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Use of Amniotic Fluid for Determining Pregnancies at Risk of Preterm Birth and for Studying Diseases of Potential Environmental Etiology

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Abstract

Amniotic fluid (AF) is a biological medium uniquely suited for the study of early exposure of the human fetus to environmental contaminants acquired by the mother before and during pregnancy. Traditional diagnostic applications of AF have focused almost exclusively on the diagnosis of genetic aberrations such as Trisomy-21 and on heritable diseases in high-risk pregnancies. Since more than 50 anthropogenic compounds have been detected in AF, there is considerable potential in utilizing fetal protein biomarkers as indicators of prenatal exposure-related health effects. Here, we focus on preterm birth (PTB) to illustrate opportunities and limitations of using AF as a diagnostic matrix. Representing a pervasive public health challenge worldwide, PTB cannot be managed simply by improving hygiene and broadening access to healthcare. This is illustrated by 15-year increases of PTB in the U.S. from 1989-2004. AF is uniquely suited as a matrix for early detection of the association between fetal exposures and PTB due to its fetal origin and the fact that it is sampled from women who are at higher risk of PTB. This critical review shows the occurrence in AF of a number of xenobiotics, including endocrine-disrupting compounds (EDCs), which are known or may reasonably be expected to shorten fetal gestation. It is not yet known whether EDCs, including bisphenol A, phytoestrogens, and polychlorinated biphenyls (PCBs), can affect the expression of proteins considered viable or potential biomarkers for the onset of PTB. As such, the diagnostic value of AF is broad and has not yet been fully explored for prenatal

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diagnosis of pregnancies at risk from toxic, environmental exposures and for the elucidation of mechanisms underlying important public health challenges including PTB.

Keywords

Preterm birth; amniotic fluid; protein biomarker; fetal origin of disease; endocrine-disrupting chemicals

Introduction

Identifying adverse human health outcomes from exposures to mixtures of anthropogenic chemicals is a recognized challenge deserving more scrutiny (Carlin et al., 2013). Environmental exposures are inherently complex and of great plasticity, varying by individual, life phase, geography, and behavior. Numerous individual and nationwide surveys, including the National Health and Nutrition Examination Survey (NHANES) and the National Children's Study, confirm that women and children in the U.S. are ubiquitously exposed to complex mixtures of persistent and non-persistent environmental contaminants (CDC, 2013), with the relevant exposures occurring prenatally (Barr et al., 2007). Fetal serum, cord blood, and meconium are all appropriate matrices for monitoring fetal environmental exposures (Barr et al., 2007). However, only amniotic fluid (AF) and certain surrogate matrices (i.e., maternal serum, plasma, urine, or placental tissue) can provide information prior to delivery to inform intervention strategies directed at improving perinatal outcomes.

Due to its fetal origin, AF is the matrix of choice for prenatal screening of risk factors of adverse health outcomes and associated molecular predictors. Formation during embryogenesis occurs by way of diffusion of maternal plasma through the fetal membranes and includes transudate through the unkeratinized fetal skin. Following keratinization of the fetal skin (mid-trimester), AF is a product of fetal urination, tracheal secretions, and intramembranous and transmembranous pathways (Sherer and Langer, 2001). Thus, AF is a principal fetal repository of metabolized environmental toxicants that can be accessed for prenatal assessments throughout gestation (Lozano et al., 2007).

Here, we review the state of science in the evolution of AF diagnosis from monitoring of genetic abnormalities, to elucidating the etiology of environmental diseases and adverse perinatal outcomes, to detecting protein biomarkers of diagnostic value for reducing the incident rate of preterm birth.

History of AF Sampling

Amniocentesis, the sampling of amniotic fluid, is a diagnostic procedure that has been practiced routinely since the 1970s for detection of various birth defects after its inception in the mid-1950s and earlier reports on AF drainage dating back over a century (CDC, 2013). The procedure is indicated in pregnancies of increased risk due to, for example, advanced maternal age, family history of genetic disorders and miscarriage, and as a follow-up procedure to early maternal serum screening. It is generally performed during the 15th-18th

week of gestation. Some of the genetic aberrations and heritable diseases that can be diagnosed prenatally with amniocentesis include fetal aneuploidies, sickle cell anemia, cystic fibrosis, muscular dystrophy, Tay-Sachs and related diseases, and open neural tube defects. In 1990, 40% of women 35 years of age elected either amniocentesis or chorionic villus (placental tissue) sampling (CVS) for prenatal diagnostics (CDC, 2013). Whereas CVS can be performed from 10 weeks onward, and thus several weeks earlier than amniocentesis, the former has been associated with transverse limb reduction defects, cytogenetic ambiguities, and comparatively higher rates of miscarriage (CDC, 2013). In the U.S. in 1990, the amniocentesis procedure was performed approximately 200,000 times, while international numbers vary by region and socioeconomic status (CDC, 2013). Complications in the U.S. occur at a rate of 0.06-0.25% (CDC, 2013; Eddleman et al., 2006).

Non-invasive prenatal testing (NIPT), used for screening and detection of a limited set of genetic abnormalities, focuses on circulating cell-free fetal DNA in maternal plasma and allows for earlier diagnosis (10-16 weeks gestation) of Trisomies 13, 18, and 21 in the first trimester; standing in the way of universal adoption of this more recent technology are its high cost and issues with sample processing (Gil et al., 2013; Wong et al., 2013). Women with a positive result from this test are advised to undergo amniocentesis or CVS for further assessment. Thus, amniocentesis will continue to be administered in clinical settings, providing access to the diagnosis of diseases of genetic and environmental etiology.

Although amniocentesis is most commonly performed to allow prenatal screening for adverse perinatal outcomes, aliquots of AF sampled in excess can be archived and reallocated for expanded diagnosis and research purposes. Analysis of newly obtained and archived samples can serve to expand the knowledge base on fetal exposures to biological and chemical agents of endogenous, exogenous and anthropogenic origin as well as to validate novel assays for genetic and metabolic disorders. The first step in determining the association between chemical exposures and adverse perinatal outcomes is to ascertain and quantify incurred body burdens of harmful contaminants extant in AF. Therefore, we will first discuss the monitoring of environmental exposures before venturing into the exploration of protein biomarkers that potentially may serve as indicators of early adverse health effects from these toxic exposures.

Monitoring of Environmental Exposures in AF

By leveraging existing amniocentesis samples and contemporary analytical tools, most notably mass spectrometry, a wide variety of environmental contaminants have been documented to occur in AF (Table 1). These include inorganic and organic compounds of both persistent and non-persistent nature.

However, the AF contaminants listed in Table 1 represent only a fraction of those known to occur in other fetal and maternal matrices, such as meconium, cord blood, and maternal blood (examined as whole blood, plasma or serum) (Barr et al., 2007). Research efforts focusing on an assessment of chemical body burden typically favor the latter matrices for multiple reasons. These alternative matrices generally can be obtained less invasively, are

more readily sampled across the general population, provide a more representative spectrum of contaminants, particularly for fat-soluble, lipophilic substances, show relatively stable and higher concentrations than those extant in AF, and provide larger sampling volumes (Foster et al., 2002).

Yet, AF represents an attractive sampling matrix featuring distinct benefits. Compared to maternal specimens (e.g., maternal blood, plasma, breast milk), contaminants detected in AF are known to be in contact with and thus bioavailable to the fetus. The spectrum of toxicants occurring in AF is broad and includes lipophilic compounds, although AF's lipid content, at 0.6-0.7 g/L, is 20-fold lower than that of maternal plasma (Foster et al., 2000). Whereas detection of contaminants with octanol-water distribution coefficient (K_{OW}) values of $>10^5$ is not favorable in AF, recent advances in mass spectrometry have facilitated quantitative monitoring of a range of lipophilic compounds including numerous hydrophobic organochlorine contaminants (Table 1).

AF-screening studies have found that the concentrations of some contaminants such as total bisphenol A (BPA) may decrease during the course of pregnancy (Edlow 2012), resulting in the less frequent detection in third trimester AF compared to second trimester AF. A similar trend has been observed for urinary pesticide metabolites, where the levels decreased throughout gestation (Castorina 2010). Together with decreasing total contaminant levels, the ratio of free-to-total contaminant levels may also change over time in AF. This ratio will be affected by contaminant deconjugation via the placental deconjugation enzymes in combination with the fetal hepatic capacity for contaminant glucuronidation (Edlow 2012).. However, current available data are too sparse and chemical properties too diverse to enable definitive judgment as to the body burden time course of xenobiotic chemicals in maternal and fetal compartments. Comparative analyses of exposure and metabolism are complicated by the changing composition of AF during gestation, and the fact that contaminant concentrations generally are not normalized to protein or lipid content, as is done for other biological matrices (Herbstman et al., 2007; Sapkota et al., 2006).

Cohorts for fetal exposure studies using AF are generally comprised of women of advanced maternal age undergoing amniocentesis due to an elevated frequency of high-risk pregnancies. This leads to a sampling bias prone to skew both the detection frequency of a given contaminant and its concentration observed in AF from corresponding values in the general population. This bias in cohort composition needs to be taken into account when extrapolating the risks from fetal exposure to environmental contaminants to the general population. Lifetime accumulated dose, fetal exposure, and associated health risks likely are higher for older expecting mothers and their neonates than the corresponding metrics for the general population, especially for lipophilic substances.

Preterm Birth

The evolution of AF from a matrix used for prenatal diagnosis of adverse perinatal outcomes to a resource for elucidating the etiology of adverse health outcomes is ongoing. Its potential diagnostic value in regards to environmental exposures and biomarker discovery is discussed in the following with an emphasis on preterm birth. Discerning the relationship

between inflammation, toxic exposures, and disease is challenging. Inflammation is known to increase the risk and incidence of disease, such as cancer. Inflammation plays a role in tissue homeostasis and repair and may be an important promotor of tumorigenesis (Rakoff-Najoum 2006), for example, tumor necrosis factor (TNF) plays a role in the regulation of tumor progression in mice (Moore et al. 1999). Environmental chemicals can have inflammatory and anti-inflammatory effects. Cigarette smoke has been linked to development of inflammation and cancer (Han 2013), and BPA has been linked with inflammation in postmenopausal women (Yang, 2009) and in rats (Braniste et al. 2009). Conversely, the chemical triclocarban was observed to trigger anti-inflammatory responses in an animal model (Lui et al. 2011). Discerning these effects is complicated by other factors such as infections, which are known to trigger immune responses and may render difficult the identification of biomarkers related to environmental exposures. Although some environmental contaminants of endocrine disrupting potency (including bisphenol A and triclocarban) have been associated with (anti-) inflammatory responses and biomarkers (Yang et al. 2009, Ben-Jonathan et al. 2009, Liu et al. 2011, Clayton et al. 2010), the topic remains largely underexplored.. Yet, data from the above-referenced work together with studies such as of perinatal immunotoxicity, whereby environmental chemicals can impede growth of the developing brain and cause growth deficiencies, provide examples for how such a link could exist and become useful for identification of disease pathways (Dietert et al. 2000).

Preterm birth (PTB) (delivery < 37 weeks of gestation) is the second leading cause of infant mortality worldwide for children aged five and under. In the U.S., 12% of neonates are born preterm (CDC, 2013) and, at 13.6%, the rate is even higher among women classified as black, African-American and African-Caribbean. Comparative rates for PTB in the developing world are less certain. The World Health Organization reports rates of PTB by geographic region (Blencowe et al., 2012; CDC, 2013; Menon et al., 2009). Rates range from a low of 5-6% in Japan and some European countries to a high of 16-18% in various African countries (Reich, 2012) . Long-term trends indicate that PTB rates have been on the increase overall, particularly in developed countries. For example, in the U.S., the rate of PTB has increased by 36% over the past 25 years (March of Dimes, 2013).

Globally, PTB encompasses 15 million deliveries per year and accounts for 70% of neonatal deaths and almost half of life-long disabilities (Chang et al., 2012). Besides the more immediate complications, an extensive body of literature suggests that PTB is associated with long-term developmental problems and greater risk for adult disease (Barker et al., 2009; Osmond and Barker, 2000). For example, nearly half of PTBs before 34 weeks of gestation are associated with long-term neurological deficits and three quarters of all neonatal mortality occurs in infants born before 34 weeks of gestation (Honest et al., 2009). Limited access to and limited quality of prenatal care are deemed to contribute to these statistics globally (March of Dimes, 2013).

In the following section, we discuss the causes and management of preterm birth, causal factors and proposed mechanisms, and the large spectrum of proteins and cell mediators detectable in AF that may aid in understanding the mechanisms and biologic pathways involved.

Etiology of Preterm Birth

Approximately 30-35% of PTBs are considered iatrogenic or medically indicated due to obstetric complications such as fetal growth restriction, preeclampsia, placenta previa and non-reassuring antenatal fetal testing. The remainder of PTB is categorized as spontaneous and follows preterm labor (40-45%) or preterm rupture of membranes (PROM) (25-30%) (Conde-Agudelo et al., 2011). Although the precise mechanisms remain elusive, intrauterine inflammation and infection are a primary contributor to the factors leading to spontaneous PTB (Goldenberg et al., 2000; Institute of Medicine, 2007).

The etiology of PTB is complex, multi-factorial, variable by geographic region, and not yet fully understood. Risk factors contributing to the rise of PTB in developed countries include increased maternal age, bacterial infections, toxicant exposure (e.g., cigarette smoke), stress, over/underweight, underlying maternal health conditions (hypertension, obesity, and diabetes), clinical depression, and multiple gestations. PTB rates in the U.S. increased from 10.6% in 1989 to 12.5% in 2004. It is suspected that some of this increase can be attributed to problems associated with reproductive technologies (increasing multiple embryo transfers), cervical cerclage, progesterone supplementation, and increase in non-medically indicated labor induction or indicated Cesarean delivery; the remainder are of unknown etiology (Chang et al., 2012). This assessment is in agreement with data compiled in a 2013 March of Dimes report reporting 40-50% of PTB in the U.S. to be idiopathic in origin (March of Dimes, 2013). There are also differences in risk factors for spontaneous PTB with intact membranes versus spontaneous PTB with PROM, suggesting differential pathophysiological pathways for the various disease phenotypes (Dekker et al. 2012).

Early diagnostic procedures for determining the risk of PTB include sampling of fetal fibronectin (fFN) in cervico-vaginal fluid, maternal serum alpha-fetoprotein and C-reactive protein (CRP), and measurement of cervical length at 24 weeks (DeFranco et al., 2013; March of Dimes, 2013). These tests are most accurate in predicting birth within 7-10 days of a woman presenting with symptoms (Boots et al., 2014; DeFranco et al., 2013). Current clinical markers are based on presence of known symptoms with subsequent treatment to stall, postpone or slow labor, including the use of therapeutic treatments such as tocolytics and corticosteroids, and performance of the cerclage procedure when indicated (Buhimschi and Buhimschi, 2012). Further exploration to identify markers in asymptomatic patients is ongoing, and these efforts will allow for a better understanding of the underlying pathophysiology and molecular mechanisms leading to initiation of preterm labor and spontaneous PTB and for early screening, leading to improved interventions (Vrachnis et al., 2012).

In the U.S., PTB rates are significantly higher in African-American compared to Caucasian women (Menon et al., 2009). The reason for the discrepancy in rates between different population groups within the U.S. is uncertain, and studies point to gene-gene and gene-environment causes for these differences (Menon et al., 2009). One study suggests that higher rates of bacterial vaginosis, chorioamnionitis, and overall genital tract infections may explain much of the disparity for African American populations (Goldenberg et al., 2000). Another study suggests that single nucleotide polymorphisms (SNPs) of genes responsible

for coding cytokine activity and mediation of the inflammatory response in amniotic fluid may be partially responsible for the differences seen in PTB rates (Vrchnis et al., 2012). For example, various SNPs identified in African-American women were associated with higher AF TNF concentrations compared with Caucasians (Menon and Velez, 2008). Further potential exists to explore variations in PTB rates for various demographic groups using biological markers such as cytokine activity. These genetic predispositions may also be mediated by environmental factors.

PTB exerts a significant toll on human health and imposes staggering healthcare costs in the U.S. and around the world. Of particular concern is the rate of very-low birth weight (<1,000 grams) neonates who are at the highest risk of extreme morbidity (e.g., cerebral palsy) and mortality; rates for these neonates have declined only marginally from 1.44% in 2011 to 1.42% in 2012 and remain a large burden to the healthcare system (CDC, 2013). These challenges are driving the study of the etiology of spontaneous PTB, and the development of new strategies for its early identification and intervention through monitoring of indicators (biomarkers) of PTB. The earlier an indicator of the likelihood of PTB, the earlier a therapeutic intervention can be implemented. Because the risk of adverse outcomes associated with PTB is increased at an earlier gestational age (<32 weeks), prediction at these earlier stages becomes a high research and clinical priority (Goldenberg et al., 2005). The ideal marker should be measurable prior to the expression of symptoms, be specific to PTB, and advance our mechanistic understanding or exhibit advantages over current diagnostic capability (Buhimschi and Buhimschi, 2012). Current challenges include low predictive value of most screening methods and, once detected, lack of effective treatments and therapies to prevent PTB (Karaer et al. 2012).

Biomarkers in AF for Early Detection of Spontaneous Preterm Birth

In this section, we discuss the large spectrum of proteins and cell mediators detectable in AF that may aid in understanding the mechanisms and biologic pathways involved in PTB. While the normal process of parturition is associated with expression of anti-inflammatory mediators, the release of pro-inflammatory mediators suggests the presence of intrauterine inflammation or infection, which is believed to be the primary causal factor in the initiation of preterm labor (PTL) leading to PTB (Vrchnis et al., 2012).

Amniotic fluid contains large amounts of genetic material and proteins produced by or contained in amnion epithelial cells, fetal tissues, fetal excretions, and placental tissues, permitting its use for diagnosing and identifying genetic disorders in a clinical setting (Cho et al., 2007). Hence, AF may also be a promising medium for the identification of candidate markers of adverse perinatal outcomes, specifically PTB. AF proteins include cytokines, chemokines, matrix metalloproteinases and antigen-specific antibodies, potentially involved in the mechanisms leading to PTB (La Sala et al., 2012). From the perspective of peptide-content, AF is thought to mimic fetal plasma during 10 to 20 weeks of gestation (Cho et al., 2007). Hence, it is suspected that fluctuations in protein levels will closely mimic or track early disease processes (Buhimschi and Buhimschi, 2012). This is important, as intrauterine inflammation and infection are frequent in early preterm delivery (<32 weeks) (Goldenberg et al., 2005).

Candidate markers are typically selected from pathways suspected to be involved in PTB, and are predominately related to inflammatory response mediation. Biomarkers of PTB risk are recruited from principal pathways and processes including: (i) inflammation and infection (including intra-amniotic, cervical, decidual, and systemic); (ii) activation of maternal/fetal response (including through stress); (iii) ischemia and decidual hemorrhage (including placental abruption); and (iv) pathologic uterine distension (encompassing multiple pregnancies, polyhydramnios, and uterine structural anomaly) (Institute of Medicine, 2007; Vrachnis et al., 2012). Figure 1 provides an overview of the above-mentioned factors serving as candidate indicators and of the pathways known or suspected to result in PTB. Each of these pathways is associated with protein molecules and levels that can serve as markers of PTB (Institute of Medicine, 2007). Conversely, potential indicators determined in maternal plasma and serum and cervico-vaginal fluids have exhibited low predictive accuracy (Buhimschi and Buhimschi, 2012; Honest et al., 2009). Hence, a fetal matrix such as AF may be more relevant for diagnosis than a maternal one. For example, neutrophils of fetal origin found in AF are known to trigger an increase in cytokines relating to inflammatory insults (Buhimschi and Buhimschi, 2012; Buhimschi et al., 2013). The fetus also is active in response to infection, with mediators of such being directly measurable in AF and aiding in elucidating pathways of disease.

Studies have effectively identified AF markers in the presence of PTL symptoms (including contractions and cervical change), and these markers include inflammatory cytokines, immune factors (white blood cells), defensins, metalloproteases, and glucose levels (Goldenberg et al., 2005). Some of these markers have also been explored as predictors in asymptomatic women. A summary of candidate markers in AF is included in Table 2.

Identifying predictive biomarkers is a tedious task and there are many requirements and evaluation stages leading up to the final selection of such an indicator. Biomarkers are evaluated based on their predictive accuracy. Studies have shown that elevated levels of inflammatory cytokines can occur even in the absence of ruptured membranes or positive results of AF culture seeking to identify microbial infections; hence, there is great value in the ability to identify these and other early markers of PTL and spontaneous PTB (Figueroa et al., 2005). To test for predictive accuracy of a marker, standard procedures involve selection of the 90th or 95th percentile range used to determine a cutoff value for an elevated marker concentration (Goldenberg et al., 2005). The likelihood of PTB in the presence or absence of the elevated marker is tested, as are other measures of association such as odds ratios of relative risk. Higher or lower marker levels in the spontaneous PTB group versus those extant in an indicated or control group provide evidence for the predictive ability of the marker. Two-by-two tables are used to calculate sensitivity (probability of a positive test given that the patient has the condition), specificity (probability of a negative test given that the patient does not have the condition), and positive and negative predictive values for each marker (Conde-Agudelo et al., 2011). These values indicate the marker's potential utility in a clinical setting. Receiver operator characteristic (ROC) curves which chart the marker's sensitivity against specificity can be used to compare various markers for screening efficiency and to determine the optimal cut-off value to distinguish between cases and controls. Measures of agreement are used to assess reliability and stability of the marker (Buhimschi and Buhimschi, 2012). In meta-analyses, data from multiple studies can be

pooled to calculate likelihood ratios for larger, more diverse study populations (Conde-Agudelo et al., 2011). Standard criteria for evaluating target sensitivity and specificity depend on how the marker will be used. For example, although a marker with a high predictive value is most useful, a marker with a good negative predictive value, such as the test for fetal fibronectin or fFN, can give confidence in knowing that the fetus will not be delivered within a certain range of time (Goldenberg et al., 2005).

The Diagnostic Value of Proteins in Amniotic Fluid

Potential candidate biomarkers that qualitatively indicate the occurrence of processes which may lead to the onset of PTB are depicted in Figure 1, while a brief overview of quantitative reference values for select markers in AF that have been most consistently associated with PTB is presented in Table 3. These markers include cytokines, chemokines, matrix metalloproteinases, and antigen-specific antibodies that have been used to diagnose early intrauterine inflammation leading to PTB (La Sala et al., 2012). Putative PTB biomarkers can be grouped according to type of mechanism, including inflammation, placental proteins/hormonal, angiogenesis, or coagulation (Conde-Agudelo et al., 2011); these are discussed individually below. For a more detailed discussion on potential PTB biomarkers in AF, readers may want to refer to recent reviews or comprehensive studies by (Buhimschi et al., 2013; Conde-Agudelo et al., 2011; Menon et al., 2011; Vrachnis et al., 2012). The lettered entries below correspond to entries in Table 2.

A. Inflammation-related markers

One of the suspected causal mechanisms of PTL and subsequent PTB is secretion of cytokines, chemokines, and protein and polypeptide products at the fetomaternal interface in response to intrauterine inflammation. Because elevated levels of white blood cells may not be present early in the disease process, fluctuations in levels of cytokines in early second trimester AF may be used instead to indicate asymptomatic, sub-acute inflammation (Ruiz et al., 2012). The primary cytokines identified in the literature to be involved in PTB include the interleukins (IL) -2, -6, -8 and -10. These compounds are upregulated in response to microbial invasion into the amniotic cavity (Vrachnis et al., 2012). Cytokines and the other principal inflammation related markers are discussed in detail below for their PTB predictive value. For a more complete list of the markers of inflammation that have been studied, refer to Table 2.

Interleukin-6—IL-6 has consistently been found to be the most predictive and best candidate as a diagnostic tool for detecting pre-clinical chorioamnion inflammation and intra-amniotic inflammation (IAI) leading to PTB (Table 2) (El-Bastawissi et al., 2000; Harirah et al., 2002; Krupa et al., 2006; Lee et al., 2011; Thomakos et al., 2010; Vrachnis et al., 2012; Weissenbacher et al., 2012; Wenstrom et al., 1997). IL-6 stimulates production of proteins involved in the inflammatory process and of enzymes involved in prostaglandin synthesis. Neutrophil infiltration to the chorionic plate, choriodecidua, and umbilical cord can be measured through IL-6 concentrations of the AF and cord blood. For example, women with IAI delivered at earlier gestational age and had elevated IL-6 concentrations, a sign of fetal inflammatory response (Buhimschi et al., 2009). Elevated levels of IL-6 in mid-

trimester AF have been associated with increased risk for spontaneous PTB (before 32 wks), acute chorioamnionitis and funisitis (Gervasi et al., 2012).

Some studies, however, suggest potential drawbacks to the use of IL-6 as a marker; IL-6 has immuno-regulatory properties that can down-regulate inflammation, limiting its ability to consistently, sensitively, and accurately quantify the magnitude of inflammatory response. The predictive ability of IL-6 was inconsistent across studies, lending possible evidence that other factors could explain the divergent results (Bamberg et al., 2012; Cobo et al., 2009; Weissenbacher et al., 2012).

Interleukin-10—Another important anti-inflammatory cytokine identified in the pathways leading to PTB is IL-10. Elevated levels of IL-10 are associated with pathways leading to parturition, as labor is recognized as an inflammatory function (Gotsch et al., 2008). IL-10 participates in down-regulation of T-cell function and inhibits the synthesis of IL-1, thereby modulating the immune response (Ruiz et al., 2012). The association between IL-10 concentrations and gestational age, IAI, and parturition (both preterm and at term) has been studied (Gotsch et al., 2008). There was no change in IL-10 levels with gestational age; however, women who experienced PTB with PTL and IAI had significantly higher levels of IL-10 than those without IAI or those who delivered at term. Additionally, those who experienced PTL and delivered preterm without IAI had significantly higher levels of IL-10 than those who delivered at term. IL-10 continues to be a promising candidate marker but further studies are needed.

Interferon-gamma-inducible protein (IP-10)—IP-10 is associated with chronic chorioamnionitis, with evidence of lesions suspected to occur in late spontaneous preterm delivery (Gervasi et al., 2012). In a recent study, IP-10 has been shown to be a significant predictor of spontaneous PTB after 32 weeks, whereas in the same study, IL-6 was a better indicator of PTB at or before 32 weeks, consistent with the proposed mechanisms of each marker (Gervasi et al., 2012). These prior findings suggest that differential mechanisms are at play in the inflammatory process leading to PTB. Further study of these potential markers could provide evidence of pathways and timing of effect (Gervasi et al., 2012).

Tumor necrosis factor (TNF)—TNF is an inflammatory cytokine involved in the initiation of preterm and term birth, perhaps due to its role in apoptosis (Vrachnis et al., 2012). TNF-alpha, along with IL-1 and IL-1 β , are some of the most potent inflammatory mediators and stimulate production of prostaglandins involved in the pathway leading to PTB. Levels of TNF-alpha, cytochrome C and cell death nucleosomes have been examined in second trimester AF for the prediction of PTL and premature rupture of membranes (PROM) (Puchner et al., 2012). Cell death nucleosomes were found to be associated with a 0.2% increased risk of delivering preterm (OR 1.002, CI 1.0-1.003, $p < 0.018$); however, results for TNF-alpha showed no association. In contrast other studies confirmed an association between elevated levels of TNF-alpha and IAI and PTB (Thomakos et al., 2010). Further, there appear to be allelic variations in TNF-alpha and the soluble forms of TNF including sTNFR1 and sTNFR1. It is suspected that these variations may account for differences in SNPs in Caucasian compared with African-American women. This may be a valuable tool in understanding disparity in disease rates by racial group. Thus TNF-alpha

likely plays a role in the initiation of preterm and term labor through inflammation pathways, and has potential in the identification of women at risk for IAI and PTB (Thomakos et al., 2010). Based on the disparate study results, more data are needed to evaluate the role of TNF as a possible marker.

B. Extracellular matrix mediators

Matrix metalloproteases (MMPs)—In addition to IL-6, MMPs have consistently been found to be predictive of infection-related mechanisms in the maternal/fetal compartment leading to PTB (Harirah et al., 2002). MMPs, and more specifically MMP-8, are zinc-dependent enzymes expressed in the inflammatory response and can be involved in the breakdown of connective tissue such as collagen (Vrachnis et al., 2012). These molecules, which play a role in the regular processes of parturition through involvement in digestion of extracellular matrices, are thought to play a role in cervical ripening and PROM. In several studies including a large meta-analysis (Conde-Agudelo et al., 2011), MMP-8 was identified as the most predictive marker for spontaneous PTB (Yoon et al., 2001). Overall, MMPs are promising compounds deserving of further study in the prediction of PTB.

C. Angiogenesis-related markers

Angiogenin—Angiogenin is a polypeptide plasma protein responsible for induction of neovascularization. It has been one of the early markers of intrauterine ischemic damage and inflammation and has been explored as a marker for PTB in mid-trimester AF (Madazli et al., 2003; Spong et al., 1997). Because angiogenin is expressed locally and not systemically, changes of angiogenin levels in maternal serum are of limited predictive value for spontaneous PTB (Madazli et al., 2003; Spong et al., 1997). The few studies available on angiogenin as a marker detectable in AF lack consistency (Yoon et al., 2001), in part due to their low sample sizes. A meta-analysis of angiogenin as a biomarker showed a sensitivity of 50% (Conde-Agudelo et al., 2011).

D. Arachidonateloxygenase metabolites

Prostaglandins (PGs) and Uterotonins—PGs and uterotonins are involved in the regulation of myometrial contractility, changes in extracellular matrix composition, and consequent cervical ripening. Biologically active PGs comprise a large percentage of mediators in AF (Institute of Medicine, 2007; Menon et al., 2011; Vrachnis et al., 2012). PGs are thought to be key mediators in mechanisms regulating the onset of PTL as PGs or uterotonin (-like proteins) can induce contractions. A proposed underlying mechanism for the diagnostic value of PGs may be cytokine-mediated response to infection which is known to trigger PG production. PGs, and specifically PGE₂ and PGF_{2α}, have been associated with PTL in several studies and warrant further exploration (Vrachnis et al., 2012).

E. Host defense-related markers

A combination of cytokines including human neutrophil proteins 1-3, and calgranulin A and B have been observed to be over-expressed in women experiencing IAI and PTB (Rüetschi et al., 2005). Exploration of the AF proteome for pathogenic mechanisms leading to PTB identified a novel protein fingerprint consisting of peaks in the 10-12.5 kDa mass range

(Buhimschi and Buhimschi, 2008). Protein profiles associated with stages of IAI included neutrophil defensin-1, -2 and calgranulin A and C; these are associated with metabolic, non-inflammatory biological processes, including protein metabolism, signal transduction and transport (Buhimschi and Buhimschi, 2008). Further study of these types of markers, and more specifically panels of these occurring in combination, may provide mechanistic insights into PTB pathways and biomarkers of utility (Buhimschi et al. 2013). Opportunities in biomarker panel development are discussed in detail in the section below titled “Marker Combinations”.

F. Cell adhesion molecules

Intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1)—Cell surface adhesion molecules, such as ICAM-1 and VCAM-1, are proteins expressed on endothelial cells that are up-regulated by inflammatory cytokines (Salafia et al., 1993; Vrachnis et al., 2012). Elevated levels of ICAM-1 in AF have been associated with shortened length of gestation (Salafia et al., 1993). Further data are needed to adequately assess the role of these molecules in elucidating pathways leading to PTB.

G. Others

Urocortin-1—Urocortin-1 is a corticotrophin-releasing-factor-related oligopeptide. It may play a role in PTB facilitated by activation of the hypothalamic-pituitary-adrenal axis (HPA) by way of paracrine effects in intrauterine tissue or endocrine effects in maternal and fetal tissues (Karaer et al. 2013). One recent study identified this mediator as a possible marker; however, further study is needed to assess the role of this promising peptide

Marker Combinations

Standard approaches for selection of possible candidate markers have included identification of a single target protein; however, evolving methodologies focus on the detection of protein panels, as done in diagnostic pattern proteomics (Buhimschi and Buhimschi, 2012). The latter methodology exploits the identification of protein regions (Buhimschi and Buhimschi, 2012), with benefits including an improved predictive accuracy and the ability to reflect multiple pathways contributing to PTB (Buhimschi and Buhimschi, 2012; Goldenberg et al., 2005; Kim et al., 2013). For example, a large range of cytokines and peptides exhibited a high predictive value for intrauterine infection; these included IL-18, IL-1 β , IL-6 and RANTES, with an area-under-the-curve ranging from 0.67-0.76 (Holst et al., 2011). In addition, poorly characterized markers can be captured as a sum using a mass restriction (MR) score, as discussed above under “Host defense-related markers” (Buhimschi et al., 2005). Further study is needed in this area as combinations of markers in AF can more accurately represent the heterogeneity of factors leading to PTB (Buhimschi and Buhimschi, 2012).

Role of Environmental Exposures in Fetal Development

To date, few strong predictors of PTB are available and many of these fail to capture the majority of women who deliver prematurely. Filling this knowledge gap will be of import for the development of preventive measures. Rising rates of PBT in developed countries

show that improved hygiene and broad access to healthcare alone are not enough to reverse or even halt these unwanted trends. *In utero* environmental exposures to complex mixtures of bioactive chemicals have the potential to interfere with fetal development and are a potentially important factors.

Recent evidence suggests that several classes of bioactive and emerging contaminants, such as endocrine-disrupting chemicals (EDCs), metals, and nanomaterials, have the potential to adversely affect fetal development and birth outcomes; these exposures are hypothesized to trigger subsequent disease in adulthood (Edwards and Myers, 2007; Meeker, 2012; Pietroiusti et al., 2011; Shimizu et al., 2009). We hypothesize that environmental exposures could interfere with the inflammation process and mediation of tissue repair and immune system modulatory responses (Moore et al. 1999, Rakoff-Nahoum 2006) that could act as precursors to PTB. An association between PTB and fetal exposure to xenobiotics (and EDCs in particular) was reported in case-control studies where *in utero* exposure to the antenatal drug, diethylstilbestrol, was associated with miscarriage and PTB (Bamigboye et al., 2003). A similar, though unsubstantiated, risk association was suggested to exist between exposure to BPA and PTB (Edlow et al., 2012). Currently, approximately 800 chemicals are suspected of being capable of interfering with the natural hormonal homeostasis (WHO, 2013).

Associations between toxicant exposure and PTB could be indicative of cause-effect relationships but such mechanistic models thus far are mostly hypothetical. The contribution of xenobiotics to PTB could be the result of an inherent ability of the pollutant to modulate factors in the maternal and fetal compartments related to inflammatory and host immune systems. In this scenario, there are ample opportunities for identifying candidate markers of PTB, discussed below in greater detail.

Potential Role of Environmental Chemicals in the Search for Candidate Markers

The role, impact, and significance of chemical exposures in PTB have yet to be fully elucidated but multiple scientific leads exist. A good example is the assessment of potential impacts of EDC exposures on reproductive health. EDCs have been implicated in the increased incidence of a wide range of adverse health outcomes, including infertility, genital tract abnormalities, breast cancer, obesity, and early onset of sexual maturation; these adverse health outcomes have been observed in U.S. and European populations over the last 50 years (Munoz-de-Toro et al., 2005; Sharpe and Skakkebaek, 1993; Skakkebaek et al., 1998). Animal studies have found links with BPA exposure and uterine, ovarian and testicular toxicity, but these results have not been duplicated in human cohorts (Peretz et al. 2014). Some human reproductive data may be interpreted as being supportive of potential adverse birth outcomes, hyperandrogenism, sexual dysfunction, and impaired implantation in women exposed to BPA. For example, reduced oocyte quality in women undergoing *in vitro* fertilization has been reported in conjunction with BPA exposure (Peretz et al. 2014). However, the interpretation of such associations found in human cohort studies is complicated by the fact that the exposure data may rely on few or only a single measurement with time-averaged exposures remaining uncertain in many cases. Adding to this uncertainty, various studies report on the variability of BPA body burdens during pregnancy

(Philippat 2013, Jusko 2014). A recent report by the World Health Organization (WHO) suggests a potential role of EDCs in the rise of PTB worldwide, though this is not yet substantiated by evidence in the literature (WHO, 2013). EDCs by definition interfere with hormone function (Diamanti-Kandarakis et al., 2009). This disruption of signaling pathways is particularly threatening during critical periods of development, the so-called windows of susceptibility (Pryor et al., 2000). In addition to their role in regulating fetal development, estrogens and xenoestrogens (such as BPA) are known to modulate a multitude of components of the immune system (Inadera, 2006). Because estrogens have been found to regulate the level of serum and uterine IgM, IgA, and IgG (Wira and Sandoe 1987), it can be hypothesized that these biomarkers, also associated with PTB, could be impacted by environmental contaminants and particularly those contaminants that mimic estrogenic activity by acting as ER- α agonists, of which BPA is the best known example. Thus of concern in the context of PTB is the possible impact of BPA on the regulation of uterine IgA and IgG secretion (Wira and Sandoe, 1987) and on the production of interleukin (Lee et al., 2003). Furthermore, fetal exposure to BPA has been hypothesized to affect the functioning of adipocytes and the risk for developing adult obesity, a known risk factor for PTB (Vom Saal et al., 2012). Phthalate plasticizers such as di-*n*-butylphthalate are EDCs of potential import in both infertility and PBT (Fennell et al., 2004; Mylchreest et al., 1998). Due to their environmental ubiquity, exposure assessments for these anthropogenic chemicals rely on detection of their characteristic metabolites rather than the parent compound itself (Table 3).

The physiological relevance of endocrine-disrupting xenobiotics remains largely unexplored in general and with respect to PTB in particular. Whereas the estrogenic potency of BPA is about three orders of magnitude lower than that of estradiol (E2; the natural estrogen to which estrogenic potency is normalized), the potency and activity of BPA can be vastly different *in vivo* (Steinmetz et al., 1997). Furthermore, dose-response relationships may not be monotonic for this and other EDCs. Presence of BPA and other EDCs in the form of a mixture adds further complexity and may alter effects in unpredictable ways (Ames et al., 1995; Vom Saal and Hughes, 2005; Welshons et al., 2003). Moreover, the deconjugation enzymes β -glucuronidase and arylsulfatase C were found to be highly active in human placental tissues, and may further increase fetal exposure to free BPA by hydrolyzing the conjugated BPA as it passes into the fetal compartment (Ginsberg and Rice, 2009). In this context it is noted that when BPA is found in AF, the more potent (“free”) variant comprises the majority (80-90%) of the sum of BPA derivatives present (Edlow et al., 2012).

Phytoestrogens are naturally occurring plant estrogens that are part of a normal diet and particularly important in Asian cuisine with phytoestrogen-rich meals, and other dietary lifestyles centered on plants (Messina and Messina, 1991). This is reflected in the high detection frequencies for daidzein and genistein, as summarized in Table 3. Even though their estrogenic potency is lower compared to human estrogens, phytoestrogens are a chemical group of concern due to their additive effect on developmental processes. Phytoestrogen levels can be 20-100 $\times$ higher than those of the natural estrogen, E2 (Engel et al., 2006). What is more, consumption of soy-rich diets has been associated with deleterious health effects, e.g., altered reproductive function (Cassidy et al., 1994) and decreased serum estrogen levels (Adlercreutz et al., 1987; Adlercreutz et al., 1992), and consumption of

EDC-contaminated fish has been associated with neurodevelopmental impairment in children (Jacobson and Jacobson, 1996). The impact of phytoestrogens together with the effects of synthetic EDCs such as BPA and polychlorinated biphenyls (PCBs) (Inadera, 2006) is of potential importance and concern for perinatal outcomes. However, studies exploring such mixture effects in the context of PTB are lacking.

However, PCBs may also pose risks to the fetus by themselves. This family of manmade EDCs has been banned in the 1970s but is still detectable in the environment and in human populations (Table 3). PCBs can impair immunologic maturation of infants. A recent longitudinal study linked *in utero* exposure to PCBs to impaired thymus development extending beyond the neonatal period (Jusko et al., 2012).

Different types of environmental contaminants, including diesel exhaust particulates (DEPs), are known to affect the immune system *in vivo* and *in vitro* in adults (Inadera, 2006). DEPs can increase IL levels *in vivo* (Miyabara et al., 1998; Takano et al., 1997), depress immune responsiveness to bacterial antigens (Yang et al., 1999), impede phagocytosis-driven bacterial clearance (Yin et al., 2002), and weaken innate immunity by inhibiting secretion of interleukins (e.g., IL-1 β) and tumor necrosis factor (e.g., TNF- α) (Yin et al., 2002).

Despite the lack of conclusive evidence of a causal relationship between the increasing exposures to EDCs and increasing PTB rates, accumulating literature on EDC-driven modulation of biological processes overlaps with PTB-inducing processes. Moreover, conflicting study outcomes could be due to a wide range of factors, including non-monotonic EDC potency, mixture effects, cohort selection, limited sample size, etc. Although none of the AF monitoring studies cited in Table 3 were performed in the framework of PTB specifically, the potential role of these contaminants in PTB etiology is plausible mechanistically and thus deserving further investigation.

Conclusions

In summary, AF is an information-rich bodily fluid of diagnostic value with untapped potential. It can serve to determine and quantify toxic, environmental exposures to the fetus and may aid in elucidation of causes of the high incidence of PTB. Already, markers detectable in AF have played a central role in the elucidation of mechanisms and pathways underlying PTB. Opportunities exist to combine the investigation of toxic environmental exposures with the quest for biomarkers of increased risk for PTB. Studies examining such a relationship are lacking, hampering progress in understanding PBT etiology and minimizing the incidence of PTB through preventive measures. As advances in analytic chemistry enable the precise and accurate measurement of low quantities of contaminants in ever decreasing volumes of samples, opportunities abound for filling important knowledge gaps with respect to the diagnosis and studying mechanistically the impact of environmental exposures on maternal and fetal health. The ultimate goal of this work is the development of preventive treatments for PTB and other diseases of yet uncertain etiology for which environmental exposures may represent a plausible, if not likely, risk factor.

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References

- Adlercreutz H, et al. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J Steroid Biochem.* 1987; 27:1135–44. [PubMed: 2826899]
- Adlercreutz H, et al. Dietary phytoestrogens and cancer: in vitro and in vivo studies. *J Steroid Biochem Mol Biol.* 1992; 41:331–7. [PubMed: 1314077]
- Ames BN, et al. The causes and prevention of cancer. *Proc Natl Acad Sci U S A.* 1995; 92:5258–65. [PubMed: 7777494]
- Bamberg C, et al. Correlation of midtrimester amniotic fluid cytokine concentrations with adverse pregnancy outcome in terms of spontaneous abortion, preterm birth, and preeclampsia. *J Matern Fetal Neonatal Med.* 2012; 25:812–7. [PubMed: 21726173]
- Bamigboye AA, et al. Diethylstilbestrol--haunting lessons. *S Afr Med J.* 2003; 93:346–7. [PubMed: 12830595]
- Barker DJ, et al. Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol.* 2009; 36:445–58. [PubMed: 19562567]
- Barr DB, et al. Concentrations of xenobiotic chemicals in the maternal-fetal unit. *Reproductive Toxicology.* 2007; 23:260–266. [PubMed: 17386996]
- Ben-Jonathan N, et al. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. *Mol Cell Endocrinol.* 2009; 304(1-2):49–54. [PubMed: 19433247]
- Blencowe H, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet.* 2012; 379:2162–72. [PubMed: 22682464]
- Boots AB, et al. The short-term prediction of preterm birth: a systematic review and diagnostic metaanalysis. *Am J Obstet Gynecol.* 2014; 210:54.e1–54.e10. [PubMed: 24021995]
- Buhimschi CS, et al. Fetal inflammatory response in women with proteomic biomarkers characteristic of intra-amniotic inflammation and preterm birth. *BJOG.* 2009; 116:257–67. [PubMed: 18947340]
- Buhimschi IA, Buhimschi CS. Proteomics of the amniotic fluid in assessment of the placenta. Relevance for preterm birth. *Placenta.* 2008; 29:S95–S101. [PubMed: 18191197]
- Buhimschi IA, Buhimschi CS. Proteomics/diagnosis of chorioamnionitis and of relationships with the fetal exposome. *Seminars in Fetal & Neonatal Medicine.* 2012; 17:36–45. [PubMed: 22100864]
- Buhimschi IA, et al. Proteomic biomarker analysis of amniotic fluid for identification of intra-amniotic inflammation. *Bjog-an International Journal of Obstetrics and Gynaecology.* 2005; 112:173–181. [PubMed: 15663581]
- Buhimschi IA, et al. Advances in medical diagnosis of intra-amniotic infection. *Expert Opin Med Diagn.* 2013; 7:5–16. [PubMed: 23530840]
- Carlin DJ, et al. Unraveling the health effects of environmental mixtures: an NIEHS priority. *Environ Health Perspect.* 2013; 121:A6–8. [PubMed: 23409283]
- Cassidy A, et al. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr.* 1994; 60:333–40. [PubMed: 8074062]
- Castorina R, et al. Comparison of current-use pesticide and other toxicant urinary metabolite levels among pregnant women in the CHAMACOS cohort and NHANES. *Environ Health Perspect.* 2010; 118, 856–63.
- CDC. Preterm birth. 2013; 2013
- Chang HH, et al. Time-to-event analysis of fine particle air pollution and preterm birth: results from North Carolina, 2001-2005. *Am J Epidemiol.* 2012; 175:91–8. [PubMed: 22167746]

- Cho CK, et al. Proteomics Analysis of Human Amniotic Fluid. *Molecular Cell Proteomics*. 2007; 6:1406–15.
- Clayton EM, et al. The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003–2006. *Environ Health Perspect*. 2011; 119(3):390–6. 10.1289/ehp.1002883 [PubMed: 21062687]
- Cobo T, et al. Predictive value of combined amniotic fluid proteomic biomarkers and interleukin-6 in preterm labor with intact membranes. *American Journal of Obstetrics and Gynecology*. 2009; 200
- Conde-Agudelo A, et al. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. *BJOG*. 2011; 118:1042–54. [PubMed: 21401853]
- DeFranco EA, et al. Improving the screening accuracy for preterm labor: is the combination of fetal fibronectin and cervical length in symptomatic patients a useful predictor of preterm birth? A systematic review. *Am J Obstet Gynecol*. 2013; 208:233.e1–6. [PubMed: 23246314]
- Dekker GA, et al. Risk Factors for Preterm Birth in an International Prospective Cohort of Nulliparous Women. *PLoS ONE*. 2012; 7:7.e39154.
- Diamanti-Kandarakis E, et al. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocrine Reviews*. 2009; 30:293–342. [PubMed: 19502515]
- Dietert RR, et al. Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. *Environ Health Perspect*. 2000; 108(Suppl 3):483–490. [PubMed: 10852848]
- Eddleman KA, et al. Pregnancy loss rates after midtrimester amniocentesis. *Obstet Gynecol*. 2006; 108:1067–72. [PubMed: 17077226]
- Edlow AG, et al. Fetal bisphenol A exposure: concentration of conjugated and unconjugated bisphenol A in amniotic fluid in the second and third trimesters. *Reprod Toxicol*. 2012; 34:1–7. [PubMed: 22516041]
- Edwards TM, Myers JP. Environmental exposures and gene regulation in disease etiology. *Environ Health Perspect*. 2007; 115: 1264–70. [PubMed: 18174959]
- El-Bastawissi AY, et al. Amniotic fluid interleukin-6 and preterm delivery: a review. *Obstet Gynecol*. 2000; 95:1056–64. [PubMed: 10808034]
- Engel SM, et al. Xenobiotic phenols in early pregnancy amniotic fluid. *Reprod Toxicol*. 2006; 21:110–2. [PubMed: 16112541]
- Fennell TR, et al. Pharmacokinetics of dibutylphthalate in pregnant rats. *Toxicol Sci*. 2004; 82:407–18. [PubMed: 15456918]
- Figueroa R, et al. Evaluation of amniotic fluid cytokines in preterm labor and intact membranes. *J Matern Fetal Neonatal Med*. 2005; 18:241–7. [PubMed: 16318974]
- Foster W, et al. Detection of endocrine disrupting chemicals in samples of second trimester human amniotic fluid. *J Clin Endocrinol Metab*. 2000; 85:2954–7. [PubMed: 10946910]
- Foster WG, et al. Detection of phytoestrogens in samples of second trimester human amniotic fluid. *Toxicol Lett*. 2002; 129:199–205. [PubMed: 11888703]
- Gervasi MT, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. *J Perinat Med*. 2012; 40:329–43. [PubMed: 22752762]
- Gil MM, et al. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. *Ultrasound Obstet Gynecol*. 2013; 42:34–40. [PubMed: 23744609]
- Ginsberg G, Rice DC. Does rapid metabolism ensure negligible risk from bisphenol A? *Environ Health Perspect*. 2009; 117: 1639–43. [PubMed: 19165397]
- Goldenberg RL, et al. Maternal infection and adverse fetal and neonatal outcomes. *Clin Perinatol*. 2005; 32:523–59. [PubMed: 16085019]
- Goldenberg RL, et al. Intrauterine infection and preterm delivery. *N Engl J Med*. 2000; 342:1500–7. [PubMed: 10816189]

- Gotsch F, et al. The anti-inflammatory limb of the immune response in preterm labor, intra-amniotic infection/inflammation, and spontaneous parturition at term: A role for interleukin-10. *Journal of Maternal-Fetal & Neonatal Medicine*. 2008; 21:529–547. [PubMed: 18609361]
- Harirah H, et al. Amniotic fluid matrix metalloproteinase-9 and interleukin-6 in predicting intra-amniotic infection. *Obstet Gynecol*. 2002; 99:80–4. [PubMed: 11777515]
- Herbstman JB, et al. Determinants of prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in an urban population. *Environ Health Perspect*. 2007;115, 1794–800. [PubMed: 18174959]
- Holst RM, et al. Prediction of microbial invasion of the amniotic cavity in women with preterm labour: analysis of multiple proteins in amniotic and cervical fluids. *BJOG*. 2011; 118:240–9. [PubMed: 21054762]
- Honest H, et al. Screening to prevent spontaneous preterm birth: systematic reviews of accuracy and effectiveness literature with economic modelling. *Health Technol Assess*. 2009; 13:1–627. [PubMed: 19796569]
- Inadera H. The immune system as a target for environmental chemicals: Xenoestrogens and other compounds. *Toxicol Lett*. 2006; 164:191–206. [PubMed: 16697129]
- Institute of Medicine. *Preterm Birth*. The National Academies Press; Washington, D.C.: 2007.
- Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med*. 1996; 335:783–9. [PubMed: 8703183]
- Jusko TA, et al. Pre- and postnatal polychlorinated biphenyl concentrations and longitudinal measures of thymus volume in infants. *Environ Health Perspect*. 2012;120, 595–600.
- Jusko TA, et al. Reproducibility of urinary bisphenol A concentrations measured during pregnancy in the Generation R Study. *J Expo Sci Environ Epidemiol*. 2014; 24(5):532–6. [PubMed: 24736100]
- Han Y, et al. Acute cigarette smoke exposure modulates the inflammatory response in pdmH1N1 and H9N2 infection (P6190). *The Journal of Immunology*. 2013; 190:189.10.
- Kim A, et al. Identification of biomarkers for preterm delivery in mid-trimester amniotic fluid. *Placenta*. 2013; 34:873–8. [PubMed: 23953866]
- Krupa FG, et al. Predictors of preterm birth. *Int J Gynaecol Obstet*. 2006; 94:5–11. [PubMed: 16730012]
- La Sala GB, et al. Protein microarrays on midtrimester amniotic fluids: a novel approach for the diagnosis of early intrauterine inflammation related to preterm delivery. *Int J Immunopathol Pharmacol*. 2012; 25:1029–40. [PubMed: 23298493]
- Lee MH, et al. Enhanced interleukin-4 production in CD4+ T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: involvement of nuclear factor-AT and Ca2+ *Immunology*. 2003; 109:76–86. [PubMed: 12709020]
- Lee SY, et al. IL-6 Trans-Signaling System in Intra-Amniotic Inflammation, Preterm Birth and Preterm Premature Rupture of the Membranes. *Journal of Immunology*. 2011; 186:3226–3236.
- Lozano J, et al. Biological matrices for the evaluation of in utero exposure to drugs of abuse. *Ther Drug Monit*. 2007; 29:711–34. [PubMed: 18043469]
- Liu JY, et al. Inhibition of soluble epoxide hydrolase contributes to the anti-inflammatory effect of antimicrobial triclocarban in a murine model. *Toxicol Appl Pharmacol*. 2011; 255:200–206. [PubMed: 21741984]
- Madazli R, et al. Mid-trimester amniotic fluid angiogenin, lactate dehydrogenase and fibronectin in the prediction of preterm delivery. *Eur J Obstet Gynecol Reprod Biol*. 2003; 106:160–4. [PubMed: 12551785]
- March of Dimes. *Preterm labor and birth*. 2013; 2013
- Meeker JD. Exposure to environmental endocrine disruptors and child development. *Arch Pediatr Adolesc Med*. 2012; 166:952–8. [PubMed: 23367522]
- Menon R, et al. Racial disparity in pathophysiologic pathways of preterm birth based on genetic variants. *Reprod Biol Endocrinol*. 2009; 7:7–62. 1477-7827. [PubMed: 19171040]
- Menon R, et al. Biomarkers of spontaneous preterm birth: an overview of the literature in the last four decades. *Reprod Sci*. 2011; 18:1046–70. [PubMed: 22031189]

- Menon R, Velez DR. Genetic regulation of amniotic fluid TNF-alpha and soluble TNF receptor concentrations affected by race and preterm birth. *Human Genetics*. 2008; 124:243–53. [PubMed: 18807256]
- Messina M, Messina V. Increasing use of soyfoods and their potential role in cancer prevention. *J Am Diet Assoc*. 1991; 91:836–40. [PubMed: 2071799]
- Miyabara Y, et al. Effects of diesel exhaust on allergic airway inflammation in mice. *J Allergy Clin Immunol*. 1998; 102:805–12. [PubMed: 9819298]
- Moore RJ, et al. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat Med*. 1999; 5:828–831. [PubMed: 10395330]
- Munoz-de-Toro M, et al. Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. *Endocrinology*. 2005; 146:4138–47. [PubMed: 15919749]
- Mylchreest E, et al. Male reproductive tract malformations in rats following gestational and lactational exposure to Di(n-butyl) phthalate: an antiandrogenic mechanism? *Toxicol Sci*. 1998; 43:47–60. [PubMed: 9629619]
- Osmond C, Barker DJ. Fetal, infant, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. *Environ Health Perspect*. 2000; 108(Suppl 3): 545–53. [PubMed: 10852853]
- Peretz J, et al. Bisphenol A and Reproductive Health: Update of Experimental and Human Evidence, 2007–2013. *Environ Health Perspect*. 2014; 122(8):775–786. [PubMed: 24896072]
- Philippat C, et al. Prenatal exposure to environmental phenols: concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. *Environ Health Perspect*. 2013; 121(10): 1225–31. 10.1289/ehp.1206335 [PubMed: 23942273]
- Pietroiu A, et al. Low doses of pristine and oxidized single-wall carbon nanotubes affect mammalian embryonic development. *ACS Nano*. 2011; 5:4624–33. [PubMed: 21615177]
- Pryor JL, et al. Critical windows of exposure for children's health: the reproductive system in animals and humans. *Environ Health Perspect*. 2000; 108(Suppl 3):491–503. [PubMed: 10852849]
- Puchner K, et al. The implication of second-trimester amniotic fluid TNF-alpha, cytochrome C and cell death nucleosomes in the prediction of preterm labor and/or premature rupture of membranes. *Arch Gynecol Obstet*. 2012; 285:37–43. [PubMed: 21526362]
- Rakoff-Nahoum S. Why cancer and inflammation. *Yale J Biol Med*. 2006; 79(3-4):123–130. [PubMed: 17940622]
- Reich ES. Pre-term births on the rise. *Nature*. 2012; 485:20. [PubMed: 22552070]
- Ruiz RJ, et al. Second trimester maternal plasma levels of cytokines IL-1Ra, IL-6 and IL-10 and preterm birth. *J Perinatol*. 2012; 32:483–90. [PubMed: 22460542]
- Rüetschi U, et al. Proteomic analysis using protein chips to detect biomarkers in cervical and amniotic fluid in women with intra-amniotic inflammation. *J Proteome Res*. 2005; 4:2236–42. [PubMed: 16335971]
- Salafia CM, et al. Circulating intercellular adhesion molecule-1 in amniotic fluid, maternal serum alpha-fetoprotein levels, and intrauterine growth retardation. *Am J Obstet Gynecol*. 1993; 169:830–4. [PubMed: 7694462]
- Sapkota A, et al. Urinary biomarkers of 1,3-butadiene in environmental settings using liquid chromatography isotope dilution tandem mass spectrometry. *Chem Biol Interact*. 2006; 160:70–9. [PubMed: 16423335]
- Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet*. 1993; 341:1392–5. [PubMed: 8098802]
- Sherer DM, Langer O. Oligohydramnios: use and misuse in clinical management. *Ultrasound Obstet Gynecol*. 2001; 18:411–9. [PubMed: 11844157]
- Shimizu M, et al. Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to brain development in the mouse. *Part Fibre Toxicol*. 2009; 6:20. [PubMed: 19640265]
- Skakkebaek NE, et al. Germ cell cancer and disorders of spermatogenesis: an environmental connection? *APMIS*. 1998; 106:3–11. discussion 12. [PubMed: 9524557]

- Spong CY, et al. Elevated maternal serum midtrimester alpha-fetoprotein levels are associated with fetoplacental ischemia. *Am J Obstet Gynecol.* 1997; 177:1085–7. [PubMed: 9396898]
- Steinmetz R, et al. The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. *Endocrinology.* 1997; 138:1780–6. [PubMed: 9112368]
- Takano H, et al. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am J Respir Crit Care Med.* 1997; 156:36–42. [PubMed: 9230723]
- Thomakos N, et al. Amniotic fluid interleukin-6 and tumor necrosis factor-alpha at mid-trimester genetic amniocentesis: Relationship to intra-amniotic microbial invasion and preterm delivery. *European Journal of Obstetrics & Gynecology and Reproductive Biology.* 2010; 148:147–151. [PubMed: 19945777]
- Vom Saal, FS.; Hughes, C. *Environ Health Perspect.* 2005. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment; p. 113p. 926-33.
- Vom Saal FS, et al. The estrogenic endocrine disrupting chemical bisphenol A (BPA) and obesity. *Mol Cell Endocrinol.* 2012; 354:74–84. [PubMed: 22249005]
- Vrachnis N, et al. Review: Impact of mediators present in amniotic fluid on preterm labour. *In Vivo.* 2012; 26:799–812. [PubMed: 22949593]
- Weissenbacher T, et al. Influence of maternal age, gestational age and fetal gender on expression of immune mediators in amniotic fluid. *BMC Res Notes.* 2012; 5:375. [PubMed: 22827842]
- Welshons WV, et al. Large effects from small exposures I Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect.* 2003;111, 994–1006.
- Wenstrom KD, et al. A placebo-controlled randomized trial of the terbutaline pump for prevention of preterm delivery. *Am J Perinatol.* 1997; 14:87–91. [PubMed: 9259905]
- Winecker RE, et al. Detection of cocaine and its metabolites in amniotic fluid and umbilical cord tissue. *J Anal Toxicol.* 1997; 21:97–104. [PubMed: 9083826]
- Wira CR, Sandoe CP. Specific IgA and IgG antibodies in the secretions of the female reproductive tract: effects of immunization and estradiol on expression of this response in vivo. *J Immunol.* 1987; 138:4159–64. [PubMed: 3584972]
- Wong D, et al. Optimizing blood collection, transport and storage conditions for cell free DNA increases access to prenatal testing. *Clin Biochem.* 2013; 46:1099–104. [PubMed: 23643886]
- World Health Organization. State of the science of endocrine disrupting chemicals - 2012. United Nations Environment Programme and the World Health Organization; 2013.
- Yang HM, et al. Effects of diesel exhaust particles (DEP), carbon black, and silica on macrophage responses to lipopolysaccharide: evidence of DEP suppression of macrophage activity. *J Toxicol Environ Health A.* 1999; 58:261–78. [PubMed: 10598952]
- Yang YJ, et al. Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women. *Environ Res.* 2009; 109(6):797–801.10.1016/j.envres.2009.04.014 [PubMed: 19464675]
- Yin, XJ., et al. *Environ Health Perspect.* 2002. Alteration of pulmonary immunity to *Listeria monocytogenes* by diesel exhaust particles (DEPs) I Effects of DEPs on early pulmonary responses; p. 110p. 1105-11.
- Yoon BH, et al. An elevated amniotic fluid matrix metalloproteinase-8 level at the time of mid-trimester genetic amniocentesis is a risk factor for spontaneous preterm delivery. *Am J Obstet Gynecol.* 2001; 185:1162–7. [PubMed: 11717651]

Figure 1.
Biologic Mechanisms of Preterm Birth and Inflammatory Mediators in Amniotic Fluid (AF),
Adapted from IOM, 2007.

Table 1
Natural and Xenobiotic Contaminants Detected in Amniotic Fluid

Xenobiotic (summedanalytes)	AF Range [ng/mL]	AF Median [ng/mL]	Det. freq. (Subjects)	Gestation [weeks]	Collection Date	Other Matrices	Reference
<i>Inorganic contaminants</i>							
Iodide	1.7-170	8.1	100% (48)	15-20	-	-	Blount 2006
Nitrate	288-8940	1500	100% (48)	15-20	-	-	Blount 2006
Perchlorate	0.057-0.71	0.18	100% (48)	15-20	-	-	Blount 2006
<i>Organic contaminants</i>							
Benzoyllecgonine	143-925	909	30% (20)	Birth	-	MU, IU, Me	Casanova 1994
	ND-152,288	-	9% (32)	-	-	UC	Winecker 1997
	51-836	277	1% (450)	13-39	1991	MS	Ripple 1992
Bisphenol A (BPA)	ND-0.75	0.47	80% (20)	T2	-	-	Chen 2011
	0.36-0.66	0.45	80% (20)	14-21	2010	-	Edlow 2012
	0.1-0.46	-	10% (20)	33-38	2006	-	Edlow 2012
	0.5-1.96	0.5	10% (21)	<20	2004	-	Engel 2006
	ND-5.62	0.26	- (200)	16.3 ± 1.0	1989-1998	MS	Yamada 2002
	-	0	- (48)	16.2 ± 1.0	1989-1998	MS	Yamada 2002
Caffeine	-	-	- (-)	16-17	-	-	Graca 2008
Carbofuranphenol	0.12-0.12	0.12*	5% (20)	18 ±2.6	-	-	Bradman 2003
Cocaine	-	-	56% (16) [†]	-	-	CB, IU, Mec, MH	Eyler 2005
	11-24	18	1% (450)	13-39	1991	MS	Ripple 1992
Cotinine	ND-531	2.2	98&percent; (300)	10-30	1980-1996	-	Jensen 2012
Σ Cyclodienes (9)	-	0.038*	17% (100)	15-20	2006-2007	MS	Luzardo 2009
Daidzein	0.5-5.52	1.08*	68.4% (57)	15-23	1999-2000	-	Foster 2002
	3.84-17.4	9.52	100% (21)	<20	2004	-	Engel 2006
p,p'-DDE	0.10-0.63	0.211*	28.3% (41)	15-23	-	-	Foster 2000
	ND-1.67	0.21*	25% (70)	14-21	1999-2000	-	Foster 2002
	ND-0.63	0.24*	20.8% (48)	15-23	1999-2000	-	Foster 2002
2,5-Dichlorophenol	0.37-0.43	0.39*	55% (20)	18 ±2.6	-	-	Bradman 2003
Diethylphosphate	0.26-0.36	0.31*	10% (20)	18 ±2.6	-	-	Bradman 2003
Dimethylphosphate	0.30-0.34	0.32*	10% (20)	18 ±2.6	-	-	Bradman 2003

Xenobiotic (summed analytes)	AF Range [ng/mL]	AF Median [ng/mL]	Det. Freq. (Subjects)	Gestation [weeks]	Collection Date	Other Matrices	Reference
Dimethylthiophosphate	0.43-0.43	0.43*	5% (20)	18 ±2.6	-	-	Bradman 2003
D-Xylitol	-	-	- (-)	16-17	-	-	Graca 2008
Ecgonine Methyl Ester	40-115	-	30% (20)	Birth	-	MU, IU, Me	Casanova 1994
	ND-11,879	-	16% (32)	-	-	UC	Winecker 1997
Enterolactone	11-34	17	1% (450)	13-39	1991	MS	Ripple 1992
Genistein	11.8-112	95.9	100% (21)	<20	2004	-	Engel 2006
	0.20-7.88	1.38	100% (21)	<20	2004	-	Engel 2006
α -HCH	0.5-4.86	0.94*	89.5% (57)	15-23	1999-2000	-	Foster 2002
	0.10-0.26	0.147*	14.6% (41)	15-23	-	-	Foster 2000
γ -HCH (Lindane)	ND-0.26	0.15*	8.3% (48)	15-23	1999-2000	-	Foster 2002
Σ HCH	-	0.003*	28% (100)	15-20	2006-2007	MS	Luzardo 2009
Hexachlorobenzene	-	0.017*	30% (100)	15-20	2006-2007	MS	Luzardo 2009
Monobutyl Phthalate (MBP) [†]	-	0.023	66% (100)	15-20	2006-2007	MS	Luzardo 2009
	ND-263.9	5.8	92.6% (54)	-	-	-	Silva 2004
	28.4-192.0	85.2	100% (64)	27.9 ±2.3	2005-2006	MU	Huang 2009
Mono- <i>n</i> -butyl Phthalate (MnBP)	NR-18.7	7.8	100% (11)	Birth	-	MU	Wittassek 2009
Monoisobutyl Phthalate (MiBP)	NR-35.7	4.2	100% (11)	Birth	-	MU	Wittassek 2009
Monobenzyl Phthalate (MBzP)	NR-2.8	1.9	100% (11)	Birth	-	MU	Wittassek 2009
Mono(carboxyisononyl) Phthalate (cx-MiNP)	NR-4.9	-	9% (11)	Birth	-	MU	Wittassek 2009
Mono[2-(carboxymethyl)hexyl] Phthalate (2cx-MMHP)	NR-0.92	0.64	100% (11)	Birth	-	MU	Wittassek 2009
Monoethyl Phthalate (MEP) [†]	ND-7.7	-	- (64)	27.9 ±2.3	2005-2006	MU	Huang 2009
Mono-(2-ethylhexyl) Phthalate (MEHP) [†]	ND-148.0	22.8	>90% (64)	27.9 ±2.3	2005-2006	MU	Huang 2009
	ND-9.0	<LOD	39% (54)	-	-	-	Silva 2004
Mono(2-ethylhexyl) Phthalate (MEHP)	NR-8.4	1.6	100% (11)	Birth	-	MU	Wittassek 2009
	ND-2.8	<LOD	24% (54)	-	-	-	Silva 2004
Mono(2-ethyl-5-hydroxyhexyl) Phthalate (5OH-MEHP)	ND-0.31	-	73% (11)	Birth	-	MU	Wittassek 2009
Mono(2-ethyl-5-carboxypentyl) Phthalate (5cx-MEPP)	NR-2.7	0.53	100% (11)	Birth	-	MU	Wittassek 2009
	ND-2.3	0.27	99% (300)	10-30	1980-1996	-	Jensen 2012
Mono(4-methyl-7-carboxyheptyl) Phthalate (7cx-MMeHP)	ND-0.91	0.07	96% (300)	10-30	1980-1996	-	Jensen 2012
1- or 2-Naphtol	0.61-4.19	0.72*	70% (20)	18 ±2.6	-	-	Bradman 2003
Σ Organochlorines (26)	-	0.027	67% (100)	15-20	2006-2007	MS	Luzardo 2009

Xenobiotic (summed analytes)	AF Range [ng/mL]	AF Median [ng/mL]	Det. freq. (Subjects)	Gestation [weeks]	Collection Date	Other Matrices	Reference
Paraxanthine	-	-	- (-)	16-17	-	-	Graca 2008
Σ PBDEs (21)	0.337-21.842	3.795*	100% (15)	T2	2009	-	Miller 2010
PCB-138	0.01	-	33.3% (6)	15-23	-	-	Foster 2010
Σ PCBs (7)	-	0.016*	7% (1000)	15-20	2006-2007	MS	Luzardo 2009
Pentachlorophenol	0.15-0.54	0.23*	15% (20)	18 ±2.6	-	-	Bradman 2003
Perfluorooctanesulfonic Acid (PFOS)	ND-4.5	1.1	98% (300)	10-30	1980-1996	-	Jensen 2012
<i>o</i> -Phenylphenol	0.10-0.17	0.13*	30% (20)	18 ±2.6	-	-	Bradman 2003
Sorbitol	-	-	- (-)	16-17	-	-	Graca 2008
Thiocyanate	ND-5860	89	90% (48)	15-20	-	-	Blount 2006

*Results skewed by cohort selection;

†Metabolites of commercially used phthalates; ND: Non-Detect; CB: Cord blood; Me: Meconium; MH: Maternal hair; MU: Maternal urine; MS: Maternal serum; IU: Infant urine

Table 2

Select Candidate Markers in Amniotic Fluid, Adapted from Vrachnis et al. (2012), Conde-Anguelo et al. (2011) and Buhimschi et al. (2013)

Marker	Outcomes
A. Inflammation-related markers Interleukins-1 β , -2, -4, -6, -8, -10, -18 IP-10	PTL, IAI, PTL w/out IAI PTB, MIAC
B. Extracellular matrix mediators Matrix-metalloprotease-3, -8 and 12 C-reactive protein, TIMP, ADAM-8 sTREM-1	PTB PTB IAI, PTB
C. Angiogenesis-related markers Angiogenin	PTL, PTB
D. Arachidonatelipoxygenase metabolites Prostaglandins/uterotonins	PTL
E. Immune- host defense markers Neutrophil defensin-1, 2; calgranulins A, B Human neutrophil peptide (HNP) 1-3 S100A12/ENRAGE	PTB IAI IAI
F. Cell adhesion molecules ICAM-1, VCAM-1	PTL
G. Others Urocortin-1 RANTES, MIP-1 α , MIP-1 β	PTB PTL

PTL= preterm labor

IAI= intra-amniotic inflammation

MIAC= microbial invasion of the amniotic cavity

ADAM-8 = A disintegrin and metalloprotease-8

TIMP = tissue inhibitor of metalloproteinases

S100A12/ENRAGE = a ligand for 'receptor for advanced glycation end products' (RAGE)

RANTES = chemokine (C-C motif) ligand 5

MIP= macrophage inflammatory protein

Table 3
Reference Values for Select Candidate Markers in Amniotic Fluid

Factor	Cutoff (ng/ml)	Author, Date	Event	Sensitivity (%)	Specificity (%)	Meas. of Assoc.	95% CId
IL-6*	24.7	Holst	MIAC	87	77	15.9 ^a	2.1-122.2
TNF-alpha	.62	2011				8.3 ^a	1.7-40.6
IL-6	.099	Thomakos	**PTB	89.6	80.3	11.4 ^b	4.8-27
TNF-alpha	.006	2010		81.3	79.2	6.2 ^b	3.3-11.9
MMP-8	5.14	Kim	**PTB	N/A	N/A	.637 ^c	.509-.776
IL-6	134.35	2013				.650 ^c	.517-.782
VEGF	24.99					.829 ^c	.722-.935
MMP-8	23	Yoon	**PTB	42	99	68.4 ^a	7.8-599
IL-6	.60	2001		42	92	7.9 ^a	2.5-25.3
Angiogenin	13.85			37	87	4.0 ^a	1.3-12.3
IL-6	1.74	Gervasi	**PTB 32	58	88	73 ^c	.6-.9
IP-10	.502	2012	**PTB>32	83	40	.64 ^c	.5-.7
MMP-8		Weiss 2007					
Uro cortin-1	.058	Karaer 2013	**PTB	81.8	40	.673 ^c	.55-.78
CRP	.110	Ghezzi 2002	**PTB <34	80.8	69.5	8.6 ^a	1.9-39.8
Cell death nucle.	NA	Puchner 2012	PTL	NA	NA	1.002 ^a	1.0-1.003
IP-10 + TNF-alpha	NA	Weissenbacher 2012	Intrauterine Inflammation	80	90	NA	NA
Angiogenin	.031	Spong 1997	**PTB 34	45.5	91	3.8 ^a	1.5-9.3
Angiogenin	.035	Madazli 2003	**PTB<37	100	91	.928 ^c	.852-1.00

* Multivariate model.
** Spontaneous.
Cutoff = limit to distinguish cases and controls.
MIAC= microbial invasion of the amniotic cavity.
VEGF= vascular endothelial growth factor.
^a Odds ratio.
^b Relative risk.
^c Area under curve.
^d 95% Confidence interval of measure of association.
NA= not available.

Exclusion criteria and adjustment for risk factors may differ by study, so values represented may not be directly comparable.