

## PRETERM BIRTH

# Prevention of preterm birth: Harnessing science to address the global epidemic

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Preterm birth is a leading cause of infant morbidity and mortality worldwide, but current interventions to prevent prematurity are largely ineffective. Preterm birth is increasingly recognized as an outcome that can result from a variety of pathological processes. Despite current research efforts, the mechanisms underlying these processes remain poorly understood and are influenced by a range of biological and environmental factors. Research with modern techniques is needed to understand the mechanisms responsible for preterm labor and birth and identify targets for diagnostic and therapeutic solutions. This review evaluates the state of reproductive science relevant to understanding the causes of preterm birth, identifies potential targets for prevention, and outlines challenges and opportunities for translating research findings into effective interventions.

## INTRODUCTION: WHY CARE?

Preterm birth (PTB), defined as birth before 37 weeks of completed gestation, is the most frequent cause of neonatal death and the second leading cause of under-five mortality worldwide. PTB accounts for an estimated 1 million neonatal deaths and affects about 1 in 10 pregnancies every year (1). Even in high-income settings, where sophisticated intensive care has improved survival of preterm infants, PTB remains the most frequent cause of infant mortality. The estimated 15 million infants who are born preterm each year face multiple short- and long-term health threats. As a result of the immaturity of multiple organ systems, preterm newborns are at increased risk of numerous neonatal complications, including intraventricular hemorrhage, necrotizing enterocolitis, and respiratory distress syndrome. Lack of full immunologic competence places preterm infants at increased risk for multiple infectious processes, including pneumonia, sepsis, and meningitis. The many long-term sequelae of prematurity include neurodevelopmental disorders and chronic lung disease (2). Preterm infants are also at increased risk of adult-onset chronic diseases, such as obesity, diabetes, and hypertension. Although infants born at the earliest gestational ages are at the greatest risk of severe complications, a growing body of evidence has found that even late preterm infants (34 to 37 weeks of gestation) have higher rates of morbidity and mortality compared with their term counterparts (3). Costs to the health care systems for care of preterm infants are substantial; in the United States alone, more than \$26 billion is spent annually on the delivery and continued care of preterm infants (4).

Despite the high global burden of PTB on childhood mortality, morbidity, and health care expenditure, few strategies are available to effectively detect women at risk and prevent preterm delivery. PTB can be iatrogenic or spontaneous. Iatrogenic, or medically indicated, PTBs are necessary when risks to the fetus or mother outweigh the benefit of continuing the pregnancy. These are often precipitated by clinical conditions

such as preeclampsia, diabetes, maternal medical conditions, and fetal problems (5). In industrialized countries, about one-third of infants born preterm are delivered by Cesarean section or induction of labor because of pregnancy complications, and these deliveries contribute to the growing incidence of late PTB. About 70% of PTBs, however, are spontaneous with or without premature rupture of membranes, and these births are the focus of this review. Uncovering the multifactorial processes and the interplay of risk factors that lead to spontaneous birth is necessary to identify effective strategies for preventing PTB, but the complex nature of pregnancy and parturition, governed by an intricate array of interrelated biological processes, creates challenges for researchers. In this article, we outline the current understanding of pregnancy, parturition, and spontaneous PTB and suggest a research agenda to elucidate the basic and translational science opportunities and barriers that need to be overcome to advance the field of PTB prevention. Because of the magnitude and complexity of the topic, not all key contributions could be referenced, and we have pointed readers to other reviews that focus more narrowly on specific topics within the PTB literature.

## CURRENT KNOWLEDGE AND THE SCIENCE NECESSARY TO MOVE FORWARD

### Risk factors

Multiple risk factors increase a woman's risk of PTB. In the United States, infants of non-Hispanic black women have PTB rates that are 40% greater than those of Hispanic and non-Hispanic white women, and this difference persists even after adjustment for maternal socioeconomic status and education (4). Increased risk of PTB is associated with extremes of maternal age (both young and old), short interpregnancy interval, multiple gestation, assisted reproductive technology, prior PTB, family history, substance abuse, cigarette use, low maternal socioeconomic status, late or no prenatal care, low maternal prepregnancy weight, bacterial vaginosis, periodontal disease, and poor pregnancy weight gain (4). However, the biological basis for many of these risk factors and the underlying mechanisms remain poorly understood. Although some of these risk factors are not modifiable, others represent potential targets for treatment and risk reduction. For example, although the mechanisms that increase the risk of PTB among smokers

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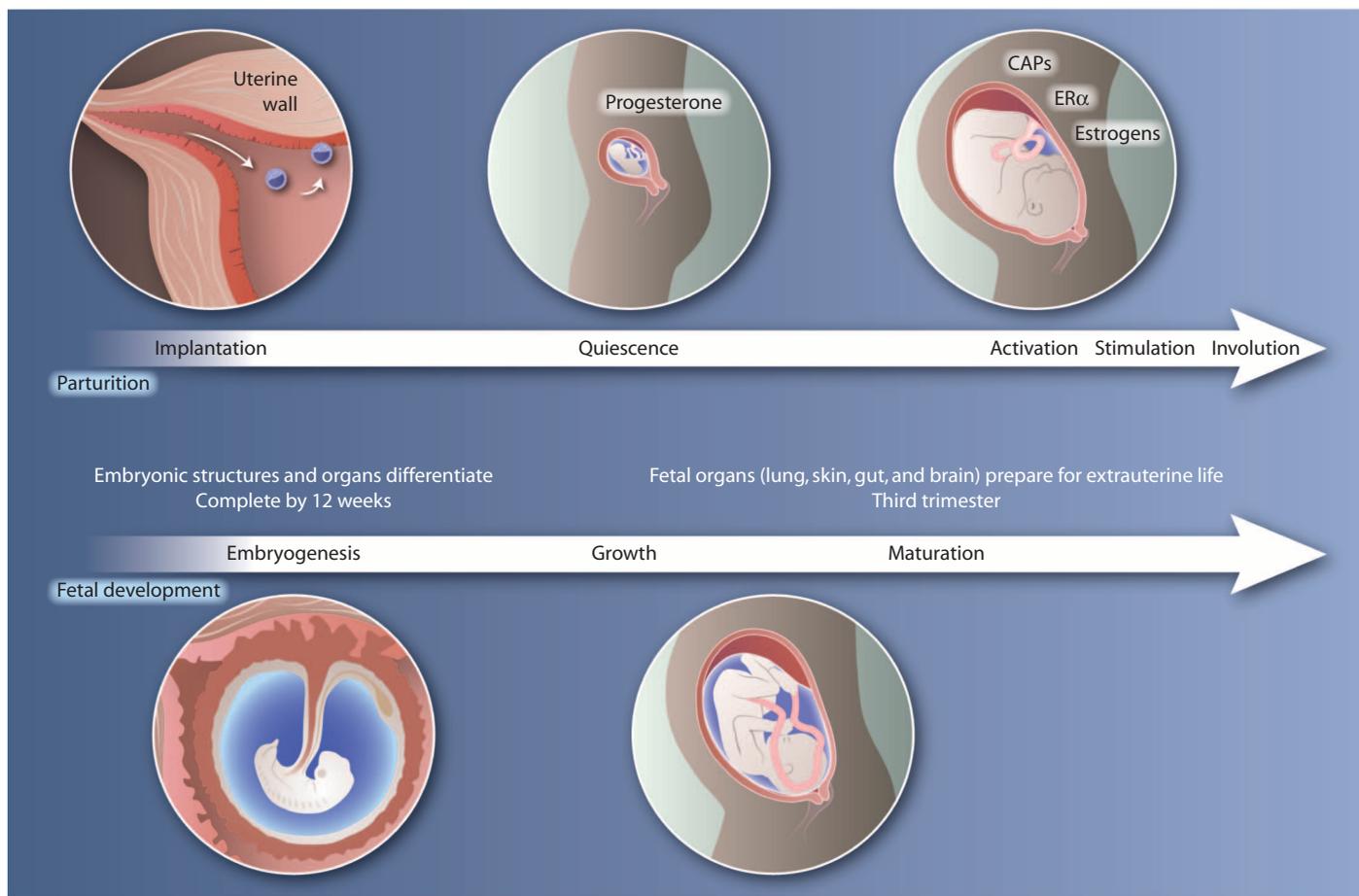
are not clear, smoking cessation interventions reduce this risk in developed countries (6). Unfortunately, clinical trials aimed at addressing other modifiable risk factors through treatment of bacterial vaginosis and periodontal disease, nutritional interventions, home uterine monitoring, bed rest, and home nursing care have failed to demonstrate efficacy. Overall, more information is needed to better understand the biology of pregnancy and how these risk factors contribute to PTB to develop effective strategies for early detection and prevention.

### Normal physiology of the contractile apparatus

Pregnancy and parturition involve a complex and progressive cascade of events beginning with implantation of the fertilized oocyte and proceeding through several well-characterized phases (Fig. 1) (7). These phases are (i) implantation, which is necessary to establish normal placentation and early development; (ii) uterine quiescence, during which rapid growth of the uterus occurs, accompanied by fetal growth; (iii) activation, which includes cellular and biochemical events that promote uterine contractility; (iv) stimulation, characterized by uterine contractility, cervical ripening, and labor; and (v) involution, whereby the uterus returns to its nonpregnant state. Less than 0.5% of pregnancy is spent in stimulation, or labor. Increased circulating levels of progesterone ( $P_4$ ) are primarily responsible for maintaining uterine quiescence.

Progesterone suppresses the expression of proinflammatory cytokines and contraction-associated proteins (CAPs), including oxytocin receptors and connexin-43. Its nongenomic and genomic actions are mediated primarily through the nuclear progesterone receptor B (PR-B). During activation, expression of a truncated nuclear PR isoform, PR-A, which has only minimal transcriptional activity compared to PR-B, increases, creating a functional progesterone withdrawal characteristic of mammalian parturition (8). Progesterone function is further reduced by increased expression of CAPs, estrogens, and estrogen receptor- $\alpha$  (9). Thus, the transition from quiescence to activation is characterized by a functional progesterone withdrawal and increased CAPs and estrogen effects, resulting in a proinflammatory milieu associated with uterine contractions. Recent evidence suggests that these changes may be mediated by microRNAs (miRNAs), as discussed below.

A critical principle is that spontaneous PTB is a complex final outcome resulting from a wide variety of pathological processes that are initiated by specific molecular pathways (4, 10). Many risk factors; biological, environmental, behavioral, and social influences; and interactions between maternal and fetal factors impinge on PTB. Historically, PTB was often defined as a single endpoint, regardless of etiology. The lack of understanding of the mechanism of PTB and parturition and the absence of more precise, etiology-based classifications have



**Fig. 1. Key phases of parturition and fetal development.** The phases of parturition, from implantation through involution in conjunction with key time points of fetal development, are shown. Research into the mechanisms

responsible for maintaining uterine quiescence and initiating activation of labor is critical for elucidating the pathways to preterm labor and designing efficacious interventions to prevent PTBs.

resulted in failure to effectively detect and treat abnormal processes and to apply successful treatments or interventions.

The major known pathways underlying parturition are described below and in Fig. 2. Regardless of how the process is initiated, these pathways ultimately lead to activation of fetal and maternal tissues, resulting in the release of mediators such as prostaglandins and interleukins that stimulate myometrial contractions and/or rupture of the fetal membranes.

### Established pathways implicated in term and preterm parturition

**Genetics.** A history of a prior spontaneous PTB increases the risk for subsequent PTBs in the same mother (11). Moreover, epidemiological analyses of large, population-based cohorts have revealed increased risk of PTB if the mother herself was born preterm or has sisters that have had preterm children (12). These observations point to the existence of genetic contributors to PTB through the maternal lineage. More formal genetic studies in offspring of twins and segregation analysis of traits in families consistently demonstrate that 30 to 40% of the variation in birth timing is due to genetic factors (13).

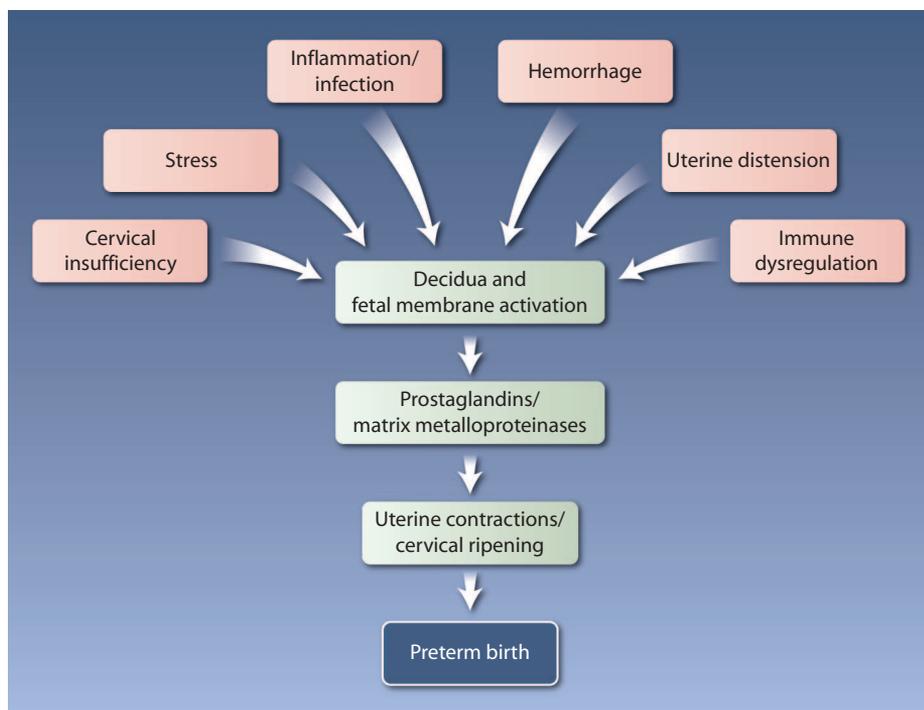
In designing genome-wide association studies to look for common variants, or genome or exome sequencing studies for detection of rare variants, a conundrum emerges: Which genome, maternal or fetal, harbors the variants in question? Analyses of birth timing of the offspring of twins suggest little or no contribution from the paternal genome. Consistent with this finding, segregation analysis shows that maternal genome effects in both the mother and the fetus are much more prominent than paternal effects (13). A comparison of the offspring of twins, full siblings, and half siblings estimated that 13% of the variation in gestational

age at delivery was explained by fetal genetic factors, and 21% was accounted for by maternal genetic factors (14). As reviewed recently, human candidate gene studies in both maternal and fetal genomes have been inconsistent in demonstrating associations across populations, although the most robust evidence suggests a role for interleukin-6 (IL-6) in the maternal genome and for coagulation protein Factor V in both maternal and infant genomes (15, 16). Genome-wide association studies have not revealed statistically significant variants in either the maternal or fetal genomes to date. However, the total number of subjects who underwent both genotyping and detailed pregnancy phenotyping remains relatively modest, fewer than about 10,000.

Two studies that used linkage analysis of Finnish families with multiple PTBs report an association of PTB with chromosomal loci harboring the genes for the insulin-like growth factor 1 receptor (*IGF1R*) and androgen receptor (*AR*) in the fetal genome. These loci were further evaluated in nuclear families from Finland, where similar associations with common variants were found (17, 18). More recently, families with highly penetrant spontaneous PTB phenotypes from Finland were subjected to whole-exome sequencing analysis and found to have an over-representation of rare variants in genes in the complement and coagulation cascade pathways (19). An extension of this analysis to nuclear families in Finland demonstrated an association of the complement receptor 1 (*CR1*) locus with PTB risk (19). The continued acquisition of well-phenotyped families for genome-wide studies to identify associations with common gene variants and enable sequencing for rare variants holds substantial promise for revealing fundamental mechanistic pathways in PTB.

**Epigenetics.** The strong familial aggregation of PTB risk, together with the limited success in identifying robust contributions of specific genetic variants, has stimulated investigation into potential epigenetic modifications of gene expression as an alternative explanation for these findings. Indeed, fetal and infant epigenetic programming, associated with impaired or excessive early life growth, leads to long-lasting alterations in physiology and metabolism in adulthood, as determined by epidemiological investigations by Godfrey and Barker (20). Transgenerational increases in the frequency of PTB and low birth weight in African American women subjected to racial discrimination, poverty, and other environmental stressors further support the notion of epigenetic programming of fetal growth and pregnancy outcomes (21, 22).

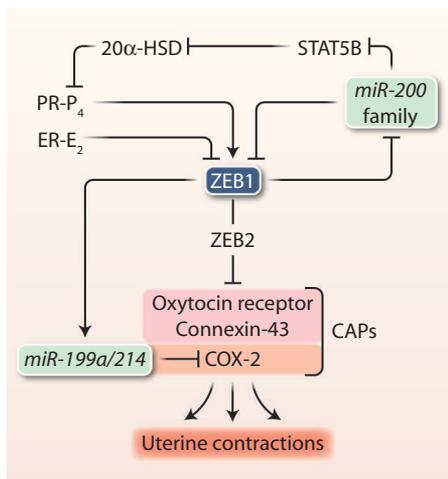
Several recent studies have provided molecular evidence for epigenetic effects related to PTB. For example, when researchers analyzed myometrial samples from pregnant women, they found that DNA methylation in regions flanking a subset of contraction-associated genes differed depending on whether the specimen was obtained at term or preterm, and with or without spontaneous labor (23). Analysis of DNA methylation of the prostaglandin E receptor 2 gene (*PTGER2*) and long-interspersed



**Fig. 2. Pathways to PTB.** The pathways to preterm labor and PTB are multifactorial and complex. Multiple molecular mechanisms are influenced by a variety of risk factors, including genetic, epigenetic, biological, behavioral, social, clinical, and environmental influences.

nuclear element 1 *Homo sapiens*-specific (*LINE-1-HS*) sequences from cervical swab samples, in the context of maternal cigarette smoke exposure and education, demonstrated changes in methylation that predicted changes in gestation length in a small cohort of Mexican women (24). Further, general *LINE-1* element methylation was measured in blood from cohorts of women during the first and second trimesters and cord blood from their infants at birth. After adjustment for maternal demographic factors, women in the highest quartile for first trimester *LINE-1* methylation had longer gestations [0.45 weeks (95% confidence interval, 0.12, 0.78)] and lower odds of PTB [odds ratio, 0.40 (0.17, 0.94)] compared to those in the lowest quartile (25). In contrast, the associations with cord blood *LINE-1* methylation were in the opposite direction. Analysis of DNA extracted from the cord blood of infants experiencing spontaneous preterm labor, preterm premature rupture of membranes, or medically indicated PTB found no differences in the methylation of nine differentially methylated regions that regulate imprinted loci (26).

**miRNA-based pathways.** miRNAs contribute to inhibition of mRNA translation into protein and to transcript degradation. Although miRNAs commonly buttress the homeostatic resilience of complex gene regulatory networks, they can also play key roles in single-gene pathways (27). The most extensive, mechanistic analysis of miRNAs in modulating parturition centered on the opposing roles of the *miR-200* and *miR-199a/214* clusters in PTB pathways in the mouse and the human (Fig. 3). This research uncovered a double-negative feedback loop between miR-200 members and the zinc finger E-box-binding homeobox (ZEB) proteins ZEB1 and ZEB2. This feedback loop, originally implicated in epithelial-to-mesenchymal transition (28), fine-tunes the contractile signals. ZEB1 is highly expressed in the myometrium under the influence of the ligand-activated PR. ZEB1 up-regulates ZEB2, and both proteins reduce the expression of CAPs and decrease uterine contractions (29). Near term, increased *miR-200* expression silences ZEB1



**Fig. 3. Uterine miRNA-based pathways that regulate myometrial CAPs.** The double-negative feedback loop between *miR-200* members and ZEB proteins, emphasizing the central role of ZEB1 in mediating the opposing effects of the *miR-200* and *miR-199a/214* clusters on the contractile apparatus (29–32). 20 $\alpha$ -HSD, 20 $\alpha$ -hydroxysteroid dehydrogenase; STAT5B, signal transducer and activator of transcription 5B; PR, progesterone receptor; P<sub>4</sub>, progesterone; ER, estrogen receptor; E<sub>2</sub>, estrogen; ZEB, zinc finger E-box-binding homeobox; COX-2, cyclooxygenase-2; CAPs, contraction-associated proteins.

and ZEB2, resulting in further up-regulation of *miR-200* members. Furthermore, *miR-200* members also silence signal transducer and activator of transcription 5B (STAT5B), which is known to repress 20 $\alpha$ -hydroxysteroid dehydrogenase (20 $\alpha$ -HSD), a progesterone-metabolizing enzyme (30). This leads to increased breakdown of progesterone (P<sub>4</sub>) to inactive products, reduced local concentration of progesterone, lower PR-dependent up-regulation of ZEB1/2, and enhanced expression of CAPs. Members of the *miR-199a/214* cluster are positively regulated by ZEB1 and are down-regulated in the uterus during parturition, allowing the up-regulation of their target, cyclooxygenase-2 (COX-2) (31). Similar changes in these miRNAs occur with the induction of preterm labor in mice by the PR antagonist RU486 or lipopolysaccharide (LPS), and in term myometrium of women in labor (32). Together, these data shed light on the opposing roles of miRNA clusters in the transition from uterine quiescence to a contractile phenotype and imply a central role for ZEB proteins in this process.

The expression of other miRNAs in the placenta, chorioamniotic membranes, or cervix has also been associated with increased risk of PTB (33, 34). The targets and mechanism of action of these miRNAs remain largely unknown.

**Hypothalamic-pituitary-adrenal axis, stress, and stress hormones.** Multiple studies link psychological and other environmental stressors with PTB (11). Black race, poverty, depression, emotional stress, and psychiatric disorders are all associated with an increased risk for PTB. The endocrine responses to psychological (requiring cognition) and physiological (perceived independently of cognition, such as hypotension or hypoglycemia) stress are mediated through the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the generation and secretion of cortisol from the adrenals. In the hypothalamus and its paraventricular nucleus, corticotropin-releasing hormone (CRH) is a primary driver of pituitary adrenocorticotropic hormone release, which subsequently stimulates adrenal glucocorticoid secretion.

The role of the HPA axis and stress hormones in humans was evaluated in the 1980s after the recognition that the human placenta produces increasing amounts of CRH as gestation progresses, in a pattern of CRH production shared only by other simians. Whether CRH itself directly promotes the onset of parturition remains unclear. Unlike its effects in the hypothalamus, cortisol stimulates, rather than inhibits, CRH production and secretion in the placenta (35). The consequence of this feed-forward regulation may be enhanced placental CRH expression in the presence of maternal stress as a consequence of HPA axis activation. Placental CRH would then have the capacity to further augment its own expression by acting on the maternal (and fetal) pituitary. This feed-forward regulation of CRH in the placenta is not the only atypical response of uterine tissues to glucocorticoids. Normally, cortisol and other glucocorticoids are potently anti-inflammatory. One target for suppression of myometrial activity is COX-2 (PTGS2), an isoform of a prostaglandin synthesis enzyme at the first committed step of the pathway. In the amnion, glucocorticoids stimulate PTGS2 synthesis and activity, increasing production of proinflammatory prostaglandins, particularly prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which stimulates myometrial contractions and promotes parturition (36). Thus, plausible molecular mechanisms can explain how stress could lead to spontaneous PTB, and these merit further intensive investigation.

**Infection/inflammation.** Inflammation, whether or not related to infection, is one of the best-studied pathways in humans and animal models of PTB. Infection and inflammation are particularly prevalent in early spontaneous PTB: chorioamnionitis is implicated in >85% of

deliveries occurring before 28 weeks of gestation (10, 37). The consequences of intrauterine infection include uterine contractions, cervical dilatation, and weakening of the chorioamniotic membrane.

Infection commonly ascends from the vagina and cervix into the choriodecidual space, affecting the myometrium, fetal membranes, and amniotic fluid. Infection may infrequently enter the uterus by the hematogenous route, through retrograde seeding via the fallopian tube, or during intrauterine procedures such as amniocentesis (38). The initiating infection may be clinical or subclinical, monomicrobial or polymicrobial, and may require high-throughput, metagenomic tools for identification of common and unusual pathogens (39–43). The most common bacteria associated with PTB are *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Streptococcus agalactiae*, *Escherichia coli*, *Fusobacterium* species, and *Gardnerella vaginalis* (44). The bacteria release lipoglycans (such as LPS), peptidoglycans, and related cell membrane products that can trigger the inflammatory response and preterm labor (44). Viruses are not commonly implicated in spontaneous PTB, but they may reduce innate maternal barriers to ascending bacterial infection, predisposing the intrauterine environment to bacterial infections and subsequent PTB (45).

Bacterial products interact with the receptors for pathogen-associated molecular patterns, such as Toll-like receptors (TLRs; mainly TLR-2 and -4) and NOD (nucleotide-binding oligomerization domain)-like receptors (NLRs), leading to tumor necrosis factor- $\alpha$  (TNF $\alpha$ )-mediated release of cytokines (such as IL-1 and IL-6) and chemokines (such as IL-8 and RANTES), the production of other proinflammatory mediators, and apoptosis (46). These cytokines stimulate uterine contractions through the production of prostaglandins, primarily by the amnion and decidua, and through stimulation of fetal or maternal neutrophils (47, 48). Similar signaling cascades may be activated by noninfectious inflammation, triggered by damage-associated molecular patterns (DAMPs), which activate signals such as the receptor for advanced glycation end products (RAGE) and resulting in the production of parturition-triggering cytokines (49). Inflammatory mediators also activate complement, which contributes to cervical remodeling and PTB (50). These diverse pathways converge on a set of well-established labor-inducing inflammatory mediators, such as prostaglandins. The role of these mediators in PTB has been established by association with PTB in humans, their ability to trigger PTB in animal models, and evidence of inhibitor-mediated attenuation of labor-promoting signals in vivo and in vitro (44, 51). Systemic maternal inflammatory response, as in sepsis, may also stimulate the innate immune response and uterine contractions through activation of granulocytes and monocytes (52). These pathways may, in turn, be inhibited by anti-inflammatory immunomodulators such as IL-10 or lipoxins within different uterine tissues, resulting in attenuation of contractile signals. Thus, the ultimate contractile phenotype is a balance between opposing proinflammatory and anti-inflammatory pathways (53, 54).

**Uterine stretch.** During pregnancy, an increase in uterine intraluminal pressure, coupled with structural and biochemical myometrial remodeling, results in increased tissue stretch. Whereas the intrauterine pressure in a normal pregnancy remains fairly stable (55), spatial differences in tissue remodeling, as well as temporal changes in the balance between uterotonics, which increase the tonicity of the uterus, and smooth muscle relaxants can trigger a cascade of second messengers such as kinases and transcription factors [MAP(mitogen-activated protein) kinase, AP-1 (activating protein 1), or C/EBP $\beta$  (CCAAT/enhancer binding protein  $\beta$ )], culminating in activation of CAPs (56, 57). More-

over, uterine stretch is associated with altered patterns of uterine activity and cervical dilatation and a higher incidence of premature rupture of the chorioamnion, as seen in pregnancies involving multifetal gestation or an excessive amount of amniotic fluid (polyhydramnios) (58).

**Decidual hemorrhage.** Uterine contractions and PTB can be instigated by bleeding between the chorion and decidua at the placental bed, causing overt or subclinical separation of the placenta from the implantation site at the uterine wall, clinically known as abruption (59). Vaginal bleeding, even as early as the first trimester, or the presence of a subchorionic hematoma, is associated with a higher incidence of PTB (60). The plasma protease thrombin plays a central role in the signaling cascade that is initiated by blood in the subchorionic space. Uterine contractions can be initiated by direct intrauterine injection of blood in animal models (61). These contractions can be attenuated by hirudin, a thrombin inhibitor (61), whereas intrauterine injection of thrombin into pregnant mice causes PTB (62). Thrombin up-regulates COX2 and PGE<sub>2</sub> production in the amnion (63), and it may also enhance cervical softening by activating matrix metalloproteinases (MMPs), which break down the cervical extracellular matrix. Thrombin also promotes membrane rupture by activating MMPs or protease-activated receptors in the amnion (64–66). Activation of the coagulation cascade that releases thrombin and other proteases may be initiated by decidual tissue factor, which can be released by infection, inflammation, hypoxia, or oxidative stress (67). In addition to thrombin, other proteases, such as elastase, may be involved in contraction signals, releasing fibronectin and promoting the breakdown of the chorion-decidual fusion (68).

**Preterm premature rupture of the membranes.** Rupture of the fetal membranes before 37 weeks of gestation and before the onset of labor complicates 1 to 2% of pregnancies, but is associated with 25 to 40% of PTBs in developed countries (11). Preterm premature rupture of the membranes (PPROM) often represents a common final pathway to PTB and is associated with infection/inflammation, cigarette smoking, uterine overdistension, decidual hemorrhage, and genetic predisposition (69). The fetal membranes consist of two distinct entities: the inner amnion, adjacent to the amniotic cavity, and the outer chorion, adjacent to the maternal decidua. The chorion contributes to strength and elasticity of the membranes, but to a lesser degree than the amnion. Regardless of initiating pathway, MMPs, proinflammatory cytokines, apoptosis, and oxidative stress are primary factors leading to loss of the extracellular matrix and collagen, reduced tensile strength of the membranes, and PPRM. Extracellular collagen is degraded by a wide range of MMPs, and MMPs can degrade many types of extracellular collagen, cleave cell surface receptors, release apoptosis-stimulating ligands (such as FAS ligand), and activate cytokines and chemokines. Apoptosis of the fetal membranes, mediated either by the TNF $\alpha$ /FAS pathway or by the p53/Bax pathway, is also associated with PPRM. Studies consistently demonstrate higher rates of apoptosis in the membranes from women with PPRM when compared to women with preterm labor and intact membranes (70, 71). Direct exposure to cigarette smoke also increases apoptosis and oxidative stress in the fetal membranes (72). Oxidative stress, which generates reactive oxygen species, is another established pathway to apoptosis and PPRM, causing cleavage of extracellular collagen, induction of MMP-9, DNA degradation, and initiation of lipid peroxidation (73). One recent study also found a correlation between infection and the down-regulation of cytokeratin genes, which are responsible for tensile strength of the

membranes (74). Finally, PPRM has been associated with genetic disorders of collagen structure, such as Ehlers-Danlos syndrome (75), and with a variety of single-nucleotide polymorphisms (76).

**Cervical insufficiency.** Although cervical dilatation may occur passively in response to contraction of the uterine corpus, active biochemical and biomechanical processes remodel the uterine cervix during pregnancy and promote cervical softening, effacement, and consequently dilatation. These processes, which take place throughout pregnancy, may be accelerated before term, predisposing to PTB or rupture of the fetal membranes (77). Cervical smooth muscle constitutes only ~10% of the cervical tissues, which means that the cervical fibrous tissue and extracellular matrix, composed of collagen (predominantly type I and type III), elastin, decorin, and viscous macromolecules (glycosaminoglycans, proteoglycans, and hyaluronic acid), are key to dynamically maintaining cervical architecture (78). The process of cervical remodeling is modulated by hormones (79) as well as inflammatory regulators. Among them, prostaglandins are central and are used clinically to stimulate cervical softening (79). Many of the biochemical changes that take place physiologically within the cervix may be different between term or preterm tissues (80). Nonetheless, the final common pathway triggered by inflammatory mediators is degradation of collagen fibers by MMPs (mainly MMP-2 and -9), with changes in collagen organization, decreased fiber density, and cervical ripening (81). How extracellular tissue remodeling within the cervix interacts with the cervical smooth muscle and fibroblasts to influence tissue architecture remains unknown.

Another regulator of the uterine cervix, relaxin, has also been implicated in PTB. The serum concentration of relaxin, which is produced by the corpus luteum early in pregnancy and later by the placenta and decidua, is highest in the first trimester of pregnancy and then maintained at a lower level throughout pregnancy (82, 83). Relaxin receptors are expressed in the human cervix, and increased relaxin expression has been associated with softening of the cervix, possibly acting through up-regulation of MMP activity (82, 83). Other hormones also directly modulate cervical ripening in pregnancy. For example, deficiency in steroid 5 $\alpha$ -reductase type 1 results in inadequate cervical ripening despite regular uterine contractions, and this is attributed to local accumulation of cervical progesterone as a consequence of deficient enzymatic activity (84). Hence, cervical changes predisposing to preterm labor and birth may be the result of passive or active physiologic pathways, which are still poorly understood.

### Emerging disease pathways

**The placenta and PTB.** How the placenta contributes to the regulation of uterine contractions and PTB in humans remains largely unknown. Although the placenta actively determines the timing of parturition in certain animal models, such as sheep (85), it remains unclear whether this applies to human parturition. Several processes in other intrauterine tissues that are associated with preterm labor, such as infection and inflammation, may also affect the placenta. Yet, the role of the placenta in initiating contractile signals is uncertain. The placental microbiome, although of low abundance and mainly consisting of nonpathogenic commensal species, is distinct in women with preterm compared to term deliveries (86), but how placental bacteria participate in the parturition process remains undefined. The human placenta also expresses CRH, as well as relaxin and other stress-related hormones. As discussed above, human placental CRH may initiate parturition through its stimulation of fetal adrenal ste-

roidogenesis, which promotes estrogen synthesis. CRH increases estrogen synthesis by stimulating production of 19-carbon precursors in the fetal adrenals, which are then aromatized to estrogens within the placenta (35). Some nonhuman primates exhibit CRH expression patterns that are similar to humans, and so new genetic engineering technologies may shed light on the role of placental CRH in nonhuman primates and consequently humans. In addition, clock genes (*Bmal1*, *Cry1*, *Per1*, *Clock*, *Cry2*, and *Per2*) are expressed in the murine placenta during pregnancy, with some exhibiting up to 2.5-fold increase and rhythmic expression patterns shortly before labor (87). Whether clock genes are involved in human parturition is unknown. Animal models such as mice and rats may be inadequate to address many of the biological questions regarding human pregnancy; more relevant gestational animal models, such as nonhuman primates, need to be identified.

**Decidual senescence.** Hirota and colleagues (88) postulated that impaired decidual health, starting early in pregnancy at the time of implantation, would result in a cascade of effects that would ultimately result in PTB. Indeed, mice with accelerated decidual senescence, initiated by gene disruption of tumor protein 53 (TP53) in the uterus, exhibit spontaneous PTB. This PTB occurs in spite of high maternal serum progesterone concentrations, analogous to human pregnancy but unlike several other mouse models of PTB. Further, mice with a uterine-specific TP53 conditional knockout exhibit enhanced sensitivity to environmental triggers of PTB (89). Decidual senescence has also been identified in human PTB, suggesting that senescence pathways such as mTORC1 (mammalian target of rapamycin complex 1) may be promising targets for intervention (89).

**Allograft rejection.** The ability of the maternal immune system to tolerate the antigenically foreign fetus (harboring both maternal and paternal antigens) has been a long-standing conundrum. The notion that breakdown of maternal tolerance of the fetus contributes to adverse pregnancy outcomes, including PTB, is a mechanistically attractive one that has recently garnered experimental support. Several experimental strategies have revealed that immune tolerance contributes to pregnancy maintenance. In pregnancies of antigenically engineered transgenic mice carrying specific fetal antigens not expressed by the mother, inflammation leads to accumulation of effector T cells that recognize fetal antigens in the myometrium and undecidualized components of the endometrium (90). These T cells do not, however, penetrate into the decidua and fetus. Epigenetic silencing of CXCR3 ligands such as CXCL9 was implicated in impairing T cell accumulation. Thus, one component of preventing allograft rejection is limiting the production of chemokines that enhance maternal T cell rejection of the fetus and preventing T cell accumulation at the maternal-fetal boundary.

In addition to the inhibition of inflammation at the maternal-fetal interface, the interactions of immune receptor–ligand pairs also determine the success or failure of pregnancy maintenance. For example, fetal trophoblast cells are in close approximation to maternal uterine natural killer cells during implantation and development, and they participate in intercellular crosstalk. Essential components of this crosstalk include killer cell immunoglobulin-like receptor (KIR) haplotype A (AA alleles) and fetal human leukocyte antigen–C (HLA-C) genotype. Interaction of this KIR with its specific ligands determines pregnancy outcomes, with deleterious results arising with certain paternal HLA-C2 alleles (91).

Beyond these innate immune and maternal-fetal interaction genotypes, pioneering studies by Rowe and colleagues have elucidated the essential functions of maternal regulatory T cells in pregnancy

maintenance. Pregnancy promotes the appearance of FOXP3<sup>+</sup> CD4 cells with fetal specificity (92). Expansion of fetus-specific regulatory T cells during a second pregnancy in a given mother is possible because of fetal-specific FOXP3<sup>+</sup> cells surviving from the prior pregnancy. Pregnancy-established FOXP3<sup>+</sup> CD4 cells thus provide protective regulatory memory to fetal antigen, and therefore may lead to improved outcomes in pregnancy stemming from a mother-father pair if the first pregnancy was successful.

### Summary and research needs

Further research is necessary to elucidate the complex pathways leading to PTB and to identify targets for interventions. It has been hypothesized that both term and preterm labor share a common pathway and that pathological stimuli of parturition, as described above, may act in concert with the normal physiological preparation for labor, especially after 32 weeks of gestation. Before 32 weeks of gestation, a greater pathological stimulus may be required to initiate labor. One fundamental difference between spontaneous parturition at term and preterm labor is that term labor results from physiological activation of all components of the common pathway, whereas preterm labor arises from pathological processes that activate one or more of the components. In 2005, Green and colleagues (93) proposed specific scientific areas of investigation to better understand PTB. Any comprehensive basic science agenda aimed at preventing PTB should address the following general questions:

- 1) What are the mechanisms that maintain uterine quiescence for greater than 95% of the total length of gestation?
- 2) What mediators are responsible for activation, and are these mediators potential targets for intervention?
- 3) What is the basis for the disparities in gestational length and risks of PTB among racial, ethnic, and socioeconomic groups, identified through the assessment of multiple social, biological, clinical, and environmental factors?
- 4) Do such differences have a modifiable biological basis, or can they be accounted for by environmental factors?
- 5) Is it possible to identify biomarkers to more accurately assess fetal maturation and the risk of PTB?

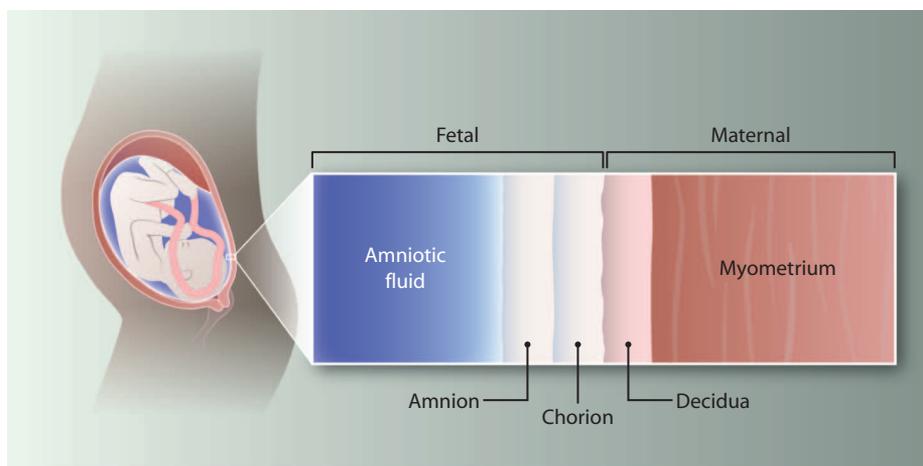
To gain insights into actionable strategies to minimize PTB risk, several obstacles must be overcome to reveal the normal physiological mechanisms for birth timing and the initiators of preterm labor and delivery. What remains problematic is that parsing PTB into specific subtypes, each reflecting a homogeneous molecular mechanism, is largely not possible at the present time. For example, one broad characteristic that could be used to divide categories of spontaneous PTB is spontaneous preterm labor versus PPROM. However, even here uncertainty exists as to whether these categories reflect different components of a common activation mechanism or distinct initiating mechanisms. Identification of biomarkers to group PTB into more homogeneous subtypes would be a substantial advance. A systems biology approach, using

sophisticated analytic methodologies, is needed to evaluate the multiple, complex, and interrelated processes regulating pregnancy, labor, and parturition.

Environmental risk factors have been strongly associated with PTB, but these only result in PTB in a minority of women exposed. It is not clear what determines this difference in susceptibility, with possible culprits being genetics (or epigenetics), background (gene-environment interactions), interactions among multiple exposures, and duration or magnitude of the exposure. Likely all these factors contribute, making the mechanisms affecting any one pregnancy challenging to define and difficult to generalize. The interval between the inciting event and the time of the preterm delivery may also vary widely depending on the causal mechanism (for example, compromised decidualization early in pregnancy may lead to later placental or uterine dysfunction). Without better objective diagnostic measures, generating more uniform cohorts of women for investigation will remain problematic.

One strategy for controlling for such complexity in other disease processes has been the creation of suitable animal models in which a known exposure, such as infection or inflammation, could be systematically introduced with the goal of interrogating the tissues and pathways involved. Unfortunately, the divergence in the endocrinology and other general characteristics (number of fetuses, ovarian versus placental steroid production, uterine morphology, etc.) of pregnancy between humans and other species has made it difficult to extrapolate mechanisms revealed by the usual model systems (rodents, ruminants, and nonhuman primates). Moreover, spontaneous idiopathic PTB is very uncommon in species other than humans. Comparative genomic strategies combined with detailed pregnancy phenotyping may turn this past limitation into a new opportunity.

Investigation of mechanisms underlying the physiology of term and preterm parturition commonly centers on discrete tissue types within the intrauterine cavity. Recent information on intercellular paracrine and endocrine communication signals, including steroid and glycoprotein hormones, as well as RNA molecules, suggests that communication among relevant tissues (Fig. 4) may be critical in initiation



**Fig. 4. Tissue types of the uterine wall.** The maternal and fetal tissue types of the uterine wall outside the placental implantation site. The myometrium and decidua are maternal tissues, whereas the chorion, amnion, and amniotic fluid are of fetal origin. Note that the maternal decidua is in direct contact with the fetal chorion. These tissues may mediate cooperative or divergent signals that lead to preterm or term parturition.

and propagation of the contractile signals. Moreover, many of the processes that lead to uterine contractions and delivery (such as inflammation, weakening, and rupture of the membranes, and cervical remodeling) are shared between physiological term birth and pathological PTB, with the only apparent difference being the timing of these changes. In contrast, infection, excessive stretch, and hemorrhage typically characterize PTB, although these can also occur at term. Investigation of intercellular communication and signal integration is needed to decipher how nonsynchronous pathological signals within one uterine tissue may be accommodated and suppressed without generalized preterm labor, and how some signals propagate into a generalized, synchronous multi-tissue cascade that leads to term or preterm parturition.

## TRANSLATING KNOWLEDGE INTO CLINICAL APPLICATION

The gaps in knowledge about the basic biology of both term and preterm pregnancies, including what constitutes normal gestational length in any given population, leave clinicians with few tools to prevent PTB. This is the fundamental reason that most prematurity intervention efforts are actually aimed at care of the woman in preterm labor and care of the preterm neonate, rather than prolonging gestation or stopping labor. Perhaps the most promising preventative treatment for PTB is the use of progesterone therapy in women with short cervix. A recent meta-analysis suggests that progesterone treatment of women with a short cervix may reduce the risk of PTB by 40% (94). However, progesterone has not been tested in developing countries and only addresses one of the many pathways leading to preterm delivery. Even if delivery of current preventative interventions were scaled up to reach high and equitable population coverage, the PTB rate would only be lowered by an estimated 0.5% in high-income countries (95). How these prevention efforts would work in low- and middle-income countries is unknown, highlighting the dearth of knowledge surrounding one of the greatest contributors to childhood mortality. Ultimately, reduction of the global PTB rate will require identification of populations at risk for spontaneous PTB and development of interventions to prevent PTB. The following sections describe the factors that need to be considered and potential scientific approaches to set the stage for translational development focused on prevention of PTB.

### Translational development priority areas

Advances in translational research for preventing PTB will not be actualized until a concerted effort is made to uncover the mechanisms of term and preterm pregnancies by scientists and funders. Paramount to the effort are a cohesive research agenda, cooperative research infrastructure, coordinated multidonor funding commitments, and a complementary set of tools, including standardized phenotypes and outcome measures, access to global research cohorts and biospecimens, and robust analytic tools for big data and systems biology research. Multiple systems and computational biology platforms will be required to analyze data and samples collected during pregnancy, from conception through labor and delivery. This approach requires longitudinal data and biological samples collected over the course of gestation in well-defined and characterized populations from different geographic regions. If possible, the studies should be standardized to allow population-specific and cross-population comparisons.

Two main areas warrant particular emphasis for addressing the prevention of PTB. The first is the development of clinical diagnostic

tools for identifying women at risk of PTB. Stratification would include a comprehensive and integrated assessment of risk, including demographics (age, race, and ethnicity), social and behavioral factors, environmental exposures, nutritional status, and comorbidities. The second is the development of effective interventions, targeted at the specific underlying mechanistic pathways, to modify potential risk. Ideally, we would be able to risk-stratify and treat pregnant women early in gestation and before the onset of threatened labor because onset of labor indicates the initiation of an irreversible cascade of events, most likely programmed earlier in gestation.

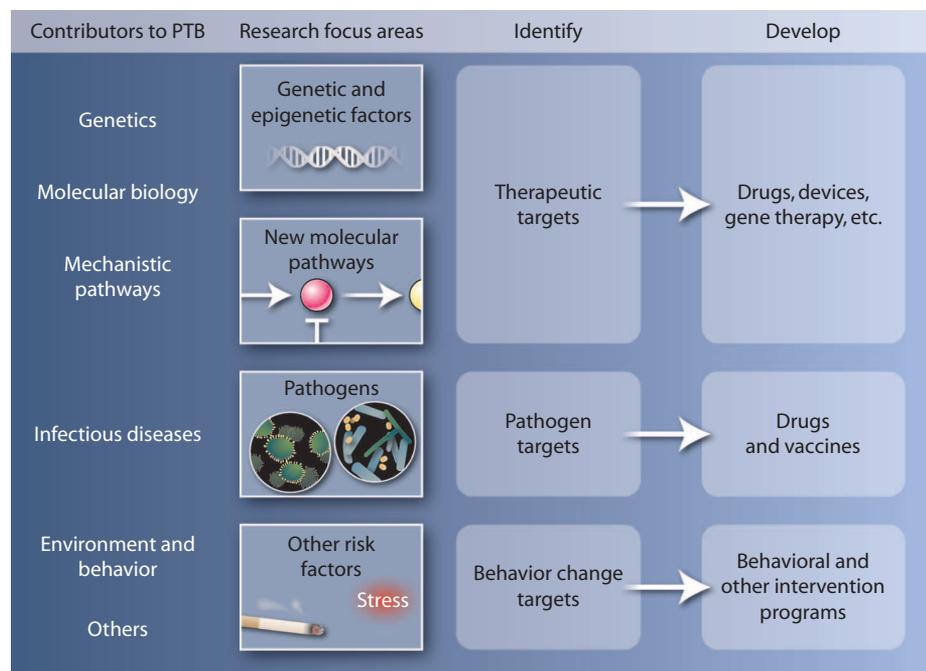
Modest efforts are currently under way to find biomarkers that can identify women at risk of spontaneous PTB with better diagnostic profiles than the currently used fetal fibronectin test (96–98). The predictive value of these biomarkers has yet to be demonstrated in large-scale clinical trials, and the technology is still very expensive for high-burden, developing country settings. Also, there are some efforts to develop therapeutic interventions that will prolong gestation for women with signs and symptoms of spontaneous PTB, where it is desirable and safe for the mother and fetus to continue the pregnancy for as long as possible. Key to this effort is ensuring that prevention of early delivery does not jeopardize the health and survival of the mother and fetus, such as in the case of intra-amniotic infection or intrauterine growth restriction. Ideally, research efforts to prevent or delay PTB would be linked to studies evaluating outcomes in later childhood and possibly even into adulthood. We anticipate that several opportunities to develop prevention interventions will emerge on the basis of studies defining the biological pathways that control healthy pregnancy, labor, and delivery.

Solutions aimed at reducing the risk of spontaneous PTB could pursue a variety of targeted approaches (Fig. 5), as follows:

- 1) Pathway-specific targeted interventions, such as treatments focusing on the microbes responsible for infection-associated PTB (pathogen targets) and the immune/inflammatory response (host targets)
- 2) Integrating the study of biochemical mediators and environmental and genetic factors to better understand the interplay between risk factors
- 3) Modifying genetic predilection by modifying gene expression
- 4) Modifying early triggers of labor and delivery caused by comorbidities, such as infection
- 5) Modifying stress-related factors, including those potentially related to racial and social determinants
- 6) Modifying behaviors associated with increased risk, such as smoking

### Critical factors to scientific success

**Improving research infrastructure.** At this time, the scientific community does not have sufficient resources to perform the basic science discovery research required to understand the mechanisms of PTB and then translate that knowledge to clinical interventions to predict and prevent PTB. Currently, much of the research on PTB is undertaken by individual scientists funded by single investigator-initiated granting mechanisms, as well as research and development efforts by a few organizations. Current study results are often discordant and difficult to compare, because different definitions and outcome measures are used, and patients are not stratified in a standard way by gestational age, phenotypic pathway, or risk factors (99). Although discovery-based, investigator-initiated research by basic scientists using relevant model systems remains essential, meaningful translational research requires access to large, well-defined pregnancy cohorts, biospecimens, and tools for conducting research, including good model systems and tools to accurately



**Fig. 5. Translational science targets.** Potential areas of focus for investigating mechanistic targets for prevention and treatment of preterm labor and birth are proposed. Research on the mechanisms behind different pathways and risk factors that contribute to prematurity should be aimed at identifying pathway-specific targets for which drugs, therapies, prevention strategies, and other intervention programs can be developed.

date gestation. There is a need for rigorous standardization of common definitions and phenotypes, large global cohorts, international biorepositories, clinical trial networks, well-defined outcomes, and transparency in data publication to prevent bias and duplication of effort. Proposed definitions and classifications of preterm labor and birth were proposed by a working group and reported by Villar *et al.* (100). Availability of data and specimens collected prospectively from human cohorts can promote the development of a more robust scientific community and attract new scientists to the field. At the same time, it will be critical to inspire the scientific community to take risks and explore translational opportunities based on study results.

**Sophistication of scientific technologies.** Utilization of new technologies will be important for monitoring pregnancy and parturition and identifying viable targets for prevention. Many investigators are using proteomics to identify potential biomarkers of PTB, with a few candidate molecules showing some promise, but many challenges remain. Because pregnancy is a dynamic process and preterm labor is multifactorial, it is likely that biomarkers will differ depending on the causal pathway and gestational age. Furthermore, biomarker profiles may change throughout pregnancy or in response to other pregnancy complications and comorbidities, such as preeclampsia or urinary tract infections. By stratifying preterm labor according to causal mechanism and gestational age during biomarker discovery, it may be possible to identify molecular phenotypes associated with specific pathways and thus inform prevention and treatment before the labor cascade is initiated. Addition of nontraditional biomarkers, including physical findings such as cervical remodeling, may increase the predictive value of a biomarker panel (101). The complexity of developing specific diagnostics will require input from multiple disciplines, including bio-

informatics, computational biology, systems biology, evolutionary biology, and bioengineering.

Investment in new technologies to detect early cervical change is also warranted. As reviewed by Feltovich and House (102), emerging tools include elastography, acoustic attenuation, light-induced fluorescence, Raman spectroscopy, cervical consistency index, and quantitative ultrasound. These technologies may help identify critical time points in gestation for further investigation of the underlying molecular events leading to cervical changes. Ultimately, the introduction of these techniques during routine prenatal care will aid clinicians in detecting changes leading to PTB and enable initiation of appropriate early monitoring and treatment.

#### Streamlining the regulatory process.

Faster regulatory decisions are required to move scientific knowledge into pre-clinical and clinical development. More streamlined mechanisms for regulatory oversight and approval are needed to accelerate diagnostic, technical, and commodity development for PTB. This is not a unique problem, but there is a critical need for increased research with

pregnant women to make meaningful advances in understanding human gestation. Unfortunately, the perception of risk and liability deters scientists and pharmaceutical companies from testing diagnostics and therapeutics during pregnancy. The Common Fund Rule sets forth regulatory requirements for including pregnant women in research, emphasizing minimal risk to the fetus and requiring sufficient preclinical research in animal models and clinical studies in non-pregnant women to assess risk before moving to trials with pregnant women (4). Institutional review boards (IRBs) and individual researchers struggle to interpret the meaning of “minimal risk” and “sufficient data,” and requirements often vary between institutions, hindering clinical investigation. Development of a standardized regulatory “map” with common guidelines, definitions, and core principles would assist investigators and IRBs. Consensus on ethical standards and guidelines for conducting research with pregnant women will be particularly important for safely conducting multicenter and international research studies. Furthermore, examining outcomes in prematurity intervention research that go beyond gestational age and include measures of short- and long-term pregnancy outcomes, as well as fetal and neonatal outcomes, is important to ensure the safety and well-being of the mother and fetus. These measures should be included as important parameters when evaluating risk and defining clinical benefit of potential interventions.

**Inclusion of all affected populations in study designs.** Research should include women from diverse racial and ethnic groups, and it should be conducted in lower- to middle-income countries to ethically and effectively address the global burden of PTB. An estimated 98% of all newborn deaths occur in these countries that often lack basic health care infrastructure. Women in these settings experience different risks

than those in high-income settings, and their pregnancies are more likely to be complicated by comorbidities such as malaria and HIV. Conducting research in low-resource settings poses many challenges, including lack of health care and research infrastructure, lack of training programs for researchers, and social and cultural barriers to accessing care. In addition, developing countries often lack the technology and tools required to conduct prematurity research. For example, accurate determination of gestational age when ultrasound is not available is a major barrier to studying PTB in low-resource settings. Development of less expensive technology or new tools for gestational age assessment is critical for moving science forward in these regions. Investigation of interventions must be practical, affordable, and culturally acceptable to different racial, ethnic, and geographic populations and communities. Despite the challenges, only a concerted global effort can effectively decrease the overall rates of prematurity.

**Increasing and coordinating funding for PTB research.** The breadth and depth of research required to elucidate the complex mechanisms regulating pregnancy and to identify prevention strategies represent a broad and costly agenda, and a mechanism for sustained long-term investment is required. One approach to this problem is the development of a consortium of organizations to fund research addressing the scientific gaps and generating new solutions. The Global Coalition to Advance Preterm Birth Research (GCAPR) is such a consortium, which was only recently formed (<http://www.gcapr.org>). Key goals of GCAPR are to identify research priorities for PTB, raise awareness of PTB, grow the financial commitments for PTB research, and facilitate the alignment of resources and research priorities among funding organizations. One priority area for GCAPR is to address the critical barriers and solutions to promote translational development of prevention and treatment strategies, especially in developing countries. These types of collaborative efforts will also reduce redundancy, address research needs, and build synergy along the continuum of discovery, development, and delivery science. Further funding opportunities that encourage rigorous, collaborative scientific investigation into the causes and prevention of prematurity are needed.

## CONCLUSION

Current research efforts in pregnancy and PTB are beginning to yield important and exciting clues to the mechanistic basis of some of the key risk factors associated with early stimulation of labor. Although traditional research methods have focused on investigation of a single or limited number of variables, more attention is now needed to investigate the multiple factors that affect pregnancy and birth outcomes and their interactions. Thus far, the lack of precision in defining PTB, as well as the lack of standardized case definitions and classification of preterm phenotypes, has resulted in inconsistent results and made it difficult to combine or compare data and results across studies. Advancing the science of the complex processes of human pregnancy will require a high level of commitment of financial and human resources, which to date have been inadequate and too poorly coordinated to achieve needed results. Collectively, a new approach to perinatal science is essential, and it should be a high priority to unravel the complexity of human pregnancy and parturition and uncover new strategies to prevent this major cause of maternal and childhood death and disability.

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# Science Translational Medicine

## Prevention of preterm birth: Harnessing science to address the global epidemic

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