Plasminogen Activator Inhibitor Activity: An Independent Risk Factor for the High Miscarriage Rate During Pregnancy in Women With Polycystic Ovary Syndrome

C.J. Glueck, Ping Wang, Robert N. Fontaine, Luann Sieve-Smith, Trent Tracy, and Sarah K. Moore

In 41 women with at least one pregnancy drawn from a group of 149 (108 never-pregnant) women with polycystic ovary syndrome (PCOS), our specific aim was to determine whether hypofibrinolysis mediated by high plasminogen activator inhibitor activity (PAI-Fx) is an independent risk factor for miscarriage. The 41 women had 77 total pregnancies with 34 miscarriages (44%) and 42 live births (55%). There were 12 women with at least one pregnancy, at least one miscarriage, and no live births (16 pregnancies and 16 miscarriages). There were 15 women with at least one pregnancy, no miscarriages, and at least one live birth (25 pregnancies and 28 live births). Of 12 women with only miscarriages and no live births, 67% had PAI-Fx greater than 16.4 U/mL (normals' 95th percentile), versus 29% of 15 women with no miscarriages and all live births ($\chi^2 = 3.8$, $P = .052$). By stepwise logistic regression, the number of pregnancies ($P = .0001$) and PAI-Fx ($P = .016$) were significant positive explanatory variables for the number of miscarriages. Age, 4G/5G polymorphisms of the PAI gene, factor V Leiden, methylenetetrahydrofolate reductase (MTHFR) gene mutations, androstenedione, testosterone, sex hormone–binding globulin, the Quetelet index, and fasting serum insulin and glucose were not significant variables in the logistic regression model. In a separate stepwise logistic regression, three nonoverlapping groups of women (12 with ≥1 pregnancy, ≥1 miscarriage, and 0 live births, 10 with ≥1 pregnancy, ≥1 miscarriage, and ≥1 live births, and 15 with ≥1 pregnancy, 0 miscarriages, and ≥1 live births) were the dependent variables. PAI-Fx was positively associated ($P = .05$) with the group with the worst pregnancy outcome (≥1 pregnancy, ≥1 miscarriage, and 0 live births). The 41 women with PCOS and at least one pregnancy were more likely than healthy normal controls to have heterozygosity and homozygosity for the 4G/5G polymorphism of the PAI-1 gene ($P = .028$), but did not differ from normals for factor V Leiden ($P > .10$) or MTHFR ($P > .09$) mutations. PAI-Fx is a predominant independent significant positive reversible risk factor for miscarriage in women with PCOS.

FAMILIAL THROMBOPHILIA, including the factor V Leiden mutation, homozygosity for the methylenetetrahydrofolate reductase gene (MTHFR) mutation, heterozygosity for the prothrombin gene mutation, hyperhomocysteinemia, and protein S deficiency, can cause miscarriage and serious complications of pregnancy.[1-8] Kupferminc et al[8] compared 110 women who had one obstetrical complication (preeclampsia, abruptio placentae, fetal growth retardation, and stillbirth) and 110 control women with at least one normal pregnancy. The factor V Leiden mutation was found in 20% of women with obstetrical complications versus 6% of controls ($P = .003$), homozygosity for the MTHFR mutation in 22% versus 8% ($P = .005$), and the prothrombin gene mutation in 10% versus 3% ($P = .03$). Overall, 52% of women with obstetrical complications had a heritable thrombophilic mutation, versus 17% of controls ($P < .001$). Thrombophilic deficiency of protein C, protein S, or antithrombin III or anticardiolipin antibodies were detected in an additional 13% of women with obstetrical complications versus 1% of controls ($P < .001$).[8] The thrombophilic antiphospholipid syndrome (anticardiolipin antibodies and/or the lupus anticoagulant) also causes obstetrical complications and recurrent miscarriage.[9-12] The documentation of thrombophilic causes of recurrent miscarriage is important.[1-12] Bick et al[10] have reported a 100% success rate in achieving normal term deliveries among women with thrombophilia who used 81 mg/d aspirin preconception and low-dose heparin 5,000 U every 12 hours after conception.

High levels of plasminogen activator inhibitor activity (PAI-Fx), the major determinant of hypofibrinolysis, can contribute to the placental alterations that occur in preeclampsia.[13,14] Moreover, in two studies of 116 and 500 patients who had early recurrent miscarriages of unknown origin, Gris et al[3,15] reported low levels of the major fibrinolysis activator (tissue plasminogen activator activity) and high levels of the fibrinolysis inhibitor (PAI-Fx). They speculated that "an impaired plasmin dependent proteolysis in women might favor recurrent abortion by promoting fibrin deposition in early placental circulation or by limiting trophoblast development, or both." Polycystic ovaries have been found by ultrasound in 36%,[16] 56%,[17] and 82%[18] of women with recurrent early miscarriage. Miscarriage rates in 21 women with polycystic ovary syndrome (PCOS) with supportive and observational care have been reported to be 18%.[16] In prospective studies of 239 women with PCOS, 39.1% of pregnancies achieved with gonadotropins miscarried, as did 17.6% of pregnancies achieved with gonadotropin-releasing hormone agonists.[19] In prospective studies of 106 women with PCOS and at least three consecutive first-trimester miscarriages, live birth rates in women receiving pituitary suppression were 52% and 63% in controls.[20] In 27 infertile women with PCOS treated with pulsatile luteinizing hormone (LH)-releasing hormone, nine (33%) had first-trimester miscarriages.[21] Miscarriage rates following in vitro fertilization are higher in infertile women with PCOS (35.8%) versus those with normal ovaries (23.6%, $P = .0038$).[22] Speculative causes for the high miscarriage rate in PCOS[16-22] include high levels of PAI-Fx (hypofibrinolysis),[3,15] testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), or LH and low levels of progesterone.[18,21,23,24]

From the Cholesterol Center, Jewish Hospital, Cincinnati; and the Molecular Diagnostics and Research Center, Cincinnati, OH.

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Address reprint requests to C.J. Glueck, MD, Cholesterol Center, 3200 Burnet Ave, Cincinnati, OH 45229.

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In women with PCOS, metformin therapy decreases fasting serum insulin, PAI-Fx, testosterone, androstenedione, and DHEAS[25-28] and restores menstrual cyclicity in most (91% to 96%) previously oligo/amenorrheic women with POCS.[26,28] In women with PCOS, metformin-induced restoration of menstrual cyclicity and subsequent conception mandates better ways to ameliorate their historically high non–metformin-treated, first-trimester miscarriage rate.[16-22] This led us to the present study of 41 women with at least one pregnancy drawn from a group of 149 (108 never-pregnant) women with PCOS. Our specific aim was to determine whether hypofibrinolysis mediated by high PAI-Fx (reversible by metformin)[25-28] has a significant independent association with miscarriage.

SUBJECTS AND METHODS

Patients

The 149 women with PCOS were entered onto the study in the temporal sequence of their referral to the Cholesterol Center without exclusions or selection bias. Criteria for the diagnosis of PCOS[25-29] are summarized for the total cohort (N = 149) and for the 41 women with at least one pregnancy in Table 1. Of the 149 and 41 patients, 148 (99%) and 40 (98%), respectively, met at least two of the first three major diagnostic criteria. Diagnostic criteria included (1) polycystic ovaries (≥10 subcapsular follicles 2 to 8 mm in diameter) diagnosed by pelvic ultrasound or laparoscopy, (2) chronic oligomenorrhea (≤six menses per year and/or amenorrhea for ≥6 months), (3) clinical hyperandrogenism (hirsutism[29] with a Ferriman-Gallwey Score ≥7),[30] LH to follicle-stimulating hormone (FSH) ratio greater than 1.5 in the amenorrheic state, (4) morbid obesity, and (5) acanthosis nigricans (Table 1).

Assessment was performed for other endocrinopathies causing oligo/amenorrhea.[25-29] The presence of congenital adrenal hyperplasia was assessed by medical history, physical examination, and 17-OH progesterone levels. Pituitary insufficiency was assessed by measurement of thyroxine (T_4), thyrotropin (TSH), fasting morning cortisol, LH, and FSH. No patients had clinical evidence of hyperprolactinemia: galactorrhea, visual-field defects, known pituitary tumors, or hypogonadotropic hypogonadism. Serum prolactin levels were not uniformly measured.

Study Protocol

The study was performed with signed informed consent, following a protocol approved by the Jewish Hospital Institutional Review Board.

At the initial visit in the morning after an overnight fast, a medical history and physical examination was performed with measurement[25-29] of height, weight, systolic and diastolic blood pressure, fasting blood glucose, glycohemoglobin, and endocrine status and assessment for other endocrinopathies (as before), lipids, PAI-Fx, 4G/5G polymorphisms of the PAI-1 gene, and factor V Leiden and MTHFR gene mutations.[28,31] None of the women had previously used metformin. A detailed history was obtained from the patients and from their doctors' records for the number of pregnancies, live births, miscarriages, and elective abortions (Figs 1 and 2). Similarly, information was obtained on previous treatments to enhance fertility. Women with at least one pregnancy were categorized into three nonoverlapping subgroups based on obstetrical outcomes (Fig 2), with subsequent group comparison of PAI-Fx, fasting serum insulin, and the other measured variables.

Two of 41 patients with at least one pregnancy were found to have type 2 diabetes mellitus at the initial visit for the present study, but both were known to have normal fasting blood glucose and glucose tolerance tests during their antecedent pregnancy.

Table 1. Diagnosis of PCOS in 149 Women, 41 of Whom Had at Least One Pregnancy

Parameter		All (N = 149)	≥1 Pregnancy (n = 41)
Polycystic ovaries by ultrasound, laparoscopy, or surgery		118/149 (79%)	37/41 (90%)
Chronic oligomenorrhea (<6 menses/yr and/or amenorrhea for ≥6 mo)		149/149 (100%)	41/41 (100%)
Hirsutism (Ferriman-Gallwey Score[29] ≥7)		145/148 (98%)	39/40 (98%)
Acanthosis nigricans		29/145 (20%)	8/40 (20%)
BMI >27[28]		126/144 (88%)	36/41 (88%)
Estradiol <69 pg/mL[28]		108/146 (74%)	24/39 (62%)
Testosterone >83 ng/dL[28]		24/147 (16%)	5/39 (13%)
Androstenedione >250 ng/dL[28]		41/144 (29%)	9/38 (24%)
LH/FSH ratio >1.5 in the amenorrheic state[28]		87/143 (61%)	21/38 (55%)
Fasting serum insulin ≥20 μU/mL[28]		87/146 (60%)	20/41 (49%)
Fasting blood glucose >126 mg/dL[28]		6/147 (4%)	2/40 (5%)
HbA_{1c} >6.4%[28]		8/140 (6%)	5/37 (14%)
PAI-Fx >16.4 U/mL[28]		65/146 (45%)	17/40 (43%)
4G/5G PAI-1 gene polymorphism	Normals (n = 234)[31]	$\chi^2 = 12$, $P = .002$	$\chi^2 = 7.1$, $P = .028$
4G4G	47 (20%)	38 (26%)	6 (15%)
4G5G	102 (44%)	78 (54%)	27 (66%)
5G5G	85 (36%)	28 (19%)	8 (20%)

Normal Controls

Studies in healthy normal controls were performed during the same period as the patients' studies.[28,31] Forty normal hospital personnel (17 men and 23 women; mean ± SD age, 37 ± 7 years) served as controls

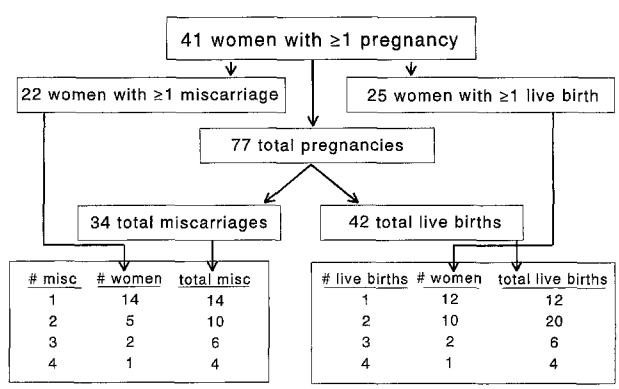

Fig 1. Pregnancy outcomes in 41 women with PCOS and ≥1 pregnancy. There were 77 total pregnancies, 34 miscarriages, 36 singleton births, 3 sets of twin births, and 4 elective abortions, for a total of 42 live births.

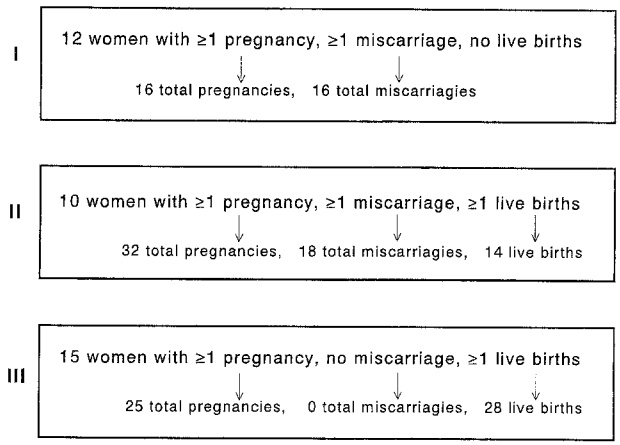

Fig 2. Three nonoverlapping groups of women with PCOS and ≥1 pregnancy characterized by pregnancy outcomes. In group III, there were 25 pregnancies with 28 live births.

for comparison to the patients' PAI-Fx; 234 healthy normal subjects[31] served as controls for PAI-1 gene polymorphisms.

Laboratory Methods

Radioimmunoassay[25-28] was used to measure LH, FSH, estradiol, testosterone, androstenedione, DHEAS, progesterone, sex hormone–binding globulin, and insulin levels. Glucose, the lipid profile, lipoprotein(a), and PAI-Fx were determined by previously established methods.[25-28]

Glycohemoglobin (HbA_{1c}) was measured using the Bio-Rad Variant Hemoglobin A_{1c} method (Bio-Rad Diagnostics, Hercules, CA). The within-day coefficient of variation for a pool with a mean HbA_{1c} of 5.66% was 0.9%, and for a pool with a mean of 12.92%, it was 0.35%. The between-day coefficient of variation for a pool with a mean of 5.77% was 1.64%, and for a pool with a mean of 10.02%, it was 1.48%.

PAI-Fx, 4G/5G polymorphism of the PAI-1 gene, and factor V Leiden and MTHFR gene mutations in the healthy normal controls were not correlated ($P > .09$) with age, sex, or race, making it unlikely that differences between patients and controls represented age, sex, or race effects.[31] Polymerase chain reaction assays were performed for two major thrombophilic gene mutations (factor V Leiden and MTHFR) and for the hypofibrinolytic 4G/5G polymorphism of the PAI-1 gene as previously described.[28,31]

Statistical Analysis

Spearman correlations[32] were calculated among the number of pregnancies, live births, and miscarriages (yes = 1, no = 0, number of miscarriages) and endocrine and coagulation measures.

Using normal limits (>95th percentile and <5th percentile for sex hormone–binding globulin)[28] for laboratory measurements as cutoff points, patient groups classified by pregnancy outcomes were compared by χ^2 tests.[32]

Stepwise logistic regression[32] was used to assess significant independent determinants of the number of miscarriages. Separately, the dependent variables were three nonoverlapping subgroups of women with PCOS, based on their pregnancy outcomes (Fig 2). Explanatory variables included age, number of pregnancies, PAI-Fx, PAI-1 gene polymorphisms, and factor V Leiden and MTHFR gene mutations. In an expanded logistic regression model, explanatory variables included age, number of pregnancies, PAI-Fx, PAI-1 gene polymorphisms, factor V Leiden and MTHFR gene mutations, androstenedione, testosterone, sex hormone–binding globulin, Quetelet index (kg/cm^2 × 1,000), fasting serum insulin, and glucose (Table 2).

The predicted probabilities for miscarriages in the PCOS population of interest were calculated from the stepwise logistic regression model. For panel 1 in Table 2, the model is detailed in the Appendix.

The stepwise logistic regression models were separately re-run after excluding the two women who were diagnosed as type 2 diabetics at the initial visit.

RESULTS

Characteristics of 149 Women With PCOS and the 41 Women With PCOS With at Least One Pregnancy

Total cohort (N = 149). Of 149 women, 148 (99%) had at least two of the first three major criteria for the diagnosis of PCOS (Table 1). High (>500 ng/dL) 17-OH progesterone was found in one of 149 women (1%).

Table 2. Significant Independent Determinants of Miscarriage by Logistic Regression

Dependent Variable	Significant Explanatory Variable	Sign	*P*
	Stepwise selection on age, no. of pregnancies, PAI-Fx, PAI gene, factor V Leiden and MTHFR mutations		
1. No. of miscarriages (40 observations used)	No. of pregnancies	+	.0001
Concordant 62%, discordant 9%	PAI-Fx	+	.016
	Stepwise selection on age, no. of pregnancies, PAI-Fx, PAI gene, factor V Leiden and MTHFR mutations		
2. Pregnancy outcome groups	PAI-Fx	+	.050
I = 12 women with ≥1 pregnancy, ≤1 miscarriage, no live births			
II = 10 women with ≥1 pregnancy, ≤1 miscarriage, ≥1 live births			
III = 15 women with ≥1 pregnancy, no miscarriage, ≥1 live births			
Concordant 61%, discordant 38%			
	Stepwise selection on age, no. of pregnancies, PAI-Fx, PAI gene, factor V Leiden and MTHFR mutations, androstenedione, testosterone, sex hormone–binding globulin, insulin, Quetelet Index, and glucose		
3. No. of miscarriages (38 observations used)	No. of pregnancies	+	.0004
	PAI-FX	+	.027

Most of the cohort was obese (88%), with a body mass index (BMI) greater than 27, and most (74%) had an estradiol level less than 69 pg/mL (lower-normal limit for midcycle estradiol).[28] High fasting serum insulin (≥20 μU/mL)[28] was common, found in 60% of the women. Type 2 diabetes was uncommon; five women (3%) had both fasting blood glucose greater than 126 mg/dL and HbA_{1c} greater than 6.4%. None had type 1 diabetes. High levels (>normals' 95th percentile)[28] of PAI-Fx, the major inhibitor of fibrinolysis, were common, found in 45% of the women. The distribution of the 4G/5G polymorphism of the PAI-1 gene, a determinant of PAI-Fx levels and of insulin resistance,[28,31] was shifted toward 4G/4G homozygosity and 4G/5G heterozygosity when compared with 234 normals ($\chi^2 = 12.2$, $P = .002$) (Table 1).

Of 149 women, 74 (50%) had at least one high-level[28] (>normals' 95th percentile) total testosterone, free testosterone, androstenedione, or DHEAS. The mean ± SD age and median age of the 149 women were 30 ± 7 and 30 years.

Women with at least one pregnancy (n = 41). Of 41 women with at least one pregnancy, 40 (98%) had at least two of the first three major criteria for the diagnosis of PCOS (Table 1). None had high (>500 ng/dL) 17-OH progesterone.

Most (88%) of the 41 women were obese, with a BMI greater than 27,[28] and most (62%) had estradiol less than 69 pg/mL (lower-normal limit for midcycle estradiol).[28] High fasting serum insulin[28] (≥20 μU/mL) was common, found in 49% of the women. Type 2 diabetes was uncommon; two women (5%) had both fasting blood glucose greater than 126 mg/dL and HbA_{1c} greater than 6.4%. At their antecedent pregnancies, these two women had normal fasting blood glucose and normal glucose tolerance. None had type 1 diabetes. High levels (>normals' 95th percentile)[28] of PAI-Fx were common, found in 43% of the women. The distribution of the 4G/5G polymorphism of the PAI-1 gene was shifted toward 4G/4G homozygosity and 4G/5G heterozygosity compared with 234 normals ($\chi^2 = 7.14$, $P = .028$) (Table 1 and Fig 3).

Of the 41 women, 17 (41%) had at least one high-level (>normals' 95th percentile)[28] total testosterone, free testosterone, androstenedione, or DHEAS. The mean ± SD age and median age of the 41 women were 35 ± 7 and 33 years.

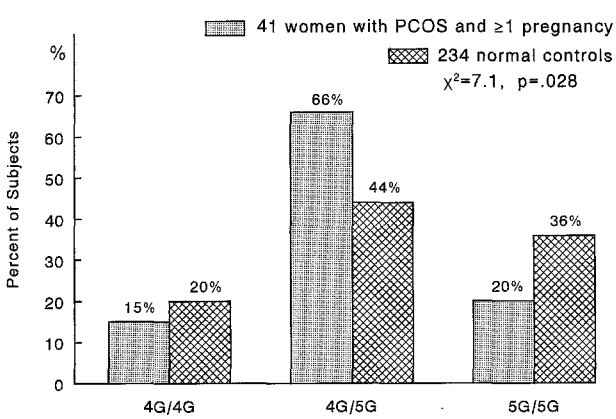

Fig 3. 4G/4G and 4G/5G polymorphisms of the PAI-1 gene along with 5G/5G (wild-type normal) in 41 women with PCOS and ≥1 pregnancy and in 234 healthy normal controls.

There were no differences ($P > .1$) in the prevalence of the factor V Leiden mutation in the 41 PCOS women (7.5%) versus 234 healthy normal controls (3%). The prevalence of MTHFR mutations in 41 PCOS women (34% wild-type normal, 56% heterozygous, and 10% homozygous) did not differ ($P = .09$) as compared with the 234 healthy normal controls (51% wild-type normal, 38% heterozygous, and 11% homozygous).

Pregnancies, Miscarriages, and Live Births

Of 149 women with PCOS, 41 had at least one pregnancy (Fig 1). Of these 41 women, 22 (54%) had at least one miscarriage and 25 (61%) had at least one live birth. The 41 women with at least one pregnancy had 77 total pregnancies which resulted in 34 miscarriages (44%), 42 live births (55%; three sets of twins), and four elective abortions. Of 34 miscarriages, 14 women had one miscarriage, five had two, two had three, and one had four. Of 42 total live births, 12 women had one, 10 had two, two had three, and one had four (Fig 1). There were 12 women who had at least one pregnancy, at least one miscarriage, and no live births (16 pregnancies and 16 miscarriages). There were 10 women with at least one pregnancy, at least one miscarriage, and at least one live birth (32 pregnancies, 18 miscarriages, and 14 live births). There were 15 women with at least one pregnancy, no miscarriages, and at least one live birth (25 pregnancies and 28 live births) (Fig 2).

Drug treatments to enhance fertility were previously used by 59% of the 41 women. The three groups of women categorized by pregnancy outcomes (Fig 2) did not differ ($P > .1$) in the percentage who used fertility-enhancing drugs, 67% for group I (worst outcome, 16 pregnancies, 16 miscarriages, and 0 live births), 70% for group II (intermediate outcome, 32 pregnancies, 18 miscarriages, and 14 live births), and 53% for group III (best outcome, 25 pregnancies, 0 miscarriages, and 28 live births).

PAI-Fx and Other Measured Variables in Groups According to Pregnancy, Miscarriage, and Live Births

Of the 12 women with only miscarriages and no live births, 67% had high PAI-Fx (>16.4 U/mL),[28] versus 29% of the 15 women with no miscarriages and all live births ($\chi^2 = 3.8$, $P = .052$). Seventy-five percent of the group of 12 women had high fasting serum insulin (≥20 μU/mL),[28] versus 40% and 30% of the groups of 15 and 10 women, respectively ($P = .069$ and $P = .035$). There were no other differences among the three groups of women ($P > .10$) for any of the other measured variables, including the PAI gene, factor V Leiden, and MTHFR mutations.

Correlations Between Live Births, Miscarriage (yes/no), Number of Miscarriages, and Other Measured Variables

In the 41 women with at least one pregnancy, the number of live births was inversely correlated with PAI-Fx ($r = -.34$, $P = 0.03$). The likelihood of miscarriage correlated positively with androstenedione ($r = .33$, $P = .04$). The number of miscarriages correlated positively with the number of pregnancies ($r = .45$, $P = .0035$) and inversely with sex hormone–binding globulin ($r = -.32$, $P = .048$). There were no significant

correlations between the number of live births, likelihood of miscarriage, or number of miscarriages and the PAI gene, factor V Leiden, or MTHFR mutations ($P > .25$).

There were no significant correlations ($P > .25$) between the use of fertility-enhancing drugs and either the likelihood of miscarriage or the number of miscarriages.

PAI-Fx was positively correlated with fasting serum insulin ($r = .52$, $P = .0005$).

Significant Explanatory Variables for the Number of Miscarriages

With the number of miscarriages as the dependent variable and explanatory variables including age, number of pregnancies, PAI-Fx, 4G/5G mutation of the PAI gene, and factor V Leiden and MTHFR mutations, stepwise logistic regression was performed. Positive explanatory variables included the number of pregnancies ($P = .0001$) and PAI-Fx ($P = .016$). The number of pregnancies can correctly predict the probability of the number of miscarriages 57% of the time (concordant), but fails (discordant) to correctly predict 15% of the time. The PAI-Fx level adds to the correct prediction 5% of the time (concordant), and reduces the failure to correctly predict 6% of the time (discordant). Together, the number of pregnancies and PAI-Fx can correctly predict the probability of the number of miscarriages 62% of the time (concordant), but fail (discordant) to correctly predict 9% of the time. The predicted probabilities for miscarriage in the PCOS population of interest can be calculated (Appendix) from the stepwise logistic regression model (Table 2). For instance, for a single pregnancy in a woman with PCOS with low-normal PAI-Fx (6 U/mL) or high PAI-Fx (32 U/mL), the predicted likelihood of miscarriage is 15% and 47%, respectively (Appendix). For four pregnancies in a woman with PCOS with high PAI-Fx (32 U/mL), the predicted likelihood of at least one miscarriage was 99%, at least two, 93%, at least three, 65%, and all four, 12%.

In a separate logistic regression model, the three nonoverlapping groups of women classified by pregnancy and miscarriage were the dependent variables. Age, number of pregnancies, PAI-Fx, 4G/5G mutation of the PAI gene, and factor V Leiden and MTHFR mutations were explanatory variables. PAI-Fx was positively associated ($P = .05$) with the groups with the worst pregnancy outcomes (group I *v* II and III and groups I and II *v* III). The PAI-Fx level can correctly predict the pregnancy outcome group 61% of the time, but fails to correctly predict 38% of the time (Table 2).

In an expanded logistic regression model, the dependent variable was the number of miscarriages and the explanatory variables included age, number of pregnancies, PAI-Fx, PAI gene, factor V Leiden and MTHFR gene mutations, androstenedione, testosterone, sex hormone–binding globulin, Quetelet index, fasting serum insulin, and glucose (Table 2). By stepwise selection, positive explanatory variables included the number of pregnancies ($P = .0004$) and PAI-Fx ($P = .027$). In the third stepwise logistic regression model, the number of pregnancies can predict the number of miscarriages correctly 53% of the time, but fails to correctly predict 17% of the time. The PAI-Fx level adds to the correct prediction 21% of the time, and increases the failure to correctly predict 5% of the time. Together, the number of pregnancies and PAI-Fx can correctly predict the probability of the number of miscarriages 74% of the time (concordant), but fail (discordant) to correctly predict 22% of the time (Table 2).

The outcomes of the stepwise logistic regression models were not substantially changed by excluding two women who were diabetic at the initial visit for the present study but were not diabetic at their antecedent pregnancies (data not shown).

DISCUSSION

In the current study of 149 women with PCOS predominantly of childbearing age, 108 (72%) had never conceived, testimony to the infertility characteristic of PCOS.[25-29] The 41 women with at least one pregnancy had 77 pregnancies that resulted in 34 mostly first-trimester miscarriages (44%) and 42 live births (55%). This 44% miscarriage rate is at the higher end of five previous studies in which 18%,[16] 18%,[19] 33%,[21] 36%,[22] and 48%[20] miscarriage rates have been reported. In the present study, the predicted likelihood of miscarriage in single pregnancy in a woman with PCOS who had either low-normal PAI-Fx (6 U/mL) or high PAI-Fx (32 U/mL) was 15% and 47%, respectively, approximating previously reported miscarriage rates (18%[16] to 48%[20]). The present report is also congruent with studies of women with habitual miscarriages, wherein polycystic ovaries have been found by ultrasound in 36%,[16] 56%,[17] and 82%[18] of women. Multiple risk factors have been implicated for the high miscarriage rate in PCOS, including high LH, testosterone, androstenedione, and PAI-Fx and low progesterone.[3,15,18,21,23,24,33] In the present study, the likelihood of miscarriage was positively correlated with androstenedione, and the number of miscarriages was correlated positively with the number of pregnancies and inversely with sex hormone–binding globulin. Sex hormone–binding globulin levels are depressed by the high serum androgens and high insulin characteristic of PCOS,[25-28] probably accounting for this correlation in the current study.

High levels of PAI-Fx, the major determinant of hypofibrinolysis, are a major risk factor for miscarriage,[3,15] probably by producing placental insufficiency via increased thrombosis as the placenta establishes arteriovenous anastomoses with the maternal circulation. In the present study, the number of live births was inversely correlated with PAI-Fx: 67% of women with only miscarriages and no live births had high PAI-Fx (>16.4 U/mL), versus 29% of women with no miscarriages and only live births. Moreover, PAI-Fx was a significant independent variable for the number of miscarriages and for the worst pregnancy outcomes.

Metformin therapy restores menstrual cyclicity in most (91% to 96%) previously oligo-amenorrheic women with PCOS,[26,28] decreasing PAI-Fx, testosterone, androstenedione, DHEAS, and insulin.[25-28] Restoration of menstrual cyclicity[26,28] mandates better ways to ameliorate the historically high, non–metformin-treated, first-trimester miscarriage rate in PCOS.[16-22] High PAI-Fx may cause thrombosis-induced placental insufficiency.[3,15] Since metformin usually normalizes or improves high PAI-Fx in women with PCOS,[25-28] we speculate that metformin therapy during pregnancy in women with PCOS would reduce their high rate of first-trimester miscarriage.

Metformin is not teratogenic, and has been shown to be safe in pregnancy.[34,35]

The major heritable thrombophilias, mediated by factor V Leiden and MTHFR mutations, have been recently shown to be risk factors for complications of pregnancy.[8] Factor V Leiden and MTHFR mutations were no more common in the 41 women with PCOS and at least one pregnancy than in healthy normal controls. In contrast, however, the 41 women with PCOS and at least one pregnancy were more likely than healthy normal controls to have heritable hypofibrinolysis with heterozygosity and homozygosity for the 4G/5G polymorphism of the PAI-1 gene. We speculate that the prothrombotic effects of thrombophilia[1-12] and hypofibrinolysis[36-40] are probably functionally similar as etiologies for both miscarriage and complications of pregnancy. We suspect that heritable hypofibrinolysis mediated by high PAI-Fx in women with PCOS[25-28] is a major determinant of their high miscarriage rate, with adverse pregnancy outcomes similar to those in women with heritable thrombophilic traits.[8] We postulate that metformin therapy during pregnancy in women with PCOS, by optimizing the endocrine milieu and decreasing PAI-Fx,[25-28] would reduce the otherwise high miscarriage rate. Within this frame of reference, in women with thrombophilia- or hypofibrinolysis-induced pregnancy complications or miscarriages,[1-12] low–molecular-weight heparin may provide another approach to ameliorate miscarriage through thromboprophylaxis.[1,10,41,42]

APPENDIX: LOGISTIC REGRESSION MODEL

The categorical response (number of miscarriages) is fitted by a logistic regression model as follows:

$$\text{Ln}\frac{\text{P(had} \geq \text{i miscarriages)}}{1 - \text{P(had} \geq \text{i miscarriages)}} = \alpha_i + C_1 \times \text{number of total pregnancies} + C_2 \times \text{PAI-Fx},$$

or equivalently,

$$\text{P(had} \geq \text{i miscarriages)} = \frac{\exp(\alpha_i + C_1 \times \text{number of total pregnancies} + C_2 \times \text{PAI-Fx})}{1 + \exp(\alpha_1 + C_1 \times \text{number of total pregnancies} + C_2 \times \text{PAI-Fx})},$$

where P (had ≥i miscarriages) is the predicted probability that a woman had ≥i miscarriages, and α_1, C_1, and C_2 are constant coefficients determined by the raw data during the regression fitting process.

The first logistic regression model in panel 1, Table 2 is

$$\text{P(had} \geq 1 \text{ miscarriage)} = \frac{\exp(-3.7778 + 1.6889 \times \text{number of total pregnancies} + 0.0613 \times \text{PAI-Fx})}{1 + \exp(-3.7778 + 1.6889 \times \text{number of total pregnancies} + 0.0613 \times \text{PAI-Fx})},$$

$$\text{P(had} \geq 2 \text{ miscarriages)} = \frac{\exp(-6.1076 + 1.6889 \times \text{number of total pregnancies} + 0.0613 \times \text{PAI-Fx})}{1 + \exp(-6.1076 + 1.6889 \times \text{number of total pregnancies} + 0.0613 \times \text{PAI-Fx})},$$

For example, if a patient had one pregnancy and PAI-Fx = 6, then the model gives the predicted probability (15%):

$$\text{P(had 1 miscarriage)} = \frac{\exp(-3.7778 + 1.6889 \times 1 + 0.0613 \times 6)}{1 + \exp(-3.7778 + 1.6889 \times 1 + 0.0613 \times 6)} = 15\%.$$

If a patient had one pregnancy and PAI-Fx = 32, then the predicted probability (47%) is as follows:

$$\text{P(had 1 miscarriage)} = \frac{\exp(-3.7778 + 1.6889 \times 1 + 0.0613 \times 32)}{1 + \exp(-3.7778 + 1.6889 \times 1 + 0.0613 \times 32)} = 47\%.$$

REFERENCES

1. Younis JS, Ohel G, Brenner B, et al: Familial thrombophilia—The scientific rationale for thrombophylaxis in recurrent pregnancy loss? Hum Reprod 12:1389-1390, 1997

2. Brenner B, Blumenfeld Z: Thrombophilia and fetal loss. Blood Rev 11:72-79, 1997

3. Gris JC, Ripart-Neveu S, Maugard C, et al: Respective evaluation of the prevalence of haemostasis abnormalities in unexplained primary early recurrent miscarriages. The Nimes Obstetricians and Haematologists (NOHA) Study. Thromb Haemost 77:1096-1103, 1997

4. Rai RS, Regan L, Chitolie A, et al: Placental thrombosis and second trimester miscarriage in association with activated protein C resistance. Br J Obstet Gynaecol 103:842-844, 1996

5. Younis JS: Factor V Leiden—A novel etiology of the long-standing thrombosis theory for recurrent pregnancy loss. Am J Obstet Gynecol 178:1107-1108, 1998

6. Richards EM, Makris M, Preston FE: The successful use of protein C concentrate during pregnancy in a patient with type 1 protein C deficiency, previous thrombosis and recurrent fetal loss. Br J Haematol 98:660-661, 1997

7. Brenner B, Maudel H, Lanir N, et al: Activated protein C resistance can be associated with recurrent fetal loss. Br J Haematol 97:551-554, 1997

8. Kupferminc MJ, Eldor A, Steinman N, et al: Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 240:9-13, 1999

9. Rand JH, Wu XX, Andree HA, et al: Pregnancy loss in the antiphospholipid-antibody syndrome—a possible thrombogenic mechanism. N Engl J Med 337:154-160, 1997

10. Bick RL, Madden J, Heller KB, et al: Recurrent miscarriage: Causes, evaluation, and treatment. Medscape Womens Health 3:2, 1998 (abstr)

11. Lima F, Khamashta MA, Buchanan NM, et al: A study of sixty pregnancies in patients with the antiphospholipid syndrome. Clin Exp Rheumatol 14:131-136, 1996

12. Birdsall MA, Lockwood GM, Ledger WL, et al: Antiphospholipid antibodies in women having in-vitro fertilization. Hum Reprod 11:1185-1189, 1996

13. Estelles A, Grancha S, Gilabert J, et al: Abnormal expression of plasminogen activator inhibitors in patients with gestational trophoblastic disease. Am J Pathol 149:1229-1239, 1996

14. Halligan A, Bonnar J, Sheppard B, et al: Haemostatic, fibrinolytic and endothelial variables in normal pregnancies and pre-eclampsia. Br J Obstet Gynaecol 101:488-492, 1994

15. Gris JC, Neveu S, Mares P, et al: Plasma fibrinolytic activators and their inhibitors in women suffering from early recurrent abortion of unknown etiology. J Lab Clin Med 122:606-615, 1993

16. Liddell HS, Sowden K, Farquhar CM: Recurrent miscarriage: Screening for polycystic ovaries and subsequent pregnancy outcome. Aust N Z J Ob Gynaecol 37:402-406, 1997

17. Clifford K, Rai R, Watson H, et al: An informative protocol for the investigation of recurrent miscarriage: Preliminary experience of 500 consecutive cases. Hum Reprod 9:1328-1332, 1994

18. Watson H, Kiddy DS, Hamilton-Fairley D, et al: Hypersecretion of luteinizing hormone and ovarian steroids in women with recurrent early miscarriage. Hum Reprod 8:829-833, 1993

19. Homburg R, Levy T, Berkovitz D, et al: Gonadotrophin-releasing hormone agonist reduces the miscarriage rate for pregnancies achieved in women with polycystic ovarian syndrome. Fertil Steril 59:527-531, 1993

20. Clifford K, Rai R, Watson H, et al: Does suppressing luteinising hormone secretion reduce the miscarriage rate? Results of a randomized controlled trial. BMJ 312:1508-1511, 1996

21. Homborg R, Armar NA, Eshel A, et al: Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. BMJ 297:1024-1026, 1988

22. Balen AH, Tan SL, MacDougall J, et al: Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. Hum Reprod 8:959-964, 1993

23. Okon MA, Laird SM, Tuckerman EM, et al: Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. Fertil Steril 69:682-690, 1998

24. Doldi N, Gessi A, Destafani A, et al: Polycystic ovary syndrome: Anomalies in progesterone production. Hum Reprod 13:290-293, 1998

25. Velazquez EM, Mendoza SG, Hamer T, et al: Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 43:647-654, 1994

26. Velazquez EM, Acosta A, Mendoza SG: Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 90:392-395, 1997

27. Velazquez EM, Mendoza SG, Wang P, et al: Metformin therapy is associated with decreased plasma plasminogen activator inhibitor-1, lipoprotein(a), and immunoreactive insulin levels in patients with polycystic ovary syndrome. Metabolism 46:454-457, 1997

28. Glueck CJ, Wang P, Fontaine R, et al: Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. Metabolism 48:511-519, 1999

29. Knochenhauer ES, Key TJ, Kahsar-Miller M, et al: Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: A prospective study. J Clin Endocrinol Metab 83:3078-3082, 1998

30. Ferriman D, Gallwey JD: Clinical assessment of body hair growth. J Clin Endocrinol Metab 21:1440-1447, 1961

31. Glueck CJ, Bell Howard, Vadlamani L, et al: Heritable thrombophilia and hypofibrinolysis: Possible causes of retinal vein occlusion. Arch Ophthalmol 117:43-49, 1999

32. Snedecor GW, Cochran WG: Statistical Methods (ed 7). Ames, IA, Iowa State University Press, 1980

33. Takeuchi T, Nishii O, Okamura T, et al: Free testosterone and abortion in early pregnancy. Int J Gynaecol Obstet 43:151-156, 1993

34. Coetzee EJ, Jackson WP: Metformin in management of pregnant insulin-dependent diabetics. Diabetologia 16:241-245, 1979

35. Coetzee EJ, Jackson WP: The management of non insulin-dependent diabetes during pregnancy. Diabetes Res Clin Pract 1:281-287, 1985-86

36. Glueck CJ, Glueck HI, Welch M, et al: Familial idiopathic osteonecrosis mediated by familial hypofibrinolysis with high levels of plasminogen activator inhibitor. Thromb Haemost 71:195-198, 1994

37. Glueck CJ, Freiberg R, Tracy T, et al: Thrombophilia and hypofibrinolysis: Pathophysiologies of osteonecrosis. Clin Orthop 334: 43-56, 1997

38. Glueck CJ, Freiberg R, Gruppo R, et al: Thrombophilia and hypofibrinolysis: Reversible pathogenetic etiologies of osteonecrosis in adults and in children (Legg-Perthes disease). AOA International Symposium on Osteonecrosis, in Urbaniak JR, Jones JP Jr (eds): Osteonecrosis: Etiology, Diagnosis, and Treatment. Rosemont, IL, American Academy of Orthopedic Surgeons, 1997, pp 105-110

39. Glueck CJ, Crawford A, Roy D, et al: Association of antithrombotic factor deficiencies and hypofibrinolysis with Legg-Perthes disease. J Bone Joint Surg Am 78:3-13, 1996

40. Glueck CJ, Brandt G, Gruppo R, et al: Resistance to activated protein C and Legg-Perthes disease. Clin Orthop 338:139-152, 1997

41. Nelson-Piercy C, Letsky EA, de Swiet M: Low–molecular weight heparin for obstetric thromboprophylaxis: Experience of sixty-nine pregnancies in sixty-one women at high risk. Am J Obstet Gynecol 176:1062-1068, 1997

42. Gris JC, Neuveu S, Tailland ML, et al: Use of a low–molecular weight heparin (enoxaparin) or of a phenformin-like substance (moroxydine chloride) in primary early recurrent aborters with an impaired fibrinolytic capacity. Thromb Haemost 73:362-367, 1995