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Prenatal Echoes: Stress Prior to Pregnancy Has Sex-Specific Effects on Fetal Health Outcomes

Did you know that frequent activation of the stress axis in response to unpredictable environmental changes establishes a persistent state of stress? This condition is associated with compromised reactions to subsequent stressors and an elevated risk of cardiometabolic and neuropsychiatric diseases. While stress is not unique to women, pregnancy, being a prevalent female-specific physiological hurdle, has the potential to exacerbate pre-existing health concerns. Despite well-documented impacts of maternal stress history on the health of offspring, there remains a lack of understanding regarding the biological mechanisms that elucidate how prior stress experiences get translated biologically and interact with pregnancy to influence long-term health consequences for mother and offspring.

In their paper, “Maternal preconception stress produces sex-specific effects at the maternal:fetal interface to impact offspring development and phenotypic outcomes,” published in *Biology of Reproduction* in 2023*,* Cisse et al. attempt to uncover the biological mechanisms behind how maternal stress prior to conception can influence pregnancy outcomes and offspring wellbeing, focusing on the placenta’s role. The placenta is a transient but highly specialized organ that develops during pregnancy. The placenta supports a fetus’s growth and development in utero by facilitating the transfer of nutrients and waste products between maternal and fetal circulatory systems. The placenta can communicate with the maternal environment by producing extracellular vesicles (EVs) (Figure 1). EVs are lipid-bound particles that cells produce to communicate via the transport of proteins, nucleic acids, and bioactive lipids. EV content changes depending on the metabolic and cellular state of the cells producing them. When analyzed through blood plasma, EVs can provide insight into biological signaling throughout the body. During pregnancy, the placenta takes over the majority of EV production that is found in maternal circulation. Analyzing the contents of EVs during pregnancy can provide insight into placental function and pathology. Specifically, analysis can highlight deficits in placental communication, leading to various pathologies for either mother or baby.

Additionally, when the pregnant mother’s internal environment changes in reaction to outside stressors, this can be communicated to the fetus through placental gene expression and nutrient flux. During pregnancy, gene expression in the placenta naturally fluctuates. As pregnancy advances, specific genes are expressed to meet the evolving needs of the developing baby. Different nutrients are transported across the placenta to ensure the baby receives what it needs to grow. Half-way through pregnancy, the fetal hypothalamus is undergoing a critical phase of development, which includes the growth and differentiation of neurons. At this point, the fetus relies solely on its mother for nutrients, so it is susceptible to changes in the maternal environment. Any disturbance in the mother’s environment, which can impact fetal hypothalamic growth, may result in adverse long-term health outcomes for the offspring. With this in mind, the researchers hypothesized that if the mother is exposed to stress before pregnancy, it would produce an unfavorable environment for fetal development due to differences in gene expression in the placenta.

Cisse et al. (2023) utilized female mice to explore their hypothesis. The mice were divided into the control and maternal preconception stress (MPS) groups. The MPS group experienced six weeks of diverse stressors, including tactile, auditory, and olfactory stimuli, followed by a period under standard conditions to distinguish immediately from lasting stress effects. Subsequently, researchers mated control and MPS female mice with experienced males. Upon confirming pregnancy, the mice were euthanized at approximately mid-gestation (12.5 days postconception), and various tissues, including the uterus, placenta, and fetal brain, were meticulously collected for further analysis.

To explore the enduring effects of past stress on the maternal environment, researchers examined extracellular vesicles (EVs) in non-pregnant female mice and during mid-gestation

pregnancy (E12.5). EVs were isolated from blood plasma and then characterized by measuring their size and concentration. The researchers also conducted proteomics, a study of the proteins within the EVs, by processing, measuring, and analyzing the samples. Analysis revealed that maternal preconception stress (MPS) did not impact the size of EVs, but pregnancy itself increased their protein concentration, regardless of prior stress experience. Further investigation into the protein content of the EVs showed distinct changes in MPS females, especially concerning cellular metabolism, glycolysis, lipid transport, and innate immunity. Interestingly, females with prior stress experience exhibited increased abundance in specific proteins, while pregnancy in control females led to changes in proteins associated with cell metabolic processes and immune functions. These results shed light on how stress history and pregnancy can influence the content of circulating EVs, potentially impacting various physiological processes.

To understand how stress history affects the body's metabolism, Cisse et al. (2023) looked at glucose tolerance—a measure of how well the body processes sugar. In non-pregnant females, stress did not impact glucose metabolism. However, during pregnancy, female mice in the MPS group had trouble clearing glucose, suggesting that stress can affect how the body handles sugar. Surprisingly, there were no differences in the weight of moms who experienced stress compared to those who did not. These results show that past stress experiences can influence how the body manages glucose levels during pregnancy, even if it does not affect overall body weight.

To better understand the changes happening at the connection between the mother and the developing baby, researchers examined the transcriptomes of different components: the placenta, the uterine tissue next to the placenta, and the fetal brain in control and MPS females. They identified patterns in groups of genes related to specific functions, giving a broader view than just looking at individual genes. This analysis showed that maternal preconception stress had a sex-specific impact on the placenta and uterine tissue but not on the fetal brain. In mothers carrying female offspring, there was an increase in genes related to cell-to-cell adhesion and immune regulation, while in mothers carrying male offspring, there was a decrease in genes related to lipid transport and steroid metabolic processes. Additionally, female offspring showed increased enrichment in genes related to insulin-like growth factor (IGF) transport and lipid metabolism, whereas male offspring displayed the opposite trend. When looking at the placenta-adjacent uterine tissue of the female offspring from MPS mothers, researchers found that genes related to nutrient transport and lipid metabolism were upregulated compared to controls.

On the other hand, in the uterine tissue of male offspring, genes linked to mitochondrial respiration and cell metabolism were decreased. Comparing the gene activity in the placenta and surrounding uterine tissue helps elucidate how stress before pregnancy can affect specific genetic processes, with notable differences between male and female offspring. Lastly, when looking at the transcriptome of fetal neuronal tissue, male and female offspring have similar gene regulation. In the female offspring of MPS mothers, genes related to neurotransmitters and ion transport were decreased compared to controls. Similarly, in male fetal brains from MPS pregnancies, gene sets linked to the development and growth of nerve cells were reduced compared to male offspring of control females.

After observing changes in gene activity and physiological responses during mid-gestation, researchers explored the long-term effects of MPS on the development and susceptibility of offspring to a metabolic challenge. The average weight of pups postnatally during the first three weeks did not vary based on maternal stress history. However, male and female MPS offspring displayed increased vulnerability to weight gain induced by a high-fat diet (HFD). Female MPS offspring on an HFD weighed more at ten weeks of age and consumed more food than female control offspring. Male MPS offspring, while showing a weight increase with HFD, did not differ in caloric intake compared to their control counterparts. These findings

suggest that maternal preconception stress may influence the long-term metabolic health of offspring in a sex-specific manner.

The study's experimental design incorporates various techniques like RNA sequencing, extracellular vesicle analysis, and glucose tolerance tests. This approach provides a thorough examination of the effects of maternal preconception stress. The inclusion of sex-specific analyses enhances the depth of understanding, acknowledging the potential for sex differences in responses to maternal preconception stress. Additionally, the study adopts a longitudinal perspective by examining mid-gestation effects and exploring the enduring impacts on offspring development. This provides valuable insight into the long-term consequences of maternal stress. Investigating various tissues, including the placenta, uterine tissue, fetal brain, and extracellular vesicles, offers a holistic view of the maternal-fetal interface and the systemic changes induced by preconception stress. Integrating transcriptomics with functional outcomes, such as glucose tolerance and offspring growth, also strengthens the study's ability to connect molecular changes with physiological consequences, enhancing the translational relevance of the findings. While this study offers valuable insights into the lasting effects of preconception stress, several aspects warrant consideration. The relatively small sample size of mice in each group may limit the study's statistical power and generalizability, urging caution in extending the findings to broader populations.

Furthermore, the study's reliance on a murine model, while instrumental for elucidating fundamental biological processes, prompts reflection on the direct applicability of results to human contexts, emphasizing the necessity for additional animal studies to validate translational implications. While the study extensively explores immediate and long-term impacts on offspring, an avenue for further exploration lies in investigating potential intergenerational effects and the transmission of stress-induced changes to subsequent generations, broadening the study's implications. Additionally, a more thorough discussion of potential epigenetic changes influencing observed outcomes would enhance the reader's ability to contextualize the findings within a broader framework. In summary, while the study contributes substantially to our understanding of maternal preconception stress and its lasting outcomes, addressing these considerations could strengthen the validity and applicability of its findings.

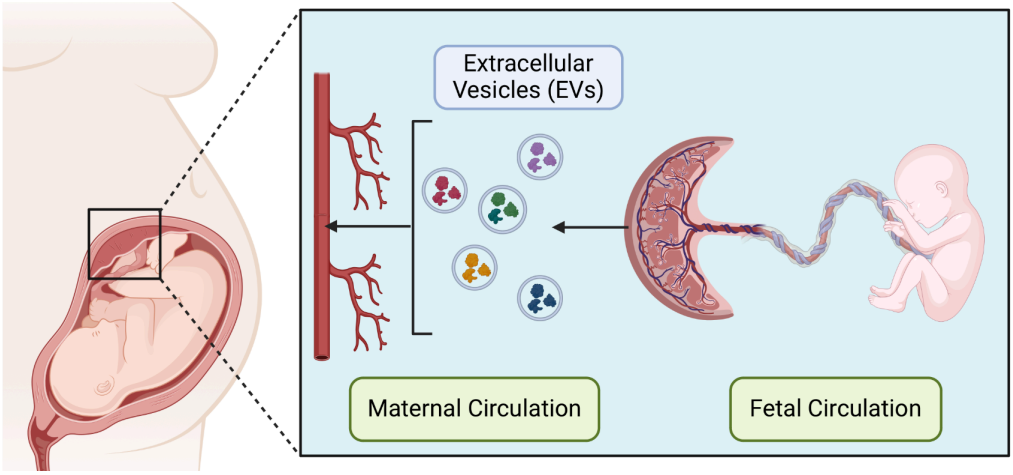

Figure 1. Placental Extracellular Vesicles Maternal-Fetal Communication. The human placenta, a unique anatomical structure, releases diverse extracellular vesicles (EVs) into maternal blood. These vesicles are vital carriers of proteins, lipids, and nucleic acids. The placenta consists of the syncytiotrophoblast, areas of syncytiotrophoblast denudation, and extravillous trophoblasts. These extravillous trophoblasts invade the uterine decidua, reaching the myometrium and enhancing the surface area for fetal-maternal interaction. This is where EVs that enter the maternal blood are produced and can influence maternal physiology.