The period of gestation during pregnancy is of immense importance for an infant's subsequent health and survival, which in humans is 37-40 weeks. The phenomenon of normal pregnancy may be compromised by a number of complications, such as threatened abortion, recurrent spontaneous miscarriage, preeclampsia, and preterm delivery (PTD) (1, 2). Preterm delivery which occurs before 37 week of complete gestation period is one of the major complications in the pregnancy globally and is the key factor associated with prenatal mortality and morbidity (3, 4). Complications associated with preterm delivery are the leading cause of death among children under 5 years of age, and is responsible for nearly 1 million deaths in 2015. Preterm delivery may predispose the baby to various medical complications due to immature lungs, intraventricular haemorrhage, immature gastrointestinal and digestive system, anaemia, retinopathy of prematurity, necrotizing enterocolitis, sepsis etc, which may be lethal for the baby. Preterm delivery is further divided into three different cohorts viz., extremely preterm (<28 weeks of gestation), very preterm (28 to <32 weeks of gestation) and moderately preterm groups (32 to <37 completed weeks of gestation) based on gestation period (5, 6). Most premature babies (almost 80–85%) are born between 32 and 37 weeks of gestation, whereas about 10% and 5% of preterm babies are born between 28 and 32 weeks, and before 28 weeks of gestation respectively, and result in high number of fetal mortality cases, especially in low income countries (7). India occupies the top most position in terms of highest incidences of preterm delivery (7). Anthropological study based data have documented the high prevalence of preterm delivery in North-eastern region of India (8). The issue of preterm delivery is increasing at alarming rate. As per world health organisation (WHO), every year an estimated 15 million babies are
born preterm (1 in 10 babies), and this number is constantly rising (7). Although many factors appear to be associated with preterm delivery, the exact cause for preterm birth in many situations is elusive and unknown; making the control of preterm delivery a challenging proposition. Preterm delivery has been presumed to be multifactorial complex disorder involving genetic, immunological including deleterious immune response of the mother toward the fetus, pathophysiological and environmental alterations (3, 9, 10); but existing data is equivocal and inconclusive.

Several pathways are known to play a critical role in sustenance and maintenance of a successful pregnancy; the folate pathway (11) and progesterone pathway (12) being the two most important ones. Therefore any defects or abnormalities in these two pathways may have adverse effect in pregnancy.

Folate is an important component of the Vitamin B9 family which is involved in different cellular biosynthetic process such as division and a growth of a cells as well as DNA synthesis and methylation (13). Folate therefore is one of the key factors essential for maternal well being as well as fetal growth and development during pregnancy and any deficiency in folate levels thus may result in adverse pregnancy outcome and complications (14). The 5-Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the folate pathway which catalyzes the conversion of 5, 10-MTHF to 5, MTHF (Methyl tetrahydrofolate) which is a circulatory form of folate that act as a methyl donor in remethylation and conversion of homocysteine to methionine (15). Defect or loss in the enzyme activity of MTHFR can be attributed to adverse pregnancy outcome because of decreased circulatory folate and increased homocysteine levels (16). The loss of enzyme activity of MTHFR (50–70% loss of activity in mutant homozygote and 30% loss in heterozygote) is a resultant of the common polymorphism
of MTHFR gene at position 677 whereby the cytosine is substituted by thymine (677C → T) (rs1801133) (17). MTHFR C677T polymorphism and deficiency of folate is thus found to increase the risk of spontaneous abortion, preterm delivery, or intrauterine growth restriction (IUGR) during pregnancy (18).

Thymidylate Synthase (TYMS) is another key enzyme of folate metabolism pathway involved in DNA synthesis and repair by participating in the denovo synthesis of thymidine nucleotide using 5, 10-MTHF as a methyl donor (19, 20). TYMS catalyzes the reaction involving the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) using 5, 10-MTHF as a methyl donor for the synthesis of thymidine (20, 21). Hyperhomocysteinemia has been established to be associated with adverse pregnancy outcome such as preeclampsia, placental abruption, recurrent pregnancy loss (RPL), IUGR in fetus, intra uterine death (IUD), low birth weight (LBW) and preterm delivery (22-24). The basic mechanism of homocysteine metabolism involves the remethylation of homocysteine to methionine by enzyme methionine synthase using substrate 5, 10-MTHF which also act as a substrate for TYMS during DNA synthesis and repair mechanism, indicating the possible role or influence of enzyme TYMS in homocysteine metabolism (13). Thus, abnormalities in TYMS gene are presumed to be associated with hyperhomocysteinemia which will influence pregnancy by affecting embryogenesis and fetal growth (13, 25). The untranslated (UTR) region of TYMS is found to have three different polymorphisms which inturn altered the activity and stability of TYMS enzyme at mRNA level. TYMS 1494del6 6bp deletion (TTAAAG) (rs16430) in 3’UTR region has been shown to be associated with the alteration of mRNA stability of TYMS gene (26, 27). Because of its participation in DNA synthesis, which is pivotal during pregnancy for fetal
development, it may be hypothesized that TYMS 1494del6 polymorphism may play a key role in the susceptibility to preterm delivery and associated complications. Available literature shows that variable TYMS genotype have been found to be associated with some adverse pregnancy outcome and congenital anomalies such as RPL (28), neural tube defect (NTD) (29) and congenital heart defects (30), but till date there is no report on the association between TYMS 1494del6 polymorphism with preterm delivery and associated complications.

Hyperhomocystenemia has been shown to be associated with adverse pregnancy outcome such as preeclampsia, placental abruption, RPL, IUGR in fetus, IUD, abortion and preterm delivery (22, 23). Increased levels of plasma homocysteine levels were found in woman with the risk of abortion and preterm delivery (24, 31). The basic mechanism of homocysteine metabolism involves the remethylation of homocysteine to methionine by enzyme methionine synthase using substrate 5, 10-MTHF indicating here the possible role of enzyme MTHFR and TYMS in homocysteine metabolism (13). Thus, abnormalities in gene associated with folate metabolism (MTHFR C677T and TYMS 1494del6 polymorphism) resulting in hyperhomocystenemia likely influence the pregnancy by affecting embryogenesis and fetal growth (13, 25).

Cellular uptake of folate is mediated by folate receptors. Folate is critically required in high amount during the pregnancy for proper growth and development of fetus and during pregnancy folate is transported by abundantly located folate receptors in placenta (32). Amongst the characterized folate receptors, the role of folate receptor alpha (FR-α) a GPI anchored protein have been proved to have critical importance. The effective role of FR-α in successful pregnancy has been demonstrated by experiments on FR-α
knockout mouse which was susceptible to embryo toxicity (33) but lacunae exists on its role in human preterm delivery and associated complications.

Another critically important pathway associated with pregnancy is the progesterone pathway. Progesterone plays a key role in establishment and maintenance of pregnancy (34). The physiological function of progesterone during pregnancy is maintained by progesterone receptor (PR) therefore any abnormalities in PR gene may results in adverse pregnancy outcome (35). Studies show the association of three linked single nucleotide polymorphism (SNPs) in exon 1, exon 4 and exon 5 of the PR gene with the recurrent spontaneous abortion (36). Further the SNP in the exon 1 is linked with the SNPs in exon 4 and 5 (36) and these in turn are found to be in linkage disequilibrium with PROGINS which is referred to as ALU insertion of 306 bp in intron G between exons 7 and 8 of the coding region of hormone binding domain. (36), this polymorphic variant of PR gene is found to be associated with the implantation failure during pregnancy (37).

Scientific evidences suggests that progesterone plays a critical role in the maintenance of pregnancy by establishing an adequate immune environment during pregnancy (38) and inhibiting myometrial contractility as the onset of labor is thought to be the result of progesterone withdrawal (34). Progesterone helps in differentiating the resting human peripheral blood T cells into Th2-type clone cells, which is required during pregnancy (39). The immunological effects of progesterone depend on the availability of progesterone receptors (PRs) which act as nuclear transcription factors, and also as modulators of cell signalling pathways (40). Deregulation in PR expression has been shown to be associated with threatened spontaneous miscarriage or preterm delivery (41, 42). The immunological function of progesterone is mediated by a lymphocyte-
derived immunoregulatory protein called progesterone induced blocking factor (PIBF) which act as a downstream effectors of progesterone (43). Being an immunoregulatory protein PIBF induces inhibitory effects on cell-mediated immune reactions (44) such as regulating the cytotoxic activity of T cells and proliferation of allogeneically stimulated lymphocytes (45, 46) and thus PIBF appears to play an important immunoregulatory role in successful pregnancy (42, 47, 48). Limited literature is suggestive that downregulation of PIBF levels in serum, urine and peripheral lymphocytes is associated with increased preterm delivery and miscarriage (10, 42).

Cellular immune effector have been proposed to be associated with pregnancy complications according to the several researches conducted in last decades. Most importantly the effect of proinflammatory and anti-inflammatory cytokines on the fetus and finally on the success or failure of pregnancy is the main highlights of the study. Increased activity of Natural Killer (NK) cells leads to fetal resorption and spontaneous abortion of unknown etiology, and PIBF has been reported to block the activity of Natural Killer (NK) cells during normal pregnancy (47, 49). Another unique sub group of lymphocytes with features of both T-cells and NK-cells which is thought to play an important role in successful pregnancy is NKT like cells (50). The aberrant activation of NKT like cells leads to the elevation of type 1 T helper (Th1) immune responses which further activates other immune cells such as NK cells in deciduas thereby resulting in increased cytotoxic activity of NK cells as well as cytokines thus resulting in unsuccessful pregnancy (51).

Successful pregnancy requires an alteration of maternal immunity within the uterus where the innate proinflammatory immune response is tightly regulated to prevent the rejection of fetal allograft (52). Th1 type maternal immune response has been shown to
have detrimental effects in the development of fetus resulting in unsuccessful pregnancy (53). Thus the aberrant production of Th1 type proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin-12 (IL-12) and interferon gamma (IFNγ) at the maternal-fetal interface is harmful to pregnancy (52), on the other hand the production of type 2 T helper (Th2) anti-inflammatory cytokines such as interleukin-10 (IL-10) imparts protective role in maintaining the pregnancy (54). The onset of labor after completion of gestation period during pregnancy is associated with the inflammatory responses in uterus and maternal fetal interface but if the inflammatory response gets activated untimely than it results in preterm labor resulting in preterm delivery of a baby (55). Several reports have illustrated the presence of elevated levels of Th1 inducing proinflammatory cytokines in the placenta of a preterm delivery and preterm premature rupture of membrane (pPROM) cases and elevated levels of Th2 type anti-inflammatory cytokines in term placenta (56). The elevated level of TNF-α have been registered in amniotic fluid (57) and choriodecidual tissues (58) of woman with preterm delivery. Proinflammatory cytokines such as TNF-α results in the production of prostaglandins and matrix metallo proteinases (MMPs) (59), via nuclear factor kappa beta (NF-κβ) resulting in the chain of pro-labor events such as contraction of uterus and rupture of fetal membrane before delivery of a baby but if this chain gets activated untimely than there is a possibility of occurrence of preterm labor (60). Inhibition of NF-κβ activation is another attractive strategy to prevent preterm labor as NF-κβ activation is central to the activation of labor-associated genes in labor (61). Activation of NF-κβ leads to a proinflammatory response in various cytokines including TNF-α (62) and IFNγ (63).
Pathological infections of different etiologies are always a critical component in deciding the fate of the pregnancy especially in developing low income countries. Pregnancy being a Th1 regulated condition, the situation predisposes the mother to several bacterial, protozoan, and viral infections, predisposing both the mother and the fetus to lethal consequences during pregnancy. Pathological study on placenta of preterm delivery cases has already established the association of intrauterine infection with the occurrence of preterm delivery (64). Apart from this urinary tract infection (UTI) comprising of different microbes (bacterial, protozoan, fungal etc) at present the viral infection has emerged out as an important aspect associated with the preterm delivery (65) with a deleterious effect in maternal as well as fetal health (66). Hepatitis virus is one of the important virus found to be associated with the complications in women during pregnancy, whereby hepatitis E virus (HEV) infection have been found to be more severe in case of pregnancy causing 20-25% of maternal mortality in the third trimester (67) as well as high rate of prenatal mortality because of its transplacental transmission ability (68, 69) compared to non HEV infected pregnant women and non pregnant women cases. Severity of HEV infection results in acute viral hepatitis (AVH) or fulminant hepatic failure (FHF) resulting in increased maternal as well as fetal mortality (70). High viral load in case of FHF compared to AVH and HEV genotype 1 has been reported to be associated with diseases severity in HEV infected pregnancy cases (71, 72). There are reports showing the association of high risk of preterm delivery as well as prematurity and LBW in HEV infected pregnant cases compared to non HEV infected pregnant woman (72-74). There are many reports from developing countries including India, showing HEV as one of the potential causes of pregnancy complications (75, 76). Differences in pregnancy with respect to (w.r.t) the
gestation period and outcome exists in HEV infected AVH and FHF cases, which is suggestive that apart from HEV genotype and viral load, host factors may play an important role in it. Limited literature from North Indian population supports that alterations in PR pathway and associated immunomodulation may play an important role in HEV infected pregnancy (73). But lacunae exists in terms of the role of differential modulations in the folate pathway, PR and associated differential immunomodulation, and the alterations at critical points resulting in preterm delivery in HEV infected cases; which also makes it a suitable model system to test the role and biomarker panel significance of our studied candidate pathways in preterm delivery with or without any underlying pathology.

Preterm delivery is a global problem, but more importantly it is a national and regional problem because of the highest global incidences and alarming increase in the incidence rate respectively. Because of poor socio-economic condition and associated poor hygiene, Northeast India is predisposed to preterm delivery and associated complications. Infections with pathogens including HEV can be detrimental for pregnancy, and may be associated with maternal and fetal mortality or morbidity. It is therefore hypothesized that “deregulation(s) in folate and progesterone pathway, the abnormality and alterations in the immunological response during pregnancy along with the pathogenic infections especially HEV infection can be the underlying mechanism for the complications during the pregnancy and preterm delivery.” Based on the global, national and regional concern about preterm delivery, the risks associated with the issue, and the lacunae in the existing knowledge on the underlying molecular aetiology associated with it; the present study focussed to delineate the mechanism(s) which
critically predisposes to preterm delivery and find out the possible targets for regulating or controlling it.