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Inflammation, Infection and Preterm Birth

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Abstract: Preterm birth is the leading cause of perinatal morbidity and mortality. Pathological processes that have been linked with preterm birth infection and / or intrauterine inflammation are most frequently found associated with their induction. Studies in animal models and human research showed prior infections to the induction of labor, the anteriority of infection over labor induction, and the existence of a subclinical latency phase between these two phenomena. The ascending route from the vagina and the cervix is preponderant but also microorganisms may access the amniotic cavity and the fetus by other pathways.

During inflammation associated to infection, Prostaglandins are released simultaneously with Nitric oxide and their overproduction could be detrimental. Prostaglandins promote uterine contractions contributing to embryonic and fetal expulsion. Therefore aberrant activation of the inflammatory response may cause premature labor and this does not seem to depend on how the microoorganisms accessed the uterus.

Keywords: Pregnancy- preterm labor- infection- inflammation.

INTRODUCTION

Preterm birth, defined as childbirth occurring at less than 37 completed weeks of gestation, is a major determinant of neonatal mortality and morbidity [1, 2, 3]. Sequelae of preterm birth are common in the neonatal period, may persist into adulthood and are inversely related to gestational age. The risk of death of a premature newborn is 120 folds higher than a baby born at term [4,5]. Excluding congenital malformations, premature birth accounts for approximately 70% of all neonatal deaths and results in nearly 50 percent of long-term neurological problems [6]. Preterm babies are at risk of short-term morbidity due to various causes (diseases of the respiratory syndrome, intraventricular hemorrhage, necrotizing enterocolitis, sepsis, retinopathy of prematurity) and long term morbidity, for reasons such as cerebral palsy, learning disabilities, blindness and respiratory diseases [7]. Globally, an estimated 13 million babies are born before 37 completed weeks of gestation annually. Rates are generally highest in low- and middle-income countries, and increasing in some middle- and high-income countries, particularly in the Americas [8]. In addition to this enormous waste of human potential, the monetary cost is estimated at tens of billions of dollars annually, and that should include the impact of neonatal intensive care, long-term cost recovery, educational programs, special social assistance, institutional care and other services for survivors with disabilities and their families.

Although survival rates of premature babies have increased in the last decade, there are still high rates of morbidity. Approximately 90% of infants born at less than 30 weeks of gestation show abnormalities in brain magnetic resonance [9], and the risk of cerebral palsy is 70 folds higher than those born at term [10]. Parturition is the process involving changes in gestational tissues that results in birth as well as the events in the maternal, placental, and fetal compartments that lead to these changes. The precise biomolecular mechanisms by which human parturition is initiated spontaneously, either at term or preterm, are not well understood. The current therapeutic approaches are directed toward stopping premature labor when it is started and they generally have been unsuccessful.

A better understanding of the mechanisms that regulate the onset of labor may enable the development of new strategies for early diagnosis, treatment or even prevention of preterm birth and neurological consequences.

BACTERIAL INFECTIONS AND PRETERM BIRTH

Preterm birth may result from either spontaneous developments or medically indicated interventions. Known causes of spontaneous preterm labor include infection (intrauterine or extrauterine), multiple gestation, placental abruption, hormonal disruptions and other factors, though a large proportion of preterm births are 'idiopathic', or without known cause [11].

Bacterial infections of pregnant women, fetus and newborn represent obstetric and perinatal problems of great importance. These are strongly related to the loss of pregnancy, including fetal abnormalities, stillbirth, premature labor, premature rupture of membranes and miscarriage. Due to perfected microbiological diagnostic methods and basic knowledge of microorganisms and their metabolic products, it has been possible to establish etiologic associations between these and various conditions that affect pregnancy outcomes and newborn. In approximately 50% of the cases spontaneous preterm birth was found associated to maternal infections [12, 13]. These infections are often asymptomatic and are caused by bacteria that ascend through the cervix from the vagina and colonize maternal-fetal tissues [14, 15, 16].

Prenatal infection and consequnet inflammation are the main causes of preterm labor and in turn, are key risk factors for the later development of cerebral palsy and neonatal sepsis [17]. The administration of *Escherichia coli* or lipopolysaccharide (LPS) provokes overtaking or childbirth and causes a variety of lesions in the white matter of the developing brain of the newborn [18], such as periventricular leukomalacia, a condition associated with the development of cerebral palsy [19], and diffuse lesions in the white matter, the most common brain abnormality associated with adverse neurological development [20]. Since 1950, when Knox and Hoerner [21] reported that maternal infections were related to prematurity, progress has been made in the study of the mechanisms in-

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volved in the early onset of labor due to infections, but has not managed to reduce the rates of preterm birth.

How bacteria invade the uterine cavity, which is normally a sterile environment, and the reasons why different species vary in their capacity to induce inflammation and preterm birth are still incompletely understood. Microorganisms may gain access to the amniotic cavity and the fetus through the following pathways: 1) ascending from the vagina and the cervix; 2) hematogenous dissemination through the placenta (transplacental infection); 3) retrograde seeding from the peritoneal cavity through the fallopian tubes; 4) accidental introduction at the time of invasive procedures such as amniocentesis, percutaneous fetal blood sampling, chorionic villous sampling, or shunting; and 5) contaminated food or mouth infections [22]. The most frequently way of micoorganisms to access the uterus is ascending from the vagina and cervix. Ascending infections extends to the choriodecidual space and may ultimately invade the membranes, amniotic fluid, and fetus [23-27].

Several studies supported that abnormal bacterial colonization, indicative of bacterial vaginosis, is strongly associated with preterm delivery. Due to an interruption in normal vaginal flora, it is characterized by a reduction in lactobacillus and an overgrowth of other anaerobic bacteria such as gardenerella, bacteroides, and mobiluncus, all vaginal organisms of relatively low virulence [28-32]. After changes in vaginal and/or cervix flora, microorganisms ascend into the choriodecidual space. Then, some microorganisms migrate between the chorion and amnion and infect the intrauterine cavity and some of the fetuses ultimately become infected [33]. Thus, only a small portion of the fetuses delivered preterm had positive blood or cerebrospinal fluid cultures at delivery and this is the most advanced and serious stage of ascending intrauterine infection [34].

Activation of the Innate Immune System

Once microorganisms have entered the cervix, uterus and/or amnion, there are several possible signaling pathways that may be initiated, which can either individually or collectively promote preterm parturition. A likely pathway is the activation of the innate immune system. First, bacteria or even bacterial by-products, such as LPS, are detected by immune system pattern recognition receptors, specifically the toll-like receptors (TLRs) [35] (Fig. **1**). TLRs are signaling receptors that activate gene expression programs including dendritic cell maturation, and the production of proinflammatory cytokines and type I interferons (INF) [36]. The TLR family are membrane-bound proteins that activate the innate immune system by recognizing specific molecular signatures of various pathogens. TLR2 mediates cellular responses to Gram-positive organisms via their membrane lipoproteins, glycolipids, and peptidoglycans [37]. TLR4, the best characterized member of this family, plays a fundamental role in the early activation of innate immunity to exogenous and endogenous ligands including bacterial LPS, heat shock proteins, and components of the extracellular matrix released after tissue damage [38-42]. TLRs, in particular TLR-2 and TLR-4, are expressed in the uterus [43], cervix [44], amniotic epithelium [45], and the decidua [46]. When TLRs detects bacteria or bacterial products, they activate several immune pathways, triggering an inflammatory response mediated primarily by cytokines interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), and IL-6 [47-49], prostaglandins [52] and nitric oxide [62]. All these molecules induce a coordinated response featuring uterine contractions, placental detachment, infiltration of inflammatory cells into gestational tissues, a series of biochemical and structural changes in the cervix known as 'ripening' and weakening of the fetal membranes.

Role of Cytokines

The pathology of infection-induced rupture of fetal membranes has been attributed to the secretion of proinflammatory cytokines, such as IL-1β and TNF-α, by gestational tissues in response to bacterial products [50]. At the same time, these cytokines may stimulate the biosynthesis of IL-6 by decidual cells [51], which is considered a responsive indicator of intrauterine infection in pregnant women [52]. The proinflammatory cytokines induce the augmentation of matrix metalloproteinases (MMP) production [53]. These changes contribute to increase proteinase-mediated degradation of the extracellular matrix within the uterine cervix [54-56]. IL-1β also upregulates cyclooxigenase-2 activity, increases prostaglandin E2 production, and promotes cervical maturation [57].

Considering that many cell types synthesize the inflammatory mediators, it becomes difficult to identify precisely their origin during preterm labor. Even some studies indicated that resident cells in the chorioamniotic membranes were responsible for the production of these mediators in the presence of bacterial products such as LPS [58, 59], more recent data shows that the main source of cytokines was the infiltrated leukocytes present in the placenta and not the local cells [60]. There is evidence that the arrival of leukocytes such as neutrophils, macrophages, monocytes, and T and B lymphocytes to the choriodecidua immediately before the beginning of labor coincides with the increase in the levels of cytokines IL-1β, IL-6, IL-8, and TNF-α, leading to the establishment of the inflammatory microenvironment during labor [61].

Role of Prostaglandins

Primary proinflammatory cytokines induce the production of prostaglandins (PGs) [62]. These in turn act synergistically to promote cervical ripening [63], and particularly PGE2 and PGF2 stimulate uterine contractility [64]. In normal parturition, PGs initiate uterine contractions, aided by a fall in progesterone's effect to maintain uterine quiescence [65, 66] (Fig. **1**).

PGs are generated from arachidonic acid by phospholipases A2 (PLA2s) followed by the action of cyclooxygenases (COX). COX isoforms, namely COX-1 and COX-2, mediate the conversion of arachidonic acid into PGH2, which is then converted to various PGs by specific synthases [67].

The metabolic pathways including PGs synthesis, degradation and transformation into other PGs, have been extensively studied and the different lines of evidences point to the participation of these mediators in the onset of term and preterm labor. In preterm labor associated to infection, proinflammatory cytokines stimulate the production and release of PGs—causing uterine contractility—and increase neutrophil activity, thereby augmenting the synthesis and release of metalloproteinases, which in turn cause the rupture of fetal membranes and the remodeling of collagen in the cervix [68-70]. A fetal response also may occur, in which infection stimulates the production of corticotropin-releasing hormone (from the fetal hypothalamus and placenta) causing an increase in fetal corticotropin and thereby fetal cortisol, also stimulating PGs production [71].

Role of Nitric Oxide

Cytokines also induce the increase of nitric oxide (NO) [72], another important mediator contributing to infection-induced adverse developmental outcome. NO is a free radical and potent smooth muscle relaxant, synthesized by a family of enzymes known as NO synthases (NOS), through the oxygen-dependent conversion of L-arginine to citrulline [73]. Three NOS isoforms have been identified: endothelial NOS (eNOS), neuronal NOS (nNOS), both constitutive and inducible NOS (iNOS), which is expressed mainly in macrophages after stimulation with cytokines like IFN-γ, TNF-α [74, 75].

During pregnancy, NO has important functions and is involved in physiological processes such as implantation, decidualization, vasodilatation and myometrial relaxation [76-79]. However, in high concentrations, such as those produced from iNOS during sepsis [80], NO becomes a toxic mediator due to the generation of free radicals [81]. In addition, in the course of infections reactive

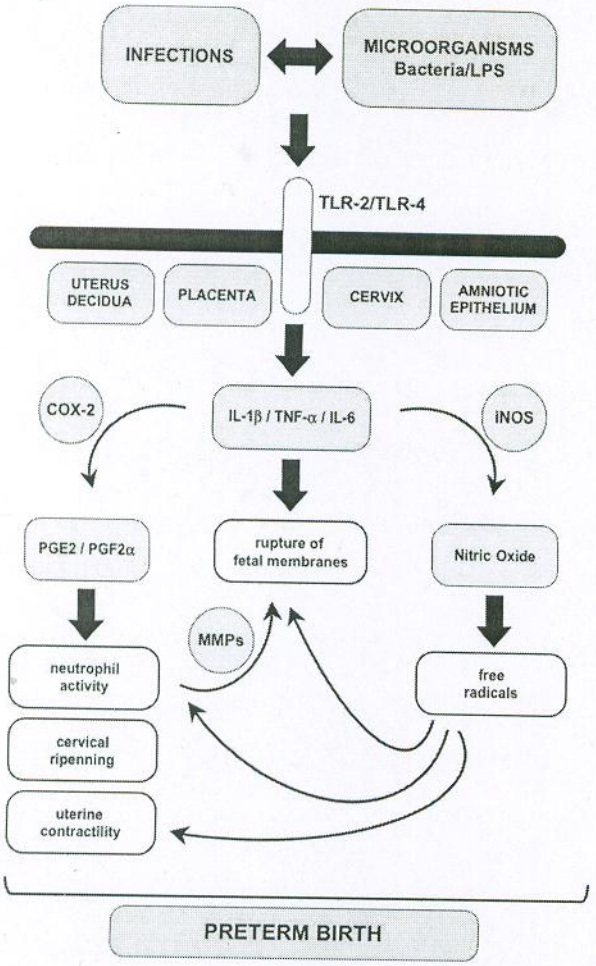

Fig. (1). Mechanism of infection-triggered preterm labor. Bacteria or bacterial by-products, such as LPS, are detected by the toll-like receptors(TLR). Activation of TLR induces local secretion of cytokines. Proinflammatory cytokines stimulate the production and release of PGs—causing uterine contractility—and increase neutrophil activity, thereby augmenting the synthesis and release of metalloproteinases, which in turn cause the rupture of fetal membranes and the remodeling of collagen in the cervix.

Cytokines also induce NO synthesis and its overproduction could increase the synthesis of PGs and contribute to uterine contractility and preterm labor.

oxygen species (ROS) production, like superoxide anion (O_2^-), is also increased [82-85]. Under this conditions, NO and O_2^- can react together to produce a more long-lived and potent pro-oxidant, peroxynitrite, a powerfull oxidant and nitrating molecule [86, 87]. The toxic effects of NO include: inhibition of the mitochondrial respiratory chain, lipid peroxidation, protein and nucleic acid nitration, DNA break, vascular injury, necrosis and apoptosis [88]. Also, is well known that LPS is able to increase leukocyte infiltration in various tissues and stimulates macrophages to generate NO and ROS production [89, 90] (Fig. **1**).

Several studies demonstrate that in normal pregnancies, NO synthesis is upregulated during gestation to maintain uterine quiescence and is then downregulated during labor [91-94]. However, large quantities of NO and ROS, like those produced in an inflammatory setting, could increase the synthesis of PGs and contribute to uterine contractility and the consequent pretem labour. ROS activate NF-κB and enhance the subsequent expression of COX-2, which leads to the orchestration of the inflammatory pathway [95, 96], and the propogation of parturition [97]. It has been reported that high concentrations of NO metabolites are present in amniotic fluid of women with intra-amniotic infection [98] and that NO may contribute to preterm labor in LPS-challenged pregnant mice [99, 100].

Mouth and Gastrointestinal Microorganisms

Although most bacteria found in the uterus in association with preterm labor are of vaginal origin, microorganisms may gain access to the amniotic cavity and the fetus by another pathways. Rarely, mouth organisms of the genus capnocytophaga, are found in the uterus in association with preterm labor and chorioamnionitis; these organisms may reach the uterus through the placenta from the circulation or perhaps by oral–genital contact [101]. The association between periodontal disease and preterm birth has gained increasing attention [102]. Periodontitis is an inflammatory disease of the supporting tissues of the teeth, caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both [103]. Periodontal disease affects up to 50% of the population, including pregnant women.

It is postulated that LPS from the anaerobic Gram negative periodontal pathogens, stimulate the release of proinflammatory cytokines which may trigger preterm labor. In rodents, subcutaneous inoculations with periodontal pathogens cause dose-dependent decreases in litter weight and elicit the production of cytokines and PGs that signal preterm labor when present in amniotic fluid [104, 105]. However, in humans, it is not clear the causal link between periodontitis and prematurity or low birth weight, and several epidemiologic studies have found no association [106-110]. *Listeria monocytogenes* is Gram-positive facultative intracellular pathogen often foodborne and found elsewhere. Listeriosis has unique preference for pregnant women. Maternal listeriosis is a diagnostic challenge, and intrauterine infections can lead to severe complications such as amnionitis, preterm labor, spontaneous abortion, stillbirth and neonatal sepsis [111-115]. So far, *Listeria monocytogenes* is the only agent known to cause fetal death or miscarriages in humans by food poisoning.

Gastrointestinal infection with Shiga toxin (Stx)-producing *Escherichia coli* (STEC) causes diarrhea and hemorrhagic colitis, and is the leading cause of hemolytic uremic syndrome (HUS), a systemic complication that is attributed to expression of Stx [116-117]. HUS is a disease characterized by hemolytic anemia, acute kidney failure and thrombocytopenia. Most cases are preceded by an episode of infectious, sometimes bloody diarrhea caused by *E. coli* O157:H7, which is acquired as a foodborne illness or from a contaminated water supply. It is a medical emergency and leads to a 5–10% of mortality; of the remainder, the majority recover without major consequences but a small proportion develop chronic kidney disease and become reliant on renal replacement therapy [113-121]. HUS is most commonly seen in young children but it is occasionally present in adults [122-124], including post-partum women. Most postpartum cases had preceeded upper respiratory or gastrointestinal symptoms [125, 126] and at least in one case, HUS was detected after a preterm delivery at 32 weeks [126]. In May 2011, an epidemic of bloody diarrhea caused by *E. coli* O104:H4 hit Germany. The consumption of sprouts was identified as the most likely vehicle of infection. The tracing of the epidemic revealed more than 4000 cases, with HUS developing in approximately 800 of the cases, with 50 of them resulting in death. Over 90% of the cases were detected in adults over 17 years old of which 68% were women [127, 128]. STEC enters to the body orally by eating contaminated food, it establishes and colonizes the colon where releases its virulence factors, including Stx. The colon is the primary site of histological lesions caused by STEC and when it is inflamed

facilitates the absorption of Stx and LPS through the intestinal barrier, which reach systemic circulation [129]. Previous studies have demonstrated that infection with STEC was able to produce placentitis and late miscarriage in sheep [130, 131].

Several authors have shown that Stx affects pregnancy in rodents. Olsen *et al.* [132] observed that Stx inhibits the development of mouse embryos in a preimplantation stage as a result of the inhibition of protein synthesis and ATP content. Yoshimura *et al.* [133] demonstrated that intravenous injection of Stx2 in pregnant mice was able to induce miscarriage by apoptosis in trophoblast before placental development and also in late stages of pregnancy, and it causes a disorder of postpartum maternal behavior. Further, he demonstrated that Stx1 and Stx2 induce apoptosis in WISH cells derived from human amniotic tissue [134]. Burdet *et al.* [135] reported that the treatment of pregnant rats on day 14–16 of gestation with Stx2 induced maternal lethality in a dose-dependent manner and premature delivery of dead fetuses at 1–2 days post-injection. They also showed that sStx2 (a combination of Stx2 and LPS) induces a significant increase in NO production and levels of iNOS protein in placental tissues from pregnant rats, suggesting that the overproduction of NO plays an important role in sStx2-induced placental toxicity and fetal mortality. Furthermore aminoguanidine, an iNOS inhibitor, completely reversed the Stx2 damages in placental tissues, but did not prevent premature delivery, thus suggesting that other mechanisms, not yet determined, could be involved [136]. Placental PGE2 and decidual PGF2α were increased by the Stx treatment and in both tissues COX-2 protein expression was stimulated. However, meloxicam, a selective inhibitor of COX-2, was unable to prevent placental toxicity and fetal mortality [137]. The mechanism of action involved in Stx-induced preterm labor could be an alteration of the balance of cytokines at the maternal-fetal interface, that is essential for reproductive success [138, 139]. Stx may act in concert with LPS to elicit cellular dysfunction [140]. Stx is able to bind to Gb3 receptors expressed on the membrane of monocytes/macrophages and leads to cellular activation and secretion of cytokines such as TNF-α, IL-1β and IL-8 that increase the endothelial susceptibility to Stx [141]. In the model of Stx-induced preterm delivery a significant increase of TNF-α was observed in serum 2 h after Stx2 treatment and this could be responsible for preterm delivery. Etanercept, a competitive inhibitor of TNF-α, blocked the TNF-α increase after Stx2 treatment and reduced the preterm delivery by approximately 30%. However, Etanarcept did not inhibit the increase of placental NOS activity caused by Stx2. When aminoguanidine was administrated together with Etanercept, preterm delivery was prevented by roughly 70%, suggesting that preterm delivery of dead fetuses induced by Stx2 is triggered by TNF-α and mediated by an increase in NOS production [137].

In humans, there is only one reported case of infection by STEC O157:H7 in Japan who developed hemolytic uremic syndrome (HUS) during pregnancy at the 32nd week of gestation. The patient had no evidence of intrauterine infection, her delivery was normal and at term, and the newborn had a normal development [142]. Although there are no reports that the Stx2 induce premature labor or mediate fetal injury or death in humans, the data obtained in this animal model suggest that STEC infections during pregnancy might be one of the causes, not yet certain, of preterm birth and/or fetal morbidity and mortality.

ANIMAL MODELS FOR POTENTIAL THERAPEUTIC INTERVENTIONS TO PREVENT PRETERM BIRTH

Because the study of preterm birth and pregnancy in humans is subject to ethical factors, the selection of an animal model most closely reflecting human physiology will provide the optimal information. Although most animal species do not have significant rates of spontaneous preterm birth, there is much interest in the use of relevant animal models to elucidate the mechanisms of preterm birth and the neonatal sequelae of prematurity and to develop rationale and efficacious treatment and prevention strategies. The use of animal models is a valuable resource that allows for strict control of environmental, genetic, pharmacologic, maternal, and fetal variables otherwise difficult to regulate in human studies. However, not all animal models are useful for the study of the various mechanisms involved in preterm birth. For example, for studying the effects of placental dysfunction on progesterone synthesis mice, rats or rabbits are not useful. In all these pregnant animals progesterone is produced by the corpora lutea and not in the placenta, and progesterone withdrawal is necessary and sufficient to evoke parturition [143, 144]. Unlike this in pregnant women, parturition occurs in the face of extremely high and unchanging or increasing maternal plasma progesterone concentrations [145, 146]. However rodents constitute efficient models for infection and/or inflammation associated preterm birth. Advantages of this model include a short pregnancy length (19 to 22 days), well-characterized genomes, and well-defined immune systems, which are similar to that of humans. Mice have the advantage of different routes of easy access for the administration of inflammatory/infectious agents [147]. More recently, increasing attention has been devoted to mice as models for parturition because of the ability to genetically manipulate single or multiple genes. Several "knockout" studies using targeted gene disruptions of endocrine/paracrine pathways provide important information regarding the regulation of parturition. Several studies in animal models have been designed to address a cause-effect relationship between the proinflammatory branch of the immune system and parturition. Experiments in normal and genetically altered mice have been used to elucidate the role of cytokines and inflammation in preterm birth associated to this process.

Various animal models have demonstrated that bacteria or bacterial by-products can induce preterm birth [100, 148, 149, 150]. The administration of LPS to mice results in elevated maternal serum and amniotic fluid concentrations of inflammatory chemokines and cytokines [150, 151]. Also LPS stimulates NOS activity [100, 152] and the synthesis of PGs [100, 152, 153, 154]. As proposed previously, a maternal response is necessary for the development of preterm birth. Maternal TLR4 recognizes LPS and is required for LPS induced preterm birth [155, 156]. Even in sonic stress-induced pregnancy loss, blocking the TLR4 receptor for LPS prevents preterm labor in mouse models [157].

Despite advancement in scientific knowledge regarding preterm labor, management to prevent it is still limited to the use of tocolytic agents to delay delivery. Tocolytic drugs delay preterm birth through various mechanisms of actions; however, none has been shown to reduce the preterm labor or improve neonatal outcomes [158]. Even findings in an animal model may not address all the pathways associated with the human preterm labor, animal models could be usefull to test effective management strategies to prevent preterm labor.

CONCLUSIONS

Preterm birth is a heterogenous entity in terms of the extent to which the birth is preterm (mild, moderate or severe) and in terms of the precipitating events (elective preterm birth or spontaneous preterm birth following either preterm labor or preterm rupture of membranes). Experimental evidence indicates that the mechanisms of labor and inflammatory processes have much in common and can overlap [52] (Fig. 1). It is now clear that the causes of preterm labor are multifactorial and vary according to gestational age. Important common pathways leading to preterm birth include stress, systemic or maternal genital tract infections, placental ischemia or vascular lesions, and uterine overdistension. These pathways differ in their initiating factors and mediators, but ultimately, they share many common features that result in preterm uterine contractions and birth. The most common cause of preterm birth is associated with intrauterine infection, characterized by increased local production of cytokines [33]. During inflammation associated to infection,

PGs are released simultaneously with NO and their overproduction could be detrimental. PGs promote uterine contractions contributing to embryonic and fetal expulsion [154, 100]. Therefore aberrant activation of the inflammatory response may cause premature labor and this does not seem to depend on how the microoorganisms accessed the uterus.

Although animal models are not without their limitations in modeling human parturition biology, they have made important contributions to our current understanding of this complex process. The use of animal models to answer specific questions related to prematurity and to describe the pathophysiological events associated with preterm birth will contribute to the development of rational and efficacious treatment and prevention strategies for preterm birth.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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