**Gestational Epigenetics**

Pregnancy can drive a range of physiological and molecular changes that have the potential to contribute to pathological conditions. There is strong evidence that both genetic and epigenetic modifications influence the course of pregnancy. In this review, we provide an overview of the epigenetic changes that influence pregnancy-related molecular programming in an effort to fill the gap in current understanding regarding interactions between the environment, the fetus, and pregnant women.

1. **Epigenetics**

Epigenetic inheritance can be defined as heritable changes in gene expression or cellular phenotypes that occur without altering the underlying DNA sequence (Bird, 2007; Richards, 2006). In most cases these epigenetic modifications produce reversible changes in gene function, influencing gene expression through several mechanisms (Best & Carey, 2013). Underlying mechanisms governing epigenetic changes include DNA methylation, which usually impacts cytosine guanine dinucleotide (CpG) motifs, and post-translational modifications of the amino-terminal (N-terminal) tails of histones (including methylation, acetylation, phosphorylation, and ubiquitination). Other epigenetic regulatory mechanisms include RNA regulation through the ability to serve as enhancers of transcription, decoy microRNA (miRNA) targets, and mediators of chromatin-modifying complex recruitment to specific locations. Several environmental alterations that have been linked to epigenetic changes include starvation and various chemical exposures. The physiological changes that occur with progression from pre-pregnancy through pregnancy to the postpartum state may include changes in methylation patterns in multiple tissues, as well as in maternal leukocytes, potentially influencing processes including implantation, trophoblastic invasion, vasculogenesis, and maternal immune tolerance (White et al., 2012).

* 1. **Maternal DNA methylation during pregnancy**

DNA methylation is the most studied type of epigenetic modification, and it has been widely assessed in pregnant women (Aldhous, Hor, & Reynolds, 2018). DNA methylation occurs through the addition of a methyl group at the C-5 position of cytosine in the context of a CpG dinucleotide. Methylated DNA is usually found in areas of the genome that are inactive or silent, whereas unmethylated regions correspond to actively transcribed DNA areas (Pozharny, Lambertini, Clunie, Ferrara, & Lee, 2010). Various cellular processes, such as genomic imprinting, chromosomal stability, chromatin structure, embryonic development, cellular differentiation, X-chromosome inactivation, and transcription are regulated by DNA methylation (Perera, 2018). Epigenetic regulation has been shown to be linked to human health, primarily because certain modifications established during early development are labile or metastable and modifiable by environmental factors. This presents an opportunity to develop therapeutic or preventative strategies for use during periods of high plasticity of the epigenome (Ávila, Echeverri, de Plata, & Castillo, 2015; Chmurzynska, 2010; Waterland et al., 2010). CpG methylation is linked with gene silencing, and clusters of CpG sites, known as CpG islands (CPI), are often methylated. Gene expression patterns can thus be modified through the methylation of the DNA encoding specific genes.

The enzymes responsible for DNA methylation include the methyltransferases (DNMTs) DNMT1, DNMT3A, and DNMT3B. DNMT1 copies the pattern of DNA methylation during genomic replication and is required for the maintenance of all methylation in the genome. DNMT3A and DNMT3B induce de novo DNA methylation and are responsible for establishing new methylation patterns (Clouaire & Stancheva, 2008; Fernández-Morera, Rodríguez-Rodero, Menéndez-Torre, & Fraga, 2010; W. Zhang & Xu, 2017).

* 1. **Pregnancy and epigenetic changes**

A growing body of evidence indicates that maternal lifestyle and prenatal factors are associated with serious health consequences and diseases later in life. Epigenetic mechanisms such as DNA methylation, chromatin modifications, and the modulation of gene expression during gestation are believed to contribute to the development of various diseases and disorders. During pregnancy, physiological changes occur to nurture the developing fetus and prepare the mother for labor and delivery. Some of these changes impact cardiac output, insulin resistance, and normal biochemical parameters whereas others may mimic symptoms of specific diseases. Thus, pregnancy is characterized by dramatic changes in metabolism. Pregnancy is coordinated by well-characterized hormonal alterations that drive key developmental processes such as placentation, ensuring that they occur at the correct time and that parturition occurs once the fetus is fully developed (Best & Carey, 2013). A complex series of epigenetic changes, together with these hormonal aspects of pregnancy, serves to coordinate the developmental timing necessary for a successful pregnancy. Both genetic and environmental factors can shape pregnancy outcomes (Dadvand et al., 2013). However, far less attention has been paid to the effects of pregnancy on the mother, although chromatin epigenetic changes are an important component of long-term responses to hormonal signaling.

During specific periods (e.g. pre-conception, oocyte fertilization, gestation, and the first few years of life), tissues and organs are particularly sensitive to numerous environmental stressors and lifestyle factors that condition the organism and shape susceptibility to disease later in life. Several studies have offered insight regarding the impact of early life stress during the developmental stages on adverse pregnancy outcomes. These factors modulate cellular function and gene expression through mechanisms including DNA methylation and histone modification. These epigenetic mechanisms can serve as central regulators of systemic physiological and biological processes, diseases, and placental development and function (Badon et al., 2018). Only recently have studies begun to explore the effect on the epigenome during gestation, although epigenetic biomarkers have been previously used to define the pathophysiology of pregnancy complications and adverse early pregnancy outcomes (Wu et al., 2018). Pregnancy-related methylation profiling and its link with methyl-group intake in a healthy population has the potential to improve current understanding of the development of pregnancy-related disorders (Pauwels et al., 2016). The most common complications of pregnancy include ectopic pregnancy, pre-eclampsia, gestational diabetes mellitus, small gestational age, and preterm birth (Zhao, Moley, & Gronowski, 2013). Additionally, physical activity-related epigenetic alterations may also be beneficial biomarkers that can aid in the identification of high-risk women during pregnancy. Badon et. al explored the link between leisure-time physical activity (LTPA) and DNA methylation and proposed that LTPA may influence maternal epigenetic biomarkers, perhaps in an offspring sex-specific manner (Badon et al., 2018).

Although there is emerging evidence regarding DNA methylation variability over time, little is known about the dynamics of DNA methylation status during pregnancy. Gruzieva et.al performed an epigenome-wide analysis evaluating temporal changes in DNA methylation associated with pregnancy. Pregnancy is characterized by substantial physiological alterations affecting processes including the immune system and the metabolism of glucose and fats. A better understanding of epigenetic variation during pregnancy may aid in the elucidation of the biological mechanisms underlying these physiological alterations and the adaptation needed to enable fetal development. Carefully orchestrated switching of DNA methylation patterns during specific gestational windows is critical to a healthy pregnancy (Barker, 2007). Certain key fetal developmental windows exist during which dietary factors can have a pronounced impact on the pregnancy-related epigenome. For instance, the epigenome is most susceptible to environmental factors during embryogenesis owing to the extremely high rate of DNA synthesis, and during this period DNA methylation patterns are established which are essential for normal development and differentiation (Faulk & Dolinoy, 2011). However, it remains unclear to what extent maternal consumption of methyl groups during pregnancy may affect maternal DNA methylation. Furthermore, researchers that assess maternal DNA methylation are generally interested in detecting pregnancy-related illnesses and preterm birth to provide a better understanding of how epigenetic variations during pregnancy can help to explain the biological mechanisms underlying important physiological alterations and adaptations needed for appropriate fetal development and to prepare the mother for childbirth and the postnatal period (Gruzieva et al., 2019). For example, Anderson et al. reported the existence of significant differences in the first trimester DNA methylation patterns of maternal white blood cells collected from pregnancies complicated by preeclampsia. Moreover, Enquobahrie et al. found that maternal DNA methylation patterns in blood samples collected after 16 weeks of pregnancy were different in women who had two consecutive pregnancies relative to women that had only one pregnancy complicated by gestational diabetes mellitus (GDM) (Enquobahrie et al., 2015). In addition, Burris et al. found that higher early pregnancy maternal LINE-1 methylation in white blood cells predicts a lower risk of preterm birth (Burris et al., 2012). Gruzieva et al. performed an epigenome-wide longitudinal DNA methylation study (EWAS) of a well-characterized sample before, during, and after pregnancy and identified 196 CpG sites displaying intra-individual longitudinal changes in DNA methylation. Most of the differentially methylated genes exhibited a decrease in the average methylation levels over the studied period. Recent evidence suggests that in addition to the genome, maternal lifestyle shapes the trajectory of pregnancy and has a lasting impact on children (Das & Maitra, 2021; Rappoport et al., 2018). For instance, the Hunger Winter Families study found that the adult mothers who were prenatally exposed to famine harbored hypomethylation patterns in the maternally imprinted insulin-like growth factor 2 (*IGF-2*) gene, a key factor involved in human growth and development (Heijmans et al., 2008; Lumey et al., 2007; Smith, Garfield, & Ward, 2006).

Many studies have revealed the ability of epigenetic modifications to induce lifelong changes in offspring exposed to unhealthy maternal nutrition and lifestyle factors such as obesity and GDM (Agarwal et al., 2018; Franzago, Fraticelli, Stuppia, & Vitacolonna, 2019; Gagné-Ouellet et al., 2017).

* 1. **Typical pregnancy-related changes in DNA methylation**

Pregnancy-related risk factors can influence maternal DNA methylation patterns, potentially increasing clinical risk and rendering mothers susceptible to long-term consequences. Immune tolerance is induced during pregnancy to ensure that mothers do not reject the sem-allogenic fetus. Accordingly, the first and early second trimesters of pregnancy are considered to correspond to an inflammatory phase (Erlebacher, 2013; Svensson-Arvelund et al., 2014). Leukocytes play a critical role in the proliferation, inflammation, immune tolerance, and maternal adaptation processes that are integral to normal placental and fetal development. White et al. found evidence of global pregnancy-related hypomethylation in maternal leukocytes relative to that observed in non-pregnant individuals, leading to the identification of candidate genes that are involved in maternal adaptation to pregnancy based on their altered methylation profiles. These included hypomethylated immunity-related genes (*IL1R2, IL1β*, and HPR), gametogenesis-related genes (*SPAG4, CCIN*), and housekeeping genes (*PC*, *NDFUS2*). The involvement of interleukin (IL)-1 family genes has previously been reported to be important in the establishment and progression of pregnancy. These authors further found that the hypomethylation of the haptoglobin-related protein (*HPR*) gene may contribute to increases in HPR expression during normal pregnancy. HPR is a secreted plasma protein associated with high-density lipoprotein (HDL) particles, which play a role in the innate immune response. Many studies have identified changes in DNA methylation as a possible mediator of the effect of prenatal stress on offspring. Babenko et al., for example, hypothesized that prenatal stress has epigenetically-regulated effects on health and diseases of the nervous system from early development to old age. For example, in placentas affected by preeclampsia, increased DNA methylation has been observed at CpG residues associated with the genes encoding glucocorticoid receptor (GR) and corticotropin-releasing hormone-binding protein (CRH-BP) compared with normal placenta tissues (Oberlander et al., 2008; Serpeloni et al., 2017).

* 1. **Epigenetic changes in the placenta**

The placenta, as an essential regulator of fetal growth, survival,and development, is likely to play a key role in controlling fetal nutrient metabolism through epigenetic mechanisms, primarily through maternal genomic imprinting. Adverse stressors such as GDM are associated with changes in placental anatomy and physiology, leading to disruptions in the supply of placental nutrition. Further research into placental epigenetics will be required to identify biomarkers of exposure, pathology, and disease risk and can provide critical insights into the biology of fetal development and pathogenesis. The identification of these epigenetic changes may also aid in disease diagnosis and prognostic evaluation, supporting the design of new preventative and therapeutic strategies.

It is becoming increasingly clear that proper epigenetic regulation is a significant mediator of placental development and function, although the specific role of epigenetic factors in the pathogenesis of hypertension-linked placental dysfunction remains incompletely understood. Epigenetic mechanisms support placental functional adaptation by altering environmental conditions. The most robust evidence regarding the impact of epigenetic factors during pregnancy is derived from foundational studies exploring human embryonic and placental methylomes, revealing that the placenta maintains a globally hypomethylated DNA profile throughout pregnancy, with such hypomethylation typically occurring in large domains known as partially methylated domains (PMDs) that cover almost 40% of the placental genome (Pacini et al., 2020). Epigenetic changes in the placenta may provide an effective mechanism that links environmental stressors to adverse pregnancy outcomes, particularly in the context of fetal malformations. Epigenetic changes have been demonstrated within placental tissues, including altered DNA methylation patterns, changes in the binding activity of DNA-binding proteins (DBPs), imprinted regions in the H19/IGF2 genes, and shifts in the methylation of histones associated with the human growth hormone gene in the placental chromatin (Maccani & Marsit, 2009). Further investigations are underway to more fully clarify the epigenomic modulation of pregnancy-related pathology, with a particular focus on the epigenetic profiles of placental resident immune cells, which are primarily responsible for the safe onset and progression of pregnancy.



Figure 1. DNA methylation changes associated with pregnancy complications.

**The epigenetics of preeclampsia**

Preeclampsia (PE) is a complex, heterogeneous disorder that affects 2-10% of pregnancies and is characterized by a combination of hypertension, proteinuria, and edema after 20 weeks of gestation, potentially resulting in maternal multi-organ dysfunction and causing 60,000 maternal deaths worldwide (Genest, Falcao, Gutkowska, & Lavoie, 2012). PE is one of the leading causes of maternal mortality. In PE placentas, altered global DNA methylation profiles are correlated with maternal blood pressure (Gao et al., 2011; Kulkarni, Chavan-Gautam, Mehendale, Yadav, & Joshi, 2011). Abnormal DNA methylation during pregnancy can contribute to the hypertensive irregularities underlying the pathogenesis of PE (Kamrani et al., 2019), with PE-related DNA methylation being particularly important in this context (Julian et al., 2015; Kamrani et al., 2019). Kamrani et al. reported that the hypermethylation of several genes plays a role in the development of PE (Kamrani et al., 2019). Separately, Anderson et al. investigated maternal peripheral white blood cells and placental chorionic tissue samples from normotensive women and individuals with PE (O. S. Anderson, Sant, & Dolinoy, 2012). Genome-wide DNA methylation analyses revealed that 64% and 36% of differentially methylated sites were associated with significant increases and reductions in methylation, respectively. Pathway analyses suggested that differentially methylated genes were associated with cell signal transduction processes pertaining to lipid binding, protease enzyme inhibition, protein-protein interaction, cell cycle processes, and adhesion. Associations with signaling pathways involving cellular metabolic processes were predominant for genes exhibiting significant reductions in methylation (C. M. Anderson, Ralph, Wright, Linggi, & Ohm, 2014). For a further summary of PE-related changes in DNA methylation, see Table 1. Several studies have established the role of methylation in normal and PE pregnancies by revealing that maternal leukocyte DNA methylation is associated with maternal adaptations critical for normal pregnancy outcomes, explaining why genome-wide methylation profiles of maternal leukocyte DNA at the time of delivery exhibit increased methylation in women affected by PE as compared to normotensive pregnant women and underscoring the potenital role of such differneital methylation in the regulation of PE-related changes in gene expression. Recent work also supports a role of PE-associated epigenetic changes by demonstrating a link between PE and the acceleration of epigenetic aging (Weng et al., 2018; White et al., 2012, 2016).

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| Target genes  | DNA methylation changes | Functional roles | Pregnancy effects |  | References  |
| *11β-HSD2* | Hypo methylation  | Plays a critical role in hypertension, plays an important role in the regulation of blood pressure by preventing the activation of mineralocorticoid receptor in tissues such as the placenta | Placental *HSD11B2* may be influenced by gestation and the chronicity of maternal distress; the effect of anxiety and depressive symptoms on *HSD11B2* is greater during the third trimester than during the first.  |  | (Majchrzak-Celińska et al., 2017)*,* (Seth, Lewis, Saffery, Lappas, & Galbally, 2015). |
| *RUNX3* | Hypo methylation | Frequently deleted or transcriptionally silenced in cancer; was selected for analysis because tumor progression and pregnancy share many common features, such as immune tolerance and invasion. | Significant increases in *RUNX3* mRNA expression levels were reported among female smokers relative to nonsmoking women. *RUNX3* has been established to be fundamental for promoting Th1 phenotypes through IL‑4 repression. |  | (Majchrzak-Celińska et al., 2017) |
| *LINE-1* | Hypo methylation | Prevents activation of the placental mineralocorticoid receptor. Associated with cardiovascular disease and with risk factors for both cardiovascular disease and preterm birth. | Significant decreases in *LINE-1* methylation levels were observed in placentas during the third trimester relative to the first trimester. Hypomethylation of *LINE-1* has been linked to pathological processes including tumorigenesis, abnormal placental function, birth defects, aging, and other chronic diseases. (DNA hypomethylation and human diseases. In utero exposures, infant growth, and DNA methylation of repetitive elements and developmentally related genes in human placenta.Transition of LINE-1 DNA Methylation Status and Altered Expression in First and Third Trimester Placentas.)  |  | (Majchrzak-Celińska et al., 2017) |
| *IGF-1* | Hyper methylation  | Involved in placental formation and fetal growth; associated with increased *DNMT1* expression. | Maternal IGF-1 levels are negatively correlated with pregnancies complicated by preeclampsia. Higher maternal IGF-1 concentrations have been reported. (IGF-1 in gynecology and obstetrics: update 2002).  |  | (Ma, Zhou, Xiong, Li, & Li, 2018) |
| *VHL* | Hyper methylation | Codes for a tumor suppressor protein that is critical for normal placental development. | Pregnancy in patients with VHL disease induces cerebellar hemangioblastoma progression and causes a high VHL disease-related pregnancy complication rate.(Pregnancy-related haemangioblastoma progression and complications in von Hippel-Lindau disease)  |  | (Alahari, Garcia, Post, & Caniggia, 2018)  |

1. **Physiological changes during pregnancy**

Premature placental aging is associated with aberrant changes in telomere length, cellular senescence, and mitochondrial dysfunction (Manna, McCarthy, & McCarthy, 2019). The mitochondria play essential roles in physiological adaptations during pregnancy, and mitochondrial functional differences have been reported between healthy and complicated pregnancies. Some studies have observed differences in mitochondrial adaptation when comparing mitochondria associated with healthy pregnancies to those of non-pregnant women (Colleoni et al., 2010; Priliani et al., 2019). The epigenetic regulation of mitochondrial function is an area of active research, and three key mechanisms have been reported to regulate gene expression within mitochondria including DNA methylation, non-coding RNAs, and post-translational changes in nucleoid-related proteins (Sharma, Pasala, & Prakash, 2019).

While prior reports have established that prenatal exposures to environmental stressors are associated with mitochondrial DNA (mtDNA) methylation, additional research has called the accuracy and biological relevance of these mtDNA methylation profiles into question. mtDNA levels in the maternal peripheral blood of women affected by PE were initially reported to be associated with high levels of oxidative stress and mitochondrial dysfunction (Qiu, Hevner, Enquobahrie, & William, 2012). More recent work exploring the existence and biological relevance of mtDNA methylation profiles has yielded inconsistent findings. Several novel studies have examined how airborne particulate matter such as cigarette smoke can influence the methylation of gDNA and/or mtDNA. Overall, it is likely that epigenetic changes in specific targeted locations on the mitochondrial chromosome may affect transcription and/or replication of mtDNA, subsequent mitochondrial gene expression, and associated oxidative phosphorylation. These changes may impact the appropriate functioning of placental trophoblasts, and may thus play distinct roles in the contexts of placental activity and fetal development.

* 1. **The relationship between cellular senescence and adverse pregnancy outcomes**

Senescence can occur in response to a range of physiological stressors and associated molecular damage (Wiley & Campisi, 2016). These stress signals include oxidative stress, which is an important contributing factor underlying the pathophysiology of complicated pregnancies. There is evidence that pregnancies affected by PE may be distinct from normotensive pregnancies with respect to epigenetic markers of aging and senescence in tissues and organs, with PE and pregnancy-related outcomes including gestational length and birth weight being linked to accelerated epigenetic aging during pregnancy in women with PE, as demonstrated by the application of the “epigenetic clock,” wheres no comparable changes are evident for normotensive pregnancies (Suvakov et al., 2021). In pregnancies affected by PE, the trophoblastic plugs open prematurely, contributing to an influx of arterial blood that increases stress within the placenta and can lead to defective placentation. Trophoblastic cell lines affect senescence regulators, cell cycle regulators, DNA damage response and epigenetic changes which causes an increase hypomethylation of cytosine (Manna et al., 2019).

There have been a limited number of studies to date suggesting that prenatal markers of maternal biological age such as telomere length, an indicator of cellular aging, may be associated with pregnancy outcomes. Many studies have explored the effects of maternal dyslipidemia on the epigenetic aging of the placenta. Placental aging is a complex process that depends on factors including genetics, epigenetics, inflammation and related diseases, sex hormone levels, oxidative stress, and external environmental factors such as diet, exercise, and socio-demographic parameters. While optimal placental aging corresponds with gestational age and complements optimal fetal growth and development, premature placental aging is associated with adverse obstetric complications including early-onset PE, low birth weight, stillbirth, and preterm birth. As such, efforts aimed at controlling dyslipidemia in early pregnancy may support the normal aging of the placenta and healthy pregnancy.

 Oxidative stress induces the activation of repair pathways, inhibits cellular proliferation through the induction of senescence or transient cell cycle arrest, and can drive apoptotic cell death. Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) and the ability of antioxidant defense mechanisms to mitigate ROS-induced damage. Pregnancy is intrinsically linked to a state of persistent oxidative stress owing to increased metabolic activity in placental mitochondria and to corresponding ROS production. While an appropriate homeostatic balance between ROS generation and antioxidant activity is maintained in normal pregnancies, the disruption of this balance can contribute to oxidative stress. Several older studies suggest that senescence and aging are related to placental apoptosis, with this relationship strengthening with the progression of pregnancy.

* 1. **activity**

The placenta ages gradually, and the presence of senescence markers such as the p21, p16, p53, and Rb proteins as pregnancies approach full-term status supports a model in which the normal placenta undergoes progressive aging. Given their importance in the context of apoptotic cell death and senescence, several studies have explored the roles of telomeres and telomerase activity in the context of various aspects of reproductive biology including fertilization, placental development, stress, and hypoxic conditions during pregnancy (Fragkiadaki et al., 2016).

During the gestational period, telomere length remains constant throughout normal pregnancy. However, certain conditions such as fetal growth restriction and uncontrolled diabetes are associated with significant reductions in telomere length. Telomere changes can be linked to increased oxidative stress and the consequent induction of DNA damage, leading to the activation of the DNA damage response (DDR) via the p53 pathway and the consequent promotion of trophoblast senescence. Franco et al. reported that malnutrition in female rats can increase superoxide free radical production. During the first trimester, trophoblasts experience low levels of oxidative stress linked to the upregulation of *HIF-1α*. Oxidative damage has been firmly established as a major component of the aging process that can drive telomere shortening and cellular senescence (Franco et al., 2003; Petrik et al., 1999; Richter & Zglinicki, 2007; Sekoguchi et al., 2007). Moreover, the activation of DDR pathways, mtDNA mutations, endogenous stress accumulation, and redox signaling can all induce changes in epigenetic modifications.

Birton-Shental et al. established that telomeres are significantly shorter in trophoblasts exhibiting reduced Human Telomerase Reverse Transcriptase (hTERT) expression with corresponding increases in telomere aggregate frequencies when comparing normal pregnancies to pregnancies complicated by PE and IUGR. Separately, Pollack et al. found that telomere lengths were shorter in women with at least one live birth relative to women with no live births. Decreased telomerase activity has been observed in normal pregnancies when comparing the early gestational period with the late gestational period, and telomerase activity has been found to be correlated with various pregnancy complications. For example, low protein diet intake during gestation can contribute to the presence of fewer large telomeres and a higher number of short telomeres. Whereas gestational diabetes or diabetes and hypoxia, increased telomerase activity, and stress exposure in intrauterine life decreased telomerase activity could be expected (Fragkiadaki et al., 2016). Further studies pertaining to telomere biology in the context of reproduction are required to improve our understanding of the importance of telomerase activity and the lifelong implications of such activity for disease risk and associated aging processes. Given the relationship between short telomeres and genetic and environmental factors, their roles in pregnancy have emerged as an exciting area for ongoing research.

## **Epigenetic modifications associated with obesity and gestational diabetes**

 GDM is the most common metabolic condition during pregnancy and may result in short- and long-term difficulties for the mother. GDM can be defined as diabetes diagnosed during the second or third trimester of pregnancy in women unaffected by overt diabetes prior to gestation, with the etiology of GDM being similar to that of type 2 diabetes mellitus (T2DM) (American Diabetes Association (ADA) Standards of medical care in diabetes - 2018) (Franzago et al., 2019). Epigenetic mechanisms can increase the risk of becoming obese and diabetic, and can be influenced by the nutritional status and physical activity patterns of the parent. It is now widely accepted that environmental insults, including poor or unhealthy nutrition, lack of exercise, tobacco smoking, alcohol consumption, exposure to environmental pollutants, psychological stress, ethnicity, and hypertension can increase an individual’s risk of metabolic diseases during their lifetime in approximately 50% of cases (Das & Maitra, 2021; De Barros, Lopes, Francisco, Sapienza, & Zugaib, 2010; Franzago et al., 2019; Gupta & Kalra, 2016). The prevalence of GDM has risen dramatically by over 30% within the last two decades in several countries, including developing countries (Zhu & Zhang, 2016). One of the possible causes underlying this increased incidence can be attributed to advanced pregnancy, which in turn is associated with the presence in pregnant women of risk factors, such as being overweight or obese, the render them more susceptible to experiencing hyperglycemia during pregnancy. As some non-obese women also develop GDM, other factors including unhealthy nutrition and low physical activity before or during pregnancy may also contribute to the risk of this metabolic disease (Agarwal et al., 2018; C. Zhang & Ning, 2011). Most studies of DNA methylation in GDM have been conducted using samples of fetal tissue such as the cord blood and placenta. Notably, placental DNA methylation has been found to be correlated with maternal glycemic levels during pregnancy, with increasing maternal glycaemia leading to the demethylation of the leptin gene in the fetus, resulting in increased transcriptional activity and higher leptin levels that may contribute to leptin resistance and the development of obesity. Many previous studies have focused on the epigenetic effects of a single gene on GDM risk, whereas a limited subset of studies have employed a genome-wide approach to assessing the different methylation profiles of the placenta and cord blood in GDM and non-GDM participant groups (Finer et al., 2014; Kang, Lee, Li, Hsu, & Lin, 2017; Ruchat et al., 2013). Kang et al., for example, compared patterns of global methylation between pregnant women and their children in the context of GDM to those of healthy maternal-offspring pairs using blood samples taken at admission, before delivery, and umbilical cord blood samples obtained shortly after delivery. They identified 151 differentially methylated genes in mothers affected by GRM, including the *SLC22A4*, *ADR*A1A, *CACNB2*, and *SERPINE1* genes associated with lipid metabolism pathways. Changes in the DNA methylation of genes associated with GDM-related metabolic pathways including the JAK (Janus activated kinase) and MAPK pathways were also noted, with these pathways traditionally being associated with innate immunity, inflammation, and environmental stress responses (Kang et al., 2017). Wu et al. further profiled genome-wide DNA methylation changes in maternal peripheral whole blood samples collected from pregnant women 12–16 weeks into pregnancy, prior to the diagnosis of GDM. Through these analyses, the authors found that Hook Microtubule-Tethering Protein 2 (*HOOK2*) and Retinol Dehydrogenase 12 (*RDH1*) were differentially methylated, as has also been reported in other studies analyzing placental and cord blood samples. *HOOK2* codes for a linker protein that mediates binding to organelles and is responsible for the morphogenesis of cilia and endocytosis. RDH12 encodes a retinal reductase, which also plays a role in the metabolism of short-chain aldehydes. Five additional CpG loci were also identified that were associated with the *COPS8, PIK3R5*, *HAAO, CCDC124*, and *C5orf34* genes, regardless of its location- CpG in the genomic context were not mentioned (Wu et al., 2018). As such, further studies are warranted to more fully clarify the mechanisms whereby GDM influences fetal metabolic programming

1. **Epigenetics modification and prenatal maternal depression**

Depressive disorders are among the leading causes of disability worldwide. Depressive indicators during pregnancy are common, and around 10% of pregnant women suffer from major depressive disorder (MDD) (Non, Binder, Kubzansky, & Michels, 2014). These frequencies may be as high as 30% when considering all depressive disorders, and a large proportion of pregnant women experience at least one major depressive event before delivery, with this phenomenon being referred to as prenatal depression. Prenatal maternal depression can cause covalent epigentic changes in the DNA of their offspring that are detectable at birth in leukocytes and that may be present in other tissues, suggesting a model wherein systemic epigenetic changes may be involved in lifelong responses to the *in utero* psychosocial environment. Maternal depression during pregnancy has been linked to an increased risk of obstetric complications such as GDM, hypertension, and PE, and to smoking, increased alcohol consumption, and inadequate nutrition. Evidence suggests that maternal stress during pregnancy induces the hypermethylation of *Hsd11b2*in the placenta.

Bagot et.al found that mothers suffering from depression during the third trimester of pregnancy exhibited the hypermethylation of the glucocorticoid receptor promoter and exon 1f. Interestingly, these effects were not reversed with antidepressant treatment. In addition, prenatal maternal depression altered DNA methylation at the serotonin transporter. Thus, prenatal stress can alter adult susceptibility to depression, in part through changes in DNA methylation (Bagot, Labonté, Peña, & Nestler, 2014; Liu & Liu, 2010). These changes affect specific genes and brain regions, highlighting the difficulty in using peripheral tissues to predict functionally relevant changes within the brain (Babenko, Kovalchuk, & Metz, 2015).

1. **Nutrition as an epigenetic stimulus during pregnancy**

Nutrition is a key factor that supports a normal pregnancy and consists of both nutrient intake and the prenatal, perinatal, and postnatal composition of the maternal diet. Accordingly, nutrition is one of the most studied and best understood environmental factors shaping epigenetic outcomes.

Nutrients can act directly by inhibiting epigenetic enzymes such as DNMT, HDAC, or HAT or by altering the availability of the substrate required for these enzymatic reactions. This, in turn, changes the expression of critical genes and impacts overall health and longevity. Mitsuya et al. studied patterns of placental DNA methylation and hydroxymethylation on a genomic scale and observed a partial but significant overlap between the genes that exhibited an increase in DNA methylation and a reciprocal decrease in DNA hydroxymethylation with increased maternal obesity, suggesting a possible decrease in the conversion efficiency of methylation to hydroxymethylation, which is governed by TET dioxygenases (Mitsuya et al., 2017). Other studies have also provided evidence in support of a connection between metabolism and epigenetics. Present work suggests that the dysregulation of adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) homeostasis is linked to gestational obesity (Reichetzeder, 2021). AMPK activation occurs in response to dropping energy levels indicated by high AMP levels and low ATP concentrations. Folic acid is a well-known one-carbon donor for the methylation and synthesis of DNA. *In vivo*, the increased consumption of folate and folate-rich foods is associated with the decreased promoter methylation of genes associated with tumor suppression (Jacobs et al., 2013). Additionally, folic acid is required for the production of S-adenosyl methionine (SAM), which serves as a methyl group donor in the context of DNA methyltransferase-mediated DNA modification (Aon, Cortassa, Juhaszova, & Sollott, 2016). Vitamin D is another micronutrient that alters epigenetic pathways. Low concentrations of vitamin D are associated with increased inflammation, and the anti-inflammatory actions of vitamin D may be attributable to changes in DNA methylation and histone modifications. Furthermore, severe vitamin D deficiency has been linked with changes in methylation in peripheral blood leukocyte DNA in humans (Wang et al., 2013).

Despite the critical importance of nutrition to pregnancy outcomes, little is known regarding the factors influencing dietary quality, particularly during the first trimester. A pregnant woman’s dietary quality, particularly in the first trimester of pregnancy, has a profound effect on subsequent pregnancy outcomes (Kind, Moore, & Davies, 2006).

1. **Significance of maternal immune responses during pregnancy**
	1. **Pregnancy-related maternal immunological adaptation**

The perception that pregnancy is related to the suppression of the immune system has created a myth of pregnancy as a condition of immunological weakness and hence of increased susceptibility to infectious diseases. The maternal immune system undergoes profound transformations during the early stages of pregnancy. Epigenetic mechanisms have been shown to regulate immune cells and to thereby influence the incidence and progression of autoimmunity. Given their high turnover rates, most immune cells are highly sensitive to environmental changes and can adapt to a range of stressors. Myeloid-derived cells, in particular, can activate stress response pathways that cause plastic changes in their transcriptional activity. During normal pregnancies, the human decidua contains a high number of immune cells including macrophages, natural killer (NK) cells, and regulatory T cells. Accordingly, immune cells infiltrate the decidua during the first trimester and accumulate around the invading trophoblast cells. As such, pregnancy is more appropriately considered a unique immunomodulatory state, rather than a state of true immunosuppression.

Pregnancy hormones are of key importance to the maintenance of pregnancies, and they can profoundly impact the associated immune response. A growing body of evidence has confirmed that sex hormones may regulate major epigenetic changes including the modulation of miRNA expression, DNA methylation status, and chromatin rearrangement. An overview of current evidence regarding the effects of sex hormones on immune system cells is provided in Table 2.

Table 2. The effects of sex hormones on immune cells.

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| Sex hormone  | DNA methylation changes | Target gene  | Biological effect  | References  |
| 17-β-estradiol - high concentration | A key component in the passive and active DNA demethylation processes both on the DNA and histones. | T helper cell | Stimulates Th2 responses: increases IL-4, IL-10, TGF-β, stimulates IRF-1, inhibits TNFα and IL-17  | (Merrheim et al., 2020; Pacini et al., 2020; Straub, 2007) |
|  |  | Treg cell | Stimulates differentiation and activity: stimulates FoxP3 and PD-1 | (Polanczyk, Hopke, Huan, Vandenbark, & Offner, 2005; Schaub et al., 2009) |
|  |  | Nk cell  | Reduces activity: increases IL-10, TGF-β | (Kovats, 2015; Straub, 2007) |
|  |  | B cell  | Promotes survival of autoreactive B cells: reduces BCR, increases CD22 | (Pacini et al., 2020) |
|  |  | Macrophages | Reduces activity: reduces IL-6, IL-1β and TNFα, increases IL-10 | (Moulton, 2018; Straub, 2007) |
|  |  | Dendritic cells | Reduces activity: reduces IL-6, IL-1β and TNFα | (Pacini et al., 2020; Straub, 2007) |
| Progesterone | DNA methylation status is still not yet confirmed. | T helper cell | Stimulates Th2 response: increases IL-4, IL-10, TGF-β and reduces IFNγ and IL-12. Reduces Th1 and Th17 responses: reduces IFNγ, IL-12, and IL-17.  | (Hughes & Choubey, 2014; Pacini et al., 2020; Tan, Peeva, & Zandman-Goddard, 2015) |
|  |  | NK cells | Reduces activity: reduces INFγ | (Pacini et al., 2020; Tan et al., 2015) |
|  |  | B cell | Reduces class-switch recombination and T-cell | (Hughes & Choubey, 2014; Pacini et al., 2020; Tan et al., 2015) |
|  |  | Macrophages  | Reduces activity: reduces nitric oxide production, TNFα and FcγR  | (Tan et al., 2015) |
|  |  | Dendritic cells | Reduces activity: reduces TLR-mediated IFNα production | (Bupp & Jorgensen, 2018) |
| PSG1a | Highly expressed in myoblasts and strongly downregulated after differentiation. |  | Enhances IL-10 and TGF-β production | (Motrán et al., 2002; Zhou & Hammarström, 2001) |

* 1. **Autophagy**

Autophagy is a process that maintains cellular homeostasis by eliminating senescent or damaged intracellular organelles and proteins, playing a key role in various pathophysiological processes. The role of autophagy in pregnancy, however, has only been studied to a limited degree. It has been reported that basal levels of autophagy contribute to the maintenance of intracellular homeostasis and are required for cellular remodeling. Autophagy plays a pivotal role in the processes of embryogenesis, implantation, and maintaining a pregnancy. Emerging evidence suggests that there exist reciprocal interactions between autophagy and pregnancy complications. The induction of autophagy in specific immune cells results in the stimulation of phagocytic activity in macrophages and the activation of T and B lymphocytes. The thymus is an important organ in which pre-T cells differentiate into mature T cells following positive and negative selection, and autophagy in the thymus has also been shown to shape the T cell repertoire.

Singh et al. were the first to demonstrate diethylstilbestrol (DES)-induced autophagy in the thymus and to highlight the potential role of epigenetic pathways in the regulation the autophagy. DES is a nonsteroidal estrogen that is classified as an endocrine disruptor, and these researchers found that DES-induced thymic atrophy was associated with increased autophagy in thymocytes and the pronounced upregulation of Becn1 and LC3. DES was additionally found to promote the downregulation of miR-30a expression and to trigger Becn1 hypomethylation, thereby triggering increased Becn1 expression and inducing autophagy in thymic cells (Singh, Miranda, Singh, Nagarkatti, & Nagarkatti, 2018). Elevated autophagic activity in the placenta during pregnancy has previously been shown to be involved in the pathophysiology of PE, with evidence of an inverse association between glucose and placental autophagy. Finer et al. suggested that placental methylated variable positions (MVPs) were most significantly altered in association with pathways related to placental function and growth such as endocytosis and mitogen-activated protein kinase (MAPK) signaling. MAPK signaling is also closely related to autophagic induction, and the underlying signaling mechanisms are commonly disrupted in the context of GDM. The differential methylation and expression of these pathways may thus be indicative of numerous physiological changes occurring in the hyperglycaemic placenta, which is consistent with a multifactorial disease model (Block & El-Osta, 2017; Mizushima & Levine, 2010; Oh et al., 2008; Signorelli et al., 2011). Although the molecular pathways governing autophagy have been well characterized, more work is required to fully elucidate the regulatory role of epigenetic pathways as regulators of pregnancy-associated autophagic activity.

To prevent the persistence of an excessive proinflammatory immune response that is deleterious to host cell survival, autophagy is downregulated by the anti-inflammatory cytokines IL-4, IL-10, and IL-13 that are released in response to inflammation. Similarly, prolonged activation of nuclear factor-κB (NF-κB), the transcription factor that activates genes coding for proinflammatory cytokines, can also inhibit autophagy (Sisti, Kanninen, & Witkin, 2016).

**7. The effects of epigenetic inheritance on pregnancy**

The role of the developmental and parental environmental exposures in shaping the ultimate metabolic characteristics of offspring has been effectively demonstrated in several human studies of populations exposed to extreme nutrient deficiencies during pregnancy. One of the best-understood instances of epigenetic inheritance pertains to the effects of maternal nurturing behaviors during the first week of life. Indeed, the evolutionary rationale for epigenetic inheritance may be to provide a means of adapting rapidly to short-term environmental changes without the need to wait for the modification of the underlying genes through mutation and selection.

While epigenetic inheritance is still a rapidly growing field, it still remains largely independent from the fundamental models of genetics that are traditionally based on the hereditary role of DNA (Tikhodeyev, 2018). Recent human research suggests that diseases resulting from disruptions of the normal epigenetic state (also known as “epimutations”) can be inherited across generations (King & Skinner, 2020). Gestational exposure to famine has been associated with several chronic diseases in adulthood. The Dutch Hunger Winter Families Study, first published in 1976, offers a robust albeit tragic human experiment regarding the effects of food restriction *in utero* on metabolism, cardiovascular health, and age-associated congenital abnormalities. In the winter and spring of 1944 after a railway strike, the German occupation limited rations such that people, including pregnant women, in the western region of The Netherlands, including Amsterdam, received as little as 400–800 calories per day. This study highlighted the importance of the timing of stress exposure on the risk of subsequent disease. Individuals born during this period of famine were at an increased risk of schizophrenia and depression, had a more atherogenic plasma lipid profile, responded more to stress, and had a higher risk of coronary heart disease. These individuals also performed more poorly in cognitive tasks, which may be a sign of accelerated aging. People exposed to famine conditions throughout the entirety of the gestational period suffered from higher rates of type 2 diabetes. Future research has the potential to offer additional insight regarding the ability of prenatal exposure to shape disease risk for multiple generations through epigenetic mechanisms.

Individuals exposed to hunger only during late pregnancy were born small and continued to be small throughout their lives, with lower obesity rates as adults than those born before and after periods of starvation. As such, the effects of famine appear to be highly dependent on its timing during gestation, and the organs and tissues undergoing critical periods of development at that time. However, as noted above, those exposed during early pregnancy experienced high rates of obesity, altered lipid profiles, and changes in cardiovascular disease. Incidence. In contrast, markers of reduced renal function were specific to those exposed to famine conditions during mid-pregnancy (Schulz, 2010). This suggests that transient environmental conditions early during human gestation can be recorded as persistent changes in epigenetic information that result in life-long phenotypic consequences. Indeed, as discussed previously, early embryonic development is of particular interest in this respect because this is a crucial period for the establishment and maintenance of epigenetic marks.

The Dutch Hunger Winter Families Study provided the first direct evidence for [epigenetic](https://www.sciencedirect.com/topics/medicine-and-dentistry/epigenomics%22%20%5Co%20%22Learn%20more%20about%20Epigenomics%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) programming through prenatal famine exposure. Men and women who had been exposed to the famine periconceptionally were found to exhibit reduced [methylation](https://www.sciencedirect.com/topics/medicine-and-dentistry/methylation%22%20%5Co%20%22Learn%20more%20about%20Methylation%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) of the *IGF2* gene compared with their unexposed same-sex siblings (Heijmans et al., 2008). Feeding mice a diet rich in methyl donors can influence their coat color, body weight, and the health of their progeny. This indicates that transient environmental conditions present early in human gestation can result in the establishment of persistent epigenetic changes. Certain inherited metabolic disorders can cause significant maternal complications during pregnancy, and modern epigenetic research raises complex questions regarding maternal responsibility for health (Preece & Green, 2002).

The genetic revolution has also focused attention on the implications of pregnancy for women who have been diagnosed with cardiomyopathy or who carry a familial mutation predisposing them to cardiomyopathy. Epigenetic inheritance can therefore be seen not just as a third evolutionary mechanism that complements mutation and natural selection, but as an actual engine of evolution that can drive long-term genetic changes and that may thus play an important role in species divergence.

**8. Conclusions and future perspectives**

Pregnancy is a dynamic state, with diverse mechanisms being engaged during different trimesters to enable and ensure the successful establishment, maintenance, and timely termination of the pregnancy. Pregnancy provides a unique platform for studying stress and stress responses, both in the case of pathological and uncomplicated pregnancies. It is clear that epigenetic regulation is a key feature of pregnancy and development, but our current understanding is rather piecemeal. Knowledge of such epigenetic mechanisms may be useful in identifying novel biomarkers for pregnancy-related exposure, burden, or disease risk. Such biomarkers may prove essential for the development of new tools for the early identification of risk factors and exposure levels. Here, we have provided a review of prior studies exploring changes in maternal DNA methylation during pregnancy and associated adverse conditions. Exploration of epigenomic changes can enable an improved understanding of the dynamic biological processes that take place during pregnancy. Clarifying how epigenetic control depends on early exposure may allow clinicians to identify women at increased risk for adverse pregnancy outcomes and develop precise, personalized, risk-specific interventions.

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