MORPHOLOGICAL AND HISTOGENETIC REMODELING OF THE SPLEEN IN POSTNATAL OFFSPRING BORN TO MOTHERS SUBJECTED TO CHRONIC EXPERIMENTAL STRESS

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Abstract

Background: Maternal exposure to chronic stress during pregnancy and lactation represents a critical epigenetic and neuroendocrine factor capable of altering fetal and postnatal organogenesis. Among immune organs, the spleen demonstrates high sensitivity to glucocorticoid-mediated stress responses due to its complex vascular architecture and lymphoid composition.

Objective: This study aimed to investigate morphological and histological changes in the spleen of offspring born to mothers subjected to chronic experimental stress during prenatal and early postnatal periods.

Materials and Methods: A total of 120 white laboratory rats were allocated into control and stress-exposed groups. Chronic stress was induced using a labyrinth-based behavioral stress model throughout gestation and lactation. Offspring spleens were examined on postnatal days 3, 7, 14, 21, and 30 using organometric measurements, histological staining (H&E and Van Gieson), and quantitative morphometric analysis. Statistical evaluation was performed using parametric methods.

Results: Offspring from stressed mothers exhibited significant retardation of spleen growth, reduction in white pulp volume, disorganization of periarteriolar lymphoid sheaths, decreased lymphoid follicle diameter, thickening of trabeculae, increased collagen deposition, and vascular wall hypertrophy ($p < 0.05$). These alterations were most pronounced during early postnatal ontogenesis and persisted through later developmental stages.

Conclusion: Maternal stress induces persistent postnatal morphological changes in the spleen of offspring, potentially impairing immune competence. These findings highlight the importance of prenatal environmental factors in shaping immune organ development.

Keywords: Maternal stress, spleen morphology, postnatal development, immune system, experimental rats

1.INTRODUCTION

Stress is defined as a universal nonspecific neuroendocrine response of the organism to adverse stimuli that threaten homeostasis. The concept of stress was first systematically formulated by Hans Selye, who described it as a general adaptation syndrome consisting of three stages: alarm reaction, resistance, and exhaustion. Prolonged activation of stress mechanisms, particularly the hypothalamic–pituitary–adrenal (HPA) axis, results in sustained elevation of glucocorticoids, which exert profound effects on immune organs.

The spleen is the largest peripheral lymphoid organ and performs essential immunological, hematopoietic, and filtration functions. It is structurally divided into white pulp (lymphoid tissue responsible for immune responses) and red pulp (involved in blood filtration and erythrocyte turnover). Due to its rich vascular supply and abundance of immune cells, the spleen is highly susceptible to hormonal and metabolic disturbances induced by stress.

Paul Ehrlich's pioneering studies established the spleen as a central organ of immune regulation, emphasizing its role in lymphocyte differentiation and antigen recognition. Subsequent experimental studies have demonstrated that glucocorticoids suppress lymphopoiesis, induce lymphoid tissue involution, and alter vascular permeability within the spleen.

Maternal stress during pregnancy has been identified as a critical factor influencing fetal programming, leading to long-term structural and functional changes in offspring organs. Excessive maternal glucocorticoids can cross the placental barrier and disrupt normal organogenesis, particularly during critical windows of immune system development. However, despite extensive research on stress physiology, detailed morphological and histogenetic studies focusing on postnatal spleen development remain insufficient.

Therefore, the present study aims to provide a comprehensive morphological characterization of spleen development in offspring born to mothers subjected to chronic experimental stress.

2.MATERIALS AND METHODS

2.1 Experimental Animals and Ethical Considerations

The study was conducted on 120 white laboratory rats (*Rattus norvegicus*), bred and maintained under standard vivarium conditions (12-hour light/dark cycle, controlled temperature, free access to food and water). All experimental procedures complied with international guidelines for the care and use of laboratory animals and were approved by the institutional ethics committee.

2.2 Experimental Design and Group Allocation

Animals were randomly divided into two groups:

- **Control group (n = 60):** Pregnant females received daily intragastric administration of 1.0 ml physiological saline using a subclavian catheter.
- **Stress group (n = 60):** Pregnant females were subjected to chronic experimental stress using a specially designed labyrinth cage that induced continuous psycho-emotional and behavioral stress.

Stress exposure was initiated after confirmation of pregnancy and continued throughout gestation and the lactation period.

2.3 Postnatal Sampling Protocol

Offspring from both groups were euthanized under ether anesthesia on postnatal days 3, 7, 14, 21, and 30, corresponding to critical stages of postnatal immune organ maturation.

2.4 Organometric Assessment

Following laparotomy, the spleen was carefully isolated and examined. The following parameters were recorded:

- Absolute spleen weight (g)
- Relative spleen weight (% of body weight)
- Length, width, and thickness (mm)

Measurements were performed using a digital caliper and an electronic analytical balance with high precision.

2.5 Histological Processing

Spleen tissue samples were fixed in 10% neutral buffered formalin, dehydrated in graded alcohol series, cleared in xylene, and embedded in paraffin. Histological sections of 8–10 µm thickness were prepared using a rotary microtome.

2.6 Staining Methods

- **Hematoxylin and Eosin (H&E):** General histoarchitectural evaluation
- **Van Gieson staining:** Visualization of collagen fibers and connective tissue components

2.7 Morphometric Analysis

Quantitative morphometric analysis was performed using digital microscopy and image analysis software. The following parameters were assessed:

- Percentage area of white and red pulp
- Diameter and density of lymphoid follicles
- Thickness of trabeculae
- Thickness of arterial walls (a. lienalis and trabecular arteries)

2.8 Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical significance was determined using Student's *t*-test. Differences were considered significant at $p < 0.05$.

3. RESULTS

3.1 Organometric Characteristics

Offspring born to stressed mothers demonstrated a statistically significant reduction in absolute and relative spleen weight across all postnatal periods studied. Growth retardation was particularly evident during early postnatal stages.

Table 1. Organometric parameters of the spleen (Postnatal day 21)

Parameter	Control	Stress
Absolute spleen weight (g)	0.48 ± 0.03	0.39 ± 0.02*
Relative spleen weight (%)	0.74 ± 0.04	0.61 ± 0.03*
Length (mm)	18.2 ± 0.7	15.9 ± 0.6*

*p < 0.05

3.2 Histological Findings

In control animals, the spleen exhibited well-differentiated white and red pulp, with clearly delineated periarteriolar lymphoid sheaths and mature lymphoid follicles.

Conversely, offspring from stressed mothers demonstrated:

- Marked reduction in white pulp volume
- Poorly developed periarteriolar lymphoid sheaths
- Decreased follicular cellularity
- Expansion of connective tissue elements within trabeculae
- Signs of vascular wall hypertrophy

3.3 Morphometric Alterations

Table 2. Morphometric parameters of the spleen (Postnatal day 30)

Parameter	Control	Stress
White pulp area (%)	42.6 ± 2.1	31.4 ± 1.8*
Lymphoid follicle diameter (μm)	215 ± 12	168 ± 10*
Trabecular thickness (μm)	42 ± 3	58 ± 4*
Arterial wall thickness (μm)	18 ± 2	27 ± 3*

*$p < 0.05$

3.4 Microscopic Evaluation

Figure 1. Control spleen (H&E, ×200): normal histological organization with prominent lymphoid follicles.

Figure 2. Stress group spleen (H&E, ×200): reduced white pulp and disrupted follicular architecture.

Figure 3. Van Gieson staining (×400): increased collagen fiber deposition in trabeculae and arterial walls.

4. DISCUSSION

The present study demonstrates that chronic maternal stress exerts profound effects on postnatal spleen morphogenesis. Reduced development of white pulp and lymphoid follicles suggests suppression of lymphopoiesis, likely mediated by prolonged glucocorticoid exposure.

Vascular remodeling and increased connective tissue deposition indicate stress-induced alterations in stromal components and microcirculation. These changes may impair antigen presentation and immune responsiveness.

The observed morphological alterations support the concept of prenatal immune programming and align with Selye's general adaptation syndrome, particularly the exhaustion phase associated with immune suppression.

5. Conclusion

Chronic experimental stress during pregnancy and lactation leads to persistent morphofunctional alterations in the spleen of offspring. These changes may compromise immune system maturation and adaptive capacity, emphasizing the importance of stress prevention during pregnancy.

6. Practical and Scientific Significance

The findings expand current understanding of stress-induced immune organ remodeling and provide a morphological basis for future investigations into stress-related immune dysfunction.

Primary Research on Maternal Stress and Spleen Development

1. **Normuradov, A. D., Akhmedova, S. M., & Nishonxo‘jayeva, Z. D.** (2025). *Morphological changes in the spleen of offspring born to dams exposed to chronic stress during gestation. Modern Education and Development,* 37(1).
— Study reports significant changes in splenic architecture (white pulp atrophy, increased red pulp, reduced lymphoid follicle size) in offspring of chronically stressed mothers.

Related Studies on Spleen Morphology and Stress

2. **Effects of early and late adverse experiences on morpho-quantitative characteristics of the rat spleen subjected to chronic stress** (female Sprague-Dawley rats).
The study showed significant changes in spleen compartments (white and red pulp) and lymphatic structures in stressed animals, suggesting chronic stress alters immune organ morphology.

3.Spleen contributes to restraint stress-induced changes in blood leukocyte distribution (mouse model).
— Demonstrates how chronic restraint stress alters leukocyte distribution and implicates spleen involvement in immunological changes due to chronic stress.

4.**Social stress mobilizes hematopoietic stem cells and alters splenic hematopoiesis** (mice) — Shows psychosocial stress influences splenic hematopoietic activity, myelopoiesis, and the immune role of the spleen.

General References on Postnatal Spleen Morphology and Development

5. **Morphological indicators of lymphoid structures of the spleen in postnatal ontogenesis in rats** (white rats).

6. — Shows psychosocial stress influences splenic hematopoietic activity, myelopoiesis, and the immune role of the spleen.

General References on Postnatal Spleen Morphology and Development

7. **Morphological indicators of lymphoid structures of the spleen in postnatal ontogenesis in rats** (white rats).

8. · **Postnatal development of splenic white pulp in the golden hamster** (interpretive study on PALS development).
— Classic work on structural stages of white pulp formation during early postnatal life in rodents.

9. · **Morphological and functional changes in the spleen of mice offspring after maternal immune stimulation** (prenatal).
— Although focused on immune stimulation rather than stress per se, it documents how maternal interventions alter postnatal spleen morphology and immunological structures

Articles for Background on Development & Environmental Influences

10.Chronic extragenital maternal pathology (e.g., hepatitis) influences offspring spleen development (rat models).
— Shows maternal systemic disease affects spleen ontogeny in early postnatal periods,

supporting the concept that maternal physiological stressors remodel lymphoid organ development.

Morphological features of spleen development at different postnatal stages (white rats).

11.Offers morphometric details of normal ontogenetic patterns, good for contrast with stress-induced remodeling. · Normuradov AD, Akhmedova SM, Nishonxo‘jayeva ZD. Morphological changes in the spleen of offspring born to dams exposed to chronic stress during gestation. *Modern Education and Development*. 2025;37(1).

12. Silva R et al. Morpho-quantitative changes in spleen compartments after chronic stress in Sprague-Dawley rats. *Journal Name*. Year;Volume(Issue).

13. [Author(s)]. Spleen’s role in restraint stress-induced leukocyte distribution changes. *Journal Name*. Year;Volume(Issue).

14. [Author(s)]. Social stress and extramedullary hematopoiesis in spleen. *Journal Name*. Year;Volume(Issue