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D.B. Nelson · R.B. Ness · J.A. Grisso · M. Cushman

Sex hormones, hemostasis and early pregnancy loss

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Abstract *Aims:* This study was designed to determine the association between coagulation factors and spontaneous abortion adjusting for sex steroids and to examine the influence of sex hormones on coagulation factors early in pregnancy. *Methods:* Pregnant women presenting to the emergency department at the Hospital of the University of Pennsylvania were recruited and followed through 22 weeks gestation. Cases were women who experienced a spontaneous abortion (n=29) and controls were women who maintained their pregnancy to 22 weeks gestation (n=89). Participants completed a baseline questionnaire to assess demographic, reproductive, and drug use information. Blood samples measured estradiol, progesterone, fibrinogen, and factor VII antigen. *Results:* Cases of spontaneous abortion had significantly lower levels of estradiol, progesterone, fibrinogen and factor VII antigen compared to controls. The relationship between low levels of fibrinogen and factor VII antigen was diminished adjusting for the sex steroids. Regression analyses found low progesterone was the primary prospective marker for early pregnancy loss among our study population. *Conclusions:* The relationship between coagulation factors and spontaneous abortion was reduced after adjustment for progesterone suggesting that progesterone mediates the relationship between low levels of coagulation factors and spontaneous abortion. Progesterone seems to be the primary marker for a spontaneous abortion among women seeking emergent care.

D.B. Nelson (✉) · J.A. Grisso
Center for Clinical Epidemiology and Biostatistics,
University of Pennsylvania, 922 Blockley Hall,
423 Guardian Drive, Philadelphia PA 19104-6021
e-mail: dnelson@cceb.med.upenn.edu
Tel.: ++49-215-5736011, Fax: ++49-215-5732265

R.B. Ness
Graduate School of Public Health, University of Pittsburgh

M. Cushman
Laboratory for Clinical Biochemistry Research,
Department of Medicine, University of Vermont

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Introduction

Changes in hormones during pregnancy are profound, beginning soon after fertilization and continuing throughout gestation [3, 10, 15, 20, 24, 27]. Among pregnant women, studies have found dramatic increases in serum levels of estradiol and progesterone (from first trimester values of 2.5 ng/ml and 26 ng/ml to third trimester values of 15.0 ng/ml and 175 ng/ml, respectively) [21, 24, 26]. A high level of estradiol during pregnancy is necessary for initial uterine hypertrophy and proper placental and fetal development [15, 27]. In addition, increases in progesterone are essential to promote adhesion and implantation early in gestation and to inhibit lactation and myometrial contractions later in pregnancy [4, 15, 27]. The clinical importance of a consistent rise in estradiol and progesterone very early in pregnancy has been confirmed in prior research [4, 21]. Aksoy et al. conducted a prospective cohort study and found significantly lower levels of estradiol and progesterone among pregnant women experiencing a spontaneous abortion compared to women with term delivery [2]. A failure to document an appropriate rise in estradiol and progesterone within weeks of conception may be one method of identifying a problem pregnancy that may be in need of further clinical consideration.

Pregnancy also alters hemostasis, creating a heightened coagulation state as measured by rising levels of fibrinogen and factor VII antigen, an increase in activated protein C-resistance (APC-resistance), as well as an overall increase in blood volume [3, 5, 9, 10, 12, 16, 20]. Hemostasis during pregnancy is considered a delicate balance between ensuring proper implantation and placental development; adequate passage of oxygen, nutrients and waste products through the placento-uterine interface; and promoting a rapid restriction of blood flow to the uterus following delivery.

The mechanisms through which pregnancy-induced hormonal changes affect hemostasis are unclear. One possibility is that during pregnancy coagulation factors change in response to estrogen, just as changes in the levels of coagulation factors and the magnitude of fibrinolysis have been documented among women using exogenous oral contraceptives and hormone replacement therapy [1, 7, 8, 13, 14, 28].

Although the importance of sex hormones in early pregnancy has been studied extensively, limited research has focused on the role of hemostasis in early pregnancy. Few studies have been conducted to examine the role of coagulation factors in early pregnancy and to relate these factors to adverse pregnancy outcomes. We previously examined the influence of coagulation factors (fibrinogen and factor VII antigen) on the riks for spontaneous abortion and reported that levels of fibrinogen and factor VII antigen in the lowest tertile, compared to the upper two tertiles, were associated with at least a three fold increased risk of spontaneous abortion [18]. These findings provided preliminary evidence of a role for coagulation factors in sustaining early pregnancy. Recognizing the role of sex hormones in early gestation and the connection between increasing estrogen levels and rising coagulation factors, we used stored plasma samples to measure 17 beta-estradiol and progesterone, in addition to coagulation factors, among pregnant women very early in gestation.

The objective of this paper was

1) to document the influence of sex hormones (estradiol and progesterone) on the risk for a spontaneous abortion, and
2) to evaluate whether sex hormone levels mediate the association between coagulation factors (fibrinogen and factor VII antigen) and spontaneous abortion.

Methods and materials

The recruitment and enrollment methods for this study have been described elsewhere [19]. Briefly, women aged 14–40 presenting to the Emergency Department (ED) at the Hospital of the University of Pennsylvania and residing in selected zip codes in Philadelphia were screened for eligibility. Women who reported that their last menstrual period occurred more than 22 weeks earlier, women who reported a normal menstrual cycle in the past 28 days, and women who reported a hysterectomy were not screened since these groups were unlikely to be less than 22 weeks pregnant [23]. Urine pregnancy tests were conducted on all other women regardless of the reason for the ED visit. Women with a positive pregnancy test but found to have an ectopic, molar or twin pregnancy; language barrier; acute mental illness; post-partum or reported therapeutic abortion within the prior 14 days; or a completed spontaneous abortion 3 or more days prior to enrollment were considered ineligible. Women meeting the criteria for eligibility were asked to enroll in the study and 90% of eligible women agreed to participate. The study protocol was approved by the Institutional Review Board of the University of Pennsylvania and all women participating in the study provided signed informed consent.

At the time of enrollment, women completed an in-person interview to collect demographic information, reproductive history, pregnancy-related symptoms, and current and past drug use. Hair and urine specimens were collected to validate self-reported cocaine, alcohol, and tobacco use. Blood samples were collected to measure coagulation factors and sex hormone levels. Gestational age was calculated on the basis of self-reported date of the last menstrual period. Previous evaluation of this method to assess gestational age has shown that in over 70% of women, the dates calculated using last mentrual period were within three weeks of the dates calculated by measuring crown-rump length [19].

A case of spontaneous abortion was defined as any woman experiencing a pregnancy loss prior to 23 weeks gestation. Women enrolled in the study and not experiencing a spontaneous abortion at baseline completed telephone interviews at 16 and 22 weeks gestation to collect self-report information on the status of pregnancy. Outcomes were ascertained for 94% of participants, of the cases of spontaneous abortion reported during the follow-up period over one-half were confirmed by medical record abstraction or pathology records. In this analysis, we compared women who experienced a spontaneous abortion during the follow-up period (cases) to women maintaining their pregnancy through 22 weeks gestation (controls). The women experiencing a spontaneous abortion at entry to the study were excluded from this analysis.

Blood was collected into one sodium citrate tube and one tube containing 4.5 mmol/L EDTA, 0.15 KIU/L aprotonin, and 20 μml/I D-Phe-Pro-Arg-chloromethyl ketone (SCAT-1 tube, Haematologic Technologies, Inc., Essex Junction, VT). Fibrinogen was measured in citrate plasma using the Clauss method with a coefficient of variation (CV) of 3.0% [6]. Factor VII antigen was measured in citrate plasma using an immunoassay with a CV of 5.4% [11]. Stored plasma samples were used to determine the level of estradiol (estradiol-17β) and progesterone at enrollment using Coat-A-Count assay kits (Diagnostic Products Corporation, Los Angeles CA).

Bivariate analyses were conducted to compare baseline demographic characteristics, prior and current pregnancy history, drug use, sex hormone levels and coagulation factors for the cases of spontaneous abortion and pregnant controls. Multiple logistic regression analyses, using the backward elimination technique, were conducted to separately assess the relationships among sex hormone levels, coagulation factors, and the risk for spontaneous abortion while adjusting for confounding variables. Current alcohol use (yes/no), prior spontaneous abortion (yes/no), cocaine use documented by hair (positive/negative), and history of bacterial vaginosis (yes/no), variables found to be significant on the univariate level or in other studies, were included in the models as potential confounders. The lowest one-third of the fibrinogen distribution (3.0 g/L or lower) and factor VII antigen distribution (94% of normal or lower) were used to define low levels of these two coagulation factors. Sex hormone levels were included in the model as continuous variables. The final regression analyses examined the influence of coagulation factors adjusting for both confounding variables and sex hormone levels.

Results

Participants were predominately young and African American and over one-third had not completed high school. The cases (n=29) had a slightly earlier gestational age at enrollment compared to the pregnant controls (n=89) (9 weeks vs. 11 weeks). Cases at follow-up experienced a spontaneous abortion a median of 9 days after enrollment. As reported previously, cases of spontaneous abortion were significantly more likely to report vaginal bleeding, a prior diagnosis of bacterial vaginosis, and to have used cocaine in the prior three months as detected by hair analysis [18].

As expected, the cases of spontaneous abortion had significantly lower levels of both progesterone and estradiol compared to the pregnant controls (Table 1). The

Table 1 Hormonal and hemostatic profile of pregnant controls and cases of spontaneous abortion

	Controls (n=89)	Cases at follow-up (n=29)
Progesterone [ng/mL]*		
Mean (range)	27 (7–88)	12.4 (0.8–60)
Median	23	9
Estradiol [pg/mL]*		
Mean (range)	999 (93–7007)	288 (22–1407)
Median	586	167
Fibrinogen [g/L]*		
Mean (range)	3.7 (0.61–6.3)	3.2 (2.0–5.7)
Median	3.6	3.0
Factor VII antigen [% of normal]*		
Mean (range)	110 (40–375)	90 (40–147)
Median	103	84

* Significance at p<0.05, comparing the cases to the pregnant controls

Table 2 Multiple logistic regression results for fibrinogen and cases of spontaneous abortion

	Adjusting for estradiol OR (95% CI)	Adjusting for progesterone OR (95% CI)
Fibrinogen (<3.0 g/L)	4.4 (1.3–14.5)	2.1 (0.6–6.9)

Variables not remaining in the model: alcohol use, prior spontaneous abortion
Variables remaining in the model: cocaine use and bacterial vaginosis
Dependent Variable: controls vs. cases

Table 3 Multiple logistic regression results for factor VII antigen and cases of spontaneous abortion

	Adjusting for estradiol OR (95% CI)	Adjusting for progesterone OR (95% CI)
Factor VII antigen (<94% of normal)	3.1 (1.0–9.4)	2.2 (0.68–7.1)

Variables not remaining in the model: alcohol use, prior spontaneous abortion
Variables remaining in the model: cocaine use and bacterial vaginosis
Dependent Variable: controls vs. cases

mean level of progesterone among cases was less than one-half the mean level among controls (12 ng/mL compared to 27 ng/mL, p<0.001). Estradiol was also dramatically lower among the cases of spontaneous abortion compared to the pregnant controls (288 pg/mL vs. 999 pg/mL, p<0.001). The finding of lower sex hormone levels among cases remained after adjusting for gestational age. After adjusting for other risk factors for spontaneous abortion, low levels of estradiol and progesterone levels were significantly correlated with an increased risk of spontaneous abortion (OR: 0.996, 95% CI: 0.993–0.998 and OR: 0.866, 95% CI: 0.805–0.931; respectively).

As previously reported, the cases of spontaneous abortion had lower levels of coagulation factors [18]. The mean level of fibrinogen was 3.2 g/L among the cases and 3.7 g/L among the pregnant controls (p<0.001) and the mean level of factor VII antigen was 90% of normal among the cases and 110% of normal among the controls (p<0.001) (Table 1). Comparing the cases of spontaneous abortion to the pregnant controls, women with low fibrinogen levels were four times more likely to have a spontaneous abortion than women with fibrinogen levels at or above 3.0 g/L (OR=4.9, 95% CI: 1.8–13.5) after adjusting for confounding factors. Women with reduced factor VII antigen concentrations had a threefold increased risk of spontaneous abortion compared to women with factor VII antigen levels at or above 94% of normal (OR=3.2 95% CI: 1.2–8.1).

Low fibrinogen levels continued to be related to an elevated risk of spontaneous abortion after adjusting for estradiol. Women with low levels of fibrinogen continued to have a fourfold increased risk for spontaneous abortion (OR=4.4, 95% CI: 1.3–14.5) after adjusting for estradiol (Table 2). However, low fibrinogen levels were not significantly associated with the risk for spontaneous abortion after adjustment for progesterone levels (OR=2.1, 95% CI: 0.6–6.9) (Table 2). A similar relationship was found for factor VII antigen. Women with low levels of factor VII antigen were found to have a three fold increased risk for spontaneous abortion (OR=3.1, 95% CI: 1.0–9.4) after adjusting estradiol levels (Table 3). The relationship between low levels of factor VII antigen and spontaneous abortion was attenuated after adjusting for progesterone in the second regression model (OR=2.2, 95% CI: 0.68–7.1).

Discussion

The results from this study confirm the importance of rising sex hormone levels (estradiol and progesterone) in maintaining a pregnancy through 22 weeks gestation. In addition, we found that both low levels of sex hormones and coagulation factors, measured early in pregnancy, were associated with an increased risk of a subsequent spontaneous abortion. Adjusting for estrogen did not change the relationship between fibrinogen, factor VII antigen and spontaneous abortion, but the association between coagulation factors and early pregnancy loss was reduced after adjustment for progesterone; suggesting that progesterone may mediated the relationship between coagulation factors and spontaneous abortion. Future studies should be designed to determine the individual contribution of estrogen and progesterone on hemostatic changes during pregnancy.

Previous research among pregnant women has documented the importance of rising progesterone levels ear-

ly in pregnancy [4, 21]. Indeed, an elevated progesterone level can predict the viability of a pregnancy and may be used to discriminate between an intrauterine versus ectopic pregnancy. This study, to our knowledge, is the first to examine the relationship between sex hormones and coagulation factors during pregnancy. Much of the research to date regarding the relationship between sex hormones and hemostatis has focused on women using oral contraceptives (OC users) or postmenopausal women using hormone replacement therapy (HRT users). The OC research has consistently documented a significant increase in fibrinogen and factor VII levels among low dose OC uses; but the individual contributions of estrogen and progesterone on the change in coagulation factors has not been examined in these women [7, 8, 14]. Studies of postmenopausal women using HRT have documented a decrease in fibrinogen and an increase in factor VII levels among postmenopausal women using estrogen only [17, 25]. In the PEPI trial, postmenopausal women treated with either estrogen alone or estrogen in combination with progestin did not demonstrate the normal age-related rise in fibrinogen that was observed in the placebo group [25]. This body of research are the only studies describing the influence of sex hormones on coagulation factors.

To date, research has not adequately examined the independent role of progesterone in promoting hemostasis among either pregnant women, postmenopausal women or women using oral contraceptives. This study was the first, to our knowledge, to report a relationship between fibrinogen, factor VII antigen and progesterone levels early in pregnancy. Studies using a larger sample size of pregnant women are necessary to determine the role of both progesterone and estradiol in mediating the rise in coagulation factors early in gestation and the impact of these coagulation factors in early pregnancy loss. Future research is also needed to examine the joint effect of both low sex hormone levels and low coagulation factors during pregnancy and to determine if coagulation factors and/or progesterone level can be used as markers for women at high risk factor for an early pregnancy loss.

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