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Trophoblast-microbiome interaction: A new paradigm on immune regulation

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Abstract

The immunologic paradigm of pregnancy led to the conceptualization of pregnancy as an organ transplant which requires, for its success, a systemic immune suppression of the maternal immune system. Growing scientific evidence suggests that in many ways the placenta functions as a tumor rather than a transplant and the immune regulation of the maternal-fetal interface is the result of the coordinated interaction between all its cellular components, including bacteria. The role of microbiota in reproduction is in its infancy, but there is growing literature that supports its relevance. We discuss a potential normal function of bacteria in the establishment of immune-tolerance and the compelling evidence that a viral infection might be the underlying cause of perturbation of homeostasis. There is compelling evidence that many infectious diseases of humans are caused by more than one microorganism and are defined as polymicrobial infections. We propose that pregnancy complications, such as preterm birth, are the result of polymicrobial infections. We examine the potential cellular and molecular mechanisms by which a viral infection of the placenta might disrupt the normal interaction between the cellular component of the implantation site and bacteria. As we better understand the normal homeostasis between the maternal immune system, placenta and commensal, we will be able to elucidate the pathogenic conditions and design better approaches to treat pregnancy complications associated with infection.

Keywords

Trophoblast; bacteria; virus; inflammation; preterm birth

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From Transplantation to tumor Immunology

The immunologic paradigm of pregnancy was described for the first time by a transplant immunologist, Sir Peter Medawar, who observed the antigenic mixture of the fetus containing paternal antigens that are not rejected by the maternal immune system[1, 2]. This unique observation led to the conceptualization of pregnancy as a "semiallograft" and it was proposed that pregnancy represents a natural model of transplantation that could help to understand the immunologic bases for transplant rejection and acceptance[2]. Consequently, the study of the immunology of pregnancy followed the immunological models associated with organ transplant. Over the years several mechanisms have been proposed to explain the immune privileged state of the fetus, all of them with the basic understanding that the maternal immune system is antagonistic to the fetus/placental unit [3]. Since the success of an organ transplant is obtained by inducing immune suppression of the host, it was postulated that pregnancy may have a natural mechanism to induce systemic suppression of the maternal immune system. This concept has been studied by numerous investigators and over many years has become the conventional wisdom [4]. Indeed, a wide array of factors in human serum have been found to possess profound *in vitro* immunosuppressive activities [5]. However, if we carefully analyze this hypothesis it is difficult to imagine how, from an evolutionary point of view, pregnancy involves a stage of profound immune suppression. Early humans were not able to wash their hands or clean their food and, with the absence of antiseptics, were continually exposed to bacteria, parasites and other microorganisms. If pregnant women were systemically immunologically suppressed they would not have survived and the human species would have become extinct. Even today, in many parts of the world, pregnant women are continually exposed to harsh, unsanitary conditions and a suppressed immune system would make it impossible for the mother and fetus to survive. Furthermore, in countries such as Africa where HIV is pandemic, HIV-positive women do not develop AIDS during pregnancy [6]. In fact, there are recent studies clearly demonstrating that the maternal antiviral immunity is not affected by pregnancy [78]. Together, these observations argue against the existence of such non-specific immune suppression.

Medawar's original observation was based on the assumption that the placenta was akin to a "piece of skin" with paternal antigens, which under normal immunological conditions, should be rejected. However, the placenta is more than just a transplanted organ. Our knowledge of placental biology has significantly increased over the last 50 years. We now know that the placenta is a complex organ, which has evolved from the original "egg cover". Pregnancy and implantation, contrary to "graft implants", has been taking place for more than 1,000,000 years. Therefore, from an evolutionary point of view it is difficult to conceive that the placenta and the maternal immune system still maintain an antagonistic status. Thus, while there should be an active mechanism preventing the potential recognition of paternal antigens by the maternal immune system, the trophoblast and the maternal immune system have evolved and established a cooperative status, helping each other against common enemies: infectious microorganisms [9–11].

Therefore, it is important to evaluate the immunologic aspects associated with pregnancy in order to further understand the potential biological reasons associated with the risk of pregnancy complications potentially triggered by infection. One wonders why the model of

transplantation may not represent the correct immunological situation of pregnancy: During organ transplantation there is a major influx of foreign antigens as a result of the introduction of a fully foreign organ. Under this circumstance the host immune system acutely reacts to the foreign antigens and mounts an immunologic response to reject the source of foreign antigens. During pregnancy the process is different. Pregnancy is a slow and gradual process where paternal/fetal antigens are released in a gradual and increasing manner as the blastocyst grows into an embryo and then into a fetus. The exposure of small amounts of foreign antigens during this process may actually induce *tolerance* rather than rejection [12]. Consequently, pregnancy, contrary to transplantation, does not require systemic immune suppression.

A second aspect that has been poorly evaluated for many years is the active role of the placenta in the modulation of the maternal immune system. Pregnant women represent an immunologically unique population because their immune system is influenced by signals originating from the placenta [13, 14]. The presence of the fetus and placenta alters maternal immunity and physiology to sustain and protect the pregnancy. We and others have shown that the placenta may function as an immune modulatory organ that regulates the immune responses of cells present both at the implantation site as well as systemically[1510, 16, 17]. However, this modulation is not suppressive, but protective. The placenta together with the decidua are responsible for establishing a unique microenvironment at the implantation site that i) prevents a pro-inflammatory cytokine storm; ii) inhibits the recruitment of T cells with cytolytic function, iii) educates the local immune system to facilitate the fetal development, iv) control bacterial growth and v) protect the fetus from viral infections.

In many ways, the placenta functions as a tumor rather than a transplant. Tumors secrete an array of factors that establish a local immunosuppressive microenvironment in which dysfunction and even death of tumor-specific T-cells can occur. This immune-regulatory effect occurs at two sites: 1) locally at the tumor-host interface where cancer cells condition the tumor stroma, and 2) systemically where cells and/or factors mediate suppression of anti-tumoral T-cells in the blood and lymphoid organs. The placenta has also two regulatory sites that are 1) locally, at the decidua where immune cells are differentiated towards a pregnancy supporting function, and 2) systemically, affecting the maternal immune system and preventing the expansion of T-cell clones that recognize paternal antigens.

The placenta and bacteria, friends or foes?

Bacterial infections are thought to pose a significant threat to a pregnancy and to the well-being of the fetus, by gaining access to gestational tissues, such as the decidua, the placenta, and the fetal membranes, through one of three major routes: By ascending into the uterus from the lower tract; by descending into the uterus from the peritoneal cavity; or via the maternal circulation [1819–2223]. There are strong clinical links between bacterial infection and preterm birth [242325, 2627]. Indeed, infections have been reported as responsible for up to 40% of preterm birth cases[28, 29]. Furthermore, 80% of preterm deliveries occurring at less than 30 weeks of gestation have evidence of infection [2627]. While many of the pathways involved are still largely undefined, growing literature suggests that the way in which a microorganism can induce a pregnancy complication, such as preterm birth, involves innate immune

responses towards the pathogen, leading to excessive inflammation and/or apoptosis at the maternal-fetal interface [3031, 32]. Indeed, experimental *in vivo* models have demonstrated that delivery of infectious components (bacteria and bacterial products) to a variety of animals triggers preterm delivery [33, 343536]. Clinical studies have correlated placental infection/inflammation with prematurity [37–39] and this is supported by experimental studies [14, 34, 40, 41]. In spite of the strong literature linking bacterial infection and pregnancy complications, targeting bacterial infections have failed to prevent pregnancy complications [42, 43]. Furthermore, growing evidence suggests bacteria are a normal component of the pregnant and non-pregnant uterus [444546, 4748]. These observations suggest that in most cases bacteria alone may not be sufficient to induce an inflammatory event leading to parturition and that the immune response to commensal bacterial product at the maternal-fetal unit is tightly controlled by regulatory mechanisms [17, 48]. When and why bacteria become detrimental for pregnancy has not been defined.

Trophoblast cells as a component of innate immune system

The placenta is in direct contact with maternal component, thus it is imperative that a high level of immune protection is present at the maternal-fetal interface to protect the fetus against any infectious agent that reaches the placenta[49]. It is well known that classical immune cells, such as macrophages and natural killer cells, are present at the interface to facilitate innate immune responses. In addition to these immune cells, previous studies demonstrated that trophoblasts, the major constituents of the placenta, are also able to sense and respond to pathogen-associated molecular patterns (PAMPs) such as LPS or peptidoglycan [41]. We have confirmed the expression of Toll-like receptors (TLRs), membranous receptors known to recognize microbial products and have demonstrated that via TLR4 trophoblasts elicit an immune response [50–53]. Similar to other innate immune cells, ligation of TLR4 in trophoblast with LPS are able to activate both the classical myeloid differentiation factor 88 (MyD88) -dependent and the MyD88-independent signaling pathways leading to the production of cytokines and chemokines [52]. This finding strongly supports that trophoblast can indeed recognize pathogen and initiate an immune response. The immune interaction between the trophoblast and bacteria will be further discussed in the later part of the review.

The placenta is not a sterile organ

Human body harbors multiple microorganisms termed “microbiota” and they are known to interact with the host and play various important roles to benefit the host. Bacterial colonization in the placenta from the patients who delivered preterm due to intrauterine inflammation or chorioamnionitis has been well reported in numerous studies [30, 54, 55]. Based on these findings, a general belief among obstetricians was that the placenta should remain a sterile organ to maintain pregnancy through term. Recent study by Aagaard *et al.* [56] on placental microbiome provided a turning point for this dogma. By application of culture-independent whole genome shotgun metagenomic technology, they were able to sequence various bacterial species in the placenta obtained following normal term pregnancy, the dominant bacteria identified being *Escherichia coli* and the genus *Escherichia* species. Furthermore, *Provotella tannerae*, *Bacteriodes* species, *Fusobacterium* species, *Streptomyces*

avermitilis, *Neisseria polysaccharea*, *Neisseria lactamica*, and *Fusobacterium* species, though low in abundance were also sequenced. In line with this, presence of bacteria in the placenta has been reported by others substantiating the concept that placenta is inhabited by commensal bacteria [43, 57, 58]. Of particular interest is the finding that microbiota composition of placenta shared a great similarity, not with vagina or gut in the proximity, but with that of the oral cavity.

Although still controversial, there is growing evidences suggesting that the implantation site might not represent a sterile environment and the commensal bacterial might play a significant role during fetal development. Nonetheless, where and how the commensal bacteria migrate from and colonize in the placenta still remains a mystery.

‘Immune regulatory’ crosstalk between the trophoblast and commensal bacteria

What is the role of microbiota in the context of immune modulation? Among various functions, such as metabolizing nutrients and enforcing mucosal barrier, microbiota have been found to take part in modulating the host immune response as a way to prevent undesired inflammatory response to commensal bacteria or end-products, thereby, maintaining tissue homeostasis [59]. Stimulation of TLR with commensal bacteria in gut epithelium has shown to suppress NF-κB pathway and persistent nucleotide-binding oligomerisation domain (NOD) 2 receptor ligation with peptidoglycan, a bacterial wall product induced down-regulation of proinflammatory cytokines [60, 61]. And the disruption of host immunological tolerance to microbiota has been implicated in the pathogenesis of proinflammatory disease entities, such as inflammatory bowel disease, pulmonary asthma, chronic hepatitis, autoimmune disease, and others [62, 63].

The placenta cannot be an exception. If bacterium is present in proximity or at the maternal-fetal interface during a normal pregnancy, it might play a physiological role by contributing to the normal tissue homeostasis as disruption of the placental homeostasis may adversely affect not only the fetus but also the mother. This fundamental process relies on a complex and coordinated set of innate and adaptive responses that selects and calibrates responses against self, commensals and pathogens in the most appropriate manner. At the implantation site, specialized cell populations have to integrate local signals, such as cytokines, chemokines and microbial factors, allowing the induction of responses in a way that preserves the integrity of the tissue and its function (Fig 1A).

However, as the presence of microbes at the maternal-fetal interface is just beginning to unravel, their contribution to the placental innate immune system is unknown. Our studies have focused on determining how constitutive sensing of commensal by the trophoblast, plays an important homeostatic role during pregnancy, whereas active responses against the flora might be associated with pregnancy complications. As mentioned earlier, expression of all 10 Toll Like Receptors (TLRs) as well as various co-receptors and accessory proteins, such as CD14, has been described in the human and mouse placenta [36, 41, 64], which provides the trophoblast the capacity to recognize and respond to bacteria and viral products [16, 65]. Trophoblast express TLR4 and are able to recognize the bacterial product LPS;

however, TLR4 ligation by LPS in the trophoblast did not induce the classical NF-κB-inflammatory response characterized by the production of inflammatory mediators, but promotes the secretion of regulatory cytokines, such as IL-10 and type I interferon-associated chemokines (Racicot et al 2015 in preparation) [14]. It is plausible that bacteria, through the induction of regulatory cytokines by trophoblast, might stimulate the maternal-fetal interface to a tolerogenic microenvironment (Fig. 1A). How the trophoblast integrates microbial-derived signals remains unclear, but new data from others and us suggests that epigenetic modifications or alternative pathways mediating the response to TLRs are responsible for this unique type of responses [66, 67].

Consequently, we postulate that, when trophoblast operate properly with the commensals, their durable homeostatic relationship contributes to the regulatory tone of the maternal fetal interface and alterations in this relationship might be the base for pro-inflammatory conditions, such as preterm labor and delivery [68].

Preterm birth as a polymicrobial disease

There is compelling evidence that many infections of humans and animals are caused by more than one microorganism [69, 70]. The mixed microbial nature of these diseases has been recognized since the early 1920s[71]; however, only more recently we have we begun to understand the mechanisms associated with this type of disease. Polymicrobial diseases can be caused by the synergistic or sequential action of infectious agents from either the same or different kingdoms, genera, species, strains or by different phenotypic variants of a single species [70, 72–74]. A growing body of evidence suggests that preterm birth is a manifestation of polymicrobial disease [14, 48]. Recently, Payne *et al.* [75] thoroughly reviewed multiple types of microorganisms, including bacteria, virus, and fungus, detected in the maternal-fetal unit and their association with preterm birth.

We have developed an animal model of polymicrobial disease that leads to preterm birth[76]. The model consists of a Herpes Viral infection of the placenta and decidua leading to increased sensitivity towards bacterial products, which then triggers a pro-inflammatory “cytokine storm” and preterm birth. Herpes Simplex Virus (HSV) is the most common sexually-transmitted infection among the adult female population worldwide [77]. Although HSV-2 is the main cause of genital herpes and is almost always sexually transmitted, HSV-1 has emerged as a principle causative agent of genital herpes and its importance is increasing in college students[78]. From the late 1970s, HSV-2 seroprevalence in the US has increased by 30%, resulting that one out of five adults is infected[79]. The greatest incidence of HSV infection occurs in women of reproductive age and the potential transmission to the fetus during pregnancy has become a major health concern [80–83]. In the US, approximately 22% of pregnant women are infected with HSV, 10% are at risk of acquiring infection from their partners and 2% are infected during pregnancy. In addition to the risk of placing the newborn at risk of infection, acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, intrauterine growth retardation and preterm labor[84]. Primary HSV1/2 during pregnancy may have detrimental effects for the mother and the fetus[8586]. However, the mechanism of how HSV1/2 infection during pregnancy may lead to preterm birth is poorly understood.

In our animal model, we use the murine gammaherpesvirus 68 (Murid herpesvirus 4 (NC_001826.2); MHV68) which is a rodents herpes virus that shares significant genomic co-linearity with human pathogens, Epstein-Barr virus (EBV) and HSV [87]. Maternal infection with MHV68 alone had no effect on pregnancy outcome including litter size, weight or gestational age at delivery[17, 76], similar to that observed in human infection with HSV. However, it had a significant effect on the response to microbial products such as LPS. Although, high doses of LPS administered to pregnant mice have been shown to trigger preterm birth, low doses have no detrimental effect or a mild effect on pregnancy outcome [88, 89]. When we infected pregnant C57BL/6 mice with MHV68 on day E8.5 followed by low dose LPS (20ug/kg) on day E15.5 we observed preterm delivery in 100% of the mice in less than 24 hours. A similar dose of LPS in control mice (who did not receive MHV68) had a mild effect on pregnancy outcome (Fig. 2). Preterm birth observed in the combination virus-LPS was characterized by a cytokine storm consisting of high levels of pro-inflammatory cytokines associated with parturition [76],[72;73;74]. Furthermore, trophoblast cells predisposed to MHV infection demonstrated hyper-responsive proinflammatory cytokine production when challenged with LPS.

These findings suggest that herpes virus infection at the implantation site has the potential to modify TLR4 response to LPS, disturbing the optimal trophoblast-microbiota interaction that was originally set at a point towards immune tolerance and predisposes the placenta to an inflammatory response leading to preterm birth (Fig 1B).

Conclusion

The role of microbiota in reproduction is in its infancy, but there is a growing literature that supports its relevance. As we understand the normal homeostasis between the maternal immune system of the host, placenta and commensal, we will be able to elucidate the pathogenic conditions and design better approaches to treat pregnancy complications associated with infection.

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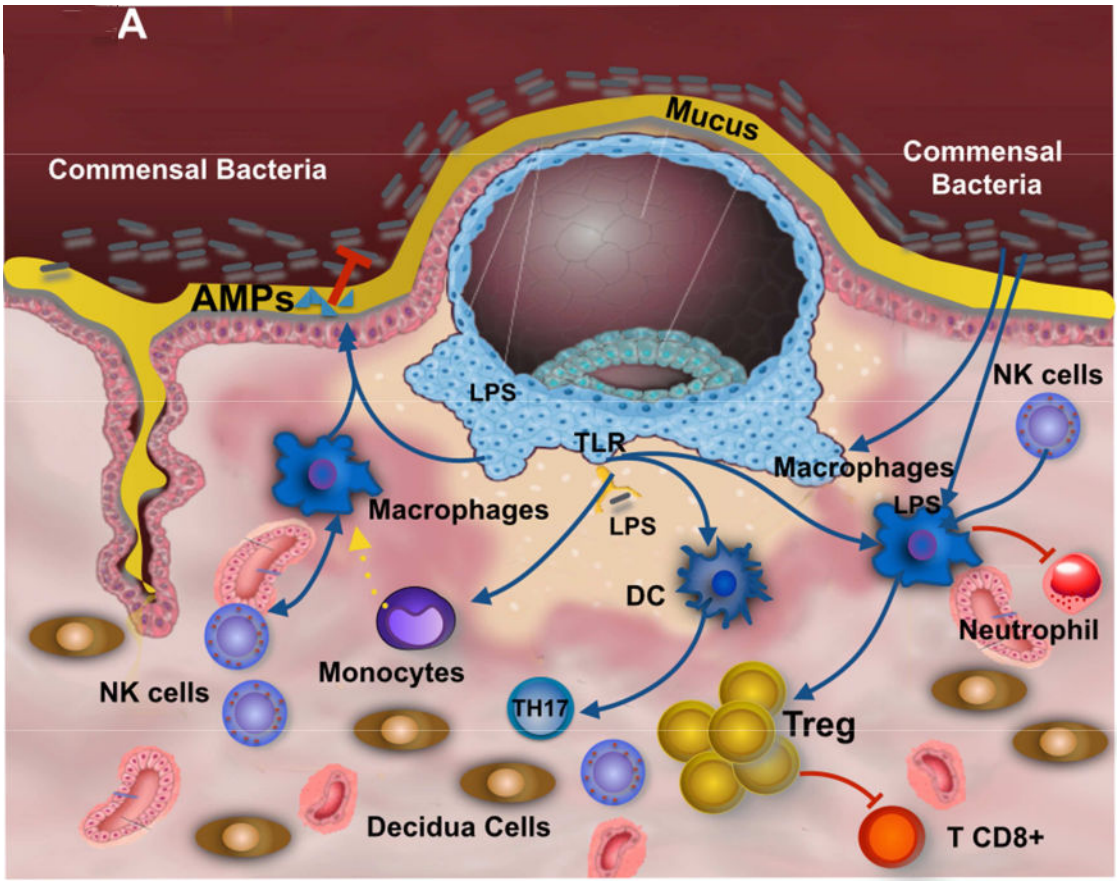

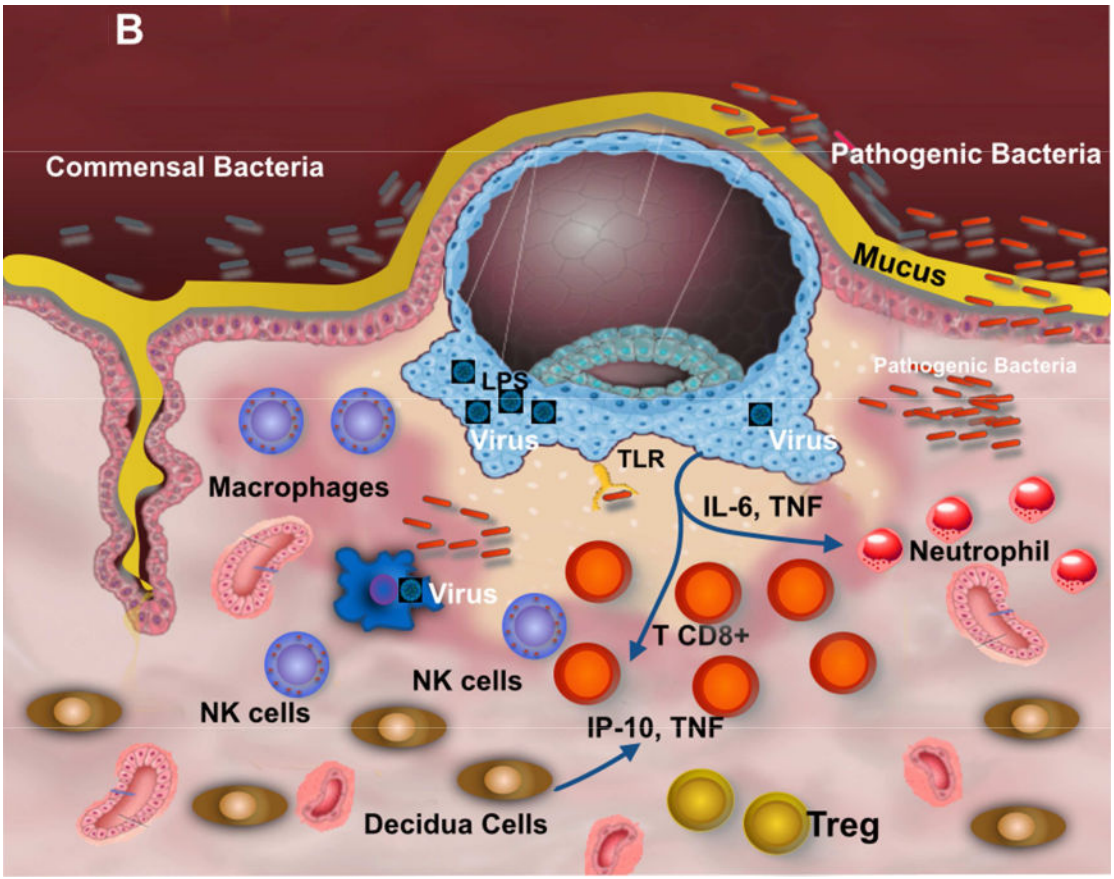

Figure 1. Contribution of the Microbiota to trophoblast-immune regulation at the implantation site

A. Commensal present at the mucus covering the epithelium of the uterus promotes the induction of regulatory factors by the trophoblast and decidual macrophages. Further, macrophages secrete anti-microbial products that control commensal overgrowth and prevent the invasion of pathogenic bacteria. The recognition of bacterial products by trophoblast enhances the expression of anti-inflammatory factors expanding T regs and promoting tolerance.

B. Viral infection at the implantation site inhibits macrophage capacity to control bacterial growth, disturbing the symbiosis between the microbiota, trophoblast and immune cells at the implantation site; leading to an inflammatory condition responsible for preterm birth.

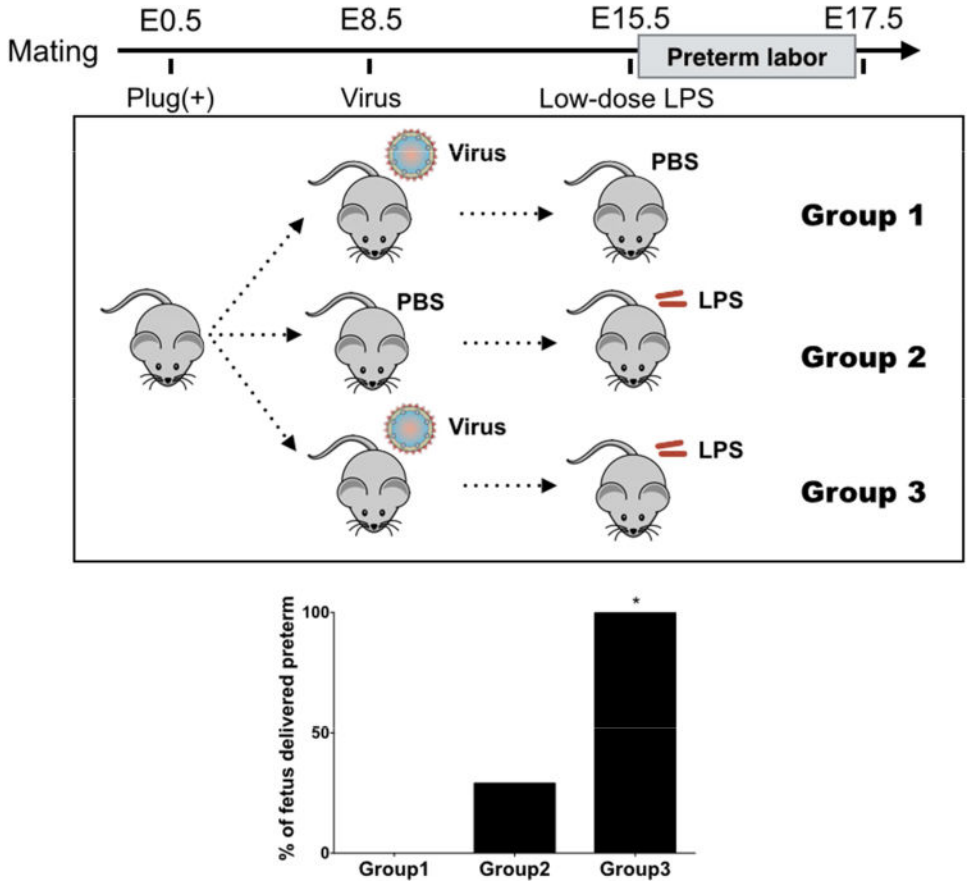

Figure 2. Murine model for preterm delivery as polymicrobial disease

C57BL/6 mice infected with murine gammaherpesvirus 68 (MHV68) followed by low dose LPS demonstrated significantly higher rate of preterm birth in comparison to MHV68 or LPS alone. (Modified from Cardenas I *et al.* AJRI 2011[14])