## Mesovarial Morphological Changes in Offspring Exposed to Maternal Cold Stress

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Abstract: Introduction: Prenatal stress has been linked to heightened allergy sensitivity in offspring. However, there is a notable absence of research on the mesovarium structure of offspring born from mothers subjected to cold stress during pregnancy. Understanding the impact of maternal cold stress on the mesovarium structure could provide valuable insights into reproductive health outcomes in offspring. Objective: This study aims to investigate structural changes in the mesovarium of offspring born from cold-stress-affected rats. Material and Methods: 20 female Westar rats weighing around 200g were chosen and evenly divided into four containers; then, 2-3 male rats were introduced to each container. The Papanicolaou method was used to estimate the spermatozoa and estrus period from vaginal swabs taken from female rats at 8:00 a.m. Female rats examined with the presence of spermatozoa during the estrous phase of the estrous cycle are defined as pregnant. Pregnant rats are divided into experimental and control groups. The experimental group was stressed using the model of severe and chronic cold stress for 30 days. They were exposed to cold stress for 3 hours each morning between 8:00 to 11:00 o'clock at a temperature of minus 15 degrees Celsius. The control group was kept under normal laboratory conditions. Newborn female rats from both experimental and control groups were selected. At 2 months of age, rats were euthanized by decapitation, and their mesovaria were collected. Tissues were fixed in 4% formalin, embedded in paraffin, and sectioned into 5µm thick slices. The sections were stained with H&E and digitized by digital microscope. The area of brown fat and inflammatory infiltrations were quantified using Image I software. The blood cortisol levels were measured using ELISA. Data are expressed as the mean ± standard error of the mean (SEM). The Mann-Whitney test was used to compare the two groups. All analyses were performed using Prism (GraphPad Software). A p-value of < 0.05 was considered statistically significant. Result: Offspring born from stressed mothers exhibited significant physiological differences compared to the control group. Specifically, the body weight of offspring from stressed mothers was significantly lower than the control group (p=0.0002). Conversely, the cortisol level in offspring from stressed mothers was significantly higher (p=0.0446). Offspring born from stressed mothers showed a statistically significant increase in brown fat area compared to the control group (p=0.01). Additionally, offspring from stressed mothers had a significantly higher number of inflammatory infiltrates in their mesovarium compared to the control group (p<0.047). These results indicate the profound impact of maternal stress on offspring physiology, affecting body weight, stress hormone levels, metabolic characteristics, and inflammatory responses. Conclusion: Exposure to cold stress during pregnancy has significant repercussions on offspring physiology. Our findings demonstrate that cold stress exposure leads to increased blood cortisol levels, brown fat accumulation, and inflammatory cell infiltration in offspring. These results underscore the profound impact of maternal stress on offspring health and highlight the importance of mitigating environmental stressors during pregnancy to promote optimal offspring outcomes.

Keywords: brown fat, cold stress during pregnancy, inflammation, mesovarium

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