortion. Rather, these findings stimulate speculation and allow more insight into the hypotheses that still need to be tested to resolve the conflict over the importance of corpus luteum failure and its treatment to inhibit pregnancy loss. There are several possibilities:

- (1) Corpus luteum failure may occur in early pregnancy despite a normal late luteal phase endometrial biopsy.
- (2) Correction of the out-of-phase endometrial biopsy without further maintenance of progesterone and estradiol at proper critical levels during the pregnancy may still result in fetal wastage.
- (3) Progesterone therapy alone, without estradiol therapy, may be insufficient to correct LPD and provide maximum prevention of spontaneous abortion. Previous studies have also found a lower mean 17-hydroxyprogesterone level in exogenous progesterone-maintained

aborters compared with non-aborters, suggesting a need for some other initial corpus luteum hormone and perhaps this was estradiol<sup>14</sup>.

- (4) Progesterone supplementation is not necessary to prevent spontaneous abortions, but estradiol maintenance is.
- (5) Some other essential hormone or substance made by the corpus luteum is still missing and supplementing estradiol and progesterone would not prove effective in lowering the abortion rate. For example, Critchley and colleagues<sup>15</sup> found a promising substance made by the corpus luteum that allows the endometrium to respond to progesterone in the production of placental protein 14.
- (6) The assumption that the rising hCG level will maintain the corpus luteum during pregnancy and thus preclude the need for hormone treatment is erroneous.
- (7) Placental failure is the cause of low estradiol levels, not the failure of the corpus luteum. Using donor oocytes, Devroey and co-workers<sup>16</sup> concluded that the placenta begins making estradiol between 6 and 7 weeks, so damage to the early placenta rather than to the corpus luteum is possible.

Previously, the only available norms for serum estradiol levels during the first trimester were published by Miyawaka and colleagues<sup>5</sup>. Our data provide more exact serum estradiol levels for weeks 4-12 based on significantly more data, and for both the infertile patient treated with just exogenous progesterone and for those treated with exogenous progesterone and ovulation inducing drugs.

Hopefully, the average estradiol levels for non-aborters presented in this study can be used in properly randomized studies in both women at risk for spontaneous abortion and women with normal risks to address some of the hypotheses proposed here.

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