HOW DOES THE PRENATAL STRESS OF WOMEN DURING PREGNANCY AFFECT IMMUNE RESPONSES WITHIN THEIR OFFSPRING

YOUREKA 2023, Faisal Altaie, Kartik Budihal, Rijwal Sangey, Tanmay Shah

Abstract

Stress is a pervasive experience for a considerable number of individuals, including expecting mothers. In fact, pregnancy can be an especially demanding period for women as they navigate the physical, emotional, and social transitions that come with nurturing a new life. Findings from research indicate that a significant percentage of pregnant women, ranging from 10-20%, experience depression symptoms during gestation, while up to 25% report anxiety symptoms (Catherine Lebel, Anna MacKinnon, Mercedes Bagshawe, Lianne Tomfohr-Madsen, and Gerald Giesbrecht). Alongside statistics, further investigating the impact of maternal stress on the offspring's immune response systems, this study employed a dataset of interleukin cytokine concentrations (pg/mL or pg/ug total protein) in the offspring of pregnant mice from an experiment conducted by the University of Iowa. In the experiment, mice were divided into two groups. Group 1 was exposed to varying degrees of thermal stress periodically, while group 2 was not subjected to any stress. Upon birthing, tissues were collected, and the cytokine concentrations were calculated in the following parts of the mother mice and offspring: maternal serum, forebrain, and placenta, across both groups. As the primary objective of the study was to investigate the relationship between mean cytokine concentrations and stress levels, six individual interleukin cytokines: IL-1, IL-2, IL-4, IL-6, IL-10, and IL-17 were used for evaluation. Using the R programming language, statistical analyses were utilized to compare mean cytokine concentrations in pregnant mice that were exposed to prenatal stress versus those that were not. Specifically, the following tests used were the Shapiro-Wilk normality test, the Levene's test, and the Wilcoxon signed-rank test. Based on the statistical analyses conducted, a

correlation was observed between the levels of stress and the mean cytokine concentrations. The analysis revealed significant differences in the cytokine concentrations between group 1 and group 2 for four out of the six interleukins examined. Our analysis revealed a significant relationship between the interleukin cytokines present in offspring and the prenatal stress experienced by their mother. Potential for our findings suggests that stress triggers the release of cortisol, which can suppress the production of white blood cells, leading to an increase in the activity of interleukin cytokines. Thus, our study provides valuable insight into how maternal prenatal stress can affect the cytokine concentrations in offspring and suggests that interventions to reduce maternal stress levels may have a positive impact on fetal development. In addition, the dataset used in this study was limited due to several factors, including the use of pregnant mice, a lack of separation between male and female offspring, and the testing of different sample types. Future studies should aim to use human subjects to provide more relevant information about the impact of maternal prenatal stress on cytokine levels in offspring. Such studies could yield valuable insights into how to mitigate the negative effects of prenatal stress on fetal development and improve overall human health.

Introduction

Despite its crucial role in physical health and response to environmental stressors, the immune system is often overlooked in the context of human psychology. However, it is vital to recognize that mental health plays a significant role in the physical well-being of pregnant women and their offspring, as they undergo numerous stressful and painful experiences. The immune system and mental health are very closely interconnected, with growing evidence suggesting that immune responses can play a significant role in the development and progression

of mental health disorders. Understanding the connection between the immune system and mental health can help further the development of novel treatment strategies for mental health disorders, such as anti-inflammatory medications, immunomodulatory therapies, or interventions targeting the gut microbiome. It also highlights the importance of considering both physical and mental health in a holistic manner, as the two are closely intertwined. According to the research by Smith (2019), there is evidence to suggest that the relationship between mental health and the immune system involves immuno-messengers and coordination. For instance, the immune protein cytokine is one of the factors affected in both women and offspring, highlighting the profound impact of mental health on the immune system.

Cytokines are a diverse group of small proteins that are involved in cell signaling. They are produced by various types of cells, including immune cells, and act as messengers between cells to regulate the immune response, inflammation, and other biological processes. Cytokines can have either pro-inflammatory or anti-inflammatory effects depending on the context in which they are produced and the receptors that they bind to. Cytokines, such as interleukins, play key roles in the immune system's response to infection. Dysregulation of cytokine production and signaling can contribute to the development of various diseases, including autoimmune disorders, chronic inflammation, and cancer. Interleukin cytokines, more specifically, act as chemical signals between white blood cells and contribute to the production of the immune response cells. As a result, cytokines are important targets for therapeutic intervention in many diseases.

High levels of stress during pregnancy can have a range of effects on offspring **(Table 2)**. Prenatal stress has been associated with alterations in brain development and behaviour in offspring, including increased anxiety, depression, and attention-deficit/hyperactivity disorder

(Vivette Glover, 2019). Epigenetic changes in the developing fetus due to prenatal stress can lead to altered gene expression and increased risk of health problems later in life (Barker, 2018; Entringer, 2019). Prenatal stress can also affect the immune system of the developing fetus, increasing the risk of allergies, asthma, and other immune-related disorders which can persist into adulthood. Here are a few types of immune differences that have been associated with prenatal stress: overall decreased immune ability and effectiveness, and the development of allergies. Moreover, prenatal stress has been linked to increased susceptibility to infections in offspring, potentially due to alterations in the developing immune system. Prenatal stress has also been linked to an increased risk of autoimmune disorders like type 1 diabetes and rheumatoid arthritis, due to alterations in the immune response (Christian, 2019; Reyes-Castro, 2019). It is important to note that the effects of prenatal stress on offspring can vary depending on a number of factors, including the timing, duration, and intensity of the stressor, as well as genetic and environmental factors. Additionally, many of these effects may not be apparent until later in life.

The primary objective of this study is to investigate the correlation between prenatal stress experienced by women during pregnancy and immune responses in their offspring. This research is crucial as there is a paucity of data on pregnancy and stress, highlighting the need for more research in this area. In today's rapidly changing world, with economic instability, and pandemic lockdowns, this study is trying to bring light to a topic that has been long neglected and ignored and try and implement a better understanding of how mental stressors can affect an individual's life before they take their first breath in the world. The data analysis from this study represents a meticulous and systematic approach to examining the impact of prenatal stress on

cytokine concentrations, and has significant implications for comprehending the intricate interplay between early life experiences, immune function, and health outcomes in pregnant women. Furthermore, cytokines that hold pivotal significance in organisms are listed in **Table 1,** and these cytokines are analyzed within this study

Therefore, we hypothesize that prenatal stress within pregnant women will significantly deregulate the concentrations of interleukin cytokines. This is justified through the countless affects that prenatal stress has on offspring, both physically and mentally. We believe that prenatal stress will cause hormonal imbalances such as increases in cortisol which will affect the immune system, therefore deregulating the concentrations of cytokines that are transferred over to offspring during pregnancy.

Methods

This study employed a dataset of interleukin cytokine concentrations (pg/mL or pg/ug total protein), in the offspring of pregnant mice from an experiment conducted by the University of Iowa. The experiment is as follows: mice were estranged into two groups, group 1 experienced degrees of thermal stress on a periodic basis, whereas group 2 didn't. During the initial stage of the experiment, the specimens in group 1 were subjected to varying degrees of thermal stress, which resulted in a range of physiological and body responses. Group 1 underwent repeated stress by confinement in a clear tube under bright light for 45 minutes on three separate occasions, with an additional exposure shortly before birthing. Tissues were collected across both groups, and cytokine concentration levels were calculated respectively upon birth of the offspring. This experiment emphasizes the concentration of several interleukin cytokines, IL-1, IL-2, IL-4, IL-6, IL-10, and IL-17, in the following parts of the mother mice and

offspring: maternal serum, forebrain, and placenta. The data collected during this phase was analyzed using a multivariate statistical comparison of mean cytokine concentrations in pregnant mice that were exposed to prenatal stress versus to those that were not. With the use of the computer language R, this study made correlations between the level of stress and the observed biochemical immune response changes within the mices' offsprings.

To ensure the validity of the statistical analysis in this study, meticulous examinations of the normality of each protein's distribution were carried out through the Shapiro-Wilk normality test. This test outputs a p-value that indicates the level of statistical significance of the deviation from normality. If the p-value is greater than the significance level (set to *0.05*), then the data can be assumed to be normally distributed. The homogeneity of variances was also thoroughly assessed using Levene's test to ensure that the variances across the mean groups were similar. In statistical analysis, variance is a measure of the spread or dispersion of a set of data points around the mean. Levene's test is a parametric test that returns the outcome of calculated variance in accordance with a p-value. Similar to the Shapiro-Wilk normality test, if the p-value is greater than the significance level (*set at 0.05*), then it can be assumed that the variances across the groups are similar.

As the data did not conform to normal distribution and equal variance across both mean concentration groups, the use of parametric tests such as an ANOVA is not appropriate due to the violation of assumptions. In this study, the Wilcoxon signed-rank test was utilized to compare the means of two related groups. This test calculates a test statistic based on the sum of ranks for positive and negative differences, and determines the p-value from the distribution of the test

statistic under the null hypothesis of no difference between paired samples. When the p-value is below the significance level of 0.05, it indicates sufficient evidence to reject the null hypothesis.

Results

Upon examining 78 pregnant mice (n = 78), each having varied cytokine concentrations, the average cytokine concentrations for the nonstressed and prenatally stressed groups are displayed in **Table 1,** with appropriate standard deviation for each.

Cytokine Type	Mean Cytokine Concentration For Nonstressed	Standard Deviation	Mean Cytokine Concentration For Prenatally Stressed	Standard Deviation
IL-1	1.216 (pg/mL)	0.7212366	2.08625 (pg/mL)	0.998083
IL-2	7.937692 (pg/mL)	4.319433	4.3775 (pg/mL)	1.707441
IL-4	79.698 (pg/mL)	12.59076	73.3325 (pg/mL)	6.165362
IL-6	5.98e-05 (pg/ug total protein)	1.55606e-05	5.22e-05 (pg/ug total protein)	5.40259e-06
IL-10	2.4325 (pg/mL)	1.702907	0.925 (pg/mL)	1.166726
IL-17	287.2375 (pg/mL)	102.0531	154.5825 (pg/mL)	9.65879

Table 1. *Referenced mean concentrations with standard deviation of cytokines for nonstressed and prenatally stressed mice groups.*

Cytokine Type	Shapiro-Wilk normality test (*p-value*, numeric)	Levene's test (*p-value*, numeric)	Wilcoxon signed-rank test (*p-value*, numeric)
IL-1	<0.05	<0.05	0.02297
IL-2	<0.05	<0.05	0.009066
IL-4	>0.05	<0.05	0.4127
IL-6	>0.05	<0.05	0.03805
IL-10	>0.05	<0.05	0.5333

Cytokine Type	Shapiro-Wilk normality test (*p-value*, numeric)	Levene's test (*p-value*, numeric)	Wilcoxon signed-rank test (*p-value*, numeric)
IL-17	<0.05	<0.05	0.02857

Table 2. *Referenced p-values for each individual cytokine with the appropriate statistical analysis: Shapiro-Wilk normality test, Levene's test, and Wilcoxon signed-rank test.*

Within Table 2, it is apparent that cytokine concentrations IL-1, IL-2, IL-6, and IL-17 exhibit statistically significant correlations with stress levels, as evidenced by their p-values falling below the designated threshold oflkj 0.05 in the Wilcoxon signed-rank test. Conversely, cytokine concentrations IL-4 and IL-10 exhibit statistically insignificant correlations with stress levels, as evidenced with p-values above the designated threshold. To further prove such correlations and visualize the data, boxplots were created using R for each individual cytokine concentration with respect to stress level. The following provide indication as to what the correlation is for each individual interleukin (**Figure 1**).

Discussion

The findings of the analysis revealed a relationship between the interleukin cytokines present in offspring and the prenatal stress experienced by their mother. By testing the concentrations of six interleukin cytokines in different offspring against mothers' stress levels, significant correlations were found for four interleukin cytokines, namely IL-1b, IL-2, IL-6, and IL-17. Hence, it can be concluded that a definite correlation exists between the two factors.

A possible reason for this result comes from the fact that with stress there is an increase in cortisol levels within the body and this reduces the production of white blood cells which are used to fight off viruses (Sunshine Clinic, 2022). This can cause interleukin cytokines to be more active in restoring the white blood cells (Cleveland Clinic, 2023) which can deviate the concentration levels of the interleukin cytokines which in turn affects the offspring.

These findings have great prevalence within the world today regarding pregnancy and the immune systems of the future generations. Within the workspace today there have been studies that show that women feel significantly more stressed due to their work, especially within women with family responsibilities such as pregnancy (iED Team, 2022). This suggests that new measures should be implemented in order to minimize the stress that pregnant women feel within the workspace as it directly affects their offspring. Furthermore, strong immune systems on a global or societal scale can prove many benefits such as preventing future epidemics and pandemics. This gives further importance to the necessity of preventing weak immune response systems within the future generations.

One of the limitations within our study was due to the subjects that were tested. The data that was studied had applied stress to pregnant mice, which could be problematic. Although there are many similarities to the reproductive systems of mice and humans, it doesn't provide a wholly accurate representation of the effects of stress within pregnant women. This is because although mice have similar biological systems to humans they also fail to mimic the phenotypes within humans, specifically within their inflammatory responses (Eric Torres, 2022). This makes it difficult to apply to humans due to the inconsistencies between humans and mice subjects. Furthermore, another limitation within our study was the lack of separation between male and female offspring. Within the dataset the cytokine concentrations of male and female offspring were split but there was not enough data within each one for them to be individually tested, therefore they were tested together. This is problematic due to the fact there are differences within the strength of inflammatory responses between males and females which would affect their concentrations of cytokine in relation to each other (Tiziana Ciarambino, Obretta Param, Mauro Giordano, 2021).

A further limitation within the study, comes from the sample types tested within the raw data. During data collection for the dataset the concentration of cytokines was measured from three different sites within the offspring or the mother: the forebrain, the maternal serum, and the placenta. Each cytokine didn't have a consistent amount of data within each sample type therefore different sample type concentrations were used for each cytokine. This makes it difficult to compare the results of each cytokine to each other as they were tested using different biological components.

Further studies could be conducted to strengthen the findings of our study. An alternative approach to better identify correlations in immune response in humans could be to conduct epidemiological studies or clinical trials. These studies can involve collecting data from human subjects with a variety of medical conditions or backgrounds, and analyzing their immune response using various methods such as blood tests, immune cell phenotyping, and cytokine profiling. By comparing the immune response of individuals with different conditions or exposures, researchers can identify potential correlations between immune function and factors such as stress, diet, and environmental toxins. This approach can provide more relevant and applicable information to human health than studying mice, although it also involves more ethical considerations and practical challenges. Another study that could be conducted is one that further analyzes common causes for stress within pregnant women which can propose solutions to the root problem that affect pregnant women offspring.

Conclusively, this study found evidence of a significant correlation between prenatal stress and changes in brain development and behavior in offspring. In our daily lives, external stressors such as financial troubles, emotional injury, and a more recent example of a global pandemic and health issues show that stress is a factor that will continuously harm individuals.

Further research is necessary to understand the underlying mechanisms and develop interventions to reduce prenatal stress and maintain healthy levels of cytokines in offspring.

Reference

sBenchSci. (2022, February 4). Mammalian Model Organisms: Contributions and Limitations. Retrieved February 6, 2023, from https://blog.benchsci.com/mammalian-model-organisms-contributions-and-limitations#:~:text=However%2C%20despite%20their%20genetic%20similarity,accurately%20mimic%20human%20disease%20phenotypes.

Christian, L. M. (2019). Psychoneuroimmunology in pregnancy: Immune pathways linking stress with maternal health, adverse birth outcomes, and fetal development. Neuroscience & Biobehavioral Reviews, 107, 96-106. Retrieved February 27, 2023

Cleveland Clinic. (2022). Cytokines. Retrieved February 12, 2023, from https://my.clevelandclinic.org/health/body/24585-cytokines#:~:text=For%20example%2C%20 cytokines%20can%20i tell,blood%20files%20to%20fight%20infection.

Dantzer, R., & Walker, A. K. (2014). Is there a role for glutamate-mediated excitotoxicity in inflammation-induced depression? Journal of Neural Transmission, 121(8), 925-932. Retrieved March 16, 2023, from https://doi.org/10.1007/s00702-014-1238-8 Berk, M., Williams, L. J., Jacka, F. N., O'Neil, A.

Entringer, S., Buss, C., & Wadhwa, P. D. (2019). Prenatal stress and developmental programming of human health and disease risk: Concepts and integration of empirical findings. Current Opinion in Endocrine and Metabolic Research, 7, 6-15. Retrieved February 28, 2023

Gumusoglu, Banu; Maurer, Sara; Stevens, Hanna (2022), "Maternal prenatal restraint stress effects on immune factors in mice", Mendeley Data, V3, doi: 10.17632/5tfj857sb2.3. Retrieved February 22, 2023

Institute of Entrepreneurship Development. (2022, January 11). Career Women and Stress: Why Women Feel More Pressure at Work. Retrieved February 19, 2023, from https://ied.eu/blog/career-women-and-stress-why-women-feel-more-pressure-at-work/.

Li, Y., Chen, M., & Chen, Z. (2021). COVID-19 Susceptibility and Severity Disparity between the Sexes: Examining the Role of Sex Hormones. Frontiers in Public Health, 9, 758803. Retrieved February 8, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8188967/#:~:text=Females%20are%20less%20susceptible%20to,molecules%20to%20reduce%20systemic%20inflammation.

NCI Dictionary of Cancer terms. National Cancer Institute. (n.d.). Retrieved March 3, 2023, from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cytokine

Pasco, J. A., Moylan, S., ... & Maes, M. (2013). So depression is an inflammatory disease, but where does the inflammation come from? BMC Medicine, 11(1), 200. Retrieved February 6, 2023, from https://doi.org/10.1186/1741-7015-11-200

Reyes-Castro, L. A., Rodriguez-Gonzalez, G. L., & Bautista, C. J. (2019). Maternal stress and its association with immunity and inflammation in the offspring: Implications for prenatal programming of chronic diseases. In Prenatal Programming of Neurodevelopment (pp. 77-96). Academic Press. Retrieved March 16, 2023

Sunshine Clinic. (2022, January 6). How Stress Affects Your Immune System. Retrieved March 6, 2023, from https://www.sunshineclinic.org/blog/how-stress-affects-your-immune-system/#:~:text=Many%20factors%20contribute%20to%20stress,normal%20white%20blood%20cell%20communication.

Appendix

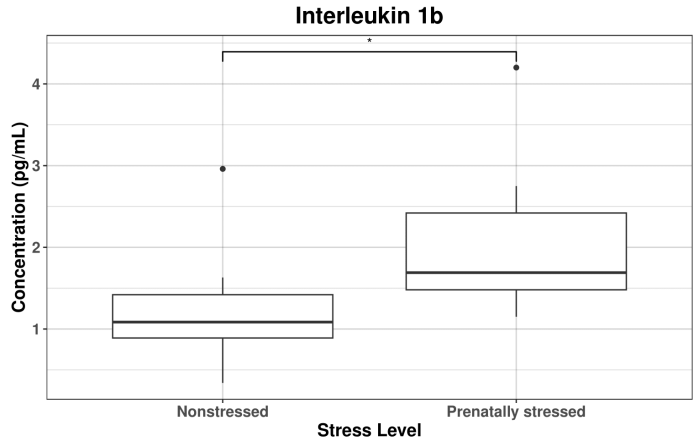
Protein Name	Description of Protein
IL-2	Interleukin-2, it is a type of cytokine, which is a protein that plays an important role in the immune system. It is produced by T cells, a type of white blood cell, and is involved in the regulation of the growth and activation of immune cells. Individuals with IL-2 deficiency can have impaired T cell function, which can result in an increased susceptibility to infections and an inability to mount effective immune responses against certain pathogens.
IL-4	Interleukin-4, is a cytokine that plays an important role in the immune system. It is produced by activated T cells, mast cells, and basophils, and is involved in the regulation of immune responses, particularly those related to allergies and parasitic infections. IL-4 deficiency can result in impaired immune responses against parasitic infections and allergies, as well as decreased production of certain types of antibodies.
IL-6	Interleukin-6, is a cytokine that plays an important role in the immune system and inflammation. It is produced by a variety of cells, including T cells, B cells, macrophages, and fibroblasts, and it is often used in response to infection, injury, and stress. IL-6 deficiency can lead to impaired immune responses against infections and an increased susceptibility to certain types of bacterial infections.
IL-10	Interleukin-10, is a cytokine that plays an important role in regulating the immune system and inflammation. It is produced by a variety of immune cells, including T cells, B cells, macrophages, and dendritic cells, in response to infection or other stimuli. IL-10 deficiency can result in an increased risk of autoimmune disorders and chronic inflammation
IL-17	Interleukin-17, is a cytokine that plays an important role in the immune system and inflammation. It is produced by a variety of immune cells, including T cells, as well as some other non-immune cells. It is involved in the recruitment and activation of immune cells, such as neutrophils, to sites of infection or injury. IL-17 deficiency can lead to an increased susceptibility to certain types of fungal infections, as well as decreased immune responses against certain types of bacterial infections.

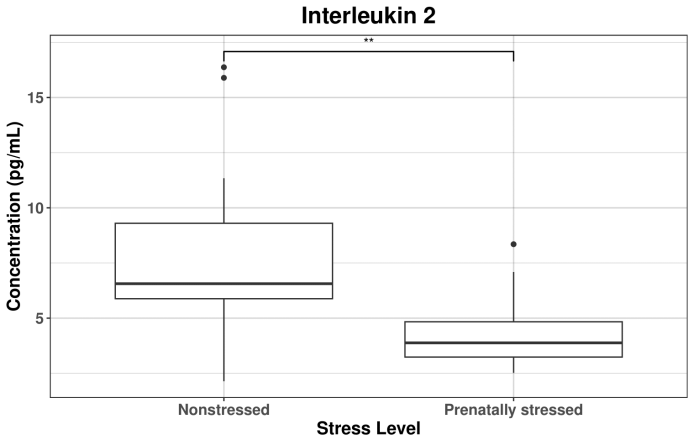
Supplementary Table 1. Referenced Cytokines Utilized And Analyzed In This Study, With Given Description And Roles Within An Organism.

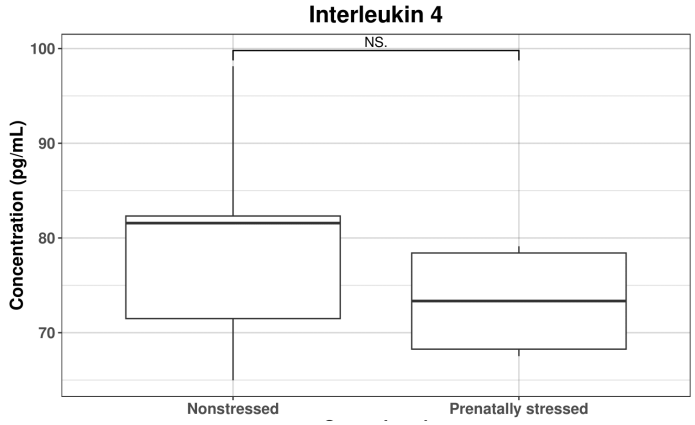
Note: IL is short for Interleukin

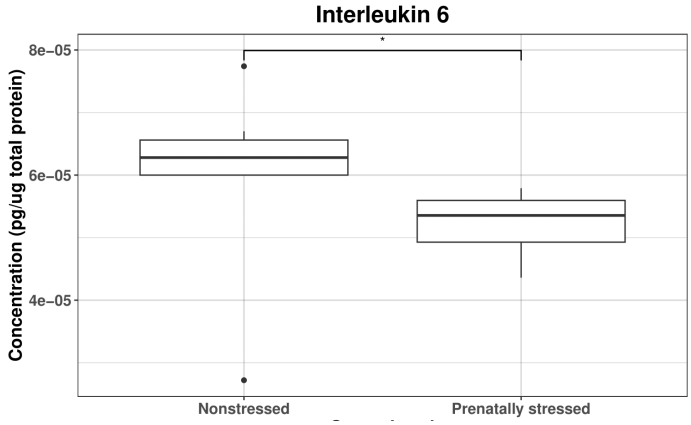
Effects	Description Of Deficiency Due To Prenatal Stress
Maternal Malnutrition	Maternal malnutrition during pregnancy can lead to a reduction in the number of immune cells in the developing fetus, as well as impaired immune function in the offspring. This can increase the risk of infections and other immune-related disorders in the offspring.
Maternal infections	Maternal infections during pregnancy can have direct effects on the developing fetus, as well as indirect effects on the immune system of the offspring. Certain infections can cross the placenta and infect the fetus, leading to congenital infections and immune deficiencies. Additionally, maternal infections can lead to the activation of the maternal immune system, which can affect fetal immune development and lead to immune dysregulation in the offspring.
Maternal stress	Maternal stress during pregnancy can lead to altered immune function in the offspring, including impaired T cell and natural killer cell function. This can increase the risk of infections and other immune-related disorders in the offspring.
Maternal smoking	Maternal smoking during pregnancy can lead to reduced immune function in the offspring, including impaired T cell and natural killer cell function. This can increase the risk of infections and other immune-related disorders in the offspring.
Maternal medication use	Medications taken by pregnant women can have immunosuppressive effects on the developing fetus, leading to immune deficiencies or dysregulation in the offspring.

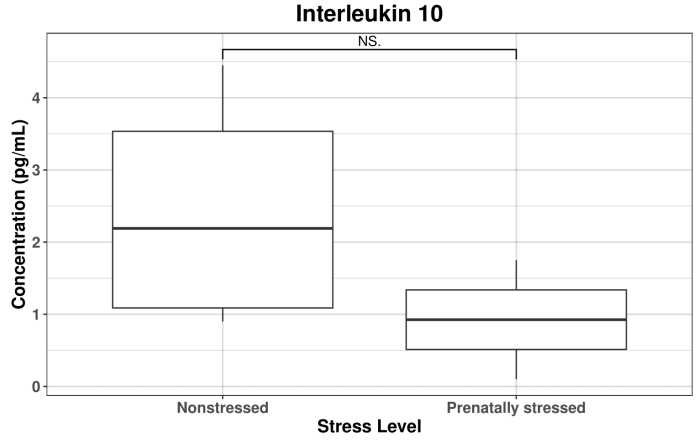
Supplementary Table 2. *Specific effects of prenatal stress during pregnancy that can cause deficiencies in offspring immunity.*

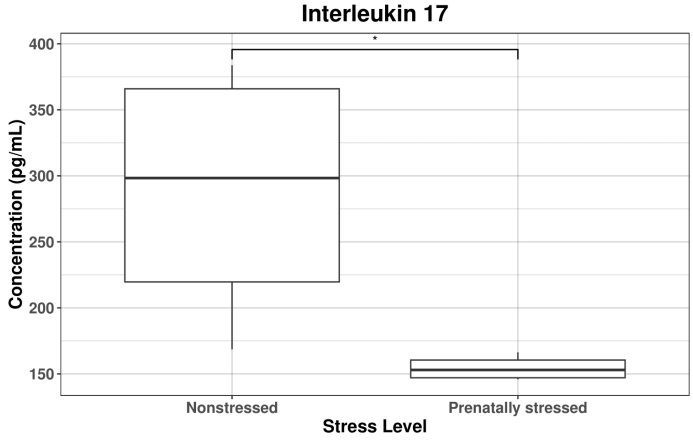

Interleukin 1b
*
Concentration (pg/mL)
4
3
2
1
Nonstressed
Prenatally stressed
Stress Level


Interleukin 2
**
Concentration (pg/mL)
15
10
5
Nonstressed
Prenatally stressed
Stress Level


Interleukin 4
NS.
Concentration (pg/mL)
100
90
80
70
Nonstressed
Prenatally stressed


Interleukin 6
*
Concentration (pg/ug total protein)
8e−05
6e−05
4e−05
Nonstressed
Prenatally stressed


Interleukin 10
NS.
Concentration (pg/mL)
4
3
2
1
0
Nonstressed
Prenatally stressed
Stress Level


Interleukin 17
*
Concentration (pg/mL)
400
350
300
250
200
150
Nonstressed
Prenatally stressed
Stress Level

Supplementary Figure 1. Boxplots showcasing comparison of concentrations for six individual interleukin cytokines with respect to stress levels.

Note. * = *0.01 < p <0.05,* ** = *p<0.01, NS. = insignificant*