Asian Journal of Medicine and Health

Volume 24, Issue 8, Page 63-88, 2026; Article no.AJMAH.163569
ISSN: 2456-8414

In Utero Epigenetic Programming of the Fetal Genome: A Critical Synthesis of Maternal Nutritional, Environmental and Psychosocial Influences

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajmah/2026/v24i81410

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/163569

Review Article

Received: 03/06/2026
Accepted: 29/07/2026
Published: 01/08/2026

Abstract

The fetal epigenome is established during periods of extensive cellular differentiation and may record, buffer or transmit information about the maternal milieu. This critical narrative review evaluates evidence that maternal nutrition and metabolic state, environmental toxicants and ambient pollution, and psychosocial conditions alter epigenetic regulation in the placenta, cord blood or fetal tissues. Literature published from January 2000 to 26 May 2026 was identified through accessible scholarly indexes, supplemented by citation searching and verification against authoritative bibliographic records. Evidence was appraised across human observational studies, natural experiments, randomised trials and mechanistic animal models. The strongest replicated human evidence concerns sustained maternal smoking, for which newborn DNA methylation signatures are consistent across cohorts and some marks persist beyond childhood. Nutritional programming

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Cite as: *Bittmann, S., Luchter, E., & Moschüring-Alieva, E. (2026). In Utero Epigenetic Programming of the Fetal Genome: A Critical Synthesis of Maternal Nutritional, Environmental and Psychosocial Influences. Asian Journal of Medicine and Health, 24(8), 63–88. https://doi.org/10.9734/ajmah/2026/v24i81410*

is mechanistically compelling, particularly for one-carbon metabolism and severe energy or protein imbalance, but human findings are most persuasive in quasi-experimental settings and at metastable epialleles; routine supplementation and dietary interventions have produced smaller, locus-specific or non-replicated effects. Air pollution, metals, phenols, phthalates and per- and polyfluoroalkyl substances are associated with placental or cord-blood epigenetic variation, yet effect sizes, loci and biological interpretation vary with exposure assessment, gestational timing, tissue composition and co-exposure. Psychosocial studies support stress-responsive placental and hypothalamic-pituitary-adrenal pathways, but candidate-gene associations have not translated into a stable epigenome-wide signature. Across domains, DNA methylation dominates measurement, whereas histone regulation, chromatin accessibility and non-coding RNA remain less comprehensively studied in humans. Most findings establish exposure-associated molecular variation rather than durable, causal programming of organ function or disease. Progress requires longitudinal and diverse pregnancy cohorts, repeated exposure measurement, cell-resolved placental and fetal models, multi-omic integration, functional perturbation and causal triangulation. Epigenetic evidence can clarify biological embedding of prenatal conditions, but it should not be interpreted as deterministic or used to individualise responsibility for structurally patterned maternal exposures.

Keywords: Developmental origins of health and disease; placenta; DNA methylation; one-carbon metabolism; prenatal exposome; maternal stress; fetal programming; causal inference.

1. Introduction

Development from conception to birth requires a single genome to generate specialised tissues while preserving the capacity for growth and physiological adaptation. This process depends on epigenetic regulation: mitotically propagated DNA methylation, post-translational histone modifications, nucleosome organisation, chromatin accessibility and regulatory non-coding RNAs that influence when, where and to what degree genes are expressed. The prenatal period is therefore biologically distinctive. Epigenetic states are being erased, re-established and refined at the same time that maternal nutrients, hormones, metabolites, inflammatory signals and xenobiotics are entering or modifying the fetal environment. Developmental epigenetic epidemiology asks whether these conditions leave molecular marks that help explain later phenotypic differences, including cardiometabolic, respiratory, immune and neurobehavioural outcomes (Waterland & Michels, 2007).

The language of "programming" carries stronger implications than the observation of an exposure-associated epigenetic difference. In this review, epigenetic programming denotes a developmentally timed alteration in a regulatory state that is sufficiently stable to influence subsequent cellular function. An exposure biomarker may satisfy none of those conditions: it may be transient, confined to a changing cell population, downstream of impaired growth, or unrelated to gene regulation. Likewise, methylation in placenta or cord blood cannot be assumed to reproduce methylation in fetal brain, liver, pancreas or adipose tissue. The distinction matters because the term is often used interchangeably for molecular responsiveness, statistical association and causal mediation. A rigorous synthesis must separate evidence that an exposure changes an epigenetic measurement from evidence that the change persists, alters transcription or chromatin function, and contributes to a later phenotype (Birney et al., 2016; Greally, 2017; Michels et al., 2013).

Two waves of extensive epigenetic reconfiguration create both opportunity and constraint. After fertilisation, much of the parental DNA methylome is remodelled while embryonic lineages acquire new regulatory identities; the prenatal germline later undergoes further demethylation before sex-specific re-establishment. Imprinted regions and selected repetitive or metastable elements show distinct resistance or timing, making them plausible sentinels of periconceptional conditions (Gkountela et al., 2015; Reik et al., 2001; Smith et al., 2014). These processes do not imply that the embryo is passively rewritten by every exposure. Redundant developmental pathways, maternal metabolism, placental transport and DNA repair buffer many perturbations. Conversely, a subtle change introduced before lineage allocation may be propagated across a large cell population, whereas the same perturbation late in gestation may remain local or reversible. Timing, dose and cellular context are therefore inseparable from claims of programming.

The placenta is central to this interpretation. It is a fetally derived, rapidly changing organ that allocates nutrients and oxygen, metabolises hormones and xenobiotics, and coordinates immune and endocrine communication. Its epigenome is unusual, with extensive partially methylated domains, imprinting features and

gestational dynamics that differ from somatic tissues (Hemberger et al., 2020; Novakovic et al., 2011; Robinson & Price, 2015). Placental epigenetic variation may be mechanistically important when it alters transport, vascular development or hormone metabolism, but it can also represent an adaptive response that protects the fetus. Treating the placenta as a passive proxy for the fetal genome obscures this dual role. The same measured mark can indicate causal dysfunction, compensatory regulation, altered cell composition or simply the developmental age of the tissue (Burton & Fowden, 2012; Burton et al., 2016). Recent single-cell and spatial multi-omic analyses further demonstrate that placental regulatory landscapes are organised by cell lineage, gestational stage and anatomical niche, reinforcing the need to interpret bulk-tissue epigenetic associations within a cell-resolved developmental context (Derisoud et al., 2024; Ounadjela et al., 2024).

Evidence spans levels that cannot be combined uncritically. Controlled animal experiments establish that maternal diet, stress and toxicants can modify offspring epigenetic regulation, sometimes with organ-specific functional consequences. Human natural experiments provide unusual leverage over timing and long-term persistence, whereas pregnancy cohorts offer more representative exposures but remain vulnerable to confounding, measurement error and selection. Randomised nutritional trials are comparatively rare and frequently underpowered for epigenome-wide outcomes. Human studies also overwhelmingly measure DNA methylation because it is stable and technically accessible, creating a literature in which the best measured mechanism can be mistaken for the only relevant mechanism. Histone modifications, chromatin looping, transcription-factor occupancy and non-coding RNAs may be equally consequential but are difficult to assay in archived birth samples.

Methodological appraisals of placental genomic and epigenomic research additionally emphasise that sampling location, cell composition, genetic variation and analytical adjustment can materially affect reproducibility and should therefore be treated as central design considerations rather than secondary covariates (Inkster et al., 2025).

Recent reviews have usually examined one component of this field: one-carbon metabolism and placental methylation, maternal mental health and infant epigenome-wide association studies, or interactions between maternal nutrition and environmental toxicants (Drzymalla et al., 2023; Shafiq et al., 2026; van Vliet et al., 2024). This specialisation has improved depth but can fragment causal interpretation. Nutritional status alters toxicant metabolism; psychosocial adversity affects diet, smoking, sleep and endocrine function; air pollution and social disadvantage co-occur; metabolic disease changes placental transport and inflammation. Reviewing each exposure in isolation risks assigning a molecular signal to a nominal exposure when it reflects an intertwined maternal exposome. An integrated review is also needed to compare evidential standards across domains. Smoking has reproducible newborn methylation signals, whereas psychosocial and endocrine-disrupting chemical studies often report non-overlapping loci. These differences may reflect true biology, but also exposure measurement, sample size, tissue choice and analytical maturity.

Recent replication work on maternal tobacco exposure has strengthened this contrast by confirming a subset of placental methylation loci while showing that persistent signals are less common than transient associations (Masdoumier et al., 2025).

1.1 Scope, Research Gap and Objectives

This review examines maternal nutritional and metabolic conditions, environmental toxicants and ambient pollution, and psychosocial exposures from the periconceptional period through pregnancy in relation to epigenetic regulation in the placenta, cord blood and fetal or offspring tissues. It integrates mechanistic animal evidence with human natural experiments, observational cohorts and intervention studies, while giving greatest interpretive weight to replicated human findings and designs that strengthen temporal or causal inference. The review focuses on DNA methylation, histone regulation and non-coding RNA where evidence is available, and considers later phenotypes only when they help evaluate biological function or persistence. Assisted reproduction, paternal preconception exposures, postnatal programming and clinical epigenetic disorders caused by primary genetic variants are outside the principal scope. The central gap is not whether prenatal conditions can correlate with epigenetic variation, but which associations meet credible criteria for developmentally timed, persistent and functionally consequential programming. The objectives are to compare evidence across exposure domains, identify mechanisms and contradictions, evaluate methodological limitations, distinguish robust

conclusions from speculation, and define research priorities that can move the field from molecular association towards causal and translational understanding.

2. Methods for Literature Selection

2.1 Review Design and Rationale

A critical narrative review design was selected because the evidence combines experimental and observational studies, natural experiments, randomised dietary trials, candidate-gene analyses, epigenome-wide association studies and mechanistic investigations with non-comparable exposures, tissues, assays and outcomes. A single pooled effect would have little biological meaning, and a conventional systematic review restricted to one exposure-outcome pair would not answer the integrative question. Narrative design was not treated as exemption from transparent searching or appraisal. The review was structured around the Scale for the Assessment of Narrative Review Articles principles, including a defined purpose, reproducible search concepts, explicit source selection, evidence-based reasoning and disclosure of limitations (Baethge et al., 2019).

2.2 Information Sources

PubMed was the principal biomedical index. The Directory of Open Access Journals, OpenAlex and Semantic Scholar were searched to identify additional peer-reviewed records and related articles. Backward reference searching was undertaken from recent high-quality reviews and influential primary studies, and forward and related-article searching was used to locate replications, null findings and methodological critiques. Bibliographic identity, publication status and Digital Object Identifiers (DOIs) were checked against PubMed records, DOI landing pages and official journal records. Searches for corrections, retractions and duplicate publications were incorporated during verification. Subscription databases for which direct, auditable access was unavailable were not represented as searched.

2.3 Search Strategy and Search Period

The principal search period was 1 January 2000 to 26 May 2026. Earlier foundational work was retained when necessary to explain epigenetic reprogramming or the intellectual basis of developmental epigenetic epidemiology. Searches combined pregnancy and developmental terms with epigenetic mechanisms, tissues and exposure domains. A representative core string was: (“pregnancy” OR maternal OR prenatal OR “in utero” OR periconception*) AND (epigenetic* OR “DNA methylation” OR hydroxymethylation OR histone* OR chromatin OR microRNA OR “non-coding RNA”) AND (placenta* OR “cord blood” OR fetal OR foetal OR embryo* OR offspring). Nutritional modules added (folate OR choline OR methionine OR “one-carbon” OR famine OR undernutrition OR diet OR obesity OR hyperglycaemia OR “gestational diabetes”); environmental modules added (smoking OR tobacco OR “air pollution” OR particulate OR metal* OR arsenic OR lead OR mercury OR phthalate* OR phenol* OR bisphenol OR PFAS); and psychosocial modules added (stress OR depression OR anxiety OR trauma OR violence OR socioeconomic OR adversity). Searches were iteratively refined with named genes, cohorts, epigenome-wide association study terms, cell composition, mediation, causal inference and longitudinal follow-up.

2.4 Eligibility Criteria

Eligible publications were peer-reviewed original studies, high-quality systematic or narrative reviews, and methodological papers directly relevant to prenatal epigenetic inference. Human pregnancy studies were prioritised. Animal studies were included when they tested developmental timing, tissue function or experimental causality that could not be resolved in humans. Studies had to measure a maternal or intrauterine exposure during the periconceptional or gestational period and an epigenetic outcome in placental, embryonic, fetal, neonatal or later offspring tissue, or provide a necessary methodological framework. English-language full texts or sufficiently complete authoritative records were required for verification. Conference abstracts, unverifiable records, retracted papers, purely postnatal exposures, non-scholarly commentary and studies reporting only gene expression without an epigenetic measure were excluded. Later health outcomes were included selectively, not as evidence of mediation unless temporal ordering and an exposure-epigenetic-outcome pathway were evaluated.

2.5 Screening, Duplicate Handling and Study Selection

Records were assessed by title and abstract for topical relevance, followed by full-text or complete-record assessment for study design, exposure timing, tissue, epigenetic assay and interpretability. Duplicate records were reconciled using DOI, PubMed identifier, title, author and cohort information. When multiple reports used overlapping participants, each article was retained only if it addressed a distinct exposure, tissue, time point or analytical question; apparent replication within the same cohort was not treated as independent confirmation. Sources lacking verifiable authorship, complete bibliographic metadata or a resolvable DOI were excluded. No screening counts or flow diagram are presented because the process was purposefully iterative and thematic rather than a prospectively enumerated systematic selection.

2.6 Critical Appraisal and Evidence Synthesis

Evidence was appraised according to design appropriateness, sample size and representativeness, exposure timing and measurement quality, tissue and cell-type validity, assay coverage, batch and multiple-testing control, treatment of maternal and fetal genotype, confounding, replication, functional corroboration, persistence and external validity. Experimental control and tissue-specific function were weighted heavily in animal work, but translational distance and exposure severity were explicitly considered. Human evidence was graded qualitatively as stronger when findings were replicated across independent cohorts, supported by objective exposure biomarkers or quasi-experimental timing, linked to transcription or phenotype, and robust to alternative causal explanations. Themes were developed around developmental architecture, nutritional and metabolic exposures, toxicant and ambient exposures, psychosocial conditions, and cross-cutting causal limitations. Contradictions were retained and interpreted rather than resolved by citation frequency.

3. Biological Architecture of Fetal Epigenetic Programming

3.1 Reprogramming, Lineage Allocation and Developmental Windows

The developmental epigenome is not a blank slate but a sequence of constrained transitions. Following fertilisation, paternal and maternal genomes undergo partly distinct demethylation kinetics, after which methylation is rebuilt as the embryo separates pluripotent, extraembryonic and organ-specific lineages (Reik et al., 2001; Smith et al., 2014). A second extensive reconfiguration occurs in primordial germ cells (Gkountela et al., 2015). These transitions create windows during which regulatory states can be sensitive to substrate availability, redox status, endocrine signalling or toxicant interference. They also remove many acquired marks. Consequently, exposure before implantation may generate broad mosaic effects if it alters an early progenitor, but only loci that escape reprogramming or are re-established through an altered developmental pathway are likely to persist. Exposure later in gestation acts on a more differentiated epigenome and may produce stronger tissue specificity.

Metastable epialleles illustrate this timing principle. Their methylation is established stochastically in the early embryo, varies between individuals and is concordant across tissues derived from different germ layers. Seasonal nutrition studies in rural Gambia identified human genomic regions with such properties and showed that periconceptional maternal biomarkers were associated with offspring methylation at environmentally sensitive hotspots (Dominguez-Salas et al., 2014; Silver et al., 2022). This design is more informative than a single nutrient questionnaire because season of conception alters a coordinated set of nutritional and environmental inputs before tissue differentiation. It does not, however, isolate one causal nutrient, and the selected loci are exceptional rather than representative of the entire methylome. Metastable epialleles support developmental sensitivity while cautioning against generalising their cross-tissue behaviour to ordinary CpG sites.

3.2 The Placenta as Sensor, Buffer and Effector

Placental development begins during the same early lineage decisions, yet the mature placenta differs markedly from fetal somatic tissues. Its methylome contains large hypomethylated and partially methylated domains, variable imprinting and gestational-age-related promoter changes (Novakovic et al., 2011; Robinson & Price, 2015). These features reflect trophoblast differentiation and rapid turnover, and they complicate conventional interpretations imported from blood or cancer epigenetics. A lower methylation value is not inherently

pathological, and an exposure-associated difference may indicate altered proportions of trophoblast, stromal, endothelial or immune cells. Placental sampling location, labour, mode of delivery, gestational age and pathology can each shift the measured profile. Even careful statistical adjustment cannot fully recover a cell-specific mechanism from heterogeneous bulk tissue.

Functionally, the placenta can amplify, attenuate or redirect maternal signals. Nutrient transporters, glucocorticoid metabolism, vascular development, mitochondrial activity and inflammatory pathways are plausible epigenetic targets because they alter the actual fetal milieu (Burton & Fowden, 2012; Burton et al., 2016). This creates two causal routes. A maternal exposure may act directly on fetal cells after crossing the placenta, or indirectly by changing placental function. The same placental alteration may also be compensatory: increased transporter expression can preserve fetal supply under maternal scarcity, while epigenetic repression may limit toxicant transfer or excessive growth. Without measurements of transport, hormone concentrations, transcription and fetal physiology, classifying a placental mark as injury or adaptation remains speculative.

3.3 Sex-specific Regulation and Resilience

Fetal sex is more than a covariate. The placenta shares the fetal sex-chromosome complement, sex hormones and autosomal regulatory differences; male and female placentas can therefore respond differently to the same maternal condition. In a mechanistic mouse model, higher placental O-linked N-acetylglucosamine transferase activity in females supported trimethylation of histone H3 lysine 27 (H3K27me3), and experimental reduction of this pathway removed female resilience to prenatal stress-related neuroendocrine outcomes (Nugent et al., 2018). The study provides unusually strong evidence because trophoblast-specific manipulation connected an epigenetic mechanism to offspring physiology. Its direct applicability to humans is uncertain: term human placental observations were limited, the stress paradigm was controlled but artificial, and H3K27me3 cannot be inferred from routine methylation arrays. The broader implication is that pooling sexes may erase genuine effects or create unstable average associations.

3.4 From Molecular Mark to Programmed Phenotype

Four inferential steps are required to establish programming. First, the exposure must precede the epigenetic difference within a biologically plausible window. Second, the difference should be attributable to regulation rather than genotype, cell mixture or developmental stage. Third, it should alter transcription, chromatin state, cellular behaviour or organ function. Fourth, the alteration should contribute to a later phenotype or persist when persistence is part of the claim. Most human studies securely address only the first step, and some measure exposure and placental methylation at delivery, leaving temporal ambiguity. Association between methylation at birth and later adiposity, for example, may identify a risk biomarker without showing that methylation caused adipose development (Godfrey et al., 2011). Mediation analyses do not supply missing biological evidence when assumptions about confounding and measurement error are implausible.

Assay choice further defines what can be concluded. Array platforms sample a small, non-random fraction of genomic cytosines, emphasising promoters and CpG islands while incompletely covering enhancers, repetitive elements and placenta-specific regions. Comparison of 450K and EPIC arrays in placenta shows broad agreement but also platform-specific coverage and technical considerations (Fernández-Jiménez et al., 2019). Bisulphite-based methods usually cannot distinguish 5-methylcytosine from 5-hydroxymethylcytosine, although these modifications can have different functions. Gene-level labels can be misleading because CpGs in promoter, gene body and distal regulatory regions may move in opposite directions. An exposure-associated CpG is therefore a coordinate, not a self-explanatory mechanism.

Table 1 synthesises the developmental windows most relevant to maternal exposure research. It shows that evidential strength depends not only on the exposure but on when an epigenetic state is established, which tissue is sampled and whether the measured mark plausibly survives later reprogramming. The recurrent asymmetry is that the periods of greatest mechanistic interest are those in which direct human tissue access is least feasible. Accordingly, a “critical window” should be treated as a mechanistic hypothesis rather than a post hoc label attached to the trimester yielding the smallest P value; repeated exposure measurements and developmental knowledge are needed to distinguish a genuine susceptible period from correlated exposure across pregnancy.

Table 1. Developmental windows, epigenetic processes and inferential constraints in fetal programming research

Developmental window	Dominant epigenetic processes	Relevant maternal inputs	Current evidential position	Principal interpretive constraint	Supporting references
Periconception and preimplantation	Genome-wide demethylation, imprint maintenance and establishment of metastable epialleles	Maternal one-carbon substrates, energy balance, endocrine milieu and early toxicant exposure	Human season-of-conception studies and embryo methylome maps support marked sensitivity at selected loci	Direct fetal sampling is unavailable; exposures are correlated and most marks are later erased or tissue-restricted	(Dominguez-Salas et al., 2014; Reik et al., 2001; Silver et al., 2022; Smith et al., 2014)
Implantation and early placentation	Trophoblast lineage specification, chromatin remodelling and development of placenta-specific methylation domains	Maternal vascular, inflammatory and metabolic signals; xenobiotic handling at the interface	Strong developmental biology; moderate human evidence linking maternal conditions with placental epigenetic variation	Placental adaptation and altered cell composition can mimic programming; sampling occurs months after the initiating event	(Hemberger et al., 2020; Novakovic et al., 2011; Robinson & Price, 2015)
Organogenesis	Tissue-specific enhancer activation, stable lineage methylation and histone-state refinement	Nutrient deficiency or excess, drugs, toxicants, hypoxia and stress hormones	Compelling animal evidence; sparse direct human fetal-tissue evidence	Cord blood and term placenta may not retain organ-specific events; exposure timing is often reconstructed coarsely	(Lillycrop et al., 2005; Nugent et al., 2018; Waterland & Michels, 2007)
Mid- to late gestation	Cell maturation, growth-related gene regulation, immune and metabolic differentiation	Maternal hyperglycaemia, adiposity, air pollution, smoking, metals and psychosocial stress	Largest human cohort evidence base, particularly for smoking and term tissues	Late gestational marks may be transient, reflect fetal growth or delivery, and are highly tissue- and cell-specific	(El Hajj et al., 2013; Joubert et al., 2014; Kotsakis Ruehlmann et al., 2023; Richmond et al., 2015)
Prenatal germline	Erasure and sex-specific re-establishment of germ-cell methylation	Direct exposure of fetal germ cells within an exposed pregnancy	Developmental maps are strong; human evidence for inherited exposure effects is weak	Effects observed in children or grandchildren of an exposed pregnant woman are not sufficient to establish true transgenerational inheritance	(Gkountela et al., 2015; Reik et al., 2001)

Note. H3K27me3 = trimethylation of histone H3 lysine 27. The classifications are interpretive and non-exhaustive; "strong" refers to evidence for developmental biology or exposure association, not necessarily causal mediation to disease

4. Maternal Nutritional and Metabolic Exposures

4.1 Undernutrition and Natural Experiments

Maternal nutrition is the oldest and most experimentally developed programming domain, but it is also vulnerable to reductionism. Nutrients provide energy and structural substrates, shape hormones and redox balance, alter placental growth, and supply cofactors for chromatin enzymes. An observed epigenetic difference cannot therefore be assigned automatically to a methyl-donor pathway. Severe undernutrition simultaneously changes glucose, amino acids, lipids, micronutrients, glucocorticoids and placental perfusion. The fetal response may include altered growth trajectories and organ allocation that secondarily change cell composition. Human evidence is strongest when exposure timing is externally constrained, but even natural experiments cannot fully isolate the molecular component responsible.

The Dutch Hunger Winter remains a pivotal quasi-experiment because an abrupt famine affected pregnancies at different gestational stages within a defined population. Adults exposed around conception showed persistent methylation differences at *IGF2* decades later, and subsequent genome-scale work identified additional loci related to growth and metabolism (Heijmans et al., 2008; Tobi et al., 2014). The findings support timing-specific persistence, particularly for early gestation, and are difficult to explain by short-term reverse causation because methylation was measured many years after exposure. Nevertheless, effect sizes were modest, exposure was inferred from historical rations rather than individual intake, survivors were selected, and wartime stress, infection and social disruption accompanied famine. The studies demonstrate an enduring molecular trace of a prenatal catastrophe, not a universal epigenetic mechanism for ordinary dietary variation.

Season-of-conception research in The Gambia offers complementary evidence. Seasonal changes in diet and biomarkers near conception predicted methylation at metastable epialleles in offspring, including loci that showed cross-tissue concordance (Dominguez-Salas et al., 2014; Silver et al., 2022). The approach combines repeated biochemical markers with a naturally varying environment and therefore strengthens temporal inference. Its limitation is precisely the richness of the exposure: folate, methionine, choline-related metabolites, infection, energy balance and agricultural workload vary together. Statistical association with a panel of one-carbon biomarkers does not prove that increasing a single supplement would reproduce the epigenetic pattern or improve health. The most defensible conclusion is that the periconceptional nutritional-metabolic environment can influence a specialised class of human epigenetic loci.

4.2 One-carbon Metabolism, Folate and Choline

One-carbon metabolism links folate, vitamins B2, B6 and B12, methionine, choline and betaine to S-adenosylmethionine, the principal methyl donor for DNA and histone methyltransferases. This biochemical connection makes methyl-donor nutrition mechanistically plausible, but the relationship is not linear. Methylation depends on enzyme activity, competing pathways, substrate ratios, cell proliferation and locus-specific recruitment of chromatin complexes. Both deficiency and excess may alter development, and a higher global methylation value is not intrinsically beneficial. Placental studies reviewed across one-carbon exposures remain heterogeneous in nutrient measurement, timing, tissue processing and genomic targets (van Vliet et al., 2024).

The agouti viable yellow mouse established a visually compelling model: maternal methyl-donor or genistein supplementation shifted methylation at a retrotransposon-linked allele, changing coat colour and metabolic susceptibility (Dolinoy et al., 2006; Waterland & Jirtle, 2003). Maternal nutrient supplementation also counteracted bisphenol A-associated hypomethylation at this locus (Dolinoy et al., 2007). These experiments demonstrate that diet can modify an environmentally responsive epiallele and that nutrition-toxicant interactions are possible. Their generalisability is limited by the unusual allele, exposure doses and mouse-specific genetic context. A locus whose phenotype is directly controlled by an inserted retrotransposon is an excellent mechanistic sensor but not a model for every human promoter or enhancer.

Choline deficiency in rodents increased global and *Igf2* methylation through altered *Dnmt1* expression, illustrating that deficiency can produce hypermethylation rather than the intuitive expectation of methyl-donor scarcity causing hypomethylation (Kovacheva et al., 2007). Human cohort evidence is similarly non-uniform. Maternal folate status and supplement use have been associated with small differences at imprinted genes and

repetitive elements, with the direction varying by locus and timing (Haggarty et al., 2013). Such findings are biologically credible but do not establish clinical harm or benefit. Folic acid prevents neural tube defects through well-established pathways, and epigenetic uncertainty should not be used to weaken proven supplementation policy. The relevant research question is whether dose, timing or nutritional context creates additional long-term effects, not whether methylation change alone invalidates supplementation.

4.3 Protein-energy Balance and Tissue-specific Programming

Maternal protein restriction models provide some of the clearest experimental links among diet, epigenetic regulation and organ function. In rats, low-protein gestation altered hepatic methylation and histone modifications at glucocorticoid receptor and peroxisome proliferator-activated receptor alpha regulatory regions, with folic acid partly preventing some changes (Lillycrop et al., 2005; Lillycrop et al., 2007). Related work reported persistence into adult offspring and, under particular designs, effects in a subsequent generation (Burdge et al., 2007). These studies benefit from controlled diet, accessible target organs and mechanistic assays. Their limitations are substantial: the diets can be more severe and compositionally simpler than typical human diets, liver findings cannot be assumed to represent other organs, and altered postnatal maternal care or lactation may contribute unless cross-fostering is used.

The animal literature also demonstrates why “global methylation” is an inadequate summary. Nutrient restriction can change different regulatory regions in opposite directions, and the same locus may show promoter methylation, histone modification and transcriptional changes that are not reducible to one scalar measure. Functional relevance depends on cell type and developmental stage. A hepatic adaptation that improves glucose production under anticipated scarcity may become maladaptive when postnatal nutrition is abundant, but this predictive-adaptive interpretation is difficult to test and should not be inferred from an adult metabolic phenotype alone. Human studies rarely have prenatal target-organ tissue, so mechanistic claims often rely on analogy rather than direct evidence.

4.4 Maternal Adiposity, Hyperglycaemia and Gestational Diabetes

At the opposite nutritional extreme, maternal obesity and gestational diabetes mellitus expose the placenta and fetus to altered glucose, insulin, lipids, adipokines, inflammation and oxidative stress. Placental glucose-transporter genes undergo dynamic methylation across gestation, indicating that nutrient transport is developmentally regulated rather than fixed (Novakovic et al., 2013). In human studies, gestational diabetes has been associated with methylation differences at *MEST*, an imprinted gene linked to growth and adiposity (El Hajj et al., 2013). These findings are compatible with metabolic programming, but the exposure is not a single state. Diagnostic thresholds, treatment, maternal pre-pregnancy adiposity, glycaemic trajectories, fetal growth and delivery timing differ across cohorts, and each may influence the epigenetic outcome.

Causal interpretation is especially difficult because fetal genotype can affect growth and placental function, maternal genotype can affect glycaemia, and shared family environments influence later obesity. Placental or cord-blood methylation measured at delivery may also be a consequence of fetal hyperinsulinaemia or accelerated growth rather than the mediator that initiated it. Associations between methylation at birth and childhood adiposity are important for prediction, but they require replication, tissue-specific functional evidence and control for baseline growth before being described as a causal pathway (Godfrey et al., 2011). Intervention studies that alter maternal glycaemia before methylation is measured offer stronger leverage, provided they are large enough and the epigenetic analyses are pre-specified.

4.5 Dietary Intervention Evidence and Translational Boundaries

Randomised trials are attractive because allocation reduces confounding, yet epigenetic endpoints introduce new problems. Trials designed for gestational weight gain or glycaemia may have modest dietary separation, imperfect adherence and biospecimens in only a selected subset. In the ROLO trial, a low-glycaemic-index intervention produced discovery-stage newborn blood methylation differences that did not replicate in an additional sample from the same trial (Geraghty et al., 2018). This negative replication is informative: it suggests that small epigenome-wide signals can arise from limited sample size, batch structure or chance even under randomisation. It also shows that a biologically sensible intervention does not guarantee a detectable methylation signature at birth.

Translation should therefore prioritise maternal and child health outcomes rather than methylation normalisation. A nutrient intervention may improve development without producing a stable cord-blood mark, and an epigenetic change may be adaptive or clinically neutral. The evidence does not support universal "epigenetic diets" or supplementation above established requirements. It supports adequate, balanced nutrition; prevention and treatment of maternal metabolic disease; and targeted trials that collect repeated nutritional biomarkers, account for baseline status and test functionally justified epigenetic outcomes. Nutrition may also modify toxicant effects, but apparent protection can be confounded by overall diet quality and socioeconomic resources (Shafiq et al., 2026).

Table 2 compares the principal nutritional and metabolic evidence streams. The pattern is not a simple hierarchy from animal to human: animal models offer causal mechanism but uncertain exposure realism, natural experiments offer timing and persistence but limited exposure specificity, and trials offer allocation but often insufficient molecular power. Nutritional studies are most persuasive when the exposure is measured biochemically, the developmental window is specified in advance and methylation is linked to transcription or physiology. Confidence is highest when designs converge on the same developmental process, and the recurrent absence of independent replication means that locus-level results should not be converted into dietary recommendations without outcome evidence.

Table 2. Maternal nutritional and metabolic exposures: mechanisms, evidential strength and limitations

Exposure domain	Representative evidence	Putative epigenetic route	Evidential judgement	Principal limitations	Supporting references
Severe energy deprivation	Dutch Hunger Winter; timing-specific adult blood methylation at *IGF2* and other growth/metabolic loci	Altered substrate supply, endocrine stress, placental allocation and lineage-specific methylation	Moderate-to-strong evidence for persistent exposure-associated marks; limited evidence that the marks mediate disease	Historical exposure proxy, survivor selection and co-occurring wartime stress	(Heijmans et al., 2008; Tobi et al., 2014)
Periconceptional seasonal nutrition	Maternal biomarkers and season of conception associated with metastable epiallele methylation	One-carbon availability during early methylation establishment	Strong evidence for sensitivity of selected loci; weak attribution to any single nutrient	Correlated nutrients, infection and workload; specialised loci may not represent the wider methylome	(Dominguez-Salas et al., 2014; Silver et al., 2022)
Methyl-donor and phytochemical supplementation	Agouti mouse coat-colour and metabolic phenotypes; mitigation of bisphenol A-associated hypomethylation	S-adenosylmethionine supply and locus-specific chromatin regulation	Strong experimental proof of principle; uncertain human generalisability	Unusual epiallele, high or non-routine doses and species-specific genetic context	(Dolinoy et al., 2006; Dolinoy et al., 2007; Waterland & Jirtle, 2003)
Protein restriction	Rodent hepatic glucocorticoid receptor and PPARα regulation, with partial prevention by folic acid	Altered methyltransferase expression, DNA methylation and histone modifications	Strong tissue-specific animal mechanism; limited direct human evidence	Diet severity, organ specificity and postnatal maternal effects	(Burdge et al., 2007; Lillycrop et al., 2005; Lillycrop et al., 2007)
Folate and choline in human pregnancy	Small locus-specific differences at imprinted genes and repetitive elements	One-carbon flux and methyl-donor balance	Plausible but inconsistent; no basis for interpreting higher methylation as uniformly beneficial	Timing, baseline status, genotype, supplement formulation and multiple testing	(Haggarty et al., 2013; Kovacheva et al., 2007; van Vliet et al., 2024)

Exposure domain	Representative evidence	Putative epigenetic route	Evidential judgement	Principal limitations	Supporting references
Maternal hyperglycaemia and gestational diabetes	Placental nutrient-transporter regulation and newborn *MEST* methylation associations	Glucose/insulin signalling, inflammation, oxidative stress and placental transport	Moderate evidence for molecular embedding; causal mediation remains uncertain	Maternal adiposity, treatment, fetal growth, genotype and delivery-related confounding	(El Hajj et al., 2013; Novakovic et al., 2013)
Dietary intervention	Low-glycaemic-index trial with discovery signals that did not replicate internally	Potential modification of glycaemia, growth and one-carbon-related pathways	Insufficient evidence for a reproducible intervention-induced newborn methylome signature	Small biospecimen subsets, adherence, modest exposure contrast and batch effects	(Geraghty et al., 2018)

Note. PPARα = peroxisome proliferator-activated receptor alpha. Evidence judgements concern prenatal epigenetic programming, not the established obstetric value of correcting nutrient deficiency or treating metabolic disease

5. Maternal Toxicant and Ambient Environmental Exposures

5.1 Tobacco Smoke as the Most Reproducible Human Model

Maternal smoking provides the clearest human example of an exposure-associated newborn methylation signature. Tobacco smoke is a complex mixture, but exposure can be measured through self-report, cotinine and smoking persistence, and effects have been examined in multiple cohorts and tissues. Early studies reported altered global and gene-specific methylation in cord blood and coordinated placental methylation-expression differences (Breton et al., 2009; Suter et al., 2011). Larger studies subsequently identified recurrent loci in pathways involved in xenobiotic response, haematopoiesis and growth, including *AHRR*, *CYP1A1*, *GFI1* and *MYO1G*. Consistency across cohorts, exposure-response patterns and objective biomarkers distinguish smoking from many other prenatal exposures (Joubert et al., 2014).

Longitudinal evidence strengthens the interpretation that some marks are durable. In the Avon Longitudinal Study of Parents and Children, methylation differences related to sustained maternal smoking were detectable from birth into later childhood, although trajectories differed by locus (Richmond et al., 2015). Adult methylation signatures can also retain information about prenatal smoke exposure (Richmond et al., 2018). Persistence does not prove that these marks mediate smoking-related disease. Blood-cell composition changes across life, postnatal second-hand smoke and personal smoking can reinforce or obscure signals, and methylation may be a stable dosimeter rather than a pathogenic mechanism. Nonetheless, prenatal smoking is the domain in which the criteria of temporal exposure, independent replication and lasting molecular association are most consistently met.

Placental microRNAs provide a second regulatory layer. Maternal smoking has been associated with lower placental expression of miR-16, miR-21 and miR-146a, molecules involved in growth, apoptosis and inflammatory signalling (Maccani et al., 2010). Such findings broaden the mechanism beyond DNA methylation but are less extensively replicated and more sensitive to tissue handling. Tobacco also affects oxygen delivery, vascular function and fetal growth; epigenetic differences may therefore reflect both chemical exposure and physiological hypoxia. Causal attribution to nicotine, polycyclic aromatic hydrocarbons or another constituent remains difficult. The public-health conclusion does not depend on that attribution: smoking cessation before or during pregnancy has clear benefits, while epigenetic biomarkers may improve exposure reconstruction and mechanistic understanding.

5.2 Ambient Air Pollution and Placental Oxidative Stress

Air pollution studies link residential particulate matter estimates with placental or cord-blood methylation, mitochondrial regulation and microRNA expression. Early-pregnancy particulate exposure has been associated with placental DNA methylation differences (Cai et al., 2017), while other work has related particulate matter to

placental mitochondrial DNA methylation (Janssen et al., 2015) and to cord-blood methylation across the *IGF2/H19* growth-regulatory cluster (Wang et al., 2020). Candidate microRNA analyses suggest gestational-window-specific differences in placental miR-20a, miR-21, miR-146a and miR-222 (Tsamou et al., 2018). Together, these findings are compatible with oxidative stress, mitochondrial dysfunction, inflammation and altered growth signalling at the maternal-fetal interface.

The main limitation is exposure reconstruction. Residential models may estimate outdoor particulate concentrations precisely at an area level while misclassifying personal exposure because of mobility, indoor sources, occupation, ventilation and time spent away from home. Trimester averages are highly correlated, so distributed-lag or week-specific peaks may be unstable. Pollution also follows roads, housing and socioeconomic disadvantage; adjustment for education or neighbourhood index may leave structural confounding. Placental samples are collected at birth, when gestational age, labour and obstetric complications can influence both epigenetic profiles and the probability of enrolment. Replication should therefore require comparable exposure metrics and genomic coordinates, not merely enrichment of broad oxidative-stress pathways.

5.3 Metals: Exposure Matrices, Sex Differences and Contradictory Results

Arsenic, lead and mercury can cross or affect the placenta and interact with redox and one-carbon pathways. Low-level arsenic exposure has been associated with differential cord-blood methylation, and studies in Bangladesh reported genome-wide associations that were more evident in boys (Broberg et al., 2014; Koestler et al., 2013). Joint analysis of arsenic and mercury identified additional cord-blood loci, emphasising that populations are rarely exposed to one metal at a time (Cardenas et al., 2015). Nutritional status, particularly folate and selenium-related pathways, can alter metal metabolism or toxicity, but effect modification is difficult to separate from correlated diet and socioeconomic conditions (Shafiq et al., 2026).

Lead illustrates why apparently inconsistent studies are valuable. An epigenome-wide study in rural Bangladesh associated prenatal lead biomarkers with lower methylation at sites in *GP6*, a platelet-activation gene (Engström et al., 2015). A larger Mexican cohort with multiple maternal, cord and bone lead measures found no cord-blood epigenome-wide association after correction, despite the study's ability to recover known methylation correlates of birth size (Heiss et al., 2020). Placental work has reported associations of lead biomarkers with both methylation and hydroxymethylation (Tung et al., 2022). The discrepancy may reflect exposure range, timing, matrix, population nutrition, tissue and array processing rather than a simple false-positive/true-negative dichotomy. It also shows that pathway plausibility cannot substitute for independent coordinate-level replication.

Different exposure matrices answer different questions. Blood captures relatively recent circulating dose, urine may reflect excretion and short-term exposure, toenails integrate a longer interval, bone lead represents cumulative stores, and placenta measures a mixture of deposition and tissue handling. Cord blood is both an exposure matrix and a heterogeneous outcome tissue. When studies select the biomarker or gestational window that yields the strongest result after examining several correlated alternatives, nominal timing specificity may be exaggerated. Pre-specified windows, mixture-aware models and replication across matrices are needed before methylation is used to infer a biologically critical period.

5.4 Phenols, Phthalates and Per- and Polyfluoroalkyl Substances

Endocrine-disrupting chemicals can alter nuclear-receptor signalling, steroidogenesis, oxidative balance and placental development, providing several routes to epigenetic change. Placental epigenome-wide analysis of prenatal phenols and phthalates has identified exposure-associated CpGs, but the number and direction of signals differ by compound, fetal sex and analytical model (Jedynak et al., 2024). For non-persistent chemicals, a single urine sample is a noisy measure of gestational exposure because concentrations vary with recent product use, hydration and metabolism. Such error usually weakens associations but can also destabilise rankings among correlated metabolites. Creatinine or specific-gravity correction introduces additional modelling choices.

Per- and polyfluoroalkyl substances (PFAS) are more persistent and can be measured in maternal plasma or cord serum, yet they occur as correlated mixtures. A prospective cohort linked early-pregnancy PFAS concentrations

with placental methylation at selected candidate genes after a small discovery sequencing analysis; the mediation finding for infant developmental delay was exploratory and requires replication (Xie et al., 2024). A larger 2026 cohort related PFAS exposure to vulnerable birth phenotypes and identified placental CpGs in a small methylation subsample, with stronger exposure-outcome associations in female infants (Zhao et al., 2026). The temporal measurement and biologically relevant tissue are strengths, but epigenetic subsamples remain much smaller than the parent cohorts. Apparent mediation can be sensitive to selection, multiple testing and unmeasured determinants of both placental methylation and infant development.

Chemical-by-chemical interpretation is increasingly untenable. Phenols, phthalates, PFAS, metals, smoking and air pollutants share sources and pathways, while diet supplies both protective nutrients and contaminants. A fish-rich diet, for example, may provide long-chain fatty acids and selenium while increasing methylmercury or PFAS exposure. Statistical mixture methods can estimate joint patterns but do not solve measurement error, collinearity or causal identification. Mechanistic experiments should prioritise environmentally realistic mixtures and concentrations, while cohorts need repeated biospecimens and exposomic calibration rather than a single high-dimensional screen at delivery.

5.5 Interpretation and Prevention

Environmental epigenetics can support prevention without requiring that each methylation mark be a validated mediator. Replicated signatures may identify exposure, susceptible windows or affected biological systems; mechanistic evidence can strengthen regulation of harmful exposures. Yet framing epigenetic marks as individual susceptibility risks shifting attention from upstream sources. Air quality, occupational protection, smoke-free environments, safe water and chemical regulation are population-level interventions. Pregnant individuals cannot reasonably avoid ubiquitous contaminants through consumer choice alone, and advice based on preliminary methylation findings may create anxiety or inequity. The evidence is most useful when it informs exposure reduction and better causal studies, not when it converts uncertain molecular associations into personalised blame.

Table 3 contrasts maternal smoking, a comparatively mature exposure domain, with emerging environmental domains that have weaker replication. It also demonstrates why null studies are needed to calibrate confidence: without them, the literature would overstate reproducibility and obscure dependence on exposure matrix and tissue. Across domains, confidence rises when exposure is measured repeatedly or by a specific biomarker, the same CpG is replicated, and methylation is connected to transcription or placental function. Broad pathway overlap without coordinate-level replication is supportive at most because enrichment can be driven by annotation density and flexible analytical choices.

Table 3. Environmental exposures and fetal or placental epigenetic evidence

Exposure	Principal tissue or matrix	Recurrent signals or pathways	Consistency of evidence	Major interpretive constraint	Supporting references
Maternal smoking	Cord blood, placenta and longitudinal offspring blood	Repeated loci include *AHRR*, *CYP1A1*, *GFI1* and *MYO1G*; placental microRNAs	Highest replication across independent human cohorts; selected marks persist	Complex smoke mixture, postnatal exposure and blood-cell composition complicate mediation	(Breton et al., 2009; Joubert et al., 2014; Maccani et al., 2010; Richmond et al., 2015; Richmond et al., 2018; Suter et al., 2011)
Particulate air pollution	Placenta, placental mitochondrial DNA and cord blood	Mitochondrial regulation, *IGF2/H19*, microRNAs and oxidative-stress pathways	Moderate evidence for exposure-associated variation; limited locus-level replication	Residential exposure error, correlated gestational windows and socioeconomic confounding	(Cai et al., 2017; Janssen et al., 2015; Tsamou et al., 2018; Wang et al., 2020)

Exposure	Principal tissue or matrix	Recurrent signals or pathways	Consistency of evidence	Major interpretive constraint	Supporting references
Arsenic and mercury	Cord blood	Genome-wide and candidate methylation signals, sometimes sex-specific	Suggestive human evidence across several cohorts	Co-exposure, one-carbon nutrition, exposure range and population-specific metabolism	(Broberg et al., 2014; Cardenas et al., 2015; Koestler et al., 2013)
Lead	Cord blood and placenta; blood, urine, bone and toenail biomarkers	*GP6* and developmental pathways; methylation and hydroxymethylation	Inconsistent: positive studies coexist with a well-characterised null EWAS	Matrix-specific timing, modest samples, mixtures and multiple correlated tests	(Engström et al., 2015; Heiss et al., 2020; Tung et al., 2022)
Phenols and phthalates	Placenta	Compound- and sex-specific CpGs involving endocrine and developmental pathways	Emerging evidence; replication remains sparse	Short half-lives, within-person variability, correlated metabolites and exposure correction choices	(Jedynak et al., 2024)
PFAS	Placenta with maternal plasma or cord-serum exposure	Angiogenesis, neuronal signalling, growth and cell-migration-related loci	Emerging prospective evidence with small epigenetic subsamples	Correlated mixtures, mediation assumptions, selection into biospecimen subsets and uncertain functional persistence	(Xie et al., 2024; Zhao et al., 2026)

Note. EWAS = epigenome-wide association study; PFAS = per- and polyfluoroalkyl substances. The table is representative rather than exhaustive

6. Maternal Psychosocial Environment

6.1 Biological Pathways from Maternal Experience to the Fetal Environment

Psychosocial conditions reach the fetus through biology and behaviour rather than through a single transferable exposure. Depression, anxiety, trauma, intimate partner violence and socioeconomic adversity can alter hypothalamic-pituitary-adrenal activity, sympathetic tone, sleep, inflammation, medication use, diet and smoking. Placental glucocorticoid metabolism is a prominent candidate pathway because 11β-hydroxysteroid dehydrogenase type 2 limits fetal exposure to active maternal glucocorticoids, while glucocorticoid receptor and FKBP5 regulation influence stress responsivity. These pathways are plausible, but they are not specific to psychosocial stress; infection, undernutrition, obesity and obstetric complications can activate the same systems. A molecular association with a stress-pathway gene therefore cannot identify the initiating exposure without careful measurement of the wider maternal context.

Animal models provide direct manipulation and target-tissue access, yet the construct validity of restraint, noise or variable stress for human depression, structural racism or intimate partner violence is limited. The placental H3K27me3 work demonstrates that trophoblast chromatin can causally modify sex-specific offspring responses to an experimental prenatal insult (Nugent et al., 2018). It does not establish that a comparable histone mechanism mediates ordinary variation in human perceived stress. Human research is necessary for lived exposures, but it lacks experimental allocation and often measures stress retrospectively or with brief symptom scales. Convergence across these designs should be sought at the level of pathway and function, not assumed from shared terminology.

6.2 Candidate-gene Evidence: Informative Beginnings, Limited Specificity

Early human studies concentrated on candidate loci in the glucocorticoid pathway. Maternal depression was associated with newborn *NR3C1* methylation and infant cortisol response in a small cohort (Oberlander et al.,

2008). Placental *NR3C1* and *HSD11B2* methylation were subsequently examined in relation to maternal mood and newborn neurobehaviour (Conradt et al., 2013), while placental *FKBP5* genetic and epigenetic variation was associated with infant neurobehavioural outcomes (Paquette et al., 2014). These studies were valuable because they linked a biologically coherent pathway to endocrine or behavioural phenotypes. Their small samples, targeted regions and multiple CpG tests make effect estimates unstable, and publication of positive candidate genes creates a literature in which denominator and null findings are difficult to reconstruct.

Studies of adversity broadened the exposure beyond psychiatric symptoms. Placental *HSD11B2* methylation varied with indicators of prenatal socioeconomic adversity (Appleton et al., 2013), and maternal intimate partner violence was associated with offspring *NR3C1* promoter methylation (Radtke et al., 2011). The latter article used "transgenerational" in its title, but the design examined mothers and their directly exposed children. In a pregnancy, both the fetus and its developing germ cells can be exposed; such evidence is intergenerational and cannot establish germline transmission beyond direct exposure. Terminological precision is essential because claims of inherited trauma can become deterministic and socially consequential despite limited molecular evidence.

6.3 Epigenome-wide Studies and the Replication Problem

Epigenome-wide association studies reduce reliance on preselected genes but increase the multiple-testing burden. Cumulative lifetime maternal stress has been associated with placental methylation at several loci, with patterns varying by infant sex and stress domain (Brunst et al., 2018). A large consortium meta-analysis of prenatal stressful life events across multiple cohorts identified only a small number of epigenome-wide signals, including a cumulative-stress association at a site annotated to *ALKBH3*, and different loci for specific stress categories (Kotsakis Ruehlmann et al., 2023). The sparse result is not evidence that stress has no fetal biological effects. It indicates that a common, large cord-blood methylation signature is unlikely under heterogeneous exposure definitions and populations.

A systematic review of maternal depression, anxiety and treatment EWAS found minimal CpG replication, overlapping differentially methylated regions mainly for depression, and medium-to-high concerns about bias (Drzymalla et al., 2023). This is a stronger basis for inference than counting the number of nominally positive articles. Exposure misclassification is considerable: symptom scales at one visit do not capture chronicity, clinical diagnosis, treatment response or acute events, while medication is confounded by indication. The epigenome-wide findings may also differ between placenta and cord blood because the placenta responds directly to maternal endocrine and immune signals, whereas cord blood reflects haematopoietic composition at birth. Non-overlap between tissues may be expected rather than a failed replication, but tissue-specific hypotheses should be stated in advance.

Epigenetic age has been proposed as an integrative outcome. Maternal stress has been associated with site-specific methylation and measures of age acceleration in maternal and newborn samples (Quinn et al., 2023). Interpretation is uncertain because clocks trained in adult blood or across gestation may capture cell composition, gestational maturity or technical structure rather than biological ageing in a newborn. A statistically accelerated clock is not equivalent to accelerated organ senescence. Validation should require association with independent developmental measures, stability across platforms and prediction of later function.

6.4 Social Patterning, Confounding and Ethical Interpretation

Psychosocial exposures are socially produced and cluster with material conditions. Housing, food security, discrimination, occupational hazards, neighbourhood pollution and access to care shape both reported stress and biological exposures. Adjusting for smoking, body mass index or education may remove part of a causal pathway, leave residual confounding or create collider bias, depending on the research question. Studies often treat these variables as interchangeable covariates without a causal model. The maternal report is also not merely measurement error: perception, coping and social support may alter physiological response, while the same questionnaire score can arise from very different experiences.

The evidence therefore supports biological embedding as a credible general process but does not support a deterministic prenatal "stress methylome". Candidate-gene studies identify plausible pathways; consortium

EWAS show that any common cord-blood signal is small and exposure-specific. Stronger inference will require repeated clinical and contextual assessment, objective endocrine or inflammatory measures, paternal or sibling negative controls where appropriate, and integration with placental function. Communication must avoid implying that maternal emotion directly damages the fetal genome. Structural prevention, mental-health care and protection from violence are justified by extensive health evidence independently of whether a particular CpG mediates their effects.

Table 4 places candidate-gene and epigenome-wide findings on the same evidential scale. Its central message is that mechanistic coherence has not yielded a stable universal molecular signature; the most credible conclusion concerns pathway responsiveness and heterogeneity rather than one locus or direction of methylation. The distinction between a plausible stress-response pathway and a validated exposure biomarker is therefore critical: a locus can be biologically relevant without being sufficiently specific, replicated or stable to support individual prediction.

Table 4. Maternal psychosocial exposures and offspring or placental epigenetic findings

Psychosocial exposure	Molecular target or tissue	Pattern of evidence	Critical interpretation	Principal limitations	Supporting references
Depressive symptoms	Cord blood *NR3C1*; infant cortisol response	Candidate-region association linked to endocrine function	Mechanistically coherent but based on small samples and selected CpGs	Residual confounding, symptom timing and selective reporting	(Oberlander et al., 2008)
Maternal mood disorder	Placental *NR3C1* and *HSD11B2*; newborn neurobehaviour	Associations within glucocorticoid regulation	Suggestive pathway evidence; limited independent replication	Placental cell composition, multiple testing and outcome subjectivity	(Conradt et al., 2013)
Socioeconomic adversity	Placental *HSD11B2*	Patterned methylation across adversity indicators	Supports social patterning of placental regulation, not a single causal exposure	Adversity indicators co-vary with nutrition, smoking, pollution and health care	(Appleton et al., 2013)
Intimate partner violence	Offspring blood *NR3C1*	Association described as transgenerational in the source article	Intergenerational association only; no evidence of transmission beyond direct fetal exposure	Retrospective exposure, small sample and imprecise inheritance terminology	(Radtke et al., 2011)
Cumulative lifetime stress	Placental EWAS	Sex- and domain-dependent methylation signals	Suggestive evidence that placental response depends on stress history and fetal sex	Lifetime and pregnancy exposure are difficult to separate; replication is sparse	(Brunst et al., 2018)
Stressful life events	Cord-blood meta-EWAS across cohorts	Few epigenome-wide loci, with domain-specific associations	Best-powered evidence argues against a large universal stress signature	Heterogeneous instruments, timing, populations and cord-blood cell composition	(Kotsakis Ruehlmann et al., 2023)
Depression, anxiety and treatment	Infant EWAS literature	Minimal CpG replication; some overlapping regions for depression	Overall evidence remains inconsistent and at risk of bias	Exposure misclassification, confounding by indication, small samples and analysis flexibility	(Drzymalla et al., 2023)

Note. EWAS = epigenome-wide association study; HSD11B2 = 11β-hydroxysteroid dehydrogenase type 2. Gene symbols denote genes; methylation location and regulatory direction vary across studies

7. Cross-Cutting Methodological and Causal Challenges

7.1 Tissue, Cell Composition and Developmental Time

Epigenetic regulation is cell-specific, so bulk-tissue association is inseparable from tissue composition. Cord blood proportions vary with gestational age, labour, infection, fetal sex and maternal exposures. Reference-based deconvolution can estimate major blood-cell fractions from methylation arrays (Houseman et al., 2012), and failure to account for heterogeneity can generate or conceal associations (Jaffe & Irizarry, 2014). Adjustment is not a universal remedy. If an exposure genuinely changes immune-cell development, cell composition may be part of the causal effect rather than a nuisance. Analyses should therefore distinguish total-tissue association, composition-mediated change and within-cell-type regulation. Placenta presents a harder problem because reference profiles are less complete, trophoblast states change across gestation, and sampling depth and location matter.

Tissue substitution remains the field's largest conceptual limitation. A cord-blood CpG can be an excellent exposure biomarker and a poor proxy for brain chromatin. Placental methylation may influence fetal development through transport or endocrine function without being reproduced in fetal organs. Conversely, the absence of a cord-blood signal does not exclude programming in pancreas, hypothalamus or liver. Cross-tissue concordance should be demonstrated empirically, as for selected metastable epialleles, rather than presumed. Studies that use later peripheral blood to infer persistence must also separate maintenance of the same cells from re-emergence of an exposure-associated signature in a different cellular context.

7.2 Exposure Measurement, Correlated Windows and Mixtures

Maternal exposures change over pregnancy, yet many studies measure them once. Short-lived chemicals and episodic stress are especially prone to within-person error, while diet questionnaires incompletely capture biomarkers and metabolism. Even persistent pollutants may have trimester-dependent transfer or mobilisation. Searching many correlated windows and reporting the strongest one inflates apparent timing specificity. Repeated measures, pharmacokinetic knowledge and pre-specified distributed-lag models are preferable. For historical exposures, natural experiments can define timing but rarely quantify individual dose. Measurement error should be modelled, not mentioned only as a generic limitation.

The maternal exposome is also a mixture. Statistical models that enter one pollutant or one nutrient at a time may estimate a proxy for a correlated source. Mixture models can capture joint effects and non-linearity, but high collinearity makes component attribution unstable and results depend on scaling and prior assumptions. Mechanistic interpretation is strongest when epidemiological mixtures are paired with toxicological experiments at realistic concentrations and when nutritional modifiers are measured directly. The objective should not be to force a single causal compound from an inseparable exposure profile, but to identify intervention-relevant sources and biological pathways.

7.3 Genotype, Confounding and Causal Direction

DNA sequence strongly influences methylation through methylation quantitative trait loci. Maternal genotype can shape behaviour, metabolism and placental environment, while fetal genotype influences methylation, growth and tissue composition. Failure to model genotype can therefore produce an exposure-methylation association through population structure or inherited liability. Socioeconomic conditions, ancestry and geography are entangled in many cohorts; genetic principal components cannot substitute for detailed social and exposure measurement (Greally, 2017). Designs that include maternal, paternal and fetal genotypes can separate some pathways, but they require adequate sample size and explicit causal diagrams.

Causal direction is similarly difficult. Methylation measured at birth can be upstream of a phenotype, a response to altered fetal growth, or a correlate of a third process. Two-step epigenetic Mendelian randomisation was proposed to test exposure-to-methylation and methylation-to-outcome paths using genetic instruments (Relton & Davey Smith, 2012). Its usefulness depends on strong, specific instruments and assumptions that are often

violated by pleiotropy, developmental changes and tissue mismatch. Paternal exposure, negative-control outcomes, sibling comparisons and randomised interventions provide complementary leverage. No single method is decisive; triangulation across methods with different biases is more credible than declaring causality from mediation coefficients.

7.4 Multiple Testing, Replication and Functional Validation

Epigenome-wide studies test hundreds of thousands of correlated CpGs, often in samples far smaller than genome-wide association studies. Batch, probe cross-reactivity, genotype at probe sites, dye bias and preprocessing choices can change results. Good practice requires a priori analysis plans, stringent quality control, appropriate multiple-testing correction and independent replication (Michels et al., 2013). Region-based analyses can increase power but introduce choices about smoothing and boundaries. Pathway enrichment is exploratory because genes differ in probe coverage and annotation density. Replication should match direction, genomic coordinate, tissue, exposure definition and developmental timing; a shared broad pathway is not equivalent.

Array coverage also constrains discovery. The 450K and EPIC platforms share many probes but differ in enhancer and regulatory coverage, and placental performance requires tissue-specific quality assessment (Fernández-Jiménez et al., 2019). Whole-genome or reduced-representation bisulphite sequencing expands coverage but can trade breadth for sample size. Standard bisulphite assays conflate methylation and hydroxymethylation, and most cohort archives cannot assess histone marks or chromatin accessibility. Multi-omic integration is valuable only when assays are performed in comparable cells and analysed with clear temporal hypotheses; combining unrelated omic layers can create impressive networks without stronger causal evidence.

Functional validation is the step most often missing. Exposure-associated methylation should be connected to allele-specific expression, chromatin accessibility, transcription-factor binding, transporter activity or cellular phenotype. Placental trophoblast stem cells, organoids and perfusion models can test whether editing a candidate regulatory region changes function under controlled exposure. Animal models can establish organ outcomes and developmental timing, but human relevance requires comparison of dose, metabolism and genomic context. The field should reward precise null results and failed replication because they limit false mechanistic narratives (Birney et al., 2016).

7.5 Persistence, Inheritance and Population Generalizability

Persistence is not a binary property. A mark may persist in one lineage, change direction during maturation or remain detectable only because exposure continues. Longitudinal sampling from birth through childhood is essential, but peripheral blood trajectories cannot establish persistence in inaccessible organs. Claims of transgenerational inheritance require even greater restraint. In an exposed pregnancy, the mother, fetus and fetal germline can all be directly affected. Human evidence from the next generation may therefore still represent direct exposure, shared environment or genetic transmission. True germline inheritance would require later generations, robust exclusion of social and genetic pathways, and a plausible mechanism through epigenetic reprogramming.

Generalisability is limited by the concentration of cohorts in Europe and North America, despite higher exposures and nutritional constraints in many other settings. Ancestry affects genetic regulation, but race and ethnicity are social as well as genetic categories; using them as simple covariates obscures the sources of exposure disparity. Diverse cohorts need local exposure measurement, culturally valid psychosocial instruments and equitable governance of biospecimens. Without such design, epigenetic biomarkers may perform poorly outside the populations in which they were developed and could reproduce inequity in risk prediction. Table 5 translates recurring methodological problems into minimum design responses and pairs each recommendation with the distortion it is intended to address. No single design can eliminate every threat; the most credible studies will combine precise exposure assessment, developmentally relevant tissues, independent replication and a second method that challenges the same causal claim from a different direction.

Table 5. Methodological threats, inferential consequences and design priorities

Challenge	How it can distort inference	Minimum design response	Priority advance	Supporting references
Bulk-tissue heterogeneity	Creates apparent methylation differences through altered cell proportions or conceals cell-specific effects	Measure or estimate cell fractions; report total-tissue and composition-adjusted models; use purified or single-cell data where feasible	Cell-resolved placental and cord-blood reference atlases across gestation	(Houseman et al., 2012; Jaffe & Irizarry, 2014)
Tissue substitution	Encourages inference from cord blood or placenta to inaccessible fetal organs	State the biological role of the sampled tissue; demonstrate cross-tissue concordance rather than assume it	Linked placenta, cord blood, organoid and longitudinal offspring studies	(Robinson & Price, 2015; Silver et al., 2022)
Exposure timing and error	Produces attenuation, unstable critical windows and post hoc timing claims	Repeat biomarkers or questionnaires; use pharmacokinetic knowledge and pre-specified window models	Wearables, personal sensors, repeated metabolomics and calibrated exposomics	(Cai et al., 2017; Jedynak et al., 2024)
Genetic and social confounding	Attributes methylation to exposure when sequence, ancestry or structured social conditions explain both	Collect maternal, paternal and fetal genotype; use causal diagrams and detailed social/exposure measures	Family-based designs and diverse cohorts with methylation quantitative trait locus integration	(Greally, 2017; Michels et al., 2013)
Causal mediation	Treats cross-sectional methylation as a mediator despite unmeasured mediator-outcome confounding or reverse causation	Establish temporality; triangulate trials, natural experiments, negative controls and genetic instruments	Two-step and multivariable causal analyses supported by functional perturbation	(Relton & Davey Smith, 2012)
Multiple testing and analytic flexibility	Inflates false-positive loci and unstable pathway narratives	Pre-register analyses, correct for testing, publish null findings and replicate exact coordinates	Consortium-scale harmonisation and prospective replication	(Birney et al., 2016; Michels et al., 2013)
Limited functional evidence	Equates methylation difference with altered gene expression or disease mechanism	Measure expression, chromatin and cellular phenotype; edit candidate regions where possible	Trophoblast organoids, placenta perfusion and CRISPR-based epigenome editing	(Nugent et al., 2018; Suter et al., 2011)
Restricted assay coverage	Misses enhancers, repetitive elements, hydroxymethylation and non-DNA-methylation mechanisms	Report platform coverage and probe filters; use orthogonal validation	Whole-genome, oxidative-bisulphite, chromatin-accessibility and histone profiling in matched cells	(Fernández-Jiménez et al., 2019; Tung et al., 2022)

Note. CRISPR = clustered regularly interspaced short palindromic repeats. Recommendations apply differently according to exposure and tissue; they are not a checklist that converts an observational EWAS into causal evidence

8. Future Directions

8.1 Immediate Priorities: Measurement, Replication and Developmental Specificity

The immediate priority is not another generation of small, single-time-point EWAS, but coordinated studies designed around developmental mechanism. Pregnancy cohorts should collect repeated exposure biomarkers before conception where feasible and in each trimester, alongside diet, medication, infection, sleep, mental health and social context. Placental sampling needs standardised anatomical location, depth, processing time and documentation of labour and pathology. Cord blood studies should report cell-composition strategies and distinguish total-tissue from within-cell inference. A common minimal metadata set would allow consortia to replicate exact CpGs and regions rather than compare incompletely harmonised pathway labels. The analytical standards proposed for EWAS should be treated as a floor, with prospective replication specified before discovery results are interpreted mechanistically (Michels et al., 2013).

Developmental timing should be built into design rather than inferred from correlated trimester estimates. Repeated exposure measures can support pre-specified lag models, but they should be paired with biological information about placental transport, organogenesis and epigenetic establishment. Periconceptional studies need recruitment before pregnancy or validated retrospective biomarkers; delivery-only recruitment cannot recover this window reliably. Longitudinal biospecimens should extend beyond birth so that persistence, reversion and postnatal reinforcement can be separated. Where target organs are inaccessible, investigators should state whether the objective is exposure biomarking, placental mechanism or systemic prediction, because each requires different validation.

8.2 Causal Triangulation and Intervention

Causal claims should be tested through triangulation. Randomised trials are appropriate for established safe nutritional or behavioural interventions, while natural experiments, policy changes and exposure regulations can strengthen inference for environmental hazards. Paternal exposure, sibling comparisons and negative-control periods can help detect familial or temporal confounding. Genetic instruments may contribute when strong tissue-relevant methylation quantitative trait loci exist, but should not be used mechanically (Relton & Davey Smith, 2012). The decisive advance would be convergence: an exposure changes a placental or fetal regulatory feature in a trial or natural experiment, the feature predicts relevant function, and targeted perturbation reproduces or reverses that function in a model.

Intervention studies need adequate epigenetic power and clinically meaningful endpoints. Discovery panels embedded in small trial subsets should be labelled exploratory, as the ROLO experience demonstrates (Geraghty et al., 2018). Trials of folate, choline or other methyl donors should stratify or model baseline status, genotype, diet and co-exposures rather than assume a uniform dose-response. Environmental interventions should prioritise source reduction, such as smoke-free policy or air-quality improvement, and evaluate whether molecular changes accompany improved birth or childhood outcomes. Epigenetic normalisation should never replace direct health benefit as the criterion for success.

8.3 Cell-resolved, Multi-omic and Functional Models

Single-cell and spatial methods can resolve whether an association arises in trophoblast, endothelium, stroma or immune cells and whether it localises to a placental microenvironment. These approaches should be applied across gestation, not only at term, and linked to reference atlases that support deconvolution in large cohorts. Whole-genome methylation, hydroxymethylation, chromatin accessibility, histone profiling, transcriptomics and metabolomics can then be integrated within the same cells or carefully matched samples. The objective is a directed mechanistic chain, not maximal omic breadth. Platform comparisons and orthogonal validation remain necessary because expanded coverage does not remove batch or annotation bias (Fernández-Jiménez et al., 2019).

Human trophoblast stem cells, placental organoids and perfusion systems offer a bridge between cohort association and animal causality. Candidate regulatory regions can be edited with locus-specific epigenome tools, followed by measurement of transport, hormone metabolism, inflammatory signalling and barrier function. Exposure concentrations should reproduce maternal and placental biomonitoring ranges, including

mixtures. Animal models remain essential for inaccessible fetal organs and lifelong outcomes, but their diets, stressors and toxicant doses should be explicitly compared with human exposure. Sex-specific placental mechanisms, exemplified by experimental H3K27me3 regulation, should be tested as hypotheses rather than discovered only after pooled analyses fail (Nugent et al., 2018).

8.4 Mixtures, Diversity and Equitable Translation

Longer-term research must move from isolated exposures to an integrated maternal exposome while preserving causal interpretability. Diet can modify toxicant absorption and redox response, psychosocial adversity can alter behaviour and endocrine function, and structural disadvantage shapes pollution, nutrition and care simultaneously. Mixture models should be paired with source-based interventions and mechanistic experiments. Diverse populations are scientifically necessary because exposure ranges, genetic regulation, diet and placental adaptation differ across settings. Cohort governance should include communities in choosing outcomes, interpreting socially patterned exposures and controlling secondary use of biospecimens.

Prediction should be pursued cautiously. A robust methylation signature of prenatal smoking may improve exposure reconstruction, but most current signatures are not ready for individual risk classification. Before clinical use, a biomarker must add information beyond exposure history and conventional predictors, perform across ancestry and socioeconomic groups, and lead to an effective action. Communication should emphasise plasticity rather than fate. Epigenetic marks are probabilistic, tissue-specific and potentially reversible; they should not be used to police pregnancy behaviour or attribute structural hazards to maternal responsibility. The most valuable translation is upstream: reducing toxicant exposure, preventing nutritional deficiency and metabolic disease, providing mental-health and violence-prevention services, and improving the material conditions of pregnancy.

9. Conclusions

Maternal conditions can influence epigenetic regulation during fetal development, but the strength and meaning of that influence differ markedly across exposure domains. The most defensible human evidence concerns maternal smoking, for which newborn DNA methylation signatures replicate and selected marks persist. Severe nutritional perturbations and periconceptional natural experiments show that developmentally timed nutrition can leave durable marks at selected loci, while controlled animal studies establish mechanistic possibilities for one-carbon metabolism, protein restriction and placenta-mediated responses. Maternal hyperglycaemia, air pollution, metals, phenols, phthalates and PFAS are associated with placental or cord-blood epigenetic variation, yet the loci and pathways are less consistent. Psychosocial evidence supports stress-responsive placental and endocrine biology but does not define a stable fetal stress methylome.

Across the literature, exposure-associated molecular variation is more firmly established than causal programming of later disease. Placenta and cord blood are biologically informative tissues, not interchangeable proxies for fetal organs. DNA methylation dominates because it is measurable, while histone states, chromatin accessibility and non-coding RNAs remain under-characterised in human pregnancy. Cell composition, genotype, exposure error, correlated mixtures, social confounding and limited replication can each convert a plausible mechanism into an overstated conclusion. Programming should therefore be reserved for changes with credible developmental timing, regulatory function and persistence or phenotypic consequence.

The field is positioned to progress through repeated exposure measurement, diverse longitudinal cohorts, cell-resolved placental analysis, functional perturbation and causal triangulation. Such work can clarify how the maternal environment becomes biologically embedded without implying genetic alteration, inevitability or maternal blame. Epigenetic evidence is most informative when it connects developmental biology to prevention and when its uncertainty is treated as a scientific result rather than an obstacle to a compelling narrative.

10. Limitations

This critical narrative review is intrinsically vulnerable to selection and interpretive bias despite a transparent search framework, explicit eligibility criteria and repeated bibliographic verification. Direct access to several subscription databases was unavailable, so the search relied on accessible biomedical and multidisciplinary scholarly indexes, citation searching and official records. English-language requirements may have excluded

relevant research, and the rapidly developing literature means that studies appearing after the specified search end date were not considered.

The evidence base itself is heterogeneous in exposure definition, gestational timing, tissue, cell composition, assay platform and outcome, preventing a valid quantitative synthesis. Human evidence is predominantly observational, geographically concentrated and subject to publication bias, residual confounding and selective biospecimen availability. Animal experiments provide mechanistic control but differ in species, dose and developmental context. The review emphasised representative and influential studies rather than an exhaustive catalogue, and sparse evidence for histone modifications, chromatin accessibility and non-coding RNA limited balanced comparison with DNA methylation. Long-term target-organ evidence and true multigenerational human data remain particularly scarce.

Consent and Ethical Approval

It is not applicable.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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