

Pregnancy outcomes and community health: the POUCH study of preterm delivery

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Summary

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In light of the social/ethnic disparity in preterm delivery (PTD) rates, the Pregnancy Outcomes and Community Health (POUCH) Study takes a broad view of the determinants of PTD by attempting to link underlying biological and psychosocial factors. The relationships between placental pathology, maternal biomarkers, and antecedent psychosocial factors are evaluated in three hypothesised pathways of PTD – one characterised primarily by infection, one by maternal vascular disease, and one by premature elevations in corticotropin releasing hormone in the absence of histological evidence of placental pathology. Within each pathway, an emphasis is placed on understanding the roles of stress and of maternal serum alpha-fetoprotein, an early biomarker associated with PTD. The POUCH Study enrolls pregnant women from five Michigan communities. Information about these women and their environments is gathered through detailed interviews and collection of biological samples including hair, urine, saliva, blood, vaginal fluid, and vaginal smear at 15–26 weeks of gestation. We have chosen to focus on the second trimester – a time when pathological processes may have evolved to a detectable stage, but generally before the onset of biological changes that accompany labour. This focus is consistent with the long-range goal of early detection/intervention and prevention of PTD.

Introduction

Preterm birth is associated with elevated risks of infant mortality,¹ cerebral palsy, mental retardation, blindness and deafness.^{2–4} Attempts to reduce the incidence of preterm delivery (PTD) through the use of risk-scoring protocols,^{5–7} frequent cervical assessment,⁸ cervical cerclage,^{9,10} home monitoring of uterine contractions,¹¹ tocolytic therapy,^{12,13} enhanced prenatal care¹⁴ and social support programmes¹⁵ have generally been unsuccessful.

The disparity in PTD rates between population subgroups within the US is striking. In 1996, 16.3% of African-American singletons, but 8.1% of non-Hispanic white singletons were born before 37 weeks' gestation.¹⁶ This ethnic disparity worsens at lower gestations; before 29 weeks, the prematurity risk for African-American singletons was elevated fourfold (2.0% vs. 0.5%).¹⁶ The higher risk of PTD among

African-Americans and among women from lower social class backgrounds^{17–20} remains unexplained, but suggests that certain biological routes to PTD are closely tied to social factors.

PTD research has frequently lacked detailed measurements of both biological and social factors in the same study. Timing has also been a problem in PTD research. Studies focusing on preterm labour itself, or on pregnancy changes in the third trimester, cannot always distinguish between biomarkers of impending delivery in the month ahead and biomarkers of

BV	bacterial vaginosis
CRH	corticotropin-releasing hormone
FFN	fetal fibronectin
G-CSF	granulocyte colony-stimulating factor
MSAFP	maternal serum alpha-fetoprotein
PTD	preterm delivery

pathology unique to PTD. In response to these limitations, we set two over-arching research goals. First, we aimed to test causal hypotheses for PTD that connect biological factors and psychosocial factors assessed at the individual and ecological levels; and second, we chose to concentrate on conditions and events prior to the third trimester, with the emphasis of the research placed on opportunities for early detection/intervention and prevention.

We began by examining the relation of PTD to maternal serum alpha-fetoprotein (MSAFP), the most consistent biomarker of PTD among those measured before the third trimester.^{21–34} Our initial investigations confirmed the strong association between unexplained high MSAFP levels and PTD in both African-American and white women.^{35,36} At the same time we discovered that surprisingly little was known about the PTD pathways marked by high MSAFP. Therefore, the goal of better understanding the MSAFP-PTD connection was incorporated into our investigations of multiple pathways to PTD.

In the current study, three hypothesised pathways to PTD are examined – infection, maternal vascular disease, and isolated (i.e. without evidence of these two pathways) elevations in corticotropin-releasing hormone (CRH) and an attempt is made to determine which of these pathways are marked by elevated MSAFP (Fig. 1). Within each pathway, we assess the link between PTD and proximate biological factors (e.g. placental pathology, biomarkers), the link between PTD and more distal psychosocial and behavioural antecedents, and the mediating role of proximate biological factors in explaining psychosocial disparities in PTD. The impact of maternal stress in these pathways is of particular interest. We hypothesise that the psychosocial link to underlying PTD pathology may be partially mediated by stress hormones, and by behaviours adopted in response to feeling stressed. In addition, gene polymorphisms are examined that may prove to be important effect modifiers of the maternal vascular disease pathway.

Development of PTD pathways

We recognise that the three distinct pathways in Fig. 1 do not represent all potential routes to PTD, and oversimplify a complex phenomenon. Pathological processes may overlap and, as discussed below, a biomarker may be linked to more than one pathway. A biomarker may be part of a common pathway to

labour, or it may be associated with inflammatory responses to different underlying aetiologies (e.g. ascending infection or placental damage unrelated to infection). Furthermore, the information conveyed by a biomarker may be specific to the time in pregnancy in which it is assessed, and to the compartment (maternal serum, amniotic fluid, vaginal fluid, cord blood) in which it is measured.³⁷ While appreciating these complexities, we thought it important to organise our thinking around a priori testable links occurring along specified PTD pathways. We view this conceptualisation as a first step in bringing order to the multitude of 'risk factors' that have been associated with PTD in previous research. Below is outlined the evidence to date that has guided our a priori hypotheses. The term biomarker is used in its broadest sense to include both markers and mediators of PTD pathways, markers being indicators of pathology and mediators being causal components along the pathway to PTD.

The infection pathway

Placental/fetal membrane pathology in the infection pathway

The link between infection and PTD has been particularly evident in studies of amniotic fluid and delivered placentas. Spontaneous preterm birth, in the presence or absence of premature rupture of membranes, is associated with culture-confirmed microbial invasion of the chorioamnion and amniotic fluid,^{38,39} and with histologically confirmed acute inflammation of the chorioamnion,⁴⁰ umbilical vessels, and chorionic vessels.⁴¹ Among very preterm infants, higher rates of placental *Ureaplasma urealyticum* infections were found in infants spontaneously delivered within 1 h of membrane rupture compared with infants delivered for pre-eclampsia or impaired fetal growth.⁴² There has even been a suggestion of a dose-response relationship in a study showing higher PTD rates with increasing severity of chorioamnionitis and funisitis.⁴³

Biomarkers of the infection pathway

Studies showing an inverse correlation between amniotic fluid cytokine levels sampled in mid-trimester and gestational age at delivery^{44,45} have suggested that PTD-related inflammation may be operating and detectable well before clinical signs of preterm labour. Following this lead, we chose to try to detect evidence

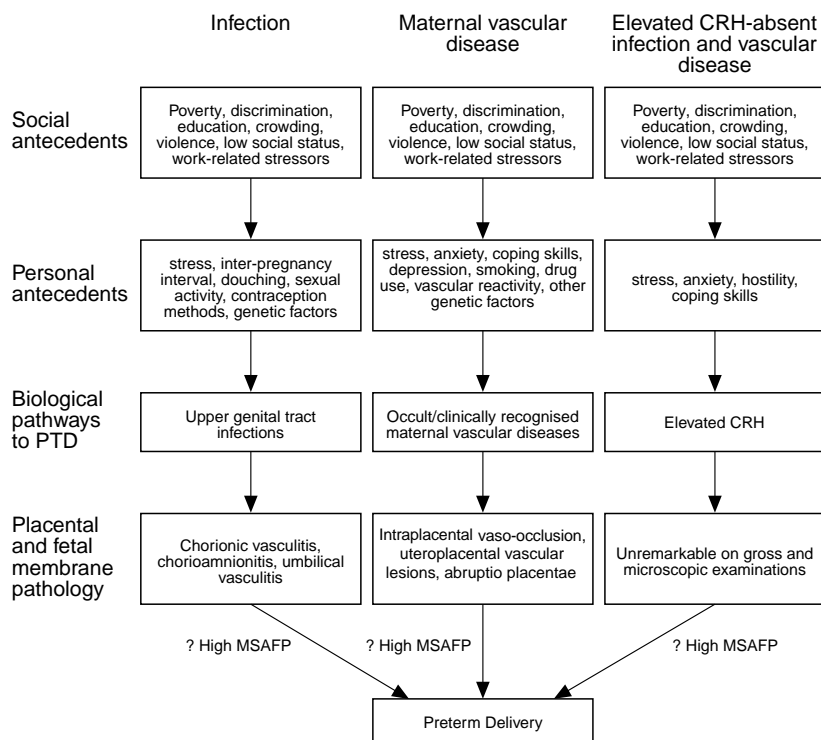


Figure 1. Hypothesised PTD pathways.

of infection in the second trimester, but only by using minimally invasive procedures such as assessment of biomarkers in maternal blood and vaginal fluid, and evaluation of vaginal flora. Examples of promising infection markers included in our study are total IgM, ferritin, interleukin-6 (IL-6), and granulocyte colony-stimulating factor (G-CSF).

In preliminary work we noted that total IgM levels above the median in mid-trimester maternal sera were strongly associated with delivery before 29 weeks' gestation (odds ratio [OR]=15.6, lower bound of confidence interval 2.5).⁴⁶ Other investigators have shown that mothers with premature rupture of membranes, preterm or term, have higher total IgM levels in sera collected only 12–24 h after membrane rupture, compared with gestational age-matched controls.⁴⁷ Elevations in total IgM may result from stimulation of B-1 cells, the predominant lymphocyte in the peritoneal cavity.⁴⁸ The B-1 cell response to T1-2 antigens resembles innate immunity in that the IgM produced lacks specificity, there is no class switching to IgG, and there is no immunological memory. Examples of T1-2 antigens include the polysaccharide components of Gram negative bacteria, organisms that have been cultured from the chorioamnion, and which have been linked to preterm birth.³⁸

Serum ferritin, an iron-binding protein that rises in the acute phase of inflammation, may also serve as a marker of infection-related PTD. High maternal ferritin levels at 24 and 26 weeks of gestation, but not 19 weeks of gestation, were linked to PTD in two separate studies.^{49,50} A longitudinal study showed that women whose ferritin levels remained above the 90th percentile from entry to prenatal care and at 28 weeks of gestation (4% of cohort) were more likely to deliver prematurely.⁵¹ In that study, the persistently high ferritin showed some relationship to clinical chorioamnionitis and to history of influenza, but a decrease in vascular expansion may have also contributed to the observed association. At least two studies have found higher serum ferritin in women with pregnancy-induced hypertension and eclampsia,^{52,53} raising some concern about the specificity of ferritin as a marker of an infection pathway. Our preliminary work detected higher maternal ferritin levels at 15–19 weeks' gestation in relation to PTD, but only in a subset of white women with high MSAFP levels.⁵⁴

Cytokines have received much attention as markers of the infection pathway, but interpretations of cytokine levels in pregnancy and labour require caution. Cytokines such as IL-1 β and IL-8 appear in increasing amounts in cervicovaginal fluid as normal

pregnancies progress,⁵⁵ and cytokines IL-1 β and IL-6 have been shown to increase in the lower uterine segment, in the placenta, and in vaginal fluid at parturition both at term and earlier.^{56,57} These findings suggest that cytokine elevations may be non-specific epi-phenomenon of labour, or that there may be multiple routes for cytokine production.⁵⁸ Assuming that cytokines play diverse roles in pregnancy and labour, the challenge is to identify cytokines that are related to specific bacterial infections and to histological chorioamnionitis.

Two cytokines, IL-6 and G-CSF, have shown potential as markers of infection, but study results have been inconsistent. High mid-trimester amniotic IL-6 levels were associated with positive amniotic cultures for *U. urealyticum*, and with fetal loss and PTD.^{44,45,59} Elevations in IL-6^{39,60} and G-CSF levels in maternal sera were detected in labour among pregnancies complicated by chorioamnionitis,⁶¹ and maternal serum G-CSF was higher in women with preterm labour and PTD, intact membranes, and chorioamnionitis.⁶² However, in another study, IL-6 levels in amniotic fluid measured at delivery were elevated in spontaneous, and not in indicated PTD, but were not associated with histological chorioamnionitis.⁶³ The preterm prediction study showed that maternal G-CSF measured at 24–28 weeks was elevated in asymptomatic women who delivered before 32 weeks, but not in women who delivered at 32–36 weeks.⁶⁴ However, the lack of information on chorioamnionitis made it difficult to determine whether the G-CSF link to very preterm delivery was indicative of an infection pathway, or was related to the shorter time from sampling to delivery in this window of gestation. In studies of vaginal fluid collected during pregnancy, IL-1 β , but not IL-6, was correlated with bacterial vaginosis (BV).^{57,65}

BV, a polymicrobial shift in the vaginal flora, has repeatedly been associated with premature delivery in observational studies,^{66–71} although antimicrobials used in randomised clinical trials to treat BV in pregnancy have not consistently lowered the risk of preterm birth.^{72–75} BV-related organisms (e.g. *Gardnerella vaginalis*, *Bacteroides* spp.) have been isolated with greater frequency in cultures of the chorioamnion and amniotic fluid in preterm than term deliveries, even excluding pregnancies complicated by prolonged rupture of membranes.^{38,39} A link between BV and asymptomatic endometritis in non-pregnant women raises the suspicion that BV-related organisms may be

capable of colonising the upper genital tract prior to pregnancy.⁷⁶ The high prevalence of BV in certain populations (10–30%),^{77–79} and the modest ORs (1.5–3) linking BV to PTD,^{66–70} suggest either that effect modifiers of the BV-PTD relationship exist, or that BV is too broadly defined and some, but not all, BV-related organisms contribute to PTD. Assessing organism virulence factors, such as sialidase production, and host defences, such as vaginal levels of secretory IgA, may improve our understanding of the relationship of BV to PTD.⁸⁰

Social and personal antecedents in the infection pathway

We hypothesise that antecedents in the infection pathway include stress hormones and behaviours that vary with resources, education, and culture. Excessive stress is related to poor living conditions, social isolation, lack of control over daily problems, discrimination, limitations in social/occupational upward mobility, and ineffective coping strategies. These conditions may have acute effects, or may represent long-standing features of a woman's life that exact a cumulative toll.⁸¹ Stress-induced alterations in the maternal immune response could lead to changes in the vaginal ecology, persistent colonisation of the upper genital tract, or progression from colonisation to local infection.

Biological plausibility for the stress-infection hypothesis is supported by the finding that environmental stressors stimulate the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system to produce hormones that affect immune responses.⁸² For example, *in vitro* studies have reported that catecholamines mobilise lymphocytes into the circulation, but decrease lymphocyte efficiency,⁸³ CRH stimulated increases in ACTH and cortisol result in decreased mitogen-induced lymphocyte proliferation,⁸⁴ β -endorphins decrease mitogen-induced lymphocyte proliferation, antibody production, and natural killer activity.^{85,86} Following antigen stimulation, cytokines such as IL-1 and IL-6 also act on the HPA axis,^{82,87,88} suggesting that the HPA axis is one of the mechanisms used by the body to down-regulate the immune response.

Recent clinical studies in non-pregnant populations lend further credence to the stress-infection hypothesis. Adults under stress while caring for relatives with dementia were found to experience delayed healing of

a punch biopsy wound,⁸⁹ and had higher mean cortisol levels that correlated with lower IgG titres in response to one of three strains of influenza vaccination.⁹⁰ However, studies of pregnant women that have looked for links between external stressors or self-reported manifestations of stress and PTD have produced mixed results. Many studies have found an excess of stressful life events in the background of women delivering prematurely,^{91–98} but other studies have not.^{99,100} While there have been reports of excess anxiety, perceived stress, depression, and concerns about the pregnancy in women delivering preterm,^{92,93,101–106} there has been little consistency in the specific emotional state described. Randomised clinical trials aimed at buffering stressors through social support interventions have, for the most part, proved unsuccessful,^{15,107} although a small trial in a subgroup of African-American women with low levels of support showed some promise in lowering rates of low birthweight.¹⁰⁸

In response to these inconsistencies, it seemed prudent to assess stress both directly and indirectly, and both in the present and in the past, by measuring stress hormones, self-reports of stressful feelings, emotional states, and antecedent stressors at the individual and ecological levels. A variety of interactions may occur between environmental stressors and host responses that will need to be considered separately in light of the different emotional responses they produce (e.g. depression, hostility) and their different effects on stress hormones.

While stress may explain the higher prevalence of BV in less-educated women and in African-American women,^{70,109} alternative explanations include behaviours such as vaginal douching, choices of contraception,^{110,111} and patterns of sexual activity.¹¹² Obstacles to seeking treatment for symptomatic BV may also put disadvantaged women at risk. Short interpregnancy intervals, often associated with increased risk of PTD,¹¹³ may deprive the upper genital tract of time needed to fully eliminate microbes that ascended during the preceding pregnancy and delivery.

The maternal vascular disease pathway

Placental/fetal membrane pathology in the maternal vascular disease pathway

Pregnant women with vascular conditions commonly defined by clinical criteria (i.e. pre-eclampsia, pregnancy-induced hypertension, and chronic hyper-

tension) are at greater risk of PTD, and this is not exclusively a result of medical intervention.¹¹⁴ Women with coagulopathies such as systemic lupus erythematosus, are more likely to deliver preterm and to have lesions related to decidual vasculopathy/coagulopathy in delivered placentas.¹¹⁵ Utero-placental vascular lesions, while more prevalent in women with clinically recognised maternal vascular disease,¹¹⁶ have also been found in excess among normotensive women with intrauterine growth retarded infants,¹¹⁷ and with spontaneous PTDs,¹¹⁸ – evidence that their pregnancies had been adversely affected by occult forms of maternal vascular disease. The proportion of PTDs mediated by maternal vascular disease is uncertain. In one study, infection-related placental pathology predominated in the earliest preterm births, but 25% of all placentas from pregnancies with preterm labour leading to PTD showed evidence of decidual vasculopathy or infarction compared with 0–4% in two groups delivered at term.¹¹⁸

Biological and physiological markers for the maternal vascular disease pathway

Results from studies of PTD-related placental pathology argue for broadly defining maternal vascular disease to include uteroplacental vascular abnormalities, atypical findings in blood pressure measures (e.g. average diastolic/systolic blood pressure, reactivity, diurnal changes), and pathology associated with coagulopathies. Biomarkers of this pathway that are sensitive to subclinical disease and/or early indicators of clinical disease would prove most useful. Two potential biomarkers, endothelin-1 (ET-1)¹¹⁹ and cellular fibronectin,¹²⁰ increase in response to endothelial damage and have been associated with pregnancies complicated by pre-eclampsia or fetal growth retardation.^{121–124} During pregnancy, ET-1 is synthesised in the decidua,¹²⁵ fetal membranes,¹²⁶ and fetal trophoblast cells,¹²⁷ as well as in endothelial cells in response to damage or inflammation. In addition to marking the vascular damage pathway, ET-1 could have a mediating role in PTD by decreasing uterine blood flow through its vasoconstrictive effects,^{128,129} or by augmenting uterine contractions as it increases intracellular calcium in the myometrium.¹³⁰

Studies of physiological measures linking maternal vascular abnormalities to PTD and low birthweight have mainly reported on overt hypertensive disorders, but there are some exceptions. In a study of

normotensive women with mid-trimester 24-h blood pressure monitoring, higher mean diastolic blood pressure within the 'normal' range was found to be associated with lower birthweight.¹³¹ Among women who had no clinically recognised maternal vascular disease, but who had spontaneous deliveries at 22–32 weeks, mean maternal blood pressure on admission was positively correlated with severity of uteroplacental vascular lesions.¹³² A third study in normotensive pregnant women found that increases in diastolic blood pressure in response to a stressful arithmetic task were correlated with an increased risk of PTD.¹³³

Social and personal antecedents in the maternal vascular disease pathway

Research on cardiovascular disease in non-pregnant adults has influenced our view that stress hormones and stress-related behaviours may be part of the maternal vascular disease pathway. We hypothesise that the antecedent stressors are the same in the infection and vascular pathways, but underlying maternal predisposition and behavioural cofactors determine which PTD pathway predominates in women who are experiencing excessive stress.

In studies of non-pregnant populations, both anxiety¹³⁴ and hostility¹³⁵ have been associated with the development of ischaemic heart disease. Anxiety and depression predicted elevated blood pressure at follow-up in the NHANES I longitudinal study, the effect being strongest in African-American women.¹³⁶ Higher systolic blood pressure was also noted in a cross-sectional study of African-American women who were more accepting of unfair treatment and less likely to acknowledge discrimination,¹³⁷ and in normotensive working women who reported greater stress at work compared with women who reported greater stress at home.¹³⁸ Evidence from the CARDIA study showed that hostility was linked to coronary artery calcification in young adults of reproductive age,¹³⁹ suggesting that uterine spiral artery calcification may adversely affect the process of trophoblast invasion and arterial conversion, or predispose to other mechanisms leading to utero-placental vascular lesions and PTD.

Stressors and the host responses may also affect the vascular pathway through stress-related behaviours. Studies of pregnant women have noted the intimate connection between physical abuse and drug use.¹⁴⁰ Hostility has been associated with smoking, greater

caloric intake,¹⁴¹ and reduced likelihood of using available social support.¹⁴² Both smoking and cocaine use have been found to be more common in women with abruptio placentae.^{143,144}

Gene polymorphisms as effect modifiers of the maternal vascular disease pathway

Gene–environment interactions may contribute to any of the PTD pathways, but we have chosen to focus on polymorphisms as effect modifiers in the maternal vascular disease pathway. Genes such as angiotensinogen (AGT), Angiotensin I-converting enzyme (ACE), Factor V (FV) Leiden, and Methylenetetrahydrofolate Reductase (MTHFR) have been reported to predispose to hypertension and thrombosis in non-pregnant populations,^{145–151} but studies of these genes in pregnant women have produced mixed results.^{152–158}

The gene for AGT is expressed in the smooth muscle of the spiral arteries during early placental development, and has been associated with hypertension.¹⁵⁹ The mutation C(–6)A has been demonstrated in the 5' regulatory sequence, and may be the mutation most likely to affect blood pressure as it lies in the promoter region and thus affects expression levels.¹⁴⁵ The T235 variant has been linked to hypertension^{146–148} and to pre-eclampsia in most,^{160,161} but not all studies.¹⁶² The association of hypertension with the T235 mutation in certain populations but not others could be explained by the high degree of linkage disequilibrium between the mutation sites.¹⁴⁷

Reports conflict about the relationship between ACE and cardiovascular disease, indicating that other genes and/or environmental factors may be important modifiers.^{163,164} The gene has a common intronic insertion/deletion polymorphism, which accounts for about 50% of the variation in serum levels.¹⁶⁵ While this gene polymorphism may have a direct effect on the cardiovascular system, it may be a marker for another closely linked gene with cardiovascular effects. The suggestion that the mutation has an effect on salt sensitivity¹⁴⁹ is intriguing and could be relevant to pregnancy in African-Americans.

The FV Leiden mutation (R506Q) results in activated protein C resistance and increased liability to intravenous coagulation.^{157,166} Carriers of this mutation appear to be at increased risk of deep vein thrombosis,^{152,167,168} and young women carriers are at increased risk of myocardial infarction, particularly if they smoke.¹⁶⁹ FV Leiden has been

implicated in poor pregnancy outcomes related to thrombotic events in the mother, the placenta and the fetus^{152–156,170,171} although it does not appear to relate to miscarriage.¹⁷² The maternal genotype affects thrombotic risk in the mother, whereas the fetal genotype may affect thrombosis in the fetus and placenta. The inter-relationship of the two genotypes has not been elucidated.

The risk of intravenous thrombosis arising from FV Leiden appeared to be even greater when an individual is also hyperhomocysteinaemic.¹⁷³ High levels of plasma homocysteine have been linked to vascular disease.^{174–176} A common polymorphism in the gene for MTHFR, (C677T), results in a thermolabile form of the enzyme. Individuals who are homozygous for the mutation have reduced enzyme levels and mild hyperhomocysteinaemia and appear to be at increased risk of thrombotic events^{150,151} The MTHFR mutation may add to, or by synergistic with, the effect of the FV mutation.¹⁷⁷ Each of the above genes are polymorphous with reasonably high allele frequencies in white and African-American women, and each can be examined with simple molecular techniques. We hypothesise that women carrying any of these polymorphisms may be at increased risk of either clinical or subclinical pregnancy-related vascular disease, when environmental stressors and biological mediators of stress are excessive.

Elevated CRH pathway (absent infection and maternal vascular disease)

CRH, produced in placental trophoblasts, fetal membranes, and decidual cells,^{178,179} gradually increases in maternal blood as pregnancy progresses, and then rises exponentially in the month preceding labour.¹⁸⁰ Researchers speculate that CRH plays a mediating role in parturition in one of two ways: either by directly stimulating the fetal pituitary-adrenal axis^{181,182} and fetal adrenal production of cortisol and dehydroepiandrosterone,¹⁸³ or through an autocrine/paracrine effect by stimulating prostaglandin production in the decidua, fetal membranes, and placenta,¹⁸⁴ and by binding to the myometrium¹⁸⁵ and potentiating the action of oxytocin.¹⁸⁶

Premature elevation in maternal CRH,¹⁸⁰ in some instances measured as early as mid-trimester,¹⁸⁷ has been associated with PTD, although questions remain as to what causes this early rise. We hypothesise that multiple triggers can stimulate placental CRH

production and release, including hormones related to the maternal stress response.

In vitro studies have reported that catecholamines and cortisol are capable of increasing placental CRH production,¹⁸⁸ although three *in vivo* studies were unable to detect a strong correlation between maternal cortisol and maternal CRH levels during pregnancy.^{189–191} One study showed a correlation between maternal and fetal cortisol levels, but it was unclear whether placental CRH was the mediator of this correlation, or whether maternal cortisol crossing the placenta was directly contributing to the fetal levels.¹⁹² High levels of maternal cortisol may be required to stimulate placental CRH because the placental enzyme 11 β hydroxysteroid dehydrogenase-2 can convert cortisol to inactive cortisone.^{193–195} The rate of this conversion is thought to be oestrogen and/or progesterone dependent, and may change as gestation progresses. In addition, progesterone competes with cortisol for steroid receptors that affect placental CRH expression.¹⁹⁶ It appears that the effect of maternal cortisol on placental CRH production may be modified by many factors, and thus prove difficult to unravel.

Biomarkers of multiple PTD pathways

Increased levels of MSAFP, maternal CRH, and cervico-vaginal fetal fibronectin (fFN) have been repeatedly associated with PTD, and these relationships most likely operate through more than one pathway. CRH¹⁸⁰ and MSAFP^{197–200} levels rise as pregnancy progresses, and errors that under-estimate gestational age therefore have the potential to exaggerate the importance of these markers in predicting PTD. In the month preceding term delivery, MSAFP levels plateau, CRH levels increase,¹⁸⁰ and the percentage of women who are fFN positive increases.²⁰¹ These findings suggest that CRH and fFN are non-specific markers of a pregnancy that is nearing labour.

Maternal serum alpha-fetoprotein (MSAFP)

Small amounts of alpha-fetoprotein (AFP), a fetal analogue of albumin, normally pass across the placenta and fetal membranes into the maternal circulation.²⁰² MSAFP was first used as a screening test for open fetal defects such as spina bifida, a situation in which AFP leaks from the fetus to the amniotic fluid, and from there crosses fetal membranes producing elevated AFP levels in maternal blood.²⁰³ Screening programmes

noted that high MSAFP levels also occur in singleton pregnancies in the absence of fetal defects, giving rise to the term 'unexplained' elevated MSAFP. In follow-up studies, the risk of PTD among women with unexplained high MSAFP levels has been 2–10 times that of women with normal MSAFP levels, the range in relative risks dependent in part on the threshold selected for high MSAFP.^{26,32,33,204} We hypothesise that excess MSAFP arising from placental and fetal membrane pathology may, under certain conditions, be a marker for either the maternal vascular disease or the infection pathways.

Placental infarcts and thromboses, insults that coincide with uteroplacental vasculopathy and coagulopathies, appear more frequently in pregnancies marked by high MSAFP.²⁰⁵ During these insults, excess amounts of AFP may enter the maternal circulation via transplacental diffusion or transfusion. The potentially transient nature of these events is consistent with the observations that women with two high MSAFP levels (1–2 weeks apart) remain at increased risk for PTD, even when MSAFP reverts to normal at a later point in mid-gestation.³¹ Others have shown that high MSAFP levels are associated with retroplacental and subchorionic haemorrhage^{206–208} and with Doppler abnormalities suggestive of uteroplacental insufficiency.^{209,210} Clinically recognised maternal vascular diseases, e.g. hypertension, pre-eclampsia, and HELLP syndrome, have also been linked to high MSAFP levels in some studies,^{22,25,26,211,212} but not in others.^{213,214} In a Doppler study of pregnancies with elevated MSAFP, 16% of women had abnormal umbilical artery velocimetry scores indicative of vascular disease, and had an extremely high rate of PTD (78.6%).²¹⁵ The PTD rates were also high (23.9%) in the remaining 84% of women with normal velocimetry scores—evidence that MSAFP may mark more than one route to PTD.

While many consider elevated MSAFP to be unrelated to infection, the presence of both plausible biological mechanisms and relevant epidemiological findings argue for a closer examination of this assumption. Chorioamnionitis may permit increased diffusion of AFP across fetal membranes. Alternatively, retroplacental haemorrhage, marked both by early gestational bleeding and by elevated MSAFP^{24,25,33} may serve as a growth-promoting medium for organisms colonising the decidua. This concept is supported by a study showing the risk of PTD was substantially increased in women with BV and concomitant early

gestational bleeding.²¹⁶ Elevated MSAFP levels have been associated with premature rupture of membranes prior to PTD,^{217,218} and with high levels of the cytokine ICAM-1 in mid-trimester amniotic fluid,²¹⁹ both of which are suggestive of underlying infection. In addition, multiple studies have reported a link between MSAFP and very preterm delivery^{35,220,221} – a subset of premature deliveries most often considered to have an infectious aetiology.²²²

Fetal fibronectin (fFN)

fFN is a glycoprotein found in the extracellular matrix at the chorio-decidual interface. In uncomplicated pregnancies, fFN is detected in cervical and vaginal secretions with decreasing frequency as pregnancy progresses, and then with increasing frequency after 36 weeks of gestation.²⁰¹ The amount of fFN present in cervicovaginal secretions may be affected by the level of production and secretion from chorion cells, by disruption of the extracellular matrix, or by ease of passage through the cervix. fFN values of > 50 ng/mL in cervicovaginal secretions were associated with PTD in women who had preterm contractions or membrane rupture,²⁰¹ in high-risk asymptomatic women,^{223,224} and in non-selected asymptomatic women screened between 24 and 36 weeks of gestation.^{225,226}

To date, fFN testing has been used primarily to rule out PTD in women with preterm labour.²²⁷ Investigators have proposed that fFN may be a marker of infection-related PTD because fFN sensitivity appears greatest for the earliest deliveries (before 32 weeks of gestation),²²⁶ fFN predicts spontaneous but not indicated PTD,²²⁸ and fFN is often^{229,230} but not always^{65,231} associated with the presence of BV. In addition, exposure of chorion cells *in vitro* to proinflammatory cytokines and lipopolysaccharides stimulates fFN production.²³²

Other evidence suggests that fFN may be a non-specific marker of cervical changes and disruptions of the choriodecidual interface that precede labour. A study of women with preterm labour and intact membranes found cervicovaginal fFN predicted PTD, but was unrelated to chorioamnionitis and positive amniotic fluid culture.²³³ The higher sensitivity of fFN in detecting early PTD may be partly explained by the inverse correlation between fFN sensitivity and the interval from fFN testing to parturition.²³⁴ A recent report showed that cervicovaginal fFN detected in the third trimester was associated with shortening of the

cervix among many risk groups including women with previous PTD and women with twin gestations.²³⁵ While acute inflammation in delivered placentas has also been found in conjunction with cervical shortening in singleton and multiple gestations, the question remains 'where does the process begin, with ascending infection or with changes in the cervix from other underlying causes'?²³⁶

Corticotropin releasing hormone (CRH)

We hypothesise that in addition to the direct stress hormone-CRH pathway, CRH shows potential as a biomarker for both the maternal vascular disease and infection pathways. *In vitro* studies have suggested that placental CRH production could be stimulated by cytokines released in response to haemorrhage, inflammation, or infection.²³⁷ *In vivo*, high CRH levels were more common in pregnancies complicated by pre-eclampsia,^{238,239} and pregnancy-induced hypertension.^{240,241} Two studies have found CRH to be specifically linked to PTD in the absence of infection,^{242,243} but at least one study has noted higher CRH levels in preterm births with microbial contamination of amniotic fluid.²⁴⁴

Methods

Study design and participant sampling

The Pregnancy Outcomes and Community Health (POUCH) Study is a prospective cohort study (goal of 2000 women) with a case-cohort component (involving an estimated 800 of the 2000 cohort women). The cohort is a stratified random sample from the universe of women screened for MSAFP at 15–20 weeks' gestation. Stratification is designed to over-sample African-Americans (25% of cohort) and women with an unexplained high MSAFP of > 2.0 MoM on the first screen (10% of cohort). Of the 75% who are non-African-American, the majority are white and a small percentage (10%) are from other ethnic groups. Study sample size is based on feasibility data showing that approximately 6960 women per year have their serum samples sent for prenatal screening to Michigan State University from the targeted communities, and the PTD rates vary by stratum, i.e. 28% in African-Americans with high MSAFP, 10% in African-Americans with normal MSAFP, 17% in non-African-Americans with high MSAFP, and 7% in non-African-Americans with normal MSAFP.

Cohort women take part in the full study protocol (i.e. interviews, collection of biological samples, medical record abstraction, linkage to census data), but testing of biological samples and detailed assessment of the placenta are reserved for case-cohort women. In the case-cohort component, cases are women who deliver before 37 weeks' gestation. The subcohort women are selected at study entry, and constitute 22% of women with normal MSAFP and 100% of women with high MSAFP. The sampling fractions for the subcohort ensure that the final case-cohort will have at least a 2:1 ratio of full-term to preterm deliveries within each MSAFP ethnic-specific stratum. Thus, each study participant is assigned a sampling weight for cohort analyses, and a second sampling weight if selected for case-cohort analyses. These sampling weights reflect the true proportion of the stratum, e.g. African-American women with normal MSAFP levels, in the original screened population.

Study population

The sampling frame for the POUCH Study includes women screened for MSAFP at participating clinics. Clinics are invited to participate if their patients deliver at one of eight hospitals in five Michigan communities and, with a few exceptions, if they send their prenatal screening samples to one of two Michigan prenatal screening programmes. The five Michigan communities are economically diverse with 1996 unemployment rates ranging from 5.2% to 9.9%, and single parent households rates from 19.6% to 30.5%. During the same period, the community rate of delivery before 35 weeks among African-Americans ranged from 6.1% to 10.5%.

Participant eligibility and recruitment

A one-page description of the POUCH study is attached to the prenatal screening requisition form at participating clinics. After screening, women are randomly sampled according to the sampling fractions described above. Those sampled receive a detailed mailing describing the study, and a follow-up telephone call for recruitment. Eligibility criteria include maternal age > 14 years, having been screened for MSAFP between 15 and 20 weeks' gestation, singleton pregnancy with no known congenital or chromosomal abnormalities, and no history of diabetes prior to screening.

Data collection protocol

At 16–26 weeks' gestation, participating women meet with a study nurse in their respective community. This encounter (part 1) includes the review and signing of consent forms, a detailed in-person interview, a self-administered questionnaire, and collection of biological samples (urine, plasma, serum, hair, vaginal smear, and vaginal fluid). Women who complete part 1 may elect to participate in part 2, an at-home data collection protocol. In part 2, women wear an ambulatory blood pressure monitor (ABPM) for 24 h, collect urine and saliva morning and night, and complete a stress checklist for three consecutive days. Approximately 80% of cohort women agree to participate in part 2, and the ABPM component is available and acceptable to one third of part 2 participants.

After delivery, case-cohort placentas are formalin-fixed and retained. Study nurses perform a detailed abstraction of prenatal, and labour and delivery medical records for the case-cohort and a less detailed abstraction of medical records for the remaining cohort. The data collected include clinical parameters of interest such as prenatal history of infections, complications, and treatments, laboratory test results, the sequence of events leading to delivery, and procedures and medication in the perinatal period. To obtain ecological level data, the residence of each participant will be geocoded and linked to census data after study enrolment is complete.

Biological samples ascertained at 15–26 weeks' gestation

Samples of serum, plasma, urine, saliva, and vaginal fluid are stored at -70°C . Selected analytes (Table 1) corresponding to the three hypothesised PTD pathways will be assessed in batches, using commercially available, standardised kits whenever possible. MSAFP levels are measured twice, 2–10 weeks apart: first, in sera submitted for prenatal screening purposes, and again in sera collected as part of the study protocol. For a majority of women, the study retains and stores the prenatal screening sera, allowing for longitudinal assessment of other biomarkers. Hair samples are used to validate self-report of illicit drug use.²⁴⁵ Vaginal smears are Gram stained and evaluated by a study microbiologist who uses the Nugent²⁴⁶ scoring system for describing BV. This is the only biological test performed in real time with results reported back to the respective prenatal care providers.

Defining PTD and its subtypes

PTD is defined as births before 37 weeks' gestation. Gestational age at delivery is determined by the gestational age estimated at the time of MSAFP screening, which is based on the date of the first day of the last menstrual period (LMP), except when a gestational age derived from early ultrasound differs from the LMP age by 2 or more weeks, in which case, the ultrasound age is given preference. PTD subtypes will be assessed according to the commonly used typology for PTD as a means of comparing our results to other studies, and to contrast results from this approach with the pathways we test in our main analyses. The common subtypes are: 1) spontaneous labour with intact membranes; 2) spontaneous labour within 12 h or less after membrane rupture; 3) rupture of membranes > 12 h; and 4) medically indicated deliveries, absent membrane rupture and spontaneous labour. In an alternative approach, we will group PTD subtypes by underlying pathology. Thus, PTDs with placental evidence of maternal vascular disease will be grouped together without regard for whether the PTD was spontaneous or medically indicated.

Placenta and fetal membrane assessment postdelivery

The study placental pathologist, who is blinded to all clinical information regarding the pregnancies, performs gross and microscopic evaluations of case-cohort fetal membranes, umbilical cords, and placental discs. Approximately 10% of case-cohort placentas are examined microscopically by a second placental pathologist to assess inter-rater reliability. Evidence of chorioamnionitis, chorionic vasculitis, or umbilical vasculitis is considered indicative of the infection PTD pathway. Evidence of uteroplacental vascular pathology and secondary villous damage (e.g. absent or incomplete destruction of muscular and elastic layers of spiral arteries, fibrinoid necrosis, atherosclerosis, abruptio placentae, retroplacental haemorrhage, villous infarcts, terminal villous fibrosis, increased syncytiotrophoblast knotting, villous hypovascularity), or coagulation (e.g. uteroplacental vascular thrombosis, perivillous fibrin deposition, thrombi in chorionic or fetal stem vessels, foci of avascular terminal villi) is consistent with the occult/clinically recognised maternal vascular disease PTD pathway. Further subdivisions of this pathway are anticipated, as some lesions may be

	Maternal sample	Frequency
Pathway 1: Infection		
BV	Vaginal smear	Once
Cytokines ^a	Vaginal fluid serum	Once
	Serum	Twice
Ferritin ^a	Serum	Twice
Immunoglobulins ^a	Serum	Twice
Pathway 2: Maternal vascular		
Cellular fibronectin ^a	Plasma	Once
Endothelin-1 ^a	Plasma	Once
Protein	Urine	Once
Markers of multiple pathways		
Fetal fibronectin ^a	Vaginal fluid	Once
Alpha-fetoprotein	Serum	Twice
Corticotropin-releasing hormone ^{a,b}	Serum	Twice
Stress markers (mediators in any of the above pathways)		
Cortisol ^a	Saliva	2/day for 3 consecutive days
Catecholamines ^a	Urine	2/day for 3 consecutive days
Beta-endorphins ^a	Plasma	Once

^aMeasured only in case-cohort.

^bIncludes pathway 3. High CRH absent gross and microscopic evidence of placental pathology.

preferentially associated with PTD. Additional subgroup analyses include women with placental pathology representing more than one pathway, and women with other forms of placental pathology, such as villitis, decidual plasma cell infiltrates, basal lymphocytic infiltrates, decidual eosinophilic infiltrates, and haemorrhagic endovasculitis.

Physiological measures

Clinically apparent forms of maternal vascular disease are based on elevations in blood pressure ascertained at routine prenatal care and during the study protocol of 24-h ambulatory blood pressure and heart rate monitoring (Part 2). There is evidence that ambulatory blood pressure measures may be more sensitive than more infrequent blood pressure measures for detecting early signs of pregnancy-induced hypertension,²⁴⁷ and vascular effects on fetal growth.²⁴⁸ Women with blood pressure abnormalities will be classified as having chronic hypertension, pregnancy-induced hypertension, and hypertension with proteinuria, as set forth by the working group on high blood pressure in pregnancy.²⁴⁹ In addition we will evaluate the risk of PTD in relation to the average measure of systolic and diastolic blood pressure, diurnal blood pressure changes, and time to baseline recovery after reactive episodes in women who do not fit the 'abnormal' categories outlined above.

Table 1. Examples of biomarkers assessed at 15–26 weeks' gestation corresponding to hypothesised preterm delivery pathways

Stress hormones

Cortisol, catecholamines, and beta-endorphins are evaluated as biomarkers of stress^{250,251} at 16–26 weeks' gestation (Part 2). Salivary cortisol levels are measured using a radioimmunoassay, and urinary catecholamines are measured using an extraction procedure followed by high performance liquid chromatography. Both are assessed in samples collected morning and night for three consecutive days. Beta-endorphins are evaluated by enzyme-linked immunoassay technique using plasma collected at one point in time. Studies of populations under stress emphasise the importance of evaluating not only high levels of stress hormones, but also unusually low levels and diurnal changes. Severe chronic stress in humans, such as post-traumatic stress disorder, can result in depressed cortisol levels.²⁵² Unemployment has been associated with higher morning and lower evening levels of cortisol,²⁵³ and relatives caring for dementia patients showed higher morning cortisol, but no change in evening cortisol.⁹⁰ In chronically stressed animals, there is often decreased variability of cortisol's natural diurnal pattern.²⁵⁴ Other studies argue for assessing stress hormones over several days, having shown catecholamine levels in lorry drivers²⁵⁵ and in pregnant physicians²⁵⁶ to be higher during work-days than non-work days.

Genetic polymorphisms as effect modifiers

DNA is extracted from white blood cells and reserved to permit assessment of gene-polymorphisms related to infection and maternal vascular disease.

Psychosocial antecedents ascertained at 16–26 weeks' gestation

Antecedents measured at the ecological level are derived from census data (Table 2). Factors such as crime rate, per capita income by ethnic group, proportion of families on welfare, percentage of substandard housing, and social disorganisation²⁵⁷ will be assessed in relation to demographic information obtained directly from the participants (e.g. occupation, income, education, ethnicity), thereby enabling us to place a woman's individual characteristics in the context of the census tract in which she lives. The interplay between individual and ecological measures has been demonstrated in studies showing that racially dissonant residential environments are associated with an increased risk of depression,²⁵⁸ whereas high income in a poor area is protective against that outcome.²⁵⁹ Controlling for individual and neighbourhood characteristics revealed higher low birthweight rates in neighbourhoods with greater

economic hardship, but lower rates in neighbourhoods with a greater percentage of African-Americans.²⁶⁰

Individual level data on psychosocial and behavioural factors are ascertained by in-person and self-administered interviews, largely composed of complete or abbreviated versions of previously validated psychosocial instruments (Tables 2 and 3). Pathway-specific PTD rates will be assessed in relation to the individual psychosocial constructs measured by the different instruments, and in relation to more complex models of stress. For example, we have defined five subtypes of stressors (restriction, impotence, loss, isolation, and uncertainty) that can overwhelm coping resources and result in a disruption of control over goals and pursuits. The disruption manifests as negative emotions such as anger, sadness (depression), shame, anxiety, and loneliness, which are specific to the stressors and the type of control lost. We will evaluate the role of the five stressor subtypes in relation to each of the three hypothesised PTD pathways, and in relation to stress hormones.

Analytic strategies and study power

In all analyses, appropriate weights will be applied to reflect group specific sampling fractions and their effects on calculated variances. In the assessment of

Table 2. Measures of social antecedents

Variable	Measure (source/persons characterised)	Time period
Social antecedents measured at the ecological level		
Economic	Income (Neighbourhood census data)	Present
Ethnicity	Segregation (Neighbourhood census data)	Present
Neighbourhood conditions	Crowding, housing quality, Index of Social Disorganisation ²⁵⁷ (Neighbourhood census data)	Present
Social antecedents measured at the individual level		
Economic status	Wealth Index (Maternal) ²⁶⁴ Indications of poverty (Maternal)	Present Growing-up and present
Education	(Maternal, paternal, maternal parents)	
Ethnicity	(Maternal, paternal, maternal parents)	
Occupation	(Maternal, paternal, maternal parents)	Before pregnancy and present
Anomie (alienation, social disarray)	Srole Anomie Scale ²⁶⁵ (maternal)	Present
Perceived social support	Modified combination of Perceived Social Support Scale ²⁶⁶ and Strogatz Social Support (Maternal) ²⁶⁷	Present
Neighbourhood conditions	Crowding, housing quality, and section VII from Korbin and Coultin Instrument (Maternal) ²⁶⁸	Present
Neighbourhood interactions	POUCH Study instrument	Present

Table 3. Measures of personal antecedents

Variable	Measure	Time period
Daily hassles	POUCH Study instrument	Present
Life stressors	POUCH Study instrument, adapted from Social Stress Indicators ²⁶⁹	Growing-up, as an adult, within past 6 months
Perceived stress	Modified Cohen's Perceived Stress Scale ²⁷⁰	Present
	Adapted from Schar Subjective Stress Scale ²⁷¹	Present
	Global Assessment of Recent Stress ²⁷²	3 consecutive days
Mastery	Modified Pearlin's Mastery Scale ²⁷³	Present
Self-esteem	Modified Rosenberg's Self-esteem scale ²⁷⁴	Present
Anger	Modified Goldstein's Anger Scale ²⁷⁵	Present
Coping style	John Henryism Active Coping Scale ²⁷⁶	Present
Depression	POUCH Study instrument	
	CES-D ²⁷⁷	Present
Hostility	Abbreviated Cook-Medley Hostility Scale ²⁷⁸	Present
Discrimination	Modified instrument from CARDIA Study ¹³⁷	
Attitudes/feelings about pregnancy	POUCH Study instrument	
Perceived respect	POUCH Study instrument	
Physical activity at work	Self-report	Present
Alcohol use	Self-report	Past, Present
Caffeine consumption	Self-report	Present
Illegal drug use	Self-report, hair samples	Past, Present
Fish consumption	Self-report	Present
Direct and passive cigarette smoke exposure	Self-report	Present
Sexual activity	Self-report	Past, Present
Contraception	Self-report	Past, Present
Vaginal douching	Self-report	Past, Present

biomarker concentrations, gestational week of sampling will be modelled as a potential confounder or effect modifier. The analytical strategies will be tailored to the research question at each level of our hypothesised causal pathways as we derive the interrelationships between covariates (i.e. placental pathology, biomarkers, antenatal factors) that predict PTD. Statistical modelling techniques will include multivariable analyses such as multivariate regression, dichotomous and polytomous logistic regression, log-linear models, and hierarchical models. For example, in the case-cohort, for whom we have antenatal biomarkers (e.g. MSAFP, fetal fibronectin, ferritin, CRH) and placental pathology, we will use multivariate regression to assess the relationships between biomarkers and placental and fetal membrane abnormalities (e.g. acute ascending infection, uteroplacental vascular pathology and secondary villous damage, coagulation) grouped according to specific biological pathways. We will also evaluate specific pathological lesions that are included within the broad groups. Using logistic regression, biomarkers will be modelled as predictors of PTD. In subsequent models, we will determine the extent to

which placental/fetal membrane abnormalities characteristic of a specific pathway explain (attenuate) the association between the biomarker and PTD. If the biomarker(s) continues to be associated with PTD after placental pathology is included in the model, there are several possible explanations that merit further exploration. First, the biomarker(s) may be linked to more than one pathway and second, mid-trimester perturbations of the placenta and fetal membranes caused by infection or maternal vascular disease may not be evident on histopathology if damage is transient or at an intracellular level.

Hierarchical models, such as those used in epidemiological research to assess contextual variables,^{261,262} will measure the magnitude of ecological variation in PTD (subtypes and combined) as it relates to variation in census tract-level predictors (e.g. concentrated poverty, racial segregation, crowding, standards of housing). This multilevel statistical model will also be used to decompose relationships between socio-demographic variables and outcomes into their individual and ecological components, and consider the context specificity (effect modification)

on all relationships between individual risk factors and outcomes.²⁶³

Calculations of statistical power for this study are based on the more simplified univariate analyses. In the entire cohort, the statistical power ranges from 70 to 99% to detect a twofold increase in the OR for PTD associated with infection in the prenatal period (based on medical records, interview), maternal vascular disease (based on medical records, interview), or socio-demographic factors (e.g. ethnicity, education level). In the case-cohort, the statistical power ranges from 48 to 99% to detect an OR of 2.0 in similar analyses that use biomarkers and placental pathology data to further define infection and maternal vascular disease.

Timeframe

Recruitment into the POUCH Study began in the first community on 8 September 1998. Although enrolment in each community developed slowly, as of 20 September 2000, all five communities are participating, enrolment is at 650 women, and average enrolment for the five communities combined is 15 women per week.

Discussion

The POUCH study has several strengths. It assesses the role of biomarkers and placental pathology in pre-hypothesised PTD pathways, and then attempts to link these biological markers and mediators to antecedent psychosocial factors measured at both the individual and ecological levels. Most data are gathered between the 15th and 26th week of pregnancy – a time when pathological processes may have evolved to a detectable stage, but generally before the onset of biological changes that accompany labour. The five-community, clinic-based sampling frame makes over-sampling of women with high MSAFP levels feasible, and avoids the bias that can occur in samples drawn from clinics at teaching or referral hospitals. Finally, the study was developed by a multidisciplinary team of scientists and social scientists whose continual interactions throughout the study bring new dimensions to understanding the biological and social determinants of PTD.

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