 doi:10.20944/preprints202211.0139.v1

Review

Chorioamnionitis and Fetal Lung: from Animal Models to Human Fetuses

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Abstract

There is disagreement on the associations between chorioamnionitis and pneumonia/sepsis, respiratory distress syndrome (RDS), and bronchopulmonary (BPD)dysplasia, three pulmonary outcomes of concern for preterm newborns. Determining clear correlations is challenging due to the intricacy of the prenatal, postnatal, and therapeutic practices that affect the diagnosis of RDS, pneumonia/sepsis, and BPD, two long-term outcomes, as well as their short- and long-term consequences. Owing to the interconnected nature of the variables, the vaguely defined fetal exposures, and the imprecise identification of disorders like RDS and BPD, multivariant studies of huge data sets are unreliable methods for defining associations. On the other hand, research using animal models offers reliable data regarding the effects of exploratory chorioamnionitis on the fetal lung. Understanding the clinical intricacy and the experimental consequences of chorioamnionitis together will help us understand on the impact on the fetal lung .

Keywords: chorioamnionitis; fetal lung; vascularization; alveolarization; lung inflammation

Introduction

Preterm birth continues to be a significant global health problem despite significant advancements in antenatal care. Preterm births make up between 5 and 12.8% of all live births in the Western world, with noteworthy regional variations. Preterm births are common, which exposes society to rising social and medical costs[1]. There are two general forms of preterm births: preterm births that were anticipated and preterm births that were followed by unplanned preterm labor or preterm prelabour membrane rupture (PPROM).

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There is no doubt that the iatrogenic direct cause in the first group is related to maternal or fetal justifications for intervention[1]. Some of them include severe prenatal growth retardation, maternal preeclampsia, hypertension, elevated liver enzymes, and low platelets (HELLP syndrome). In contrast hand, in the bigger number with spontaneous premature labor and PPROM, chorioamnionitis or intrauterine infection is commonly linked to preterm deliveries.

RDS, which is brought on by the infant lung's functional and anatomic immaturity, is strongly linked to preterm birth. Intrauterine exposure has long been known to have a moderating effect on lung immaturity. The intriguing ambiguous relationship between chorioamnionitis and respiratory morbidity was initially demonstrated by Waterberg et al. After chorioamnionitis in ventilated preterm infants, they were capable of demonstrating a drop in RDS but an increase in chronic lung injury (bronchopulmonary dysplasia, or BPD)[2]. Other researchers have since confirmed the connection between intrauterine inflammation and a lower risk of RDS, as was done earlier.

respiratory distress syndrome (RDS) reduction has been observed to be further impacted by fetal chorioamnionitis symptoms, indicating a "dose-dependent" response. A number of early human studies have found that chorioamnionitis is connected to a counterintuitive increase in chronic lung disease of prematurity despite a reduction in acute respiratory problems[3].

Meanwhile, a recent study shows a large variation in this relationship. Some of the disparity between studies may be explained by the ambiguous RDS and BPD diagnoses. The relationship between BPD and chorioamnionitis is also influenced by a number of confounding factors. The most obvious one is gestational age, which has an adverse connection with the prevalence of both disorders. Therefore, fetuses exposed to chorioamnionitis may have an increased risk of developing BPD due to their lower gestational ages[4]. The concept of a "secondary hit" is supported by recent epidemiological research, which provides an interesting expansion."According to these specific investigations, chorioamnionitis was linked to a lower rather than a higher incidence of BPD in preterm newborns. The impact was restored when mechanical ventilation was

 doi:10.20944/preprints202211.0139.v1

taken into account, although infants exposed to chorioamnionitis who were continuously ventilated for longer than a week had a higher risk of developing BPD[4].

As a result, as gestational age decreases, the proportion of preterm infants exposed to chorioamnionitis rises, reaching up to 80% below 28 weeks. Despite the fact that bacteria are only occasionally found in the uterine cavity, it is believed to be the most prevalent site of infection. *Mycoplasma* and *ureaplasma* species are two mild pathogenic pathogens that are frequently linked to chorioamnionitis[5]. According to connections between immunoregulatory gene polymorphisms and the likelihood of chorioamnionitis and premature labor, both the maternal and fetal immune systems play a significant role in chorioamnionitis.

Causative agents, Diagnosis and complications, Treatment:

The pathogens most strongly associated to very early gestational births are ureaplasma *Parvum* and *Mycoplasma hominis*. In 67% of reproductive-age women, these organisms are part of the typical vaginal flora. However, *Ureaplasma* was also found in roughly 12% of the 433 amniotic fluid samples from healthy pregnancies that were gathered for genetic testing before 20 weeks of gestation. Only 7% of amniotic fluid samples that tested positive for Ureaplasma were linked to preterm birth[5]. This human pregnancy-related *Ureaplasma* behavior is comparable to an experimental infection of fetal sheep. The chance that the several other organisms linked to preterm will expose fetuses for a protracted period of time is unknown. The fetuses of women who experience preterm labor that proceeds to ruptured membranes and delayed delivery are probably exposed to pathogens for days to months.

Both general (increased C-reactive protein, leukocytosis, and fever) and local symptoms are clinically discernible signs of more severe chorioamnionitis (vaginal discharge and uterine tenderness). The concordance with the diagnostic evaluation of chorioamnionitis has been found to be rather minor, despite the fact that a combination of these symptoms is frequently described as having "clinical chorioamnionitis" as a syndromic description[6]. It is thought that the more serious end of the continuum for chorioamnionitis is

 doi:10.20944/preprints202211.0139.v1

marked by the presence of fetal indicators of inflammation, such as funisitis or elevated interleukin-6 (IL-6) levels in the cord blood. When there is intrauterine inflammation, the fetus may be exposed through amniotic fluid contact or placental-fetal circulation.

There is strong evidence that chronic lung illness in fetuses is significantly influenced by inflammation. There have been reports of postnatally higher levels of neutrophils, macrophages, cytokines, and other mediators in the bronchoalveolar fluid of preterm infants with chronic lung disease of prematurity. Further studies have revealed that inflammatory cells produce greater messenger ribonucleic acid (RNA) of several cytokines in the bronchoalveolar fluid of fetuses with chronic lung disease of preterm.[7].

When entire lung lobe segments from preterm children with acute respiratory distress syndrome were studied postmortem, considerable interstitial inflammation was also discovered throughout the lung tissue. However, it is still unclear when this process begins and how inflammatory reactions behave inside the uterus.
Higher amounts of interleukin (IL) 1 and IL-6 were seen in tracheobronchial aspirates taken from newborns soon after delivery following a prolonged rupture of amniotic membranes, indicating that the inflammatory response may begin prior to birth. A higher IL-8 concentration in amniotic fluid, which may be related to hypothesized subclinical intrauterine inflammation, is a potential risk factor for the postnatal onset of chronic lung disease. There is currently no proof that chorioamnionitis contributes to the IL-8 generation in the lungs during pregnancy.[7].

The conventional belief is that chorioamnionitis and fetal inflammation are symptoms of a more serious inflammatory illness. Even though there is now little evidence connecting amnionitis to neonatal respiratory prognosis, a recent study contends that amnionitis may be another sign of severe intrauterine inflammation[8]. The origin of BPD was initially connected by Matsuda and colleagues to a significant fetal inflammatory response. Since then, numerous studies have examined the idea that fetal inflammation may have additional consequences on the fate of the infant. Fetal inflammation can manifest as funisitis, chorionic vasculitis, umbilical vasculitis, the "fetal reaction," or polymorphonuclear leukocyte (PMN) infiltration of the chorionic

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plate or the umbilical cord. In two studies, the prevalence of RDS was further lowered in comparison to infants with only maternal inflammatory symptoms, while no further impact was observed in the third. Multivariate research consistently confirmed the relationship between fetal inflammation and decreased RDS incidence; this effect appears to be additive to that of chorioamnionitis alone. None of the studies found that fetal inflammation increased the risk of developing BPD, as opposed to just maternal inflammation.

Furthermore, despite adjusting for potential confounders, prenatal involvement had no overall impact on the incidence of BPD, but one study observed a correlation between intense fetal engagement and a lower risk of BPD in nonwhite infants. Each of these histological abnormalities was connected to a reduced risk of RDS when compared to comparable patients without placental inflammation. It's crucial to note that only the subacute chorioamnionitis group saw a substantial rise in the prevalence of BPD. This shows that the timing and length of inflammatory stimulation throughout intrauterine life may have an impact on how the respiratory system of the newborn develops. However, because the definition of BPD was not specified, it was more challenging to assess the results. Furthermore, no association between mononuclear infiltrates and the outcomes of newborns has been found by other researchers[9].

Maternal betamethasone is the approved medication to lower RDS and infant mortality in women who are at threat of preterm labor. In addition, the majority of women who suffer early fetal uterine contractions with histologic chorioamnionitis do not show any clinical symptoms of the disease. The majority of women using corticosteroids experience birth delays of several days to weeks. As a result, although the order of exposures may differ[10], fetal exposure to prenatal corticosteroids in conjunction with chorioamnionitis is common. Depending on the precise timing of the betamethasone therapy, IA LPS injection, and premature birth, betamethasone reduces LPS-induced chorioamnionitis in sheep. When betamethasone and LPS were administered concurrently, the inflammation caused by LPS in the lungs and chorioamnion was reduced; nevertheless, it returned after 5 and 15 days.

However, IA LPS-induced inflammation and thymic cell activation were reduced when maternal betamethasone was administered before to IA LPS. Betamethasone did not decrease the inflammation brought on by IA LPS when it was administered afterward. These studies demonstrate that the anti-inflammatory effects of betamethasone are time-dependent. It's noteworthy to observe that mice subjected to IA LPS first,

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then maternal betamethasone, had less lung maturation than animals treated to IA LPS first, then betamethasone. These findings imply that in the chorioamnionitis paradigm, lung maturation is primarily driven by inflammation[10].

Animal model One can employ newly isolated trophoblastic cell lines, trophoblast cells, or placental explants to study a variety of pathogenic processes that take place during pregnancy. However, such human material does not completely replicate the intricate in vitro systems. CA(Chorioaminoitis) is governed by intricate immuno-inflammatory cascades that involve reactions from both the mother and the fetus. The only way to comprehend such complicated processes is at the mechanistic level in integrated systems, like an animal model of CA[11].

There have already been several animal models of CA in different species, including sheep, rabbits, monkeys, and guinea pigs. Murine models, however, are the most usually utilized in the first line for practical and financial reasons. Invasion of the placenta by hematogenous agents, including, Cytomegalovirus, Herpes Simplex, Toxoplasmosis, Rubella, and other agents, has been documented. Due to this, it was decided to concentrate on the ascending route in this study and use the mouse and rat models of CA. At 50 days of gestation, sheep fetuses exposed to intra-amniotic ureaplasma exhibit high titers of organisms that survive continuously for 100 days until term with a 20% fetal loss. Notwithstanding the organism's endurance, chorioamnionitis does not affect all mammals.

Intrauterine injections of agonists or live bacteria have been used to create mice and rabbit models of chorioamnionitis. Another way to cause chorioamnionitis is to inject several agonists into the sheep's amniotic fluid, including live *Ureaplasma parvum*, IL-1ß, and IL-1. The Rhesus macaque develops chorioamnionitis when Group B *Streptococci, Ureaplasma parvum*, IL-1ß, or TNF are injected intra-amniotically. While poly I:C (Polyinosinic:polycytidylic acid), a TLR3 ligand, had no impact, PamCysK4, a TLR2 ligand, caused modest fetal lung inflammation when delivered intraamniotically to sheep. It's worth noting that intra-amniotic injections of TNF, IL-6, or IL-8 did not trigger preterm labor in rhesus macaques or lung inflammation in fetal

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sheep. These assays demonstrate the comparative potency or specialized nature of responses to different inflammatory agents[11].

Mechanism of lung Injury

The intrauterine infection that causes chorioamnionitis is accompanied by elevated levels of granulocytes, proinflammatory cytokines such as tumor necrosis factor (TNF)-a, interleukin (IL)-1, interferon (IFN) IL-6 and IL-8, and granulocyte colony-stimulating factor (G-CSF). Bronchopulmonary dysplasia is more likely to occur in children whose mothers' amniotic fluid contains higher than normal levels of proinflammatory cytokines (BPD).

The number of circulating neutrophils in the lungs rises soon after birth in newborns who have previously experienced chorioamnionitis. Furthermore, large levels of, IL-8, IL-6, TNF-a, IL-1 GM-CSF (granulocyte-macrophage colony-stimulating factor), and other substances, such as proteolytic enzymes and profibrotic proteins like fibronectin, are present in bronchoalveolar lavage samples [12].

High levels of proinflammatory cytokines and inflammatory mediators during pregnancy impair the developing fetal lungs and may even be the cause of the injury that leads to CLD, according to growing evidence. According to studies, chorioamnionitis results in inflamed intrauterine lungs in newborn humans. Neutrophil and macrophage infiltration as well as increased IL-8 mRNA levels in the interstitial tissue and bronchoalveolar epithelium are associated with this inflammation. Studies have also examined the effects of chorioamnionitis on the developing lungs of sheep and rabbits. [12].

Inflammation was seen in the fetal lungs of rabbits according to histological analysis, and the proinflammatory cytokines IL-1b, IL-8, and TNF-a were more frequently expressed in the fetal lung tissues of sheep. Additionally, research employing a number of animal models has shown that lung maturation is expedited, the production of surfactant proteins is changed, and lung tissue surfactant is enhanced despite efficient release when fetuses are subjected to inflammatory mediators in the amniotic fluid[13].

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Exposure to chorioamnionitis, which has high levels of cytokines and other chemicals produced by inflammatory cells in the amniotic fluid, may have an impact on apoptosis and proliferation in the fetal lung. For instance, it is well known that the pro-apoptotic proteins TNF-a and IFN-c are present in the amniotic fluid of pregnant women with chorioamnionitis, and that G-CSF promotes proliferation. Adult lung diseases such as interstitial pulmonary fibrosis, chronic obstructive pulmonary disease, acute respiratory distress syndrome, and CLD have all been convincingly linked to apoptosis or proliferation. Caspases 8 and 9 are crucial for the intracellular transmission of proapoptotic signals. They take involved in the two main apoptosis signaling pathways[14]. The apoptotic cascade initiated by death receptors depends on caspase-8. On the other hand, stimuli that do not require the death receptor can cause apoptosis by activating caspase-9 through the mitochondrial pathway.

Studies using both human and animal models strongly imply that chorioamnionitis and fetal lung damage occur earlier in the gestational period. Elevated levels of IL-6, IL-8, IL-1, and tumor necrosis factor (TNF) in the amniotic fluid of preterm deliveries may be used to predict the chance of developing bronchopulmonary dysplasia. The amniotic fluid of preterm newborns who exhibited histological evidence of chorioamnionitis had higher levels of IL-6, IL-8, IL-1, and tumor necrosis factor(TNF).[15]. Even though these significant associations signal long-term harm to the growing lung tissue, the relationship between chorioamnionitis and pediatric asthma has not been fully explored. Moreover, it is not yet known whether the intensity of the connection is influenced by birth gestational age or race/ethnicity.

Those who are predisposed to developing asthma will have recurrent wheezing, shortness of breath, tightness in the chest, and coughing fits. In allergic asthma, the allergic cascades is essential for the development of airway inflammation. Inhaled allergens that evade mucociliary clearance are taken up and processed by antigen presentation cells (APCs), which are located all through the respiratory system, from the nasal mucosa to the lung pleura[15].

These APCs then proceed to the draining lymph nodes where they transmit the processed allergen to T and B cells that are specific to that allergen. Various factors, including the presence of cell-bound costimulatory molecules and released cytokines, influence how these cells interact with one another to produce responses.

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Cytokines are created when T helper (Th) cells are stimulated by APCs. These cytokines regulate the B cells' isotype switch, which regulates the production of immunoglobulin (Ig) E. Before engaging with basophils in peripheral blood or mast cells in tissue, which carry the high-affinity IgE receptor FcRI, IgE antibodies are generated and circulate in the circulation[16].

An upsurge in pulmonary surfactant lipids and surfactant-associated proteins A, B, and C is one of the startling and unanticipated results of intra-amniotic injection of LPS or IL-1 in fetal sheep. "Clinical lung maturation" is the phrase used to describe the improvement in preterm lung mechanics brought on by a growth in airway surfactant and changes to lung shape. The chorioamnionitis-induced lung maturation is more powerful than the effect of the betamethasone that the mother of the sheep administered to the sheep throughout pregnancy[16]

However, fetal intratracheal injection of LPS or IL-1 resulted in faster lung development, indicating that lung development only requires rapid airway interaction with LPS or IL-1. LPS intravenous infusion and tracheal closure prevented lung development. The precise signaling that triggers the development of the fetal lungs is uncertain[17]. However, intramuscular anti-CD18 antibody injection significantly lowered lung maturation and inflammatory cell influx in the fetal sheep. Similar to this, human IL-1 receptor antagonists inhibited IA LPS (Intra amniotic) induced lung maturation.

These studies on fetal sheep show that IL-1 signaling is essential for lung development brought on by LPS. Because the amount of inflammatory cells drawn to the lung is correlated with the extent of the surfactant lipid pool, it may be difficult to discriminate between the pro-inflammatory impacts of these agonists and the beneficial lung development effects. Despite the advantageous lung maturation, proper lung development is hampered by IA injection of pro-inflammatory agonists.

Sheep exposed to IA LPS had fewer alveoli, thinner alveolar septae, and larger alveoli. Alveolarization depends critically on the generation of elastin, which identifies secondary septation sites. IA LPS changed and decreased the expression of elastin. LPS-exposed fetal mouse lung explants displayed a reduced growth of terminal respiratory units. LPS reduced the expression of FGF-10 in this explant model, which required

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macrophage NF-B signaling[18]. The preterm fetal sheep's VEGFR2(Vascular endothelial growth factor receptor 2) and NOS III((Nitric Oxide Synthase 3) genes, among others, were all inhibited by IA LPS in addition to other genes. IA LPS induced adventitial fibroblast proliferation in the sheep lung embryo and smooth muscle hypertrophy in the resistance arterioles. The pulmonary vascular resistance increased as a result of these alterations in vascular remodeling.

Immunomodulation and alveolar count

It has long been known that human and rodent immune systems develop at different rates. In contrast to G8, which corresponds to 10 weeks of gestation in mice, hematopoiesis and immune cell differentiation in humans begin in utero at 5 weeks of gestation. Inflammatory cytokines and chemokines, which are released by trophoblastic cells, invading macrophages, and PMNs(Polymorphonuclear neutrophils), are primarily found in the placenta. These proteins aid in the inflammatory response in developing fetuses[19].

It is still unknown, though, whether the harmful fetal neuroinflammatory cascade develops in the placenta, the mother, or a mix of these two sources. Inoculation with an infectious pathogen causes premature birth in mice 24 hours after injection. Given the rapidity of work, it is challenging to examine the processes of inflammatory reactions[19]. The absence of premature delivery in rats enables cesarean sections to be performed 24, 48, 72, and even up to 5 days following injection. This option enables detailed investigations of inflammatory reactions.

While the fetus can start an inflammatory response to LPS at a preliminary phase of gestation, the response is not completely developed because fetal sheep did not produce an immune response to TNF-a and TLR agonists. The development of innate immunological resistance and sensitivity to the intra-amniotic endotoxin happened at the same time, it was found. Until late in gestation, alveolar macrophages and interstitial monocytes are virtually missing from the fetal lung. The fetal lung's monocytes also produce little to no cytokines or reactive

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oxygen species in reaction to endotoxin or TNF-a, which is consistent with their immaturity. Intra-amniotic LPS treatment caused a cascade of alveolar macrophage maturation in the fetal lung, as well as the production of granulocyte-macrophage colony-stimulating factor and transcription factor PU.1 in the monocytes[20]. The lung monocytes/macrophages were unaffected by endotoxin or other proinflammatory stimuli when stimulated in vitro. Fetal cells were simultaneously growing and acquiring LPS tolerance. Additional TLR agonists and the proinflammatory reactions induced by LPS were also included in the tolerance. For a fetus to live in a pro-inflammatory environment like chorioamnionitis, the inertness of the inflammatory response may be crucial. Only recently have scientists begun to understand the biological origins and clinical implications.

The lung monocytes/macrophages were unaffected by endotoxin or other proinflammatory stimuli when stimulated in vitro. Fetal cells were simultaneously growing and acquiring LPS tolerance. Additional TLR agonists and the pro-inflammatory reactions induced by LPS were also included in the tolerance[21]. A fetus' ability to improve in a pro-inflammatory environment like chorioamnionitis may depend on the inflammatory response's inertness. Experts are only now studying and comprehending the clinical importance and biological foundation of these phenomena.

Alveolar macrophages are the lung's sentinel immune cells. Macrophages are found in the gaps just next to the alveolar hypo-phase in adult humans and other animals. Most of the time, fetuses lack alveolar macrophages. While sheep and nonhuman primates rarely have mature macrophages in the fetal lung, mice can have macrophages observable in the lung interstitium as soon as during the first trimester[21]. When postnatal air-breathing starts, mature alveolar macrophages considerably overpopulate the lung in all species. Preterm lambs' immature lung monocytes react to an in vitro challenge with LPS only sporadically (IL6 release), but not to TNF. IA LPS, on the other hand, promotes the production of GMCSF and PU, which leads the lung monocytes to develop.

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These monocytes are able to enter the embryo's alveolar spaces and have a strong response to TNF and LPS in a test tube. IA LPS may cause the fetus to acquire an innate immune resistance.

Adult animals and people both exhibit endotoxin endurance, which is the reduction of LPS signaling accomplished through a highly complicated regulation of inflammatory responses. Anti-inflammatory, antibacterial, and order-to-provide-feedback genes are either generated more or less than previously, but pro-inflammatory cytokine expression is downregulated[22]. IA LPS 2d causes the fetal lung to produce a significant number of cytokines in preterm lambs prior to birth. The fetal lung is resistant to the second injection, nevertheless, if the fetus receives two identical IA LPS injections at 7 and 2 days before delivery.

In vitro LPS treatment showed no impact on blood and lung monocytes, which is significant to note[22]. Lung and blood monocytes exhibit larger amounts of IRAK-M, a bad regulator of Toll/IL-1 signaling, even if the precise mechanisms are still unknown. Contrary to adult monocytes with endotoxin endurance, which only sometimes express IL-10, IL-1RA and TGF-ß1, the fetal lung with endotoxin tolerance showed practically full down-regulation of these cytokines. When fetal sheep received two injections of IA LPS, other TLR agonists such as, PamCysK4 (TLR2), CpG-DNA (TLR9) and flagellin (TLR5) had no impact on the lung and blood monocytes.

Conclusion

Studies involving human fetuses are extremely uncommon, and the issues addressed do not fully reveal how chorioamnionitis and fetal lung growth are related. Animal models, in most cases given LPS injections to cause chorioamnionitis, have confirmed the results of those investigations. Not all gestational ages have been thoroughly researched. Important aspects like vascularization and alveolarization in humans have not been sufficiently described. More research should be done on developing fetuses. Animal models show numerous organs exhibiting tiny organ injury responses that are not visible using standard imaging methods like X-rays or ultrasound. Despite the minor increases in inflammation that can be seen in the blood, the damaging response in the preterm fetus can be exceedingly detrimental. It is also complex because immunological changes and inflammation are stacked upon growing organs. Fetal surgical studies have revealed the

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significance of lung inflammation brought on by chorioamnionitis. Since the disease is responsible for substantial numbers of preterm births, the

research in the area discussed holds a potential advantage, as it would help in reducing the incidents of such cases.

Conflict of Interest Statement:
The authors declare no conflict of interest

Funding sources:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 1- Details of a few important studies, which were taken into consideration.

Nature of study	**Objectives of the study**	**Major outcomes**	**References**
Review paper	To elucidate reasons and epidemiology of preterm births.	Reasons- intauterine growth, eclampsia and pre eclampsia	Goldenberg at al., 2008
Review paper	To determine the essentiality of choriominotis in BPD development	substantial relation between both exists.	Thomas and speer, 2014
Review paper	Assessing the impact of microbiota on immunity of host	Gut microbiome has a major impact on immunity	Inavov, 2012
Experimental	assesing impact of ureplasma colonization	ureplasma exposure can supress LPS induces choriominoits	Synder at al., 2018
Exploratory	Developing animal model for intrapartum inflammation	Brain injury follows isolated sytematic maternal inflammation	Dell' ova et al., 2015
Observational	To determine impact of choriominoits on ploiferation and apoptosis	through caspase-8 pathway apoptosis is induced	May M et al., 2008

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Exploratory	To determine concentration of amniotic cytokines in neonates in case of BPD	Antenatal exposure to Cytokines can lead to BPD development	Yoon et al., 1997

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