REVIEW

Parenting from before conception

Michelle Lane, Rebecca L. Robker, Sarah A. Robertson*

At fertilization, the gametes endow the embryo with a genomic blueprint, the integrity of which is affected by the age and environmental exposures of both parents. Recent studies reveal that parental history and experiences also exert effects through epigenomic information not contained in the DNA sequence, including variations in sperm and oocyte cytosine methylation and chromatin patterning, noncoding RNAs, and mitochondria. Transgenerational epigenetic effects interact with conditions at conception to program the developmental trajectory of the embryo and fetus, ultimately affecting the lifetime health of the child. These insights compel us to revise generally held notions to accommodate the prospect that biological parenting commences well before birth, even prior to conception.

Our constitution at birth informs how we respond to stressors and challenges, and the risk of disease, in childhood and through adult life. Experience in utero is a major determinant (1), but earlier life phases, commencing with oocyte and sperm, are also important. At conception, the gametes deliver the genetic material to form an embryo, plus a legacy of additional information, reflecting the exposures and experiences of both parents—not just mother, but father, too. If emerging concepts in transgenerational epigenetic inheritance (2, 3) are correct, the early embryo is exquisitely sensitive to signals from gametes and the environment. Here, we explore how events before and at conception shape our development and life-course trajectory.

The sensitive and adaptable early embryo

Strategies to discern how different pregnancy stages affect infant health reveal a crucial window in early embryonic development (2). During fertilization and the first zygotic divisions, the embryo is highly sensitive to signals from the mother's reproductive tract (Fig. 1). The oviductal fluid surrounding the embryo varies according to maternal nutritional, metabolic, and inflammatory parameters (4), providing a microcosm that reflects the outside world. In responding to these environmental cues, the embryo exerts a high degree of developmental plasticity and can, within a discrete range, modulate its metabolism, gene expression, and rate of cell division. In this way, the maternal tract and the embryo collaborate to generate a developmental trajectory adapted to suit the anticipated external environment, to maximize survival and fitness of the organism (2). But if the resulting phenotype is a poor match for conditions after birth, or if adaptation constrains capacity to withstand later challenges, offspring are at risk (1).

Maternal diet at conception has a major impact on the developmental program (5). Reduced protein content for just the first 3 days of embryogenesis retards cell proliferation and skews the balance of cell lineage differentiation in the blastocyst (6). The effect of nutritional disturbance at conception persists through implantation and influences placental development and nutrient transfer capacity (7), then after birth, the neonate gains weight more rapidly, developing higher systolic blood pressure and elevated anxiety (6).

Maternal inflammation at conception also can influence adult phenotype. Female mice given bacterial lipopolysaccharide (LPS) on the first day of pregnancy, mimicking a mild infection, deliver pups that develop abnormally increased body fat and reduced exploratory behavior (8). Offspring have reduced sensitivity to LPS challenge in adulthood, with a blunted cytokine response (8). This suggests that infection, or even noninfectious causes of inflammation in a mother, could lead to altered immune function in her child.

In vitro fertilization (IVF) techniques further illustrate the impact of physiochemical manipulation of the conception environment, since the embryo is exposed to physical conditions not encountered in vivo. Mice conceived by IVF display

The Robinson Research Institute and School of Paediatrics and Reproductive Health, The University of Adelaide, Level 3, Medical School, South Adelaide, SA, 5005 Australia.
*Corresponding author. E-mail: sarah.robertson@adelaide.edu.au
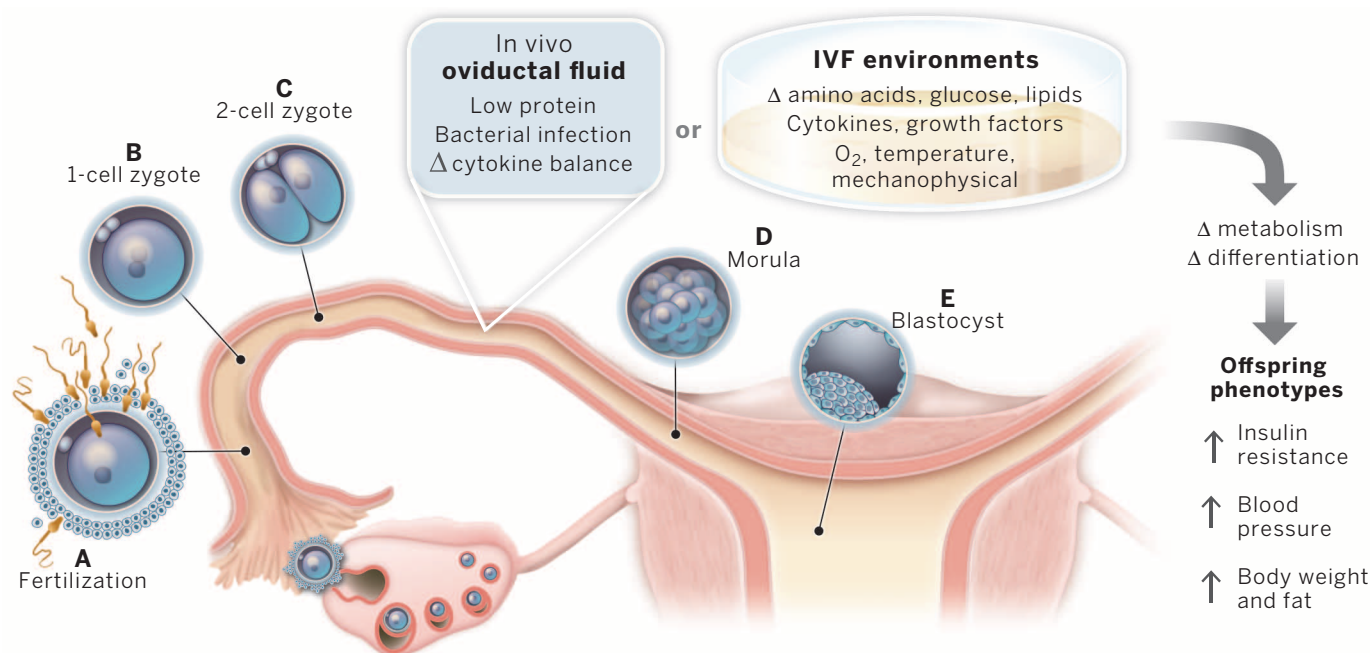
Fig. 1. Environmental effects on embryogensis. During progression from conception, through first cleavage to morula and blastocyst stages [(**A**) to (**E**), respectively], a preimplantation embryo is vulnerable to perturbations in its nutritional, biochemical, and physical environment. Influences exerted in the oviduct in vivo, or the culture dish in vitro, operate via epigenetic pathways to program embryo developmental trajectory, resulting in an altered adult phenotype.

increased fasting glucose, impaired glucose tolerance, and altered insulin signaling compared to naturally conceived controls (9). More rapid postnatal growth and fat deposition after IVF conception are associated with altered gene expression in liver, adipose tissue, pancreatic islets, and muscle (10), plus vascular stiffness, higher arterial blood pressure, and signs of endothelial dysfunction (11). Notably, adverse effects are retained if embryos are transferred to healthy recipients at the two-cell stage, implicating disruption of very early developmental events. Thus, at least in mice, conception by IVF alters later placental and fetal development, growth trajectory after birth, and metabolic parameters and behavior in adult life. In vitro–cultured embryos show changes to blastocysts and fetal growth that mimic many aspects of in vivo dietary and inflammatory insults (12), suggesting that endogenous cell stress may be a common pathway driving adverse impacts on offspring. Although the protocols implemented in animals are more aggressive than clinical IVF, emerging data suggest that in IVF-conceived children, blood pressure and fasting glucose are higher (13), and vascular dysfunction can be evident (14).

Epigenetic reprogramming at conception

The periconception influences on development are believed to occur through environment-induced modification of the embryo's epigenome. A dynamic phase of epigenetic remodeling begins at fertilization, when most epigenetic marks are cleared from the oocyte and sperm genomes before fusion of the chromatin at syngamy, and is completed just before implantation when remethylation of the embryonic genome occurs (15). Altered methylation of cytosine residues, or loss of parental-specific imprinted marks, may be attenuated by the chromatin structure, including nucleosome positioning, and altered histone acetylation or assembly, which modulate the availability of DNA for transcription. Epigenetic marks are carried forward into daughter cells, where despite further modification by the developmental program, they permanently affect gene expression in resulting adult tissues (15).

Maternal nutrition at conception is a major influence on resetting of the epigenome in the early embryo—a compelling example is epigenetic control of the agouti viable yellow (A^{vy}) locus, which determines coat color in mice and is highly sensitive to methyl grou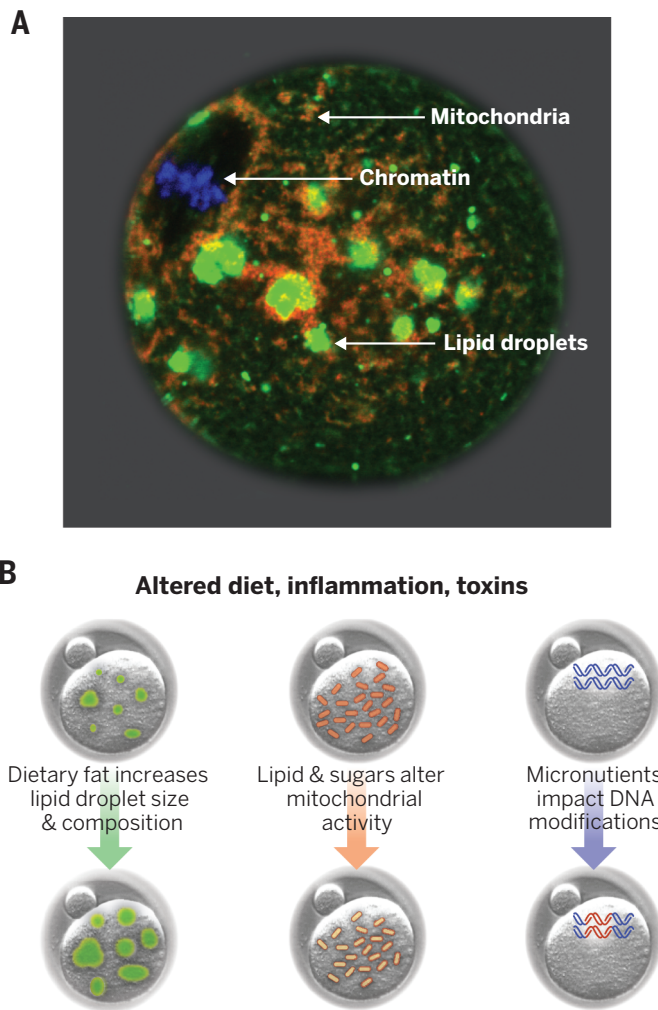ps in the diet (3, 16). DNA methylation in human infants was recently associated with seasonal variation in diet (17); similar epigenetic marks were present in different tissues, indicating that persistent systemic changes were established at conception.

Altered methylation patterns are also evident in embryos conceived by IVF or exposed to stress-inducing culture conditions (16, 18, 19). After IVF, mouse blastocysts show disrupted expression of the epigenetic regulator *Txnip* and enriched histone acetylation at its promoter, which are maintained into adulthood (10). Vascular dysfunction evident in IVF-conceived mice is associated with altered methylation of genes in the aorta (11)—but causal relationships between epigenetic changes and phenotypic alterations have not been demonstrated and are difficult to prove.

Specific classes of elements in the genome appear particularly sensitive to epigenetic dysregulation, including transposons (which control expression of the A^{vy} locus) and genomically imprinted genes, which normally survive the global erasure of epigenetic marks at conception (16). Although the impact of IVF on transposons is not known, there is an increased incidence of imprinting disorders in IVF children, suggesting that maintenance of imprinted genes may be disturbed (20). However, genome-wide analysis of methylation shows no epigenetic changes attributable to IVF (21).

Intriguingly, males are consistently more vulnerable to most dietary, culture-induced, and physiochemical models of metabolic programming (2, 5, 6, 8, 12). Female embryos consume relatively more glucose, and male embryos develop more quickly to the blastocyst stage (22). Sex-dependent transcriptional differences in molecular pathways controlling glucose metabolism, protein metabolism, DNA methylation, and epigenetic regulation (23) likely cause sex-specific differential responses to environmental insults.

Ex ovo omnia: All things come from eggs

Effects on oocytes contribute to the effects of maternal environment on offspring phenotype. Studies to isolate preconception effects from later pregnancy demonstrate that maternal nutrition during oocyte maturation influences offspring phenotype (Fig. 2). In sheep, maternal overfeeding generates offspring that accumulate fat (24), while in mice, a protein-deficient diet for 3.5 days before conception leads to hypertension (25).

Developing oocytes are suspended in follicular fluid that provides a unique nutritional environment which reflects maternal physiological states—for instance, adiposity (26). As the oocyte matures, it accumulates epigenetic marks, both on histones and DNA, until the final phases of maturation before ovulation. Although generally these marks are erased at conception, there is evidence that at some loci, oocyte epigenetic marks are not cleared, allowing the possibility of transgenerational inheritance. As well as maternally imprinted loci, epigenetic marks established in response to environmental cues may also be resistant (3, 27). This is difficult to definitively demonstrate, because the complexity of the human genome makes it impossible to clearly distinguish genetic and epigenetic heredity (27).

Attributing effects to transgenerational inheritance requires experiments in inbred genetic backgrounds, and the use of oocyte transfer or cross-fostering to ensure that effects are truly transmitted through the germ line (28). Evidence from mice exposed to preconception zinc

Fig. 2. Maternal nutrition affects oocyte provisioning. (**A**) The maternal environment influences oocyte stores of mitochondria and metabolites. Lipid droplets are stained green with BODIPY 493/503 in a mouse oocyte, and mitochondria are stained with MitoTracker Orange. Chromosomal DNA aligned at metaphase II is stained blue with Hoescht dye. (**B**) Cytoplasmic constituents respond to maternal nutrition and in turn alter conceptus development.

deficiency is convincing, because embryos from mice fed a zinc-deficient diet for just 5 days before conception generated smaller fetuses prone to neural tube defects even after embryo transfer (29), and methylation of histones and chromatin was decreased in oocytes and retained in the maternal pronucleus after fertilization (30). Increased oocyte lipid content and cellular stress are also evident in mouse studies showing poor embryo and fetal development after maternal preconception diabetes or obesity (31, 32).

Maternal nutritional influences on oocyte mitochondria are emerging as a pathway of lasting consequence to offspring (33). Embryogenesis is an energy-demanding process, and oocyte-derived mitochondria are required to support blastocyst formation (34). Alterations in maternal dietary protein affect mitochondrial localization and dampen mitochondrial activity in two-cell embryos (35) associated with later disturbances to fetal brain gene expression (36). In diabetic or obese mice, oocyte mitochondria fail to support normal embryo development (31, 32). Promisingly, these defects are modifiable by diet—oocyte quality, mitochondrial function, and fertility in aged mice can be restored by caloric restriction (37) or an omega-3–enriched diet (38).

Paternal programming—a new consideration

Paternal smoking, age, and occupational chemical exposure are well known to be linked with increased risk of cancer and neurological disorders in children (39, 40). It is less well appreciated that the father's body mass has a greater impact than the mother's on body fat and metabolic measures in prepubertal children (41). As well as sperm DNA damage, in some instances there is accumulating evidence for pathways of paternal transgenerational epigenetic effects, attributable to sperm and seminal fluid (42, 43). Interest in paternal epigenetic contributions stems from human epidemiological studies, relating a grandfather's food availability to mortality in grandsons (44) and associating paternal smoking with increased body mass index in male children (44). Paternal obesity is associated with changes to methylation in cord blood from offspring, at the demethylated region of IGF2 and possibly other imprinted genes (45). Although this can be interpreted as evidence for an epigenetic pathway, as for all human cohort studies, the possibility of shared genetic or nongenetic programming contributions cannot be discounted (27).

Rodent models have been developed to assess epigenetic transmission of metabolic and other phenotypes via the paternal line (42). For example, male mice fed a low-protein diet fathered offspring with decreased hepatic cholesterol esters and altered hepatic expression of lipid and cholesterol biosynthesis genes, associated with altered epigenetic marks (46). Male mice born to undernourished mothers sired offspring with reduced birthweight and impaired glucose tolerance (47). Other rat studies showed that nutritional cues from the father result in female offspring with impaired metabolic health (48), associated with altered gene methylation and transcriptome changes within pancreas and adipose tissues (48, 49). Rats exposed to the environmental toxin vinclozolin during development in utero have impaired spermatogenesis, which is transferred to male offspring (50). When male mice were conditioned to respond to a specific odor associated with a fear stimulus and then mated, their offspring inherited increased behavioral responses to the same odor (51). Similar transmissible effects are seen in the offspring of fathers exposed in early life to stress imposed by maternal separation (52). These intriguing studies raise the exciting prospect of specificity in paternal transmission and the possibility of targeted transmission of acquired characteristics; but to date, no biologically plausible mechanism has emerged.

Fathers transmit DNA modifications to offspring

Genetic and epigenetic transmission mechanisms may be intertwined in sperm to transmit environmental exposures to the next generation (Fig. 3). Sperm development involves extensive DNA strand repair and chromatin remodeling in which histones are largely, but not completely, replaced by protamines (43). Both sperm nucleosome and histone-bound regions are conserved among mammalian species at loci of developmental importance—including promoters for early embryo development and imprinted regions (53). Compared with protamine-bound regions, genes in histone-bound regions appear more susceptible to DNA damage (54) due to smoking, obesity, and aging (55), compounded by the incapacity of sperm to repair DNA damage due to oxidative stress (56).

Histone-bound regions appear vital for paternal DNA replication following fertilization as well as activation of paternal genome transcription in the early embryo. Whereas the paternal protamines are replaced by maternal histones in the first 4 to 6 hours after fertilization, the retained paternal histones are not replaced; therefore, epigenetic marks to these histones are likely inherited by the embryo (57). Expression of SIRT6, a class III histone deacetylase, is regulated by metabolic state and is decreased in the testes germ cells of mice with diet-induced obesity, associated with increased DNA damage in transitional spermatids as well as mature sperm (58). This may explain why sperm from obese fathers can alter the developmental capacity of the embryo in vitro, altering rates of mitosis and early differentiation events (59), resulting in reduced pluripotency and metabolic function.

Environment/lifestyle insult
Toxins
Endocrine disrupters
Smoking
Obesity

Insult affects sperm during development in testes or during maturation in the epididymis

△ Histone-bound DNA

△ MicroRNA

↑ DNA breaks

Altered gene expression in zygote

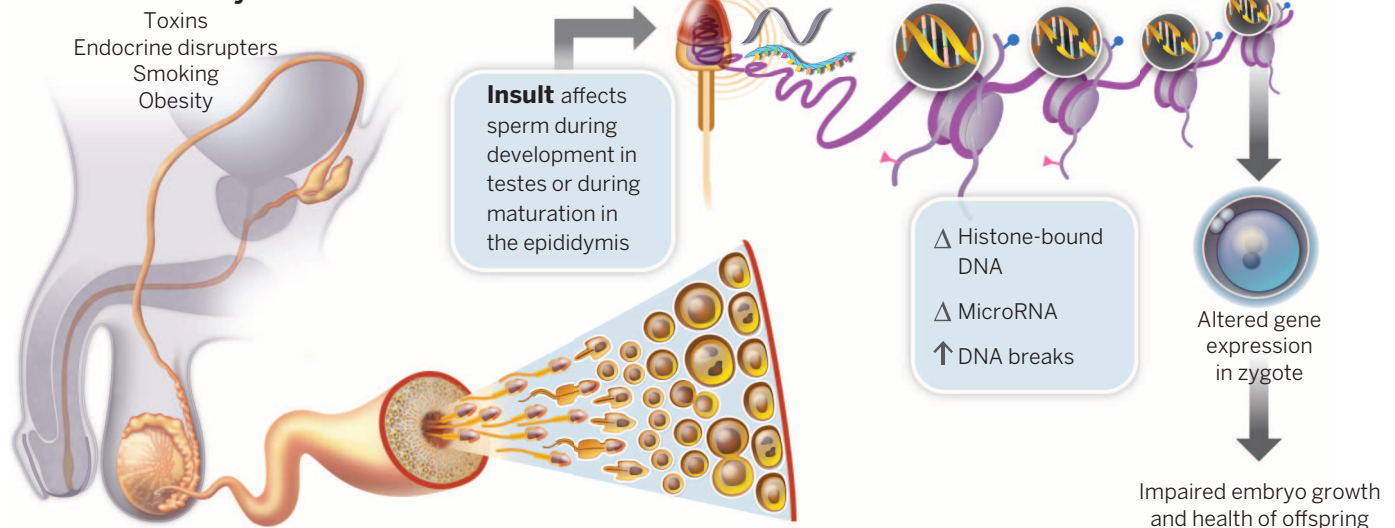Impaired embryo growth and health of offspring

Fig. 3. Environmental effects on paternal nongenetic contributions. Postulated modes of action of environment or lifestyle factors on sperm function, imparted either during spermatogenesis or epididymal transit, and pathways for impact on the development of the embryo.

Although the sperm chromatin is substantially erased after conception, epigenetic marks are not completely reset. Mouse models of diet-induced paternal obesity produce sperm that are hypomethylated (*60*). When mothers are nutritionally restricted for the period of gestation in which reacquisition of methylation occurs in primordial germ cells of male fetuses, subsequent analysis of sperm from the adult F$_1$ offspring shows an altered germline DNA methylome, with hypomethylation of discrete loci associated with differential expression of genes involved in lipid oxidation in the fetal liver (*61*). Accompanying retention of nucleosomes instead of protamines in hypomethylated regions shows germline transmission in a chromatin context (*61*). Exposure in utero of male fetuses to environmental toxins alters the differentially methylated regions of sperm DNA (*62*) whereas in men, exposure to endocrine disruptor bisphenol A alters methylation of sperm DNA (*63*). An epigenetic pathway is implicated in transmission of paternal behavioral conditioning to offspring—in the case of the olfactory stimuli, both the father and his male offspring displayed hypomethylation of the *Olfr151* gene in sperm (*51*).

Novel roles for sperm noncoding RNA

In addition to their tightly packaged DNA, sperm carry microRNAs, endogenous small interfering RNAs, and piwi-interacting RNAs (*64*). These noncoding RNAs can mediate epigenetic inheritance in lower organisms (*65*) and have the potential to influence developmental trajectory in mammalian embryos. Because each microRNA potentially regulates hundreds of mRNA transcripts, small shifts in microRNA profiles can be amplified through the molecular signaling cascades that regulate embryogenesis (*66*). Microinjection of miR-124 microRNA into the mouse pronuclear embryo can alter resultant offspring phenotype, causing cardiac hypertrophy and increased growth trajectory (*67*). The microinjected microRNAs persist only briefly, but altered gene expression is evident days later at the blastocyst stage, and change in chromatin structure in the promoter region of *Sox9* is inherited by the next two or three generations. In humans, smoking alters the microRNA profile in sperm, and in mice diet-induced obesity and early-life stress both alter sperm microRNA, persisting in the sperm of male offspring (*68*).

That oocytes with a null mutation in *Dgcr8*, a key subunit of the microRNA processing complex, generate normal blastocysts (*69*) initially suggested that microRNAs are unimportant in early embryo development. Although maternal microRNAs may be dispensable, some sperm-borne microRNAs appear capable of modulating embryo development. Most notably, psychosocial stress in early life altered mouse sperm microRNA, and injection of sperm RNAs from traumatized males into fertilized wild-type oocytes reproduced the behavioral and metabolic alterations in the resulting offspring (*52*). Sperm microRNA-34c was reported to be essential for the first cell division in mouse, through suppressing induction apoptosis (*70*), but a more recent report claims no change to male fertility in miR-34 null mutant mice (*71*).

A contribution by seminal fluid?

As well as the sperm epigenome, information may be transmitted to offspring via the non-sperm fraction of the seminal fluid. Seminal plasma can alter offspring phenotypes through postejaculatory effects on sperm survival and functional competence, plus indirect actions on female factors that in turn regulate embryo development (*72*). Surgical excision of male accessory glands producing seminal plasma causes reduced fertility and is associated with impaired embryo development, largely attributable to sperm DNA damage due to oxidative injury in the female tract (*73, 74*). An unexpected result of accessory sex gland excision seen in hamsters was altered postnatal growth and elevated anxiety in offspring (*73*). An epigenetic mechanism may be involved, as reduced acetylation in male pronuclei and retarded kinetics of demethylation and re-methylation in cleavage-stage embryos were associated with dysregulated expression of paternally expressed *Igf2* and *Dlk1* in offspring of sex gland–excised males (*75*).

Even more surprising is the observation that seminal plasma may affect offspring independently of sperm. Seminal fluid directly stimulates the female reproductive tract to produce embryotrophic cytokines and growth factors that protect embryos from cell stress, and to suppress production of embryotoxic signals (*72*). When this cytokine balance is disrupted by ablation of seminal fluid signaling, altered programming of future fat deposition and metabolic phenotype occurs in offspring (*74*). The effect was particularly evident in male progeny, which showed a substantial increase in central fat and other hallmark characteristics of programmed metabolic syndrome. Given that infection and other exposures can alter seminal fluid signals (*76*), the prospect exists that male-to-female seminal fluid signaling can transmit information about paternal experiences.

Summary

These emerging observations support the conclusion that parental influences begin before conception and compel us to further explore preconception pathways by which parents contribute more than genetic material to offspring. As well as effects of parental exposures on the genomic integrity of gametes, there is now clear evidence of epigenetic parental impact. From animal models allowing the temporal isolation of insults, we can confidently attribute outcomes on offspring of nongenomic effects mediated during maturation of the gametes, as well as effects of reproductive tract mediators on the preimplantation embryo.

Key questions to resolve are how exposures at specific stages of gamete development influence epigenetic marks in oocytes and sperm, just how early in development this begins, and the means by which these epigenetic marks survive zygotic reprogramming to be retained within the embryo. Investigating the extent to which epigenetic pathways are established and affected by interactions with noncoding RNA will likely be informative. Several questions are now pressing: Can programming conferred at or before conception be further modulated in offspring by later life events and insults? Or can it be influenced by the parents' or fetal genetics? What factors confer susceptibility or resilience to these interactions? Is this compounded or diluted in subsequent generations? Most excitingly, can acquired characteristics be transmitted by epigenetic pathways? These questions require careful analysis in appropriate models, with due consideration of confounding factors. Ultimately, once pathways are defined and prioritized according to importance for health outcomes, it will be possible to define how prospective parents can attenuate their lifestyle choices and adopt interventions to protect children from adverse outcomes.

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REVIEW

Preterm labor: One syndrome, many causes

Roberto Romero,[1,2,3]* **Sudhansu K. Dey,**[4] **Susan J. Fisher**[5]

Preterm birth is associated with 5 to 18% of pregnancies and is a leading cause of infant morbidity and mortality. Spontaneous preterm labor, a syndrome caused by multiple pathologic processes, leads to 70% of preterm births. The prevention and the treatment of preterm labor have been long-standing challenges. We summarize the current understanding of the mechanisms of disease implicated in this condition and review advances relevant to intra-amniotic infection, decidual senescence, and breakdown of maternal-fetal tolerance. The success of progestogen treatment to prevent preterm birth in a subset of patients at risk is a cause for optimism. Solving the mystery of preterm labor, which compromises the health of future generations, is a formidable scientific challenge worthy of investment.

P reterm birth, defined as birth before 37 weeks of gestation, affects 5 to 18% of pregnancies. It is the leading cause of neonatal death and the second cause of childhood death below the age of 5 years (*1*). About 15 million preterm neonates are born every year, and the highest rates occur in Africa and North America (*2*). Neonates born preterm are at an increased risk of short-term complications attributed to immaturity of multiple organ systems as well as neurodevelopmental disorders, such as cerebral palsy, intellectual disabilities, and vision and hearing impairments (*3*). Preterm birth is a leading cause of disability-adjusted life years [the number of years lost because of ill health, disability, or early death (*4*)], and the annual cost in the United States is at least $26.2 billion per year and climbing (*5*).

Two-thirds of preterm births occur after the spontaneous onset of labor, whereas the remainder is medically indicated because of maternal or fetal complications, such as preeclampsia or intrauterine growth restriction (*6*). Herein, we propose that preterm labor is a syndrome caused by multiple pathologic processes, summarize important strategies in the prevention of spontaneous preterm birth, and highlight promising areas for investigation.

Preterm labor: Not just labor before term

A tacit assumption underlying the study of parturition is that preterm labor is merely labor that starts too soon. In other words, the main difference between preterm and term labor is when labor begins. This is perhaps understandable given that both involve similar clinical events: increased uterine contractility, cervical dilatation, and rupture of the chorioamniotic membranes (*7*). These events represent the "common pathway" of labor. The current understanding of this process is that the switch of the myometrium from a quiescent to a contractile state is accompanied by a shift in signaling from anti-inflammatory to pro-inflammatory pathways, which include chemokines [interleukin-8 (IL-8)], cytokines (IL-1 and -6), and contraction-associated proteins (oxytocin receptor, connexin 43, prostaglandin receptors). Progesterone maintains uterine quiescence by repressing the expression of these genes. Increased expression of the microRNA-200 (miR-200) family near term can derepress contractile genes and promote progesterone catabolism (*8*). Cervical ripening in preparation for dilatation is mediated by changes in extracellular matrix proteins, which include a loss in collagen cross-linking, an increase in glycosaminoglycans, as well as changes in the epithelial barrier and immune surveillance properties (*9*). This decreases the tensile strength of the cervix, key for cervical dilatation. Decidual or membrane activation refers to the anatomical and biochemical events involved in withdrawal of decidual support for pregnancy, separation of the chorioamniotic membranes from the decidua, and eventually membrane rupture. Increased expression of inflammatory cytokines [tumor necrosis factor–α (TNF-α) and IL-1] and chemokines, increased activity of proteases [matrix metalloprotease 8 (MMP-8) and MMP-9], dissolution of extracellular matrix components such as fibronectin, and apoptosis have been implicated in this process (*10*, *11*) (Fig. 1).

In our view, the common pathway is activated physiologically in the case of labor at term, whereas several disease processes activate one or more of the components of the common pathway in the case of preterm labor. This conceptual framework has implications for the diagnosis, treatment,

[1]Perinatology Research Branch, Program for Perinatal Research and Obstetrics, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, Bethesda, MD, USA. [2]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA. [3]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI, USA. [4]Division of Reproductive Sciences, Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA. [5]Department of Obstetrics, Gynecology and Reproductive Sciences, Department of Anatomy, and Center for Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA.
*Corresponding author. E-mail: romeror@mail.nih.gov