

Review

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Review

Parental Dysbiosis and Nutritional Imbalance as Modified Drivers of Recurrent In Vitro Fertilization Failure: Microbiome-Epigenome Interactions in Early Embryonic Programming

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Abstract

In vitro fertilization (IVF) often requires multiple cycles before successful implantation and live birth are achieved. Although embryo quality and technical factors have traditionally been emphasized, emerging evidence suggests that parental microbiome composition and nutritional status are important, but under-recognized, determinants of reproductive success. Dysbiosis of the vaginal and endometrial microbiota in women is associated with reduced implantation rates, chronic low-grade inflammation, and altered endometrial receptivity, while maternal gut microbiota imbalance may contribute to systemic metabolic inflammation, oxidative stress, and epigenetic modifications affecting early embryonic development. Paternal factors are increasingly recognized as equally important, as altered gut microbiota, obesity, insulin resistance, and micronutrient deficiencies may affect sperm DNA integrity, methylation patterns, and small noncoding RNA profiles, potentially influencing embryonic developmental programming. This review aims to evaluate the interconnected mechanisms linking parental microbiome composition, nutritional and metabolic status, inflammation, oxidative stress, and epigenetic regulation with assisted reproduction and recurrent IVF failure. A comprehensive literature search was performed using PubMed/MEDLINE, Scopus, and Web of Science, covering the period from 2000 to 2025, with predefined keywords and Medical Subject Headings related to in vitro fertilization, recurrent implantation failure, maternal gut microbiome, reproductive tract dysbiosis, paternal gut-testis axis, oxidative stress, sperm epigenetics, DNA methylation, short-chain fatty acids, and preconception nutritional optimization. We considered peer-reviewed articles published in English, including original research, meta-analyses, and high-impact reviews providing mechanistic and translational insights. The evidence indicates that parental dysbiosis and nutritional and metabolic imbalances may converge on inflammatory, oxidative, mitochondrial, and epigenetic pathways, creating a biological environment that compromises implantation and may influence early embryonic programming. Importantly, these potentially modifiable factors support preconception microbiome modulation, nutritional optimization, and metabolic conditioning for both partners before IVF. In conclusion, an integrated preconception approach addressing parental microbiome, nutritional, metabolic, inflammatory, and epigenetic factors may represent a promising strategy for improving reproductive outcomes and potentially supporting the long-term health of the offspring.

Keywords: assisted reproduction; recurrent implantation failure; parental microbiome; epigenetic programming; nutritional optimization; IVF outcomes

1. Introduction

Infertility represents a major global public health challenge, affecting approximately 10–15% of couples of reproductive age worldwide and contributing to significant psychosocial, economic, and demographic consequences [1]. Assisted reproductive technologies (ART), particularly in vitro fertilization (IVF), have revolutionized the management of infertility and enabled millions of couples to achieve pregnancy who would otherwise remain childless. Despite continuous technological improvements in ovarian stimulation protocols, embryo culture systems, genetic screening methods, and embryo transfer techniques, the overall success rate of IVF remains relatively limited. In many clinical settings, multiple treatment cycles are required before successful implantation and live birth are achieved, highlighting the persistent biological complexity underlying human reproduction [2].

Repeated implantation failure and unsuccessful IVF cycles remain among the most challenging clinical problems in reproductive medicine. Traditionally, the determinants of IVF success have been attributed primarily to embryonic morphology, chromosomal abnormalities, and laboratory-related technical factors [3]. Morphological grading systems, blastocyst development rates, and, more recently, preimplantation genetic testing for aneuploidy (PGT-A) have been widely used to assess embryo viability and improve embryo selection [4]. Although these approaches have improved clinical outcomes to some extent, they cannot fully explain the large proportion of implantation failures observed even after transfer of morphologically optimal or genetically euploid embryos. These observations suggest that additional biological mechanisms—beyond embryonic genetics—play important roles in determining implantation success [5].

Increasing evidence indicates that implantation represents a highly coordinated biological process involving complex interactions between embryonic competence, endometrial receptivity, immune tolerance, metabolic homeostasis, and systemic parental physiology [6]. Successful embryo implantation requires precise synchronization between the developmental stage of the embryo and the transient period of endometrial receptivity known as the “window of implantation” [7]. During this period, a finely balanced molecular environment characterized by controlled inflammatory signaling, hormonal responsiveness, angiogenesis, and immune tolerance is required for successful embryo attachment and invasion. Even subtle disturbances in this tightly regulated microenvironment may compromise implantation and lead to early pregnancy failure [8].

In recent years, growing attention has been directed toward the role of the human microbiome as an important regulator of reproductive physiology. The human microbiome consists of complex microbial communities inhabiting mucosal surfaces throughout the body, including the gastrointestinal tract, reproductive organs, skin, and respiratory system [9]. These microbial ecosystems interact dynamically with host physiology through metabolic, immunological, and endocrine pathways. The microbiome contributes to the regulation of immune responses, epithelial barrier integrity, nutrient metabolism, and inflammatory signaling, all of which are fundamental processes influencing reproductive health [10]. Alterations in microbial composition, commonly referred to as dysbiosis, have been associated with a wide range of chronic diseases, including metabolic disorders, autoimmune conditions, inflammatory diseases, and reproductive dysfunction [11].

Within the context of reproductive medicine, the microbiome of the female reproductive tract has emerged as a critical factor influencing fertility and IVF outcomes [12]. In healthy reproductive-age women, the vaginal microbiota is typically dominated by *Lactobacillus* species, which maintain an acidic vaginal environment through the production of lactic acid and antimicrobial peptides [13]. This microbial dominance inhibits the growth of pathogenic organisms and contributes to mucosal immune tolerance within the reproductive tract. Disruption of this microbial balance, resulting in reduced *Lactobacillus* dominance and increased microbial diversity, has been associated with bacterial vaginosis, chronic endometrial inflammation, and adverse reproductive outcomes [14]. Recent

studies using high-throughput sequencing technologies have demonstrated that dysbiotic vaginal and endometrial microbial communities may impair embryo implantation through multiple mechanisms, including local inflammatory activation, altered cytokine signaling, and disruption of endometrial receptivity pathways [15].

The concept of the endometrial microbiome has attracted particular interest in recent years. Although the uterus was traditionally considered a sterile environment, accumulating molecular evidence suggests that low-abundance microbial populations may exist within the endometrial cavity [16]. These microbial communities appear to interact with the local immune system and may influence endometrial function, tissue remodeling, and embryo implantation. Dysbiosis within the endometrial microbiota has been associated with chronic endometritis, altered expression of implantation-related genes, and reduced success rates in assisted reproductive treatments [17]. While the precise composition and functional significance of the endometrial microbiome remain areas of active investigation, emerging data suggest that microbial imbalance within the uterine environment may represent an underrecognized contributor to implantation failure [18].

Beyond the reproductive tract itself, the maternal gut microbiome represents another important regulator of reproductive physiology [19]. The intestinal microbiota influences host metabolism, immune function, and endocrine signaling by producing bioactive microbial metabolites, including short-chain fatty acids (SCFAs), bile acid metabolites, and tryptophan-derived metabolites. These microbial metabolites interact with host signaling pathways involved in immune homeostasis, inflammation, and metabolic regulation. Gut dysbiosis has been associated with metabolic disturbances, including obesity and insulin resistance, as well as reproductive disorders such as polycystic ovary syndrome (PCOS) [20]. Metabolic syndrome and related metabolic dysfunction may negatively affect reproductive function and IVF outcomes, including ovarian response and the number of retrieved oocytes and available embryos in women with PCOS [21]. Gut microbiota-derived SCFAs have also been associated with clinical pregnancy outcomes in women undergoing IVF/ICSI-ET, with fecal propionate levels showing associations with insulin resistance and pregnancy outcomes [22].

Importantly, the influence of the microbiome on reproductive outcomes is not limited to maternal physiology. Increasing evidence indicates that paternal biological factors can also influence embryo development and pregnancy outcomes. Male factors contribute substantially to infertility, although the mechanisms through which paternal health affects preimplantation development remain incompletely understood [23]. Recent evidence indicates that systemic metabolic status, diet, and microbial composition may influence sperm quality and the molecular information carried by spermatozoa. In particular, obesity has been associated with alterations in sperm DNA methylation and chromatin organization, supporting the concept that paternal metabolic status can modify the epigenetic state of the male germline [24,25]. In parallel, experimental studies of the gut–testis axis have demonstrated that disruption of the intestinal microbiota can alter systemic and testicular metabolism and adversely affect male reproductive function, while more recent experimental evidence indicates that paternal gut microbiome perturbation can modify sperm molecular profiles and subsequently affect offspring development. Importantly, these latter findings are predominantly experimental and should not yet be interpreted as proof of an equivalent gut microbiome–sperm–embryo pathway in humans [26].

In addition to affecting sperm quality, environmental and metabolic factors may influence the epigenetic landscape of spermatozoa [27]. Epigenetic modifications—including DNA methylation, histone modifications, and small non-coding RNAs—play essential roles in regulating gene expression during early embryonic development. These epigenetic signals carried by sperm are delivered to the oocyte at fertilization and contribute to the regulation of embryonic gene expression before activation of the embryonic genome [28]. Emerging evidence suggests that paternal diet, metabolic health, and environmental exposures can alter sperm epigenetic patterns, potentially affecting embryo development and long-term offspring health [29].

The early embryo undergoes an extensive period of epigenetic reprogramming during the preimplantation stage, characterized by dynamic remodeling of DNA methylation and other chromatin

modifications that are closely associated with successful embryonic development. Parental epigenetic information is extensively remodeled shortly after fertilization through coordinated active and passive DNA demethylation processes, followed by the establishment of developmentally appropriate epigenetic states. This period represents one of the most epigenetically dynamic phases of early development, with substantial changes in DNA methylation and histone modifications occurring as the embryo progresses from the zygote through cleavage stages to the blastocyst [30]. The precise regulation of these processes is essential for appropriate lineage specification, genomic imprinting, and embryonic developmental competence [31].

The preimplantation embryo is therefore particularly sensitive to alterations in its metabolic and environmental milieu. Oxidative stress can interfere with the molecular systems required for the establishment and maintenance of DNA methylation, including pathways involving S-adenosylmethionine, methionine metabolism, glutathione, and TET-mediated DNA demethylation. Such disturbances may compromise epigenetic homeostasis during early embryonic development and may adversely affect developmental competence. Although microbial metabolites are increasingly recognized as epigenetically active molecules, direct evidence demonstrating that parental gut-derived microbial metabolites alter epigenetic reprogramming specifically in human preimplantation embryos remains limited. This distinction is important when extrapolating mechanistic observations from experimental models to human reproductive biology [32].

Nutrition represents a central modifiable factor influencing both microbiome composition and systemic metabolic regulation in men and women. Dietary patterns strongly shape the composition, function, and diversity of the gut microbiome and influence the production of biologically active microbial metabolites involved in metabolic and immune regulation [33]. Diets characterized by high consumption of saturated fats, refined carbohydrates, and highly processed foods are associated with adverse alterations in the gut microbiota and increased inflammatory signaling, whereas dietary patterns rich in plant-derived fiber and diverse plant foods can support microbial diversity and metabolite production associated with metabolic homeostasis [34]. In contrast, Mediterranean-type dietary patterns, characterized by high intake of plant foods, fiber, polyphenol-rich foods, and unsaturated fatty acids, have been associated with favorable modulation of the gut microbiota and its metabolic activity [35]. Nutritional status may therefore play an important role in reproductive health, with dietary patterns associated with both male and female reproductive parameters; however, the strength of evidence varies according to the specific dietary exposure and reproductive outcome examined [36].

Micronutrients such as folate, vitamin B12, zinc, selenium, and omega-3 fatty acids are particularly important for reproductive health due to their roles in DNA synthesis, antioxidant defense, and epigenetic regulation [37]. Deficiencies in these nutrients have been associated with impaired spermatogenesis, reduced oocyte quality, and altered methylation patterns in germ cells [38]. Nutritional imbalance before conception may therefore contribute to oxidative stress and epigenetic instability during early embryogenesis. Because nutritional factors are modifiable, they represent promising targets for preconception interventions aimed at improving reproductive outcomes [39].

Taken together, these findings support the emerging concept that recurrent IVF failure may not solely reflect embryological or procedural limitations but may also arise from broader systemic biological disturbances involving parental microbiome dysbiosis, metabolic inflammation, and epigenetic dysregulation [40]. The integration of microbiome research with reproductive medicine has therefore opened new perspectives for understanding implantation failure and suboptimal IVF outcomes [41].

In this context, increasing attention is being directed toward the concept of preconception health optimization. Strategies aimed at improving microbiome balance, dietary quality, metabolic status, and inflammatory regulation before assisted reproductive treatment may be promising approaches to enhance endometrial receptivity, improve gamete quality, and support optimal embryonic development [42]. Such strategies may include targeted nutritional interventions, microbiome-modulating

therapies, such as probiotics and prebiotics, and personalized metabolic management for both partners before the initiation of the IVF cycle [43].

This review aims to synthesize current evidence on the interplay between parental microbiome composition, nutritional status, and epigenetic mechanisms in the context of assisted reproduction. This review emphasizes the idea that dysbiosis and metabolic imbalance in both partners may contribute to recurrent IVF failure through microbiome–epigenome interactions that affect early embryonic programming. Understanding these complex biological relationships may provide a foundation for developing novel preconception strategies to improve implantation success, optimize IVF outcomes, and promote long-term health in future generations.

2. Literature Search Strategy and Study Organization

2.1. Search Strategy

We conducted a comprehensive literature search to identify relevant studies investigating the effects of parental microbiome composition, nutritional status, metabolic inflammation, and epigenetic mechanisms on assisted reproduction and recurrent IVF failure. The search was carried out across major electronic databases, including PubMed/MEDLINE, Scopus, and Web of Science, covering the period from 2000 to 2025. The search strategy employed a combination of keywords and Medical Subject Headings (MeSH) terms to ensure broad coverage: "in vitro fertilization," "recurrent implantation failure," "maternal gut microbiome," "reproductive tract dysbiosis," "paternal gut-testis axis," "oxidative stress," "sperm epigenetics," "DNA methylation," "short-chain fatty acids," and "preconception nutritional optimization." Following database screening, duplicate records were removed, and records were screened for thematic relevance. Only peer-reviewed articles published in English were included. We focused on original research, meta-analyses, and high-impact reviews that offered mechanistic insights into microbiome–epigenome interactions and early embryonic programming. Among the identified records, selections were made based on their scientific rigor, relevance to molecular signaling, and contribution to the integrative framework discussed in this review.

2.2. Study Organization

The present review is structured into thematic sections to provide a coherent overview of the molecular and clinical aspects of parental dysbiosis and assisted reproduction. Following this methodological section, the paper is organized as follows: Section 3 explores the clinical and maternal determinants of repeated IVF failure. Section 4 examines the molecular and immunological mechanisms underlying reproductive tract dysbiosis and implantation failure. Section 5 focuses on the maternal gut microbiome, metabolic inflammation, and early embryonic programming. Section 6 discusses the paternal microbiome, nutrition, oxidative stress, and sperm epigenetics. Section 7 evaluates the nutritional patterns of both partners as modifiable risk factors, followed by concluding remarks.

3. Clinical and Maternal Determinants of Repeated IVF Failure

In vitro fertilization (IVF) remains one of the most widely applied and technologically sophisticated strategies for addressing human infertility, yet its clinical success continues to be constrained by repeated implantation failures and suboptimal pregnancy rates. Despite significant advances in ovarian stimulation, embryo culture, preimplantation genetic testing, and transfer techniques, a substantial proportion of women—approximately 60–70 %—fail to achieve pregnancy after the first embryo transfer, and a notable fraction experience recurrent implantation failure (RIF) [44]. Such repeated failures compel clinicians and patients to pursue multiple IVF cycles even when embryos are morphologically optimal and chromosomally normal, underscoring that maternal factors—including hormonal milieu, immune modulation, oxidative balance, metabolic state, and molecular signaling—play a decisive role in implantation success [45].

The implantation process is inherently complex, involving a precisely orchestrated crosstalk between the blastocyst and the maternal endometrium. Central to this interaction is endometrial receptivity, defined as the capacity of the endometrial lining to accept and support an implanting blastocyst [46]. This receptive state is temporally restricted to a narrow window of implantation (WOI), usually spanning days 19–24 of the menstrual cycle, corresponding to approximately seven days post-LH surge in natural cycles or five days post-progesterone administration in medicated cycles [47]. Transcriptomic studies reveal that this period may last as little as 48 hours, during which tightly synchronized molecular communication between maternal and embryonic tissues is required [48]. Misalignment of this maternal–embryo dialogue, even in the presence of high-quality embryos, can lead to implantation failure. In women with RIF, molecular profiling often shows WOI displacement and disrupted expression of hundreds of genes involved in adhesion, growth factor signaling, extracellular matrix remodeling, and immune regulation, indicating that temporal misalignment substantially contributes to repeated failure [49,50].

Endometrial receptivity is shaped by hormonal, immune, metabolic, and microenvironmental factors. Estrogen and progesterone orchestrate stromal decidualization, angiogenesis, and immune tolerance necessary for embryo intravasation. Disruption of these hormonal cues—whether from stimulation protocols, luteal insufficiency, or intrinsic endocrine defects—can compromise receptivity, requiring repeated cycles to achieve synchrony between embryo and endometrium [51]. At the maternal–fetal interface, immune tolerance must be balanced against pathogen defense. Populations of innate lymphoid cells, uterine natural killer (uNK) cells, macrophages, dendritic cells, and regulatory T-cells contribute to tissue remodeling, angiogenesis, and controlled inflammation critical for implantation [52]. Dysregulation of immune cell populations, as well as elevated pro-inflammatory cytokines (IL-6, IL-8, TNF- α), correlates with impaired endometrial conditions and adverse outcomes if present outside the WOI context. Disruption of cytokine networks and immune cell profiles impairs trophoblast invasion, decidual proliferation, and vascular remodeling, thereby directly affecting implantation competence [53].

Oxidative stress represents an additional barrier. Defined as an imbalance between reactive oxygen species (ROS) and antioxidant defenses, oxidative stress affects cellular signaling, protein function, and genomic integrity within reproductive tissues. In conditions such as polycystic ovary syndrome, increased ROS in endometrial tissue correlates with impaired receptivity and higher implantation failure rates [54]. ROS-induced apoptosis, lipid peroxidation, and DNA damage in stromal cells may impede decidualization and establishment of a supportive microenvironment. Oxidative stress also interacts with immune pathways, fostering pro-inflammatory states that further compromise endometrial receptivity [55].

Metabolic and systemic physiology significantly influence implantation [56]. Insulin resistance, obesity, and dyslipidemia are associated with chronic low-grade inflammation, altered endocrine signaling, and impaired angiogenesis. These changes affect hormone receptor expression and progesterone responsiveness in the endometrium, reducing implantation competence. Consequently, multiple IVF cycles may be required until the maternal environment is optimized, either spontaneously or through targeted interventions [57].

Even when maternal receptivity appears adequate, limitations in embryo assessment still necessitate repeated cycles. Morphology-based scoring systems assess blastocyst expansion, inner cell mass quality, and trophectoderm grade, but they fail to capture chromosomal integrity, mitochondrial function, epigenetic programming, and metabolic capacity [58]. Morphologically competent embryos may still harbor chromosomal abnormalities or metabolic insufficiencies that preclude implantation. Advanced approaches such as time-lapse imaging, preimplantation genetic testing, and metabolomic profiling improve predictive accuracy but cannot eliminate failed implantation among “good-quality” embryos [59].

Molecular mechanisms provide additional insight into why repeated cycles are often required. Blastocyst–endometrium signaling involves a spectrum of cytokines, growth factors, and adhesion molecules that must align precisely within the WOI [60]. Leukemia inhibitory factor (LIF) is critical

for implantation, activating the JAK/STAT3 pathway via gp130 receptors, thereby regulating cytoskeletal remodeling and expression of adhesion ligands essential for blastocyst attachment [61]. Interleukins such as IL-6, IL-8, IL-11, and IL-1 β coordinate decidualization and controlled inflammatory responses, while integrins (α v β 3, α 4 β 1), laminin, and fibronectin mediate bi-directional adhesion signaling and cellular polarization required for trophoblast invasion [62]. Steroid hormones modulate these pathways: estrogen promotes receptor expression and cellular proliferation, while progesterone activates homeobox transcription factors (HOXA10, HOXA11) and integrin expression, establishing the receptive phenotype [63]. Human chorionic gonadotropin (hCG) from the embryo further modulates endometrial signaling, including microRNA networks (e.g., miR-126-3p), thereby influencing PI3K/Akt pathways, VE-cadherin-mediated vascular stability, and uNK cell distribution to promote immune tolerance [64]. Extracellular matrix remodeling via matrix metalloproteinases and prostaglandin-mediated modulation of vascular permeability complement these processes, collectively enabling stable implantation [65]. Disruption at any molecular node—hormonal, cytokine, integrin, epigenetic, or oxidative—can preclude implantation, explaining why multiple IVF cycles are often necessary to overcome these barriers and achieve successful pregnancy [66].

The stochastic nature of implantation, determined by the interplay among embryo quality, endometrial timing, immune modulation, oxidative balance, and molecular signaling, ensures that success in one cycle does not guarantee subsequent success, and failure does not preclude later implantation [67]. Even minor improvements in endometrial synchrony, immune balance, or oxidative stress may convert a previously non-receptive environment into a receptive one [68]. Up to two-thirds of implantation failures are attributable primarily to maternal endometrial factors rather than embryonic defects, driving research into personalized assessment tools and interventions, including transcriptomic receptivity assays, cytokine profiling, and metabolic optimization to reduce unsuccessful cycles [69].

4. Molecular and Immunological Mechanisms Underlying Reproductive Tract Dysbiosis and Implantation Failure

Implantation of the human embryo is orchestrated by a highly coordinated network of molecular, immunological, endocrine, and metabolic signals that establishes functional synchrony between the developing blastocyst and the receptive maternal endometrium. The reproductive tract microbiome has increasingly been recognized as a potential component of this microenvironment, although its precise contribution to implantation remains incompletely defined. Recent studies indicate that Lactobacillus-enriched microbial communities, particularly those characterized by Lactobacillus crispatus, are generally associated with more favorable reproductive outcomes. In contrast, women with repeated implantation failure and impaired endometrial receptivity have been reported to have reduced Lactobacillus dominance and increased microbial diversity [70].

Microbial composition may influence reproductive function through interactions with epithelial integrity, local inflammatory signaling, immune-cell activity, and the biochemical environment of the reproductive tract. In ART populations, vaginal communities enriched in L. crispatus have been associated with higher probabilities of clinical pregnancy and live birth, supporting the concept that microbial composition may contribute to the biological conditions surrounding embryo transfer and implantation [71].

Microbial metabolites, including short-chain fatty acids (SCFAs), provide an additional mechanistic link between microbial activity and host-cell signaling. SCFAs can influence epithelial-barrier function, immune responses, and cellular metabolism through receptor-mediated signaling and epigenetic mechanisms such as histone-deacetylase inhibition. However, the extent to which gut- or genital-tract-derived SCFAs directly regulate human endometrial decidualization and implantation remains an emerging area of investigation rather than an established clinical mechanism [72].

Dysbiosis, characterized by depletion of Lactobacillus spp. and enrichment of anaerobic taxa such as Gardnerella, Atopobium, Prevotella, and Streptococcus, has been associated with an altered endometrial inflammatory environment and impaired reproductive outcomes. In women with

repeated implantation failure, reduced endometrial *Lactobacillus* abundance and increased representation of taxa including *Gardnerella* have been associated with impaired expression of molecular markers of endometrial receptivity [73]. Similarly, dysbiotic endometrial microbiota profiles have been associated with increased concentrations of inflammatory mediators, including IL-1 β , IL-6, and COX-2, together with reduced levels of anti-inflammatory factors such as IL-10 [74].

Microbial pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS) and lipoteichoic acid, can activate innate immune receptors expressed by endometrial cells. Experimental studies in human endometrial stromal cells demonstrate that LPS stimulation activates TLR4-associated inflammatory signaling, including NF- κ B and p38 MAPK pathways, and promotes the production of inflammatory cytokines and chemokines. Importantly, decidualization itself attenuates these responses through increased expression of I κ B α , indicating that the inflammatory response to microbial signals is dynamically regulated during acquisition of endometrial receptivity [75].

Persistent inflammatory activation may further interact with oxidative and cellular stress pathways. Experimental evidence indicates that excessive ROS generation can promote oxidative damage and disturb mitochondrial and endoplasmic-reticulum homeostasis, while dysregulated ER-stress responses involving PERK, IRE1 α , and ATF6 may contribute to inflammatory signaling, apoptosis, and impaired stromal-cell function. Recent work emphasizes that successful decidualization requires coordinated regulation of oxidative stress, mitochondrial metabolism, and the unfolded-protein response; therefore, persistent or excessive activation of these pathways may compromise the cellular adaptations required for implantation [76].

Progesterone and estrogen orchestrate endometrial receptivity through the coordinated regulation of transcriptional programs required for epithelial receptivity, stromal cell differentiation, and implantation. Key regulators include the homeobox transcription factor HOXA10, which is essential for endometrial receptivity, embryo implantation, and stromal-cell decidualization; dysregulation of HOXA10 has been associated with several endometrial disorders and infertility [77]. Recent experimental evidence further demonstrates that post-translational regulation of HOXA10 can directly influence endometrial epithelial receptivity and the expression of genes involved in implantation and cellular adhesion [78]. Dysbiosis may also influence reproductive endocrine signaling by altering microbial estrogen metabolism. In particular, gut microbial β -glucuronidase participates in the deconjugation of estrogen metabolites and their enterohepatic recycling, although the extent to which this mechanism directly regulates human endometrial receptivity remains incompletely established [79]. Consistent with this possibility, a recent study of women with infertility and repeated implantation failure found increased β -glucuronidase activity and ER β expression in dysbiotic endometrial biopsies, together with increased inflammatory mediators, providing preliminary evidence for an association between endometrial dysbiosis, altered estrogen-related signaling, and impaired reproductive function [80]. Progesterone resistance may further compromise endometrial function by reducing progesterone receptor responsiveness and impairing decidualization. In endometrial stromal cells from women with recurrent implantation failure, increased EHD1 expression was shown experimentally to inhibit progesterone-receptor activity and progesterone responsiveness, whereas suppression of EHD1 increased the decidualization markers prolactin and IGFBP1 [81,82].

Endometrial immune populations, including uterine natural killer (uNK) cells, macrophages, dendritic cells, and regulatory T cells (Tregs), form a highly coordinated immune network that contributes to endometrial receptivity, maternal immune tolerance, trophoblast invasion, and vascular remodeling. Their abundance and phenotype change dynamically during the implantation window, indicating that the immune composition of the endometrium is an integral component of the receptive state [83]. uNK cells are particularly abundant in the peri-implantation endometrium and early decidua and contribute to trophoblast invasion and spiral artery remodeling through the secretion of angiogenic mediators, including vascular endothelial growth factor (VEGF) and other cytokines and growth factors. Human uNK cells are functionally heterogeneous and differ substantially from circulating NK cells; therefore, their role in implantation cannot be reduced to a simple cytotoxic versus non-cytotoxic classification [84]. In women with recurrent implantation failure, alterations in uNK-

cell abundance and phenotype have been reported, although findings across studies remain heterogeneous and the clinical significance of uNK-cell measurements is not yet fully established [85]. Endometrial macrophages also contribute to implantation by regulating tissue remodeling and trophoblast invasion. Recent single-cell and functional studies demonstrate that uterine macrophages present around implantation retain pro-invasive properties and can directly promote extravillous trophoblast invasion, supporting their role in early placental development [86].

Metabolic cues integrate with immune and epigenetic regulation [87]. Dysbiosis can alter the availability of microbial metabolites such as succinate, which participates in microbiota–host signaling and can influence inflammatory responses through metabolic-sensing pathways [88]. Short-chain fatty acids (SCFAs), particularly butyrate and propionate, can influence immune-cell differentiation and epigenetic regulation through inhibition of histone deacetylases, thereby modulating transcriptional programs involved in regulatory T-cell differentiation and immune tolerance [89]. Tryptophan metabolism provides an additional link between microbial metabolism and immune regulation. Altered tryptophan availability can reshape the intestinal microbiota and regulatory T-cell compartment through changes in microbiota-derived tryptophan metabolites and aryl hydrocarbon receptor (AhR) signaling [90]. Kynurenine-pathway activity provides an additional mechanism through which tryptophan metabolism can influence AhR-dependent immune regulation and regulatory T-cell responses [91]. In parallel, metabolic and inflammatory stress can modify chromatin regulation through histone-modifying enzymes. Increased EZH2 activity and H3K27me3 have been implicated in hyperglycemia-induced oxidative and inflammatory transcriptional programs, demonstrating how metabolic disturbances can be translated into epigenetic changes in gene regulation [92]. Collectively, these mechanisms provide a biologically plausible link between microbiome-derived metabolic alterations, inflammatory signaling, immune regulation, and epigenetic processes that may influence tissue homeostasis and reproductive function [93].

Extracellular matrix (ECM) remodeling is tightly coordinated by matrix metalloproteinases (MMPs) 2 and 9 and their tissue inhibitors of metalloproteinases (TIMPs), which regulate ECM degradation and trophoblast invasion at the maternal–fetal interface [94]. Dysregulated inflammatory conditions can alter MMP activity through inflammatory signaling pathways, including NF- κ B, leading to abnormal ECM remodeling and altered trophoblast invasion [95]. Oxidative stress can additionally impair trophoblast migration and invasion and modify invasion-associated molecules, including MMP-2 and MMP-9, thereby compromising the cellular remodeling required for successful implantation [96].

Collectively, reproductive tract dysbiosis establishes a molecular milieu integrating chronic inflammation, oxidative stress, steroid hormone imbalance, immune dysregulation, and altered extracellular matrix remodeling [97]. These converging perturbations may contribute to impaired or unstable endometrial receptivity and implantation failure, although the causal relationships between microbiome composition and reproductive outcomes remain incompletely defined [98]. Clinical studies using 16S rRNA sequencing and other molecular approaches have demonstrated associations between reproductive tract microbial profiles and markers of endometrial receptivity, including HOXA11, integrin α v β 3, leukemia inhibitory factor, MMP-9, and VEGF, as well as inflammatory mediators [99]. Interventional approaches including probiotics and antimicrobial treatment have therefore been investigated as potential strategies for microbiome modulation; however, clinical evidence remains inconsistent, and randomized trials have not consistently demonstrated improvement in reproductive outcomes following probiotic treatment [100].

The intricate interplay of the microbiome, immune system, metabolic and oxidative cues, hormonal regulation, and epigenetic modifications underscores the molecular and immunological complexity underlying implantation failure [101]. A comprehensive understanding of these pathways is critical for the development of precision reproductive medicine strategies aimed at identifying specific mechanistic disturbances in women with recurrent implantation failure and tailoring investigation and treatment accordingly. However, the clinical utility of many proposed biomarkers and interventions remains under evaluation, and further validation is required before molecular and

immune profiling can be routinely integrated into individualized management of recurrent implantation failure [102].

5. Maternal Gut Microbiome, Metabolic Inflammation, and Early Embryonic Programming

Maternal gut microbiome functions as a highly integrated metabolic–immune–endocrine–neuroactive interface that converts environmental and dietary inputs into a continuous stream of bioactive metabolites capable of regulating systemic physiology across multiple organizational levels [103]. Its functional architecture is determined by interdependent ecological networks of anaerobic microorganisms, primarily within *Firmicutes* and *Bacteroidetes* phyla, whose collective enzymatic repertoire enables fermentation of complex polysaccharides into short-chain fatty acids (SCFAs), bile acid derivatives, indole metabolites, lipopolysaccharide fragments, and tryptophan catabolites [104]. These metabolites do not act in isolation but form an interconnected signaling network that is dynamically shaped by dietary composition, circadian rhythms, intestinal oxygen gradients, mucosal immune tone, epithelial barrier integrity, and host endocrine state [105].

SCFA production represents only one layer of microbial metabolic output, but it is central due to its systemic bioavailability and pleiotropic signaling capacity [106]. Acetate, propionate, and butyrate are absorbed via monocarboxylate transporters (MCT1/SLC16A1, SMCT1/SLC5A8) and distributed through portal and systemic circulation, reaching not only metabolic organs but also immune compartments, vascular endothelium, and reproductive tissues [107]. Their systemic half-life and tissue-specific uptake are influenced by albumin binding, hepatic clearance efficiency, and local enzymatic degradation [108].

At the receptor level, SCFAs signal through FFAR2/GPR43, FFAR3/GPR41, and GPR109A, which are expressed in immune cells, endothelial cells, adipocytes, and epithelial cells of reproductive tissues [109]. These receptors couple primarily to Gi/o proteins, leading to suppression of adenylate cyclase, decreased cAMP production, modulation of PKA activity, and downstream regulation of transcription factors such as CREB, NF- κ B, and FOXO1 [110]. Parallel β -arrestin-mediated signaling introduces receptor biasing effects that fine-tune inflammatory versus metabolic responses depending on ligand concentration and cellular context [111].

Beyond membrane receptor signaling, SCFAs exert profound nuclear effects through inhibition of histone deacetylases (HDAC1, HDAC2, HDAC3, HDAC8), resulting in hyperacetylation of histones and chromatin relaxation [112]. This epigenetic remodeling increases accessibility of regulatory regions controlling immune tolerance genes, metabolic regulators, angiogenic pathways, and stress-response elements [113]. Importantly, HDAC inhibition is not uniform; it is context-dependent and influenced by intracellular acetyl-CoA availability, NAD⁺/NADH ratio, and mitochondrial metabolic state, thereby linking nuclear epigenetics with cellular energy metabolism [114].

Butyrate is uniquely positioned as both an epigenetic modulator and metabolic substrate [115]. In immune cells, it stabilizes regulatory T cell identity not only via FOXP3 acetylation but also through metabolic rewiring that shifts cellular ATP production from glycolysis toward oxidative phosphorylation [116]. This involves activation of AMPK, inhibition of mTORC1, and increased mitochondrial biogenesis through PGC-1 α and NRF1 signaling [117]. This metabolic–epigenetic coupling is essential because T cell fate is metabolically encoded; glycolysis supports effector phenotypes, while oxidative metabolism stabilizes tolerance-inducing lineages [118].

Innate immune cells exhibit parallel metabolic plasticity under SCFA influence [119]. Macrophages exposed to butyrate undergo epigenetically reinforced polarization toward M2-like states characterized by enhanced oxidative phosphorylation, increased arginase activity, and suppression of inducible nitric oxide synthase (iNOS) [120]. This shift is mediated through inhibition of NF- κ B nuclear translocation, altered STAT signaling balance (STAT6 predominance), and increased histone acetylation at anti-inflammatory gene loci [121]. In contrast, SCFA deficiency promotes succinate accumulation, HIF-1 α stabilization, and glycolytic reprogramming, thereby sustaining pro-inflammatory M1 polarization [122]. Dendritic cells respond to SCFAs by reducing antigen presentation

efficiency, altering interferon regulatory factor (IRF) activity, and decreasing IL-12–driven Th1 polarization, thereby reshaping adaptive immune priming [123].

A critical additional layer of SCFA action involves mitochondrial signaling [124]. SCFAs regulate mitochondrial dynamics not only through AMPK–PGC-1 α pathways but also via direct modulation of mitochondrial fusion and fission proteins (MFN1, MFN2, DRP1). They influence mitochondrial membrane potential stability, calcium buffering capacity, and reactive oxygen species signaling thresholds [125]. In reproductive cells such as oocytes and granulosa cells, mitochondrial dysfunction directly affects spindle assembly checkpoint integrity, chromosomal segregation accuracy, and cytoplasmic maturation competence [126]. In early embryos, mitochondrial metabolic flexibility determines blastocyst formation potential and lineage allocation between trophectoderm and inner cell mass [127].

Metabolic inflammation introduces a parallel but synergistic pathogenic axis [128]. Increased intestinal permeability, driven by dysbiosis-associated disruption of tight junction proteins (occludin, claudin-1, ZO-1), allows translocation of lipopolysaccharide (LPS), bacterial DNA fragments, and peptidoglycan components into systemic circulation [129]. LPS engages CD14–TLR4–MD2 complexes, initiating MyD88-dependent and TRIF-dependent signaling pathways that converge on NF- κ B, AP-1, and IRF3 transcriptional programs [130]. This dual pathway activation generates both pro-inflammatory cytokine cascades and type I interferon responses, amplifying systemic immune activation [131].

Free fatty acids, particularly saturated long-chain fatty acids, act as metabolic danger signals that independently activate TLR4 signaling and amplify inflammasome activation via NLRP3 complex assembly [132]. This promotes caspase-1 activation and maturation of IL-1 β and IL-18, linking lipid overload to innate immune activation [133]. Concurrently, chronic metabolic inflammation disrupts insulin receptor signaling through activation of stress-responsive inflammatory pathways, particularly JNK and IKK β . These kinases promote inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1), resulting in impaired PI3K–AKT signaling, reduced GLUT4 translocation, and diminished cellular glucose uptake. Persistent activation of these inflammatory pathways establishes a self-amplifying metabolic–inflammatory loop characterized by insulin resistance, lipid overload, mitochondrial dysfunction, and altered metabolic homeostasis, which may negatively influence the function of reproductive tissues and early embryonic development [134,135].

Within reproductive organs, these systemic signals produce multilayered dysfunction [136]. In ovarian follicles, inflammatory cytokines impair granulosa cell mitochondrial respiration, reduce steroidogenic enzyme expression (CYP19A1, STAR), and increase apoptotic signaling via intrinsic mitochondrial pathways involving cytochrome c release and caspase-9 activation [137]. Oocyte quality is compromised through altered mitochondrial distribution, spindle checkpoint instability, and epigenetic dysregulation of maternal RNA stores essential for early embryogenesis [138]. In the endometrium, chronic inflammatory signaling disrupts progesterone receptor co-activation pathways, attenuates cAMP–PKA–CREB signaling required for decidualization, and alters extracellular matrix remodeling through dysregulated MMP/TIMP balance [139]. Endothelial dysfunction further impairs spiral artery transformation by reducing nitric oxide bioavailability due to eNOS uncoupling and oxidative inactivation [140].

The gut–endometrial axis integrates these signals into a bidirectional communication system involving microbial metabolites, immune cell trafficking, endocrine signaling, and modulation of the endometrial microenvironment [141]. SCFAs, particularly butyrate, may participate in microbiome–endometrium communication by influencing endometrial epithelial function, cellular receptivity, and epigenetic regulation. Experimental evidence has shown that butyrate enhances endometrial epithelial-cell receptivity through increased histone H3K9 acetylation, providing a mechanistic link between a microbial metabolite and chromatin-dependent regulation of uterine receptivity [142]. Although these findings support a potential role for SCFA-mediated epigenetic regulation in implantation, direct evidence that gut-derived SCFAs specifically modify histone acetylation at the PRL, IGFBP1, or HOXA10 loci in the human endometrium remains limited. Conversely, LPS-mediated

inflammatory signaling can impair the endometrial environment required for implantation. In human endometrial epithelial cells, LPS exposure increased inflammatory mediators including CXCL1 and IL-8 while reducing integrin $\beta 3$ and $\beta 5$ expression, resulting in impaired trophoblastic spheroid attachment and outgrowth [143].

Hormonal regulation is deeply intertwined with microbial metabolism through the estrobolome, a functional collection of gut microbial genes encoding enzymes, particularly β -glucuronidases, that participate in estrogen deconjugation and enterohepatic recycling [144]. Alterations in gut microbial composition and β -glucuronidase activity can modify estrogen metabolism and influence systemic estrogen availability. However, the magnitude and clinical consequences of these effects in humans remain incompletely defined [145]. Endometrial receptivity is tightly dependent on coordinated estrogen and progesterone signaling, with progesterone regulating transcriptional programs involved in decidualization, cellular adhesion, extracellular matrix remodeling, and the establishment of the window of implantation. Disturbances in progesterone exposure or endometrial progesterone-responsive gene expression may therefore compromise synchronization between endometrial maturation and embryo implantation [146].

Metabolic syndrome represents a chronic amplification of interconnected metabolic and inflammatory mechanisms, encompassing insulin resistance, central adiposity, dyslipidemia, and persistent low-grade inflammation [147]. It is increasingly recognized that these metabolic abnormalities are accompanied by alterations in cellular energy metabolism and immune signaling, creating a systemic environment in which metabolic and inflammatory pathways become mutually reinforcing [148]. Dysbiosis in this context is associated with reduced microbial diversity, altered SCFA production, impaired intestinal barrier integrity, and increased exposure to microbial products such as lipopolysaccharide, contributing to metabolic endotoxemia and sustained inflammatory signaling. These disturbances may extend to reproductive tissues, where metabolic dysfunction, oxidative stress, and altered endocrine signaling can interfere with ovarian function and fertility [149].

The early embryo undergoes an extensive period of epigenetic reprogramming during the pre-implantation stage, characterized by dynamic remodeling of DNA methylation and chromatin states that is closely associated with successful embryonic development. In human embryos, substantial changes in DNA methylation and hydroxymethylation occur during the transition from the zygote through the cleavage stages to the blastocyst, and abnormal DNA methylation patterns have been associated with impaired early embryonic development [150].

Shortly after fertilization, parental epigenetic information undergoes extensive remodeling through coordinated DNA demethylation and subsequent establishment of new methylation patterns. This period represents one of the most epigenetically dynamic phases of human development, during which the embryo is particularly sensitive to environmental and metabolic conditions. Experimental evidence indicates that environmental exposures affecting gametes and early embryos can interfere with epigenetic regulation and may consequently influence developmental trajectories [151].

At the molecular level, metabolic substrates and cofactors provide an important interface between cellular metabolism and epigenetic regulation. Metabolites can influence the activity of DNA- and histone-modifying enzymes, while alterations in cellular metabolic pathways can modify the availability of substrates required for chromatin remodeling and DNA methylation. These interactions provide a mechanistic basis through which changes in the metabolic environment surrounding conception may affect epigenetic programming during early embryogenesis [152].

Nutritional status may further influence this process through one-carbon metabolism, which provides methyl groups required for DNA methylation. Folate, choline, betaine, methionine, and vitamin B12 participate in interconnected pathways regulating S-adenosylmethionine availability and DNA methyltransferase activity. A recent systematic review of human studies demonstrated that one-carbon metabolites and nutritional exposures can be associated with site-specific DNA methylation in placental tissue, particularly in genes involved in growth and development, although substantial heterogeneity exists between studies [153].

Collectively, maternal metabolic status, nutritional availability, inflammatory signaling, mitochondrial function, and epigenetic remodeling form an interconnected biological network in which disturbances during the preconception and early developmental periods may influence embryonic and placental development. Because preimplantation development involves extensive epigenetic reprogramming, alterations in the maternal metabolic and environmental milieu during this window may have consequences for developmental competence and subsequent placental and fetal programming. However, direct causal evidence linking maternal gut microbiome alterations specifically to epigenetic programming of the human preimplantation embryo remains limited, and this relationship should therefore be regarded as an emerging mechanistic hypothesis rather than an established human causal pathway [154] (Figure 1).

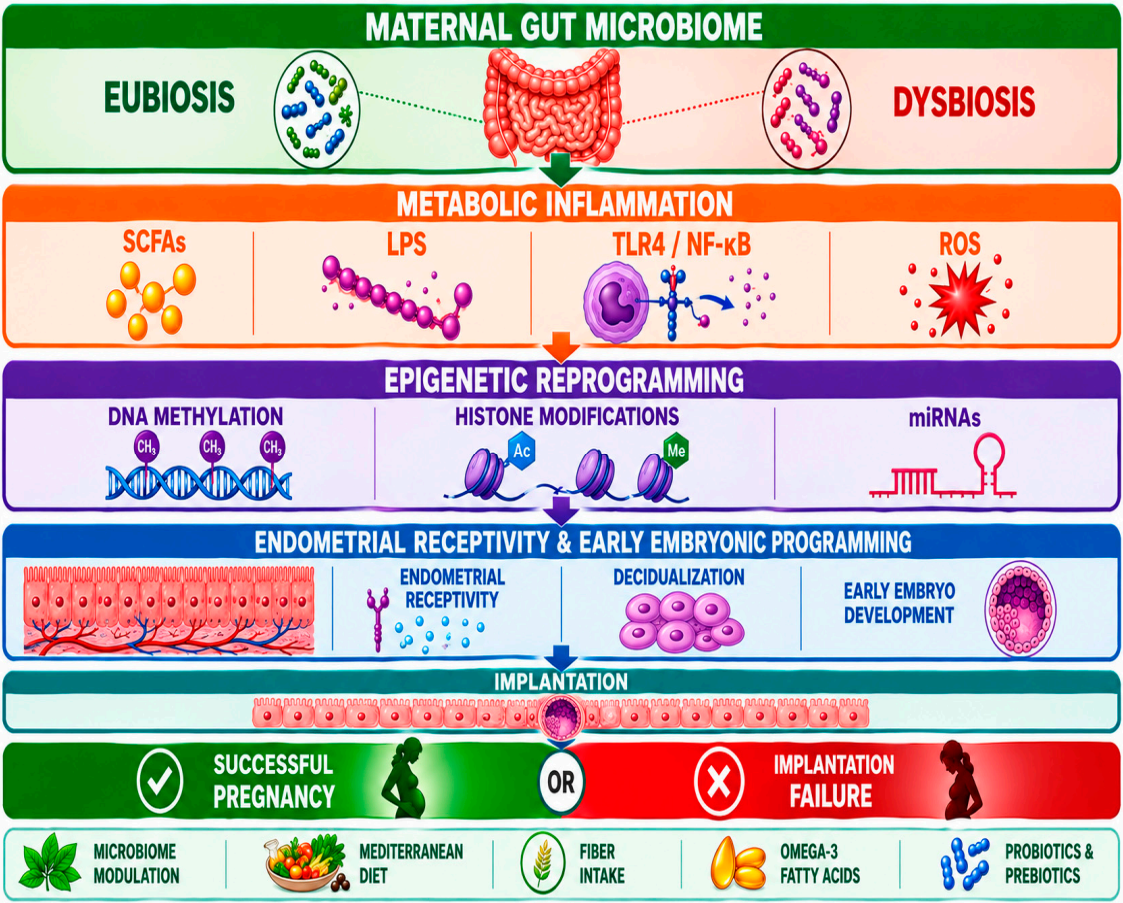


Figure 1. Maternal gut microbiome, metabolic–inflammatory signaling, epigenetic reprogramming, and implantation outcome.

The figure illustrates the proposed biological pathway linking maternal gut microbiome composition with endometrial receptivity, early embryonic programming, and implantation outcome. The upper panel contrasts eubiosis and dysbiosis of the maternal gut microbiome, emphasizing that alterations in microbial composition and function may modify the production and availability of microbiota-derived metabolites and microbial components. The subsequent metabolic inflammation stage depicts several interconnected mediators, including short-chain fatty acids (SCFAs), lipopolysaccharide (LPS), Toll-like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB) signaling, and reactive oxygen species (ROS), representing metabolic and inflammatory pathways through which alterations in the gut microbial environment may influence systemic maternal physiology. The next stage, epigenetic reprogramming, presents three major regulatory mechanisms—DNA methylation, histone modifications, and microRNAs (miRNAs)—through which metabolic and inflammatory signals may be associated with changes in gene regulation without altering the underlying DNA sequence. These processes are then linked to endometrial receptivity and early embryonic programming, including

the functional state of the endometrial epithelium, decidualization, and early embryo development, all of which are important components of the biological environment preceding and accompanying implantation. The implantation stage illustrates the interaction between the developing embryo and receptive endometrium, leading either to successful pregnancy or implantation failure. The lower panel highlights potentially modifiable factors that may contribute to microbiome modulation, including a Mediterranean dietary pattern, adequate fiber intake, omega-3 fatty acids, probiotics, and prebiotics, thereby presenting a conceptual framework in which nutritional and microbiome-related interventions may influence metabolic, inflammatory, epigenetic, endometrial, and embryonic pathways relevant to reproductive outcome.

6. Paternal Microbiome, Nutrition, Oxidative Stress, and Sperm Epigenetics

The contribution of the male partner to reproductive success extends far beyond the delivery of intact paternal DNA at the moment of fertilization. Contemporary reproductive biology increasingly recognizes that paternal metabolic health, nutritional status, microbiome composition, systemic inflammatory burden, mitochondrial integrity, and epigenetic programming collectively shape sperm quality, embryo developmental competence, implantation potential, placental development, and long-term offspring health. Within the context of assisted reproductive technologies (ART), especially recurrent implantation failure and repeated unsuccessful IVF cycles, the paternal organism must therefore be regarded as an active biological determinant of reproductive outcome [155].

One of the most important emerging concepts in reproductive medicine is the existence of the gut–testis axis, a complex bidirectional communication network linking intestinal microbial ecology with endocrine, metabolic, immune, and reproductive function. The intestinal microbiome regulates multiple physiological systems involved in spermatogenesis, including systemic inflammation, oxidative balance, mitochondrial metabolism, hormonal homeostasis, intestinal permeability, nutrient absorption, and immune signaling pathways. Alterations in microbiome composition may therefore profoundly affect sperm quality through interconnected molecular and cellular mechanisms [156].

The healthy intestinal microbiota is characterized by high microbial diversity and balanced abundance of beneficial bacterial taxa involved in short-chain fatty acid (SCFA) production, mucosal barrier maintenance, antioxidant defense, and immunological tolerance. However, modern lifestyle factors including Western dietary patterns, obesity, physical inactivity, chronic psychological stress, environmental pollutants, smoking, excessive alcohol intake, sleep disturbances, and metabolic disease contribute to progressive microbial dysbiosis. This dysbiotic state is typically characterized by reduced microbial diversity, depletion of beneficial commensal bacteria, overgrowth of pro-inflammatory microorganisms, impaired intestinal barrier function, and increased systemic exposure to endotoxins and inflammatory mediators [157].

One of the central consequences of gut dysbiosis is the development of chronic low-grade systemic inflammation. Increased intestinal permeability, frequently referred to as “leaky gut,” facilitates translocation of bacterial endotoxins such as lipopolysaccharide (LPS) into the systemic circulation. Circulating LPS activates innate immune pathways through Toll-like receptors (TLRs), particularly TLR4, triggering downstream activation of nuclear factor kappa B (NF- κ B), inflammasomes, and pro-inflammatory cytokines including TNF- α , IL-1 β , IL-6, and interferon- γ . Persistent inflammatory signaling induces oxidative stress, endothelial dysfunction, mitochondrial injury, and disruption of endocrine homeostasis, all of which negatively affect the male reproductive system [158].

Oxidative stress represents one of the most important mechanisms through which microbiome dysbiosis impairs sperm quality. Oxidative stress occurs when reactive oxygen species (ROS) generation exceeds endogenous antioxidant defense capacity. Under physiological conditions, low concentrations of ROS are necessary for normal sperm maturation, capacitation, hyperactivation, and acrosome reaction. However, excessive ROS production becomes highly detrimental to spermatozoa due to their unique structural and metabolic characteristics [159].

Sperm cells are exceptionally vulnerable to oxidative injury because they possess minimal cytoplasmic antioxidant reserves and extremely high concentrations of polyunsaturated fatty acids within

plasma membranes. These lipids are highly susceptible to lipid peroxidation initiated by ROS. Once oxidative membrane damage occurs, sperm membrane fluidity decreases, mitochondrial membrane integrity becomes compromised, ion transport mechanisms become dysregulated, and fertilization capacity declines. Lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal further amplify oxidative damage by impairing mitochondrial enzymes and inducing DNA injury [160].

Mitochondria play a central role in sperm motility and energy production. The sperm midpiece contains densely packed mitochondria responsible for ATP synthesis required for flagellar movement and fertilization competence. Dysbiosis-associated systemic inflammation disrupts mitochondrial homeostasis through increased ROS generation, impaired electron transport chain activity, altered membrane potential, and mitochondrial DNA damage. As mitochondrial efficiency declines, ATP production decreases, resulting in reduced sperm motility, impaired progressive movement, diminished capacitation, and decreased fertilization potential [161].

Oxidative stress also directly damages sperm DNA integrity. Reactive oxygen species induce single-strand and double-strand DNA breaks, oxidative base modifications, chromatin cross-linking, telomere shortening, and impaired chromatin condensation. Unlike somatic cells, mature spermatozoa possess extremely limited DNA repair capacity, making accumulated oxidative injury particularly harmful. Elevated sperm DNA fragmentation has been strongly associated with infertility, recurrent implantation failure, poor embryo morphology, impaired blastocyst formation, recurrent miscarriage, and adverse pregnancy outcomes [162].

Importantly, conventional semen analysis often fails to detect oxidative molecular damage. Men may present with apparently normal sperm concentration, motility, and morphology while simultaneously harboring significant oxidative DNA fragmentation, mitochondrial dysfunction, and epigenetic abnormalities. This observation has major implications for unexplained infertility and repeated IVF failure, where subtle paternal molecular pathology may remain clinically undetected despite normal standard semen parameters [163].

The testicular microenvironment itself is highly sensitive to inflammatory and oxidative disturbances. Chronic systemic inflammation may impair Sertoli cell function, disrupt Leydig cell steroidogenesis, alter blood–testis barrier integrity, and interfere with germ-cell maturation. Pro-inflammatory cytokines and oxidative stress may reduce testosterone biosynthesis and impair hypothalamic–pituitary–gonadal axis regulation. Obesity-associated dysbiosis further contributes to endocrine imbalance through increased aromatase activity within adipose tissue, resulting in elevated estrogen levels and reduced androgen bioavailability [164].

Diet-induced dysbiosis exerts particularly profound effects on sperm quality. Diets rich in saturated fats, trans fats, refined carbohydrates, processed meats, sugar-sweetened beverages, and ultra-processed foods promote intestinal dysbiosis and oxidative stress while simultaneously reducing antioxidant intake. Such nutritional patterns decrease abundance of beneficial SCFA-producing bacteria and increase pro-inflammatory microbial taxa associated with endotoxemia and metabolic dysfunction [165].

Short-chain fatty acids, including butyrate, acetate, and propionate, represent critical microbiome-derived metabolites involved in immune regulation, mitochondrial function, and epigenetic signaling. Butyrate possesses potent anti-inflammatory and antioxidant properties and functions as a histone deacetylase inhibitor, influencing chromatin remodeling and gene expression. Reduced SCFA production in dysbiotic states contributes to increased inflammation, impaired mitochondrial metabolism, oxidative injury, and altered epigenetic regulation within germ cells [166].

The relationship between oxidative stress and sperm epigenetics is particularly important within the context of reproductive programming. Spermatogenesis requires highly coordinated epigenetic remodeling processes, including DNA methylation, histone modification, chromatin condensation, and non-coding RNA regulation. These processes are exquisitely sensitive to metabolic and inflammatory disturbances [167].

DNA methylation abnormalities induced by oxidative stress may alter the expression of genes involved in embryogenesis, placental development, metabolic regulation, neurodevelopment, and

immune function. Reactive oxygen species interfere with methyl donor metabolism, DNA methyltransferase activity, and chromatin organization, resulting in aberrant methylation patterns within spermatozoa. Such epigenetic alterations may persist after fertilization and influence early embryonic developmental trajectories [168].

Paternal obesity and microbiome dysbiosis have been associated with altered methylation of imprinted genes, including IGF2, H19, MEG3, PEG3, and MEST, all of which are critically involved in fetal growth regulation and placental function. Disturbances in these epigenetic pathways may impair trophoblast invasion, angiogenesis, nutrient transport, and embryo implantation. Increasing evidence suggests that paternal metabolic health before conception may therefore shape not only fertility outcomes but also long-term offspring susceptibility to obesity, insulin resistance, cardiovascular disease, immune dysregulation, and neurodevelopmental disorders [169].

Small non-coding RNAs within spermatozoa constitute another major epigenetic mechanism influenced by microbiome composition and oxidative stress. Sperm cells contain microRNAs, PIWI-interacting RNAs, and transfer RNA-derived fragments that regulate post-transcriptional gene expression during early embryogenesis. Experimental studies demonstrate that high-fat diets and metabolic inflammation alter sperm RNA cargo, thereby affecting embryo metabolism and developmental programming [170].

Oxidative stress additionally impairs protamination and chromatin packaging within sperm nuclei. During spermatogenesis, histones are largely replaced by protamines to achieve dense chromatin condensation and genomic protection. Inflammatory and oxidative insults may disrupt this transition, leaving DNA more vulnerable to fragmentation and epigenetic instability. Abnormal protamine ratios have been associated with poor fertilization rates, impaired embryo quality, and recurrent IVF failure [171].

The seminal microbiome itself may further contribute to reproductive dysfunction. Seminal plasma contains bacterial communities capable of influencing sperm motility, viability, inflammatory status, and oxidative balance. Dysbiotic seminal environments have been associated with leukocytospermia, increased ROS production, elevated inflammatory cytokines, impaired mitochondrial activity, and reduced sperm function. Furthermore, seminal inflammatory mediators may influence maternal immune responses within the reproductive tract and alter endometrial receptivity during implantation [172].

Nutritional deficiencies exacerbate oxidative and epigenetic damage within spermatozoa. Folate deficiency impairs one-carbon metabolism and methyl donor availability necessary for DNA methylation. Zinc deficiency compromises antioxidant enzyme activity, DNA stabilization, and testosterone synthesis. Selenium deficiency reduces glutathione peroxidase activity and increases oxidative vulnerability, while inadequate vitamin B12 intake interferes with mitochondrial metabolism and epigenetic regulation. Collectively, these deficiencies may significantly impair sperm quality and reproductive potential [173].

Conversely, Mediterranean dietary patterns rich in fruits, vegetables, legumes, olive oil, fish, nuts, antioxidants, polyphenols, omega-3 fatty acids, and dietary fiber are associated with improved sperm concentration, motility, morphology, mitochondrial activity, and reduced oxidative stress. Increased dietary fiber intake promotes microbial diversity and SCFA production, while omega-3 fatty acids improve membrane fluidity, mitochondrial function, and anti-inflammatory signaling pathways. Polyphenols and antioxidant micronutrients neutralize reactive oxygen species and protect sperm DNA integrity [174].

These findings collectively support the concept that paternal preconception health represents a modifiable biological determinant of IVF success. Microbiome imbalance, chronic inflammation, oxidative stress, mitochondrial dysfunction, and epigenetic instability form an interconnected pathogenic network capable of impairing spermatogenesis, fertilization, embryo viability, and implantation competence. Importantly, many of these processes appear partially reversible through targeted lifestyle and nutritional interventions [175].

Future reproductive medicine will likely increasingly integrate paternal microbiome profiling, oxidative stress biomarkers, sperm DNA fragmentation assessment, metabolic evaluation, and epi-genetic analysis into comprehensive fertility diagnostics. Personalized preconception interventions aimed at restoring microbial balance, reducing oxidative stress, improving mitochondrial function, and optimizing nutritional status may ultimately enhance IVF outcomes and improve long-term off-spring health. The paternal organism must therefore be recognized as a central biological participant in reproductive programming, embryonic development, and intergenerational health trajectories rather than merely a source of paternal genetic material [176] (Figure 2).

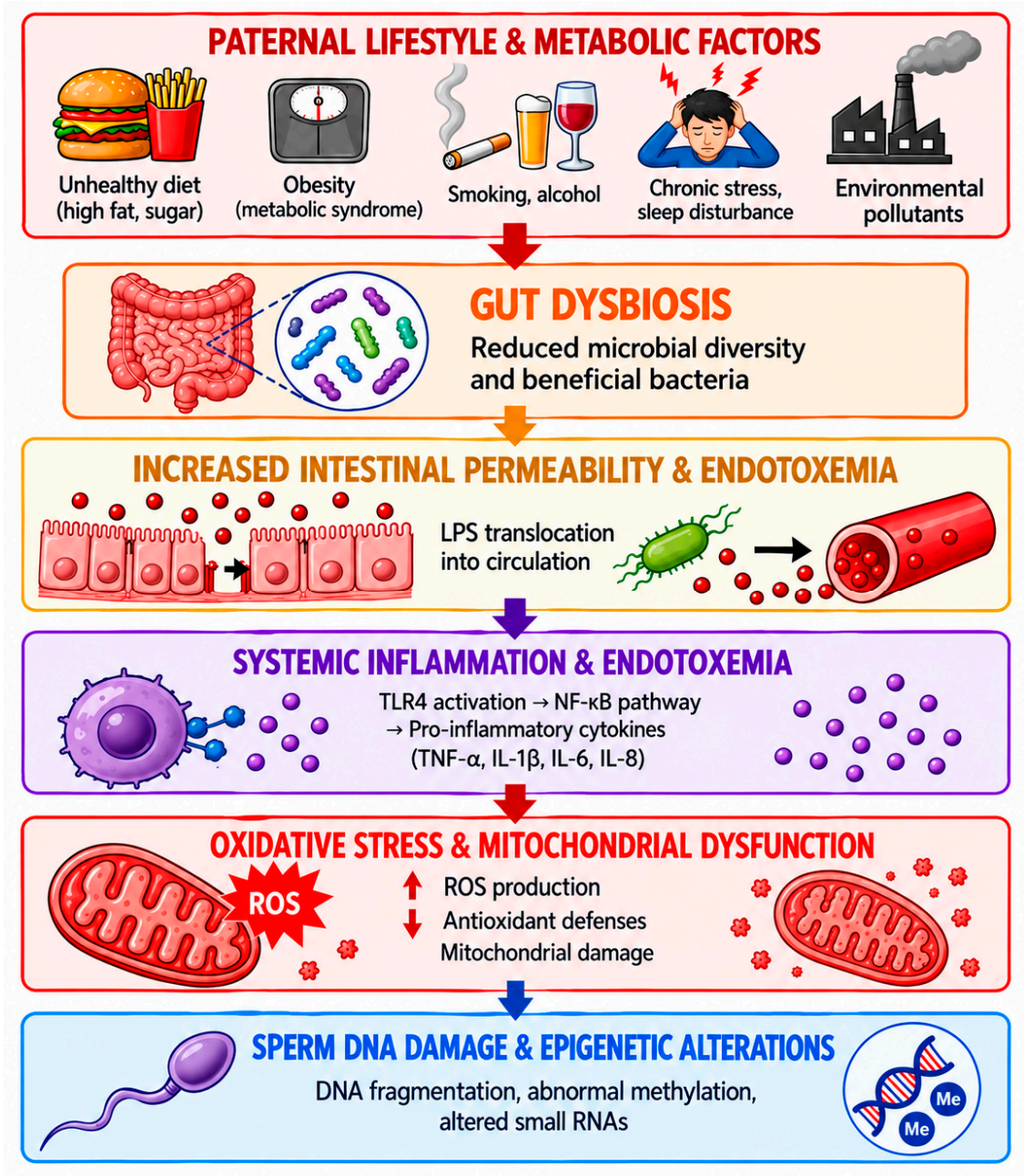


Figure 2. Paternal Lifestyle–Gut Microbiome–Sperm Axis: Pathways Linking Metabolic and Environmental Factors to Sperm Damage and Epigenetic Alterations.

Adverse paternal lifestyle and metabolic factors, including unhealthy diet, obesity, smoking and alcohol consumption, chronic stress, sleep disturbance, and environmental pollutants, may promote gut dysbiosis, characterized by reduced microbial diversity and loss of beneficial bacteria. Impaired intestinal barrier integrity may facilitate lipopolysaccharide (LPS) translocation into the circulation,

contributing to endotoxemia and systemic inflammation through Toll-like receptor 4 (TLR4) activation and nuclear factor kappa B (NF- κ B) signaling, with increased production of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and interleukin-8 (IL-8). Increased reactive oxygen species (ROS) production and reduced antioxidant defenses may subsequently promote oxidative stress and mitochondrial dysfunction, contributing to sperm deoxyribonucleic acid (DNA) fragmentation, abnormal DNA methylation, and alterations in small ribonucleic RNAs (RNAs), thereby potentially affecting sperm quality and epigenetic stability.

7. Nutritional Patterns of Both Partners as Modifiable Risk Factors

The preconception period represents a critical epigenetic window during which the nutritional status of both partners functions as an important determinant of reproductive and offspring health trajectories [177]. Contemporary molecular insights indicate that parental diet should not be viewed merely as a source of basal energy, but also as a modulator of gamete quality, sperm epigenetic programming, and subsequent embryonic development. Clinical evidence indicates that nutritional patterns in both women and men may influence outcomes of assisted reproductive technologies, while paternal nutritional status may additionally influence sperm epigenetic integrity and offspring metabolic health [178].

The divergence between Western and Mediterranean dietary patterns serves as a significant marker of systemic metabolic and inflammatory status that may influence reproductive outcomes [179]. The Western model, defined by high consumption of energy-dense and ultra-processed foods and a predominance of saturated fatty acids, is associated with adverse metabolic profiles and may negatively affect reproductive function. This metabolic environment may impair mitochondrial function and redox homeostasis in reproductive cells [180]. At the cellular level, chronic nutrient excess can influence nutrient-sensing pathways, including mTOR and AMPK signaling, which regulate cellular metabolism, autophagy, and mitochondrial homeostasis [181]. The accumulation of advanced glycation end products (AGEs) under conditions of metabolic dysfunction can activate the receptor for AGEs (RAGE) and promote inflammatory and oxidative signaling through NF- κ B-dependent pathways. These mechanisms provide a plausible molecular connection between metabolic dysfunction, oxidative stress, and impaired reproductive cellular function [182]. In males, dietary patterns characterized by high intake of saturated fat have been associated with poorer sperm parameters and increased sperm DNA damage—a direct consequence of systemic oxidative stress that disrupts the blood-testis barrier. Conversely, the Mediterranean pattern, rich in monounsaturated fatty acids, polyphenols, vegetables, fruits, legumes, whole grains, nuts, and olive oil, has been associated with potentially more favorable outcomes in assisted reproduction. Polyphenol-rich foods may additionally contribute to antioxidant defense and modulation of inflammatory signaling. Through these combined nutritional effects, a Mediterranean dietary pattern may provide a more favorable metabolic environment for gametogenesis and early reproductive processes [183].

Micronutrient availability influences DNA synthesis and epigenetic programming, with folate, choline, and vitamin B12 representing important components of one-carbon metabolism [59]. Folate and vitamin B12 participate in pathways involved in methionine metabolism and the generation of methyl donors required for DNA methylation, while choline contributes both to methyl-group metabolism and phosphatidylcholine synthesis [184]. The biological importance of these pathways is particularly relevant during the preconception and early developmental periods, when epigenetic programming is extensively remodeled [60]. The availability of methyl donors can influence DNA methylation patterns in the placenta and developing tissues, providing a potential mechanistic pathway through which parental nutritional status may affect developmental trajectories [185]. Experimental evidence further indicates that paternal folate deficiency can alter sperm histone modifications and that some of these alterations may persist into the preimplantation embryo and affect embryonic gene expression [53].

Zinc and selenium are also important micronutrients in male reproductive physiology because of their roles in antioxidant defense, cellular metabolism, and sperm function [186]. Selenium is incorporated into selenoproteins involved in protection against oxidative damage, including glutathione peroxidases, whereas zinc contributes to numerous enzymatic and structural processes relevant to reproductive cells [61]. Disturbances in antioxidant micronutrient availability may therefore increase susceptibility of spermatozoa to oxidative injury [58,61]. Iron and copper are essential trace elements but require tightly regulated homeostasis because excessive redox-active metal availability can promote oxidative reactions [187]. Consequently, nutritional adequacy rather than indiscriminate supplementation represents the appropriate physiological principle for micronutrient management during the preconception period [188].

Vitamin D signaling is also relevant to reproductive physiology because the vitamin D receptor is expressed in reproductive tissues and immune cells and participates in the regulation of cellular and immune processes [64]. However, although associations between vitamin D status and reproductive outcomes have been reported, the clinical evidence remains heterogeneous and does not establish that supplementation universally improves fertility or implantation [189]. Iodine sufficiency is particularly important before and during pregnancy because maternal thyroid hormone production is required for early fetal development, including neurodevelopment, before the fetal thyroid becomes fully functional [65]. Adequate nutritional status of these micronutrients should therefore be considered within the broader framework of preconception nutritional assessment rather than as isolated determinants of fertility [190].

The ratio of omega-6 to omega-3 polyunsaturated fatty acids regulates the availability of substrates for lipid mediator biosynthesis and therefore participates in inflammatory signaling. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are incorporated into cellular membranes and can influence membrane composition, lipid mediator production, and inflammatory responses [191]. Clinical data indicate that omega-3 intake may be relevant to both male and female reproductive outcomes: in women undergoing ART, higher EPA and DHA intake has been associated with greater probability of live birth and lower risk of pregnancy loss, whereas in men, higher omega-3 intake has been associated with sperm count, concentration, and motility. EPA and DHA can also serve as precursors for specialized pro-resolving mediators, including resolvins, protectins, and maresins, which participate in the active resolution of inflammation [192]. DHA is particularly abundant in reproductive tissues and sperm membranes, where membrane lipid composition contributes to sperm function and fertilization competence. These observations support adequate intake of omega-3 fatty acids as one component of a broader nutritional strategy for reproductive health, although clinical intervention evidence remains insufficient to establish an optimal omega-6/omega-3 ratio specifically for IVF success [190].

Finally, dietary fiber intake represents an important determinant of gut microbial metabolism. Fermentation of dietary fiber by intestinal microorganisms produces short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate, which participate in metabolic and immune regulation [193]. SCFAs act through G-protein-coupled receptors including FFAR2/GPR43 and FFAR3/GPR41 and can also influence gene expression through inhibition of histone deacetylases. Butyrate has been particularly associated with regulatory T-cell differentiation and maintenance of intestinal immune homeostasis. Through these mechanisms, dietary fiber and its microbial metabolites provide an important connection between nutrition, microbiota composition, immune regulation, and epigenetic signaling [194].

Fiber fermentation also influences the intestinal bile acid pool through microbial transformation of primary bile acids into secondary bile acids. Bile acids function not only in lipid digestion but also as signaling molecules through receptors including the farnesoid X receptor (FXR) and the G-protein-coupled bile acid receptor TGR. Microbiota-dependent changes in bile acid composition can consequently influence metabolic regulation, intestinal immunity, and inflammatory signaling [195]. A further metabolic axis involves microbial metabolism of dietary tryptophan into indole derivatives, which can activate the aryl hydrocarbon receptor (AhR) and contribute to intestinal barrier integrity

and mucosal immune regulation. Tryptophan-derived microbial metabolites therefore represent another mechanism linking dietary composition with microbiome-dependent immune signaling [196].

Chronic low-fiber dietary patterns may contribute to alterations in gut microbial composition and intestinal barrier function, potentially increasing exposure to microbial products and inflammatory signals. SCFAs and other microbiota-derived metabolites participate in maintaining epithelial barrier integrity and regulating mucosal immune responses, while disruption of these interactions can contribute to metabolic and inflammatory dysfunction. Lipopolysaccharide (LPS) derived from Gram-negative bacteria can activate Toll-like receptor 4 (TLR4)-dependent inflammatory signaling and thereby contribute to systemic metabolic inflammation [197]. Experimental evidence additionally supports an interaction between the gut microbiota and the male reproductive system, with high-fat dietary exposure capable of altering the gut and testicular metabolome and impairing sperm quality. These findings provide biological support for the concept of a gut–testis axis, although translation of experimental findings into clinical recommendations for human infertility requires further investigation [198].

By strategically modulating these nutritional factors, clinical practice can shift from reactive prenatal care to proactive preconception optimization. Addressing nutritional status, metabolic health, dietary quality, and microbiome-related factors as potentially modifiable determinants provides a framework for reducing nutritional and metabolic risks before conception [199,200]. This shift in clinical paradigm implies a corresponding shift in the timing and structure of preconception counseling: rather than addressing nutritional deficiencies only once pregnancy is confirmed, nutritional assessment should ideally begin before conception, when gametogenesis and early developmental processes remain potentially modifiable [201,202]. Operationalizing this framework requires identifying measurable, clinically accessible biomarkers that serve as proxies for the underlying molecular processes described above, including nutritional biomarkers, metabolic parameters, fatty-acid profiles, and selected measures of gut microbial composition and metabolite production. Importantly, these markers are not isolated targets but interconnected nodes within a broader metabolic and biological network [203]. Consequently, a comprehensive rather than single-marker approach may provide a more informative nutritional risk profile for each couple, allowing individualized counseling and monitoring during the preconception interval [204]. In this sense, the modifiable risk factors discussed throughout this section become components of a preventive, couple-centered model of reproductive care, in which both partners are recognized as active participants in shaping the metabolic and epigenetic conditions that influence gamete quality, implantation, early embryogenesis, and ultimately offspring health [205] (Figure 3).

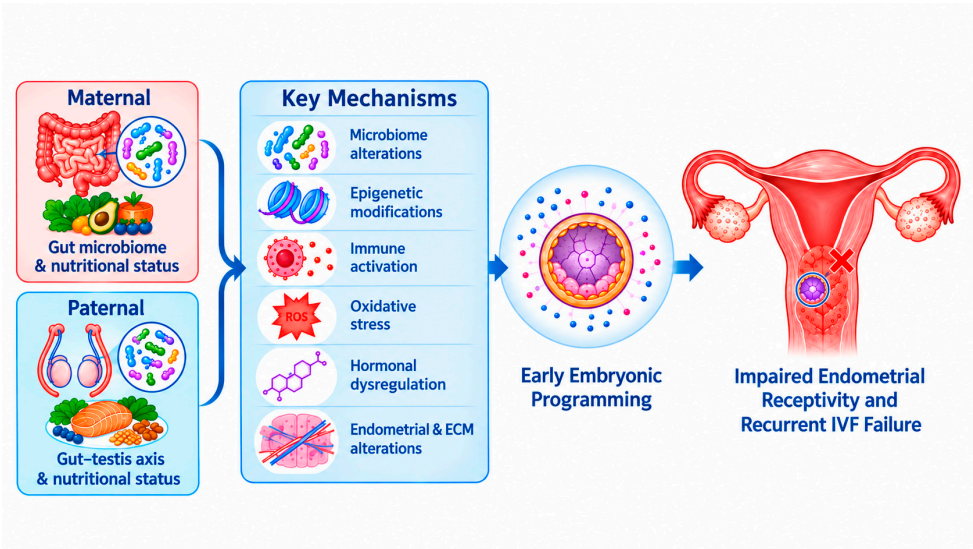


Figure 3. Parental dysbiosis and nutritional imbalance as potential modifiers of recurrent in vitro fertilization failure: microbiome–epigenome interactions in early embryonic programming.

The figure presents a conceptual framework linking maternal and paternal microbiome- and nutrition-related factors with molecular and cellular mechanisms that may influence early embryonic programming, endometrial receptivity, and recurrent in vitro fertilization (IVF) failure. Maternal factors include the gut microbiome and nutritional status, including dietary patterns and intestinal microbial composition, whereas paternal factors include the paternal microbiome, the gut–testis axis, and nutritional status. Together, these factors may contribute to reproductive and early developmental processes through interconnected metabolic, immune, oxidative, hormonal, and epigenetic pathways.

Maternal and paternal dysbiosis and nutritional imbalance may be associated with alterations in microbial composition and microbial-derived signaling, potentially affecting host metabolic and immune homeostasis. These changes may contribute to epigenetic modifications, including altered DNA methylation, histone modifications, and microRNA (miRNA)-mediated regulation, with potential consequences for gene expression and early developmental programming. Immune activation and altered inflammatory signaling may influence the maternal reproductive environment and embryo–maternal interactions. At the same time, oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) generation and antioxidant defenses, may affect cellular homeostasis, gamete quality, and early developmental processes.

Altered endocrine and metabolic signaling may further contribute to hormonal dysregulation and interfere with reproductive function. Changes in endometrial signaling and extracellular matrix (ECM) organization may affect the molecular and structural conditions required for endometrial receptivity and embryo implantation. The convergence of microbiome-derived, nutritional, metabolic, immune, oxidative, hormonal, and epigenetic signals may therefore influence early embryonic programming and may be associated with impaired endometrial receptivity and recurrent IVF failure.

Conversely, a balanced microbiome and adequate nutritional status may support metabolic and immune homeostasis and regulatory and epigenetic processes associated with reproductive function. These potentially modifiable parental factors may therefore represent components of a broader biological network linking preconception health with reproductive and early developmental outcomes.

This framework represents a conceptual synthesis of proposed biological relationships rather than an established causal model. The depicted connections indicate potential biological interactions and should not be interpreted as evidence that all pathways have been causally established in humans. The strength of evidence varies among individual mechanisms, with some relationships supported by human observational studies, others primarily by experimental or mechanistic evidence, and several remaining hypothetical and requiring further clinical investigation.

8. Clinical Implications: Preconception Microbiome Optimization Before IVF

The growing recognition of the microbiome as a critical regulator of reproductive physiology has profound implications for clinical practice, particularly in managing couples undergoing assisted reproductive technologies (ART) [206]. Traditionally, infertility treatment has focused primarily on anatomical abnormalities, hormonal disturbances, gamete quality, and laboratory-based optimization of fertilization and embryo culture procedures. However, accumulating evidence indicates that recurrent implantation failure, poor embryo development, suboptimal pregnancy outcomes, and even certain forms of unexplained infertility may reflect broader systemic disturbances involving the microbiome, immune system, metabolism, oxidative stress, and epigenetic regulation [207]. These observations have increased interest in preconception interventions aimed at restoring biological homeostasis before initiating IVF treatment [208].

The preconception period represents a unique therapeutic window during which targeted interventions may improve reproductive competence by influencing gamete quality, endometrial receptivity, embryo developmental potential, and maternal immune adaptation [209]. Unlike many genetic factors, microbiome composition is highly dynamic and responsive to environmental modification, making it an attractive target for preventive and therapeutic strategies. Consequently, optimization

of microbiome health before IVF has emerged as a promising component of personalized reproductive medicine [210].

Current evidence suggests that successful implantation depends not only on embryo quality and endometrial receptivity but also on the establishment of a systemic physiological environment characterized by balanced immune function, metabolic stability, adequate mitochondrial activity, controlled oxidative stress, and favorable microbial-host interactions [211]. Disturbances in any of these interconnected systems may compromise reproductive outcomes despite technically successful ART procedures. Therefore, preconception assessment and optimization of microbiome-related factors may represent an important opportunity to improve IVF success rates [212].

One of the most extensively investigated approaches to microbiome modulation involves the use of probiotics. Probiotics are defined as live microorganisms that confer health benefits when administered in adequate amounts. Their beneficial effects are mediated through multiple mechanisms, including the restoration of microbial diversity, competitive inhibition of pathogenic microorganisms, enhancement of epithelial barrier integrity, modulation of immune responses, production of beneficial metabolites, and reduction of inflammatory signaling [213].

Within the reproductive context, probiotic supplementation has been investigated primarily for its ability to restore microbial balance within the gastrointestinal and female reproductive tracts [214]. *Lactobacillus* species are of particular interest because they represent the dominant microorganisms within a healthy vaginal microbiome. *Lactobacilli* contribute to the maintenance of a low vaginal pH through lactic acid production, inhibit colonization by pathogenic bacteria, modulate local immune responses, and support epithelial integrity. Vaginal microbiomes characterized by *Lactobacillus* dominance have been consistently associated with improved reproductive outcomes, enhanced implantation rates, and increased IVF success [215].

The potential benefits of probiotics extend beyond the reproductive tract. Gut microbiota influence systemic inflammation, metabolic homeostasis, oxidative stress, and immune regulation, all of which are critically important for reproductive function [216]. Probiotic supplementation may enhance the production of short-chain fatty acids (SCFAs), improve intestinal barrier integrity, reduce endotoxemia, suppress pro-inflammatory cytokine production, and promote the differentiation of regulatory T cells. These effects may contribute to the establishment of an immunological environment favorable for embryo implantation and pregnancy maintenance [217].

Prebiotics represent another promising strategy for microbiome optimization. Unlike probiotics, which introduce live microorganisms, prebiotics are selectively fermented substrates that promote the growth and activity of beneficial microbial populations already present within the host. Common prebiotics include inulin, fructooligosaccharides, galactooligosaccharides, resistant starches, and various forms of dietary fiber. Fermentation of these substrates by intestinal bacteria results in increased production of SCFAs, particularly butyrate, acetate, and propionate [218].

SCFAs exert broad biological effects relevant to reproductive success. Butyrate enhances intestinal barrier function, suppresses inflammatory signaling pathways, supports mitochondrial metabolism, promotes regulatory T-cell development, and functions as a histone deacetylase inhibitor capable of influencing epigenetic regulation. Through these mechanisms, prebiotic supplementation may indirectly improve reproductive outcomes by promoting systemic immune tolerance and metabolic stability [219].

The concept of synbiotic therapy, combining probiotics and prebiotics, has gained increasing attention because of the potential synergistic effects on microbial ecology and host physiology. By simultaneously introducing beneficial microorganisms and providing substrates necessary for their growth, synbiotic interventions may produce more sustained improvements in microbiome composition and function. Although further clinical studies are needed, this approach represents a promising avenue for future reproductive medicine [220].

In addition to direct microbiome-targeted interventions, anti-inflammatory dietary strategies have emerged as a cornerstone of preconception optimization. Chronic low-grade inflammation represents a common biological denominator linking obesity, metabolic syndrome, insulin resistance,

dysbiosis, oxidative stress, and infertility. Consequently, dietary interventions capable of reducing inflammatory burden may provide substantial reproductive benefits [221].

The Mediterranean dietary pattern remains one of the most extensively studied anti-inflammatory nutritional models. Rich in fruits, vegetables, legumes, whole grains, olive oil, nuts, fish, and polyphenol-containing foods, this dietary pattern promotes microbial diversity, enhances SCFA production, improves metabolic health, and reduces inflammatory signaling [222]. Polyphenols activate antioxidant pathways including Nrf2, suppress NF- κ B activation, improve mitochondrial function, and modulate immune responses [223]. Simultaneously, dietary fiber supports beneficial microbial populations and contributes to the production of microbiome-derived metabolites with anti-inflammatory and epigenetic regulatory properties [224].

Omega-3 polyunsaturated fatty acids represent another important component of anti-inflammatory nutritional strategies [225]. Through the generation of specialized pro-resolving mediators such as resolvins, protectins, and maresins, omega-3 fatty acids actively promote the resolution of inflammation and the restoration of tissue homeostasis. These mechanisms may improve ovarian function, sperm quality, endometrial receptivity, placental development, and embryo implantation [226].

The reduction of ultra-processed foods, refined sugars, excessive saturated fats, and trans fats represents an equally important component of preconception nutritional optimization. Such dietary components contribute to dysbiosis, endotoxemia, oxidative stress, and metabolic dysfunction, thereby creating a physiological environment unfavorable for successful conception and implantation [227,228].

An additional clinical concept receiving increasing attention is metabolic conditioning before ART cycles. Metabolic health exerts profound effects on reproductive physiology through its influence on endocrine function, mitochondrial activity, inflammatory signaling, oxidative balance, and epigenetic regulation. Conditions such as obesity, insulin resistance, metabolic syndrome, and non-alcoholic fatty liver disease are associated with impaired fertility, reduced IVF success rates, and adverse pregnancy outcomes [229].

Metabolic conditioning refers to targeted interventions aimed at improving metabolic status before the initiation of fertility treatment. Such interventions may include weight optimization, improvement of insulin sensitivity, dietary modification, physical activity, sleep regulation, stress reduction, and the correction of nutritional deficiencies. Importantly, the goal is not merely weight loss but the restoration of metabolic flexibility and the reduction of systemic inflammatory burden [230].

Mitochondrial function occupies a central position within metabolic conditioning strategies. Both oocytes and spermatozoa depend heavily on efficient mitochondrial energy production. Mitochondrial dysfunction contributes to impaired gamete quality, defective fertilization, abnormal embryogenesis, and reduced implantation potential. Nutritional interventions that support mitochondrial biogenesis, enhance oxidative phosphorylation efficiency, and reduce oxidative stress may therefore improve reproductive competence before ART [231].

9. Conclusions

Successful implantation and assisted reproduction outcomes depend on a dynamic balance between a *Lactobacillus*-dominant microbiome, the suppression of chronic inflammation, and preserved mitochondrial function of gametes. Oxidative stress and metabolic disorders disrupt the epigenetic profile and DNA integrity of both partners, potentially compromising early embryonic development and endometrial receptivity. Preconception optimization through targeted nutrition and micronutrient therapy acts as a key modulator that helps restore eubiosis and systemic homeostasis, creating optimal conditions to prevent recurrent implantation failure.

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