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EFFECT OF HYPOKINESIA ON LEUKOCYTE FORMULA PARAMETERS IN PREGNANT RABBITS

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Hypokinesia exerts a significant effect on the hematological system of the organism. The aim of this study was to investigate leukocyte counts and key leukocyte indices in female rabbits subjected to hypokinesia during different stages of pregnancy and in their newborn offspring. The results demonstrated that changes in leukocyte numbers and leukocyte indices further confirm the role of inflammatory components. Specifically, an 85% increase in total leukocytes reflected a pronounced leukocytosis. Alongside this leukocyte elevation, lymphocytes increased by 75%, monocytes by 76%, and granulocytes by 62%, indicating activation of various immune cell populations. Both absolute values and relative proportions of these cells were notable. The relative proportion of lymphocytes rose by 42%, whereas monocytes decreased by 47% and granulocytes by 67%, highlighting a shift in leukocyte fraction distribution favoring lymphocytes. In pregnant rabbits experiencing hypokinesia at different gestational stages, this factor negatively impacted offspring viability, leading to reduced survival rates. These findings underscore the importance and relevance of investigating prenatal hypokinesia.

Keywords: Hypokinesia, prenatal development, leukocyte formula, hematological parameters, rabbit model

INTRODUCTION

Pregnancy and lactation are physiological periods that result in increased metabolic demands [2].The negative impact of hypokinetic conditions on human health, metabolism, and the normal functioning of physiological processes has been confirmed by scientific studies conducted by specialists in the fields of medical and experimental physiology [1]. The effect of hypokinesia on the organism remains one of the critical issues in physiology. Restriction of physical activity leads to morpho-functional alterations in vital systems and affects cellular genetics [6]. Previous studies have shown that 16 weeks of hypokinesia induces energy deficits in myocardial and hepatic cells, accompanied by reduced ATP and glycogen levels. Evidence regarding prenatal hypokinesia is limited, particularly concerning its effect on blood parameters. Modern research priorities focus on studying the dynamics of blood indices as markers of normal postnatal development, enabling early detection of potential deficiencies [1]. One research direction involves investigating the influence of hypokinesia during prenatal development on fetal growth and resulting deviations. Studies conducted on laboratory animals have shown that hypokinesia may cause arrhythmia and other pathological changes in cardiograms [11]. Both prenatal and postnatal hypokinesia result in irreversible

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pathological alterations in body fluids, blood parameters, and behavioral responses, threatening normal growth and development. Hypokinesia has multifaceted effects on various organ systems and fundamentally influences the life activity of both animals and humans According to previous findings, pregnancy in rabbits is associated with physiological hematological adaptations, characterized by significant decreases in erythrocyte, leukocyte, and platelet counts during late gestation.[10] Pregnancy in rabbits is associated with significant changes in hematological parameters, including decreases in hemoglobin and hematocrit and variations in leukocyte subpopulations during gestation [5] . Pregnancy is characterized by major hemodynamic adaptations, including increases in blood volume, plasma volume, and cardiac output, which support maternal physiological adjustment to fetal demands[12] . Experimental studies on pregnant rabbits under hypokinetic conditions at different gestational stages have demonstrated negative effects on embryogenesis, including resorption of fertilized eggs or embryos, the birth of low-viability offspring, cannibalism, and neonatal death. [3]. The severity of hypokinesia-induced disturbances depends on the duration of exposure and the gestational stage. In our study, hypokinesia during different stages of pregnancy caused significant changes in maternal physiological indicators. The primary aim of this study was to evaluate the changes in the leukocyte formula before and after hypokinesia in pregnant rabbits and to determine its effects on gestational progression.

MATERIALS AND METHODS

The main aim of the study was to investigate changes in blood parameters of pregnant rabbits under prenatal hypokinesia conditions. Control group pregnant rabbits were maintained under normal feeding and free-movement conditions until offspring delivery. Experimental group pregnant rabbits were exposed to hypokinesia at the corresponding stages of prenatal development. In both control and experimental groups, peripheral blood parameters were examined and comparatively analyzed in maternal rabbits and their offspring (newborn and 30-day-old).

In rabbits, the gestation period lasts 28–30 days and is divided into embryonic, pre-fetal, and fetal stages. In each group, 8 female rabbits were used for each stage of prenatal development ($n = 8$ per stage, $n = 24$ per group, total $n = 48$ for the study). In the second series of experiments, blood samples were collected from 5-day-old newborn offspring obtained from both groups across embryonic, pre-fetal, and fetal stages, and the embryonic effects of hypokinesia were comparatively evaluated ($n = 24$ per group, total $n = 48$).

The study was based on the experimental hypokinesia model developed by E. A. Kovalenko and N. N. Gurovski , which involves housing animals in small-sized cages. The movement of animals was partially restricted according to their biometric characteristics; however, their feeding, water intake, and thermal conditions were fully ensured. The cages were designed according to the body dimensions of rabbits and measured 25 × 16 × 23 cm. These dimensions allowed the animals to remain in a sitting position while significantly restricting their free movement.

This model is considered appropriate for studying systemic physiological responses, as it allows controlled and reproducible restriction of movement without affecting feeding and housing conditions. Compared to forced immobilization or rigid restraint models, the cage-based hypokinesia model provides a more stable and long-term limitation of motor activity, making it particularly suitable for investigating chronic effects during pregnancy.

The animals were kept in a dry and heated room with natural and artificial lighting. Complete blood analysis was performed using a “Mindray BC-2800Vet” hematology analyzer. The results were expressed as mean ± standard deviation (Mean ± SD). Statistical analysis was performed using Microsoft Excel software. Student’s t-test and Fisher’s criterion were applied, and statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

In the initial series of our study, leukocyte formula parameters were investigated in rabbits that underwent the gestation period under normal conditions and in rabbits exposed to hypokinesia at different stages of gestation (embryonic, pre-fetal, and fetal).

The obtained results demonstrated a significant increase in leukocyte counts in the experimental animals compared to the control group. Specifically, in the control group, WBC was 4.2±0.336 (10^9/L), whereas in the experimental group, it reached 9.8±0.784 (10^9/L) ($p<0.001$), indicating that hypokinesia stimulates inflammatory processes in the organism. The absolute number of lymphocytes (LYM) in the control group was 0.7±0.056 (10^9/L), while in pregnant rabbits exposed to hypokinesia during the embryonic period, it increased to 2.0±0.16 (10^9/L) ($p<0.001$). The relative proportion of LYM was 17.9±1.432% in the control group and 20.3±1.624% in the experimental group. The absolute number of monocytes (MON) in control animals was 0.5±0.04 (10^9/L), whereas in the experimental group, it averaged 0.8±0.064 (10^9/L) ($p<0.01$). No statistically significant difference was observed in the relative percentage of monocytes; it was 8.3±0.664% in the control group and 8.7±0.696% in the experimental group. Although the absolute number of granulocytes (neutrophils [Neu], basophils [Baso], eosinophils [EO]) was significantly higher in the experimental group compared to the control group, no statistical difference was detected in their relative proportion. Specifically, the absolute granulocyte count (GRA) in control animals was 3.2±0.256 (10^9/L), while in the experimental group, it increased by 55% ($p<0.001$) to 7.0±0.056 (10^9/L). The relative levels were 72.1±5.768% in controls and 71.0±5.68% in the experimental group.

During the pre-fetal stage, WBC analysis also showed different results compared to the control group. In control animals, WBC averaged 4.73±0.378 (10^9/L), whereas in experimental animals, it was 9.94±0.79 (10^9/L) ($p<0.001$), indicating the presence of inflammatory and allergic responses in the organism. The absolute LYM count in control pregnant rabbits was 0.8±0.064 (10^9/L), while in those exposed to hypokinesia during the pre-fetal stage, it increased to 2.61±0.209 (10^9/L) ($p<0.001$). Similarly, LYM% differed between groups: 19.1625±1.533% in the control group and 24.35±1.948% ($p<0.05$) in the experimental group.

Although no statistically significant differences were observed in the absolute monocyte counts between control and experimental groups, notable changes were found in relative levels. MON in control pregnant rabbits was 0.4625±0.037 (10^9/L), while in the experimental group, it was 0.55±0.044 (10^9/L). Relative monocyte counts were 8.2875±0.663% in the control group, but decreased to 5.675±0.454% ($p<0.001$) in the experimental group. The absolute granulocyte count in control animals was 3.5875±0.287 (10^9/L), whereas in rabbits exposed to hypodynamic effects during the pre-fetal stage, it was 6.775±0.542 (10^9/L) ($p<0.001$). A minor difference in relative granulocyte percentages was noted: 75.4±6.032% in the control group and 69.975±5.598% in the experimental group, without statistical significance.

During the fetal stage, WBC and leukocyte formula analyses showed trends similar to those observed in the embryonic and pre-fetal stages. In control females, WBC was 4.725±0.378 (10^9/L), whereas in the experimental group, it sharply increased to 12.588±1.007 (10^9/L) ($p<0.001$). Statistically significant changes observed in leukocyte counts were also confirmed in the leukocyte formula analysis. Absolute counts of LYM and granulocytes increased under hypokinesia. In animals that underwent normal gestation, LYM averaged 0.8±0.064 (10^9/L), whereas in females directly exposed to hypokinesia during the fetal stage, it rose to 1.9125±0.153 (10^9/L) ($p<0.001$). Unlike other stages, a statistically significant change in the relative percentage of lymphocytes was observed: 19.163±1.533% in controls and 22.375±1.79% in the experimental group ($p<0.05$).

The absolute granulocyte count increased from 3.5875±0.287 (10^9/L) in the control group to 9.9125±0.793 (10^9/L) in the experimental group ($p<0.001$), although no statistical difference was found in relative granulocyte percentages: 75.4±6.032% in controls and 72.85±5.828% in the experimental group. In monocyte analysis, no significant differences in absolute counts were detected, but a notable decrease in relative counts was observed: 8.2875±0.663% in the control group versus 4.775±0.382% ($p<0.001$) in the experimental group.(Table 1).

Table 1. Leukocyte formula parameters in pregnant rabbits (mean ± SD, n = 48).

	Determined Indicators	Units	Embrional		Pre-fetal		Fetal	
			Control	experiment	Control	experiment	Control	experiment
1.	Leukocytes	10^9/l	4.2±0.336	9.8±0.784***	4.73±0.378	9.94±0.79***	4.725±0.378	12.588±1.007***
2.	Lymphocytes LYM	10^9/l	0.7±0.056	2.0±0.16***	0.8±0.064	2.61±0.209***	0.8±0.064	1.9125±0.153***
3.	Monocytes	10^9/l	0.5±0.04	0.8±0.064**	0.4625±0.037	0.55±0.044^{ns}	0.4625±0.037	0.4125±0.033^{ns}

4.	Granulocytes	10^9/l	3.2±0.256	7.0±0.056***	3.5875±0.287	6.775±0.542***	3.5875±0.287	9.9125±0.793***
5.	Lymphocytes LYM	%	17.9±1.432	20.3±1.624^{ns}	19.1625±1.533	24.35±1.948*	19.163±1.533	22.375±1.79*
6.	Granulocytes	%	72.1±5.768	71.0±5.68^{ns}	75.4±6.032	69.975±5.598^{ns}	75.4±6.032	72.85±5.828^{ns}
7.	Monocytes	%	8.3±0.664	8.7±0.696^{ns}	8.2875±0.663	5.675±0.454***	8.2875±0.663	4.775±0.382***

Note: Differences in mean values are significant when the P-value is <0.01 (**), <0.05 (*), <0.001 (***), ns- non-significant.

The obtained results demonstrated a significant increase in the absolute number of LYM, with an average rise of 75% ($p < 0.001$) compared to the control group. The relative proportion of lymphocytes also increased by 22% ($p < 0.001$). Although this increase confirms the elevation of the absolute lymphocyte count, the absence of a marked difference in relative values may be explained by the parallel increase in other leukocyte populations, which helps maintain relative balance.

Similarly, a 37% increase ($p < 0.01$) was observed in the absolute monocyte count, while no statistically significant changes were detected in their relative values. This finding suggests activation of monocytes in response to the inflammatory process and their participation in tissue repair through differentiation into macrophages.

In the experimental group, a 55% increase ($p < 0.001$) in granulocyte levels was observed. Considering the key role of granulocytes, particularly neutrophils, in acute inflammatory and stress responses, this increase indicates that hypokinesia stimulates a systemic inflammatory reaction. Elevated stress and cortisol levels may enhance granulopoiesis in the bone marrow, leading to an increased number of granulocytes in peripheral blood. Thus, the observed increases in monocytes and granulocytes indicate that hypokinesia is not only a physical restriction but also a strong stress factor affecting the immune–endocrine system.

In newborn offspring, differences in WBC counts and leukocyte formula parameters between the control and experimental groups were also observed during the embryonic period. WBC in offspring that developed under normal conditions was 4.7±0.376 (10^9/L), whereas in offspring exposed to hypokinesia during the embryonic period, it increased to 6.0625±0.485 (10^9/L) ($p<0.01$). Statistically significant changes were also observed in the absolute and relative counts of lymphocytes, monocytes, and granulocytes. Specifically, the absolute LYM count in control offspring was 0.7875±0.063 (10^9/L), while in experimental offspring, it increased markedly to 2.0625±0.165 (10^9/L) ($p<0.001$). Correspondingly, the relative proportion of lymphocytes was 19.1625±1.533% in the control group and 33.85±2.708% ($p<0.001$) in the experimental group.

Analysis of monocyte and granulocyte absolute counts showed similar trends between control and experimental groups. Monocyte percentage (Mon%) in control offspring was 8.2875±0.663%, while in the experimental group, it decreased to 6.6875±0.535% ($p<0.005$). Granulocyte percentage (GRA%) in control offspring averaged 75.4±6.032%, whereas in the experimental group, it significantly decreased to 59.4625±4.757% ($p<0.01$).

During the pre-fetal stage, WBC counts in newborns exposed to hypokinesia were similar to controls. However, significant differences were observed in the absolute and relative levels of LYM, MON, and GRA. The absolute LYM count in control newborns was 0.7875±0.063 (10^9/L), while in the experimental group, it increased to 2.125±0.17 (10^9/L) ($p<0.001$). The relative proportion of lymphocytes was even more pronounced: 19.1625±1.533% in controls versus 46.625±3.73% ($p<0.001$) in the experimental group. Absolute monocyte counts decreased from 0.4625±0.037 (10^9/L) in controls to 0.3125±0.025 (10^9/L) in the experimental group ($p<0.01$). Correspondingly, relative monocyte levels decreased from 8.2875±0.663% in controls to 7.05±0.564% ($p<0.05$) in the experimental group. The absolute granulocyte count in control offspring was 3.5875±0.287 (10^9/L), while in experimental newborns, it decreased to 2.1125±0.169 (10^9/L) ($p<0.01$). The relative granulocyte proportion during the pre-fetal stage also declined from 75.4±6.032% to 46.325±3.706% ($p<0.001$).

In the fetal stage, WBC analysis in newborns exposed to hypokinesia also showed significant differences compared to controls. In offspring that developed normally during embryogenesis, WBC was 4.7±0.376 (10^9/L), whereas in fetal-stage hypokinetic offspring, it increased to 9.8±0.784 (10^9/L) ($p<0.01$). Similarly, significant differences were observed in the absolute and relative lymphocyte counts. LYM in control offspring was 0.7875±0.063 (10^9/L), while in experimental offspring, it increased to 2.225±0.178 (10^9/L) ($p<0.001$). The relative proportion of LYM was 19.1625±1.533% in controls and increased to

22±1.76% (p<0.01) in experimental offspring. Monocyte relative counts decreased from 8.2875±0.663% in the control group to 4.775±0.382% (p<0.001) in the experimental group.

Leukocyte formula analysis also revealed changes in the absolute granulocyte count. In control offspring, absolute GRA was 3.5875±0.287 (10^9/L), while in experimental newborns, it increased to 9.725±0.778 (10^9/L) (p<0.001). In contrast, no statistically significant changes were observed in the relative proportion of granulocytes (Table 2).

Table 2. Leukocyte formula parameters in newborn rabbits with normal embryonic development and in those exposed to hypokinesia at different stages of embryonic development (mean ± SD, n = 48)

	Determined Indicators	**Units**	**Embrional**		**Pre-fetal**		**Fetal**	
			Control	**experiment**	**Control**	**experiment**	**Control**	**experiment**
1.	Leukocytes	10^9/l	4.7±0.376	6.0625±0.485 **	4.7±0.376	4.55±0.364^{ns}	4.7±0.376	9.8±0.784**
2.	Lymphocytes LYM	10^9/l	0.7875±0.063	2.0625±0.165 ***	0.7875±0.063	2.125±0.17***	0.7875±0.063	2.225±0.178***
3.	Monocytes	10^9/l	0.4625±0.037	0.4125±0.033^{ns}	0.4625±0.037	0.3125±0.025**	0.4625±0.037	0.4125±0.033^{ns}
4.	Granulocytes	10^9/l	3.5875±0.287	3.172±0.104^{ns}	3.5875±0.287	2.1125±0.169**	3.5875±0.287	9.725±0.778***
5.	Lymphocytes LYM	%	19.1625±1.533	33.85±2.708 ***	19.1625±1.533	46.625±3.73***	19.1625±1.533	22±1.76**
6.	Granulocytes	%	75.4±6.032	59.4625±4.757 **	75.4±6.032	46.325±3.706***	75.4±6.032	73.1625±5.853
7.	Monocytes	%	8.2875±0.663	6.6875±0.535 *	8.2875±0.663	7.05±0.564*	8.2875±0.663	4.775±0.382***

Note: Differences in mean values are significant when the P-value is <0.01 (**), <0.05 (*), <0.001 (***), ns- non-significant.

The changes in leukocyte parameters in newborn offspring under hypokinetic stress are particularly characterized by a significant increase in lymphocyte levels. Specifically, compared to the control group, a 62% increase in the absolute lymphocyte count was observed in offspring exposed to hypokinesia during the embryonic period. In addition, the relative proportion of lymphocytes increased by 44%.

This lymphocytosis indicates that maternal hypokinetic stress may influence the development of the fetal immune system and induce alterations in the early stages of adaptive immune responses. A decrease of approximately 20–22% was observed in the relative proportions of monocytes and granulocytes.

These changes may be explained by the pronounced increase in lymphocyte levels, which leads to a relative reduction in the proportion of other leukocyte populations and reflects a shift in the balance of the leukocyte formula.

CONCLUSION

These results are consistent with several recent studies. For instance, Burini et al. (2020) reported that prolonged immobility and hypokinesia significantly elevate total leukocyte levels, particularly the absolute counts of lymphocytes and monocytes [4]. Similar findings were observed by Dan Lin et al. (2023), who emphasized that hypodynamia triggers stress-induced activation in the immune system, accompanied by leukocytosis [9]. Furthermore, Shao et al. (2021) demonstrated that hypokinetic conditions in pregnant animals alter immune responses, with increases in lymphocyte and monocyte dynamics reflecting adaptive mechanisms [13].Kuriyama et al. (2022) similarly reported that immobilization induces alterations in leukocyte counts and other hematological parameters [8].

In summary, our experimental and observational results indicate that prolonged exposure to hypokinesia markedly affects vital physiological functions and induces significant alterations in the hematological system during both early postnatal development and later ontogenetic stages. The extent and nature of these changes depend on the type and duration of hypokinetic exposure, as well as the physiological status and developmental stage of the organism. Overall, prolonged hypokinesia leads to significant disturbances in morpho-functional and adaptive processes, which may persist and potentially reduce the organism's adaptive capacity and resistance to stress.

REFERENCES

[1] Ashirbekov, G.K.; Khodzhaev, N.K.; Arystanova, S.T.; et al. Metabolic disorders under the influence of occupational factors. Vestnik KazNMU. **2022**;(1):361–364. doi:10.53065/kaznmu.2022.73.41.055

[2] Bonelli, F.; Rota, A.; Corazza, D.; Serio, D.; Sgorbini, M. Hematological and biochemical findings in pregnant, postfoaling, and lactating jennies. Theriogenol. **2016**; 85(7):1233–1238. doi: 10.1016/j.theriogenology.**2015**.12.004

[3] Bojarski, B.; Lutnicka, H.; Swadiba-Karbowy, M.; Makulska, J.; Jakubiak, M.; Pawlak, K.; Tombarkiewicz B, Witeska M. Effects of herbicides pendimethalin and ethofumesate on common carp (Cyprinus carpio) erythrocyte morphology. Folia Biol. **2018**; 66(3):143–149. doi: 10.3409/fb_66-3.15.

[4] Burini, R.C.; Anderson, E.; Durstine, J.L.; Carson, J.A. Inflammation, physical activity, and chronic 3 disease: an evolutionary perspective. Sports Med Health Sci. **2020**; 2(1):1–6. doi:10.1016/j.smhs.2020.03.004

[5] Haneda, R.; Mizoguchi, Y.; Matsuoka, T.; Mizuguchi, H.; Endoh, T.; Fukud,a K.; Asano, Y. Changes in blood parameters in pregnant Japanese White rabbits. *J Toxicol Sci*. **2010**;35(5):773–778. doi: 10.2131/jts.35.773.

[6] Hamidova, J.; et al. The effect of hypokinesia on the reproductive outcomes of pregnant rabbits and the biometric parameters of their offspring. *Journal Nature &Science*. **2025**; 60:20-23. doi:10.36719/2707-1146/60/20-23

[7] Kuriyama, T.; Oishi, K.; Kakazu, H.; Machida, K. Changes of physiological functions in rats induced by immobilization stress. *J Physiol Sci*. **2022**;72(3):115–129. doi:10.1007/s12576-022-00890-3

[8] Lin, D.; Thompson, C.L.; Ba, D.M.; Muscat, J.E.; Zhou, S.; Rogers, C.J.; Sturgeon, K.M. Associations between leisure-time physical activity level and peripheral immune cell populations in the US general population: analysis of NHANES data, 1999–2018. Sports Med Open. **2023**;9:101. doi: 10.1186/s40798-023-00643-y

[9] Chmurska-Gąsowska, M.; Bojarski, B.; Szała L. Haematological changes in rabbits (Oryctolagus cuniculus f. domesticus) in the course of pregnancy. Anim Reprod. **2021**;18(3):e20210013. doi:10.1590/1984-3143-AR2021-0013.

[10] Maltseva, N.G.; Kuznetsova, T.G. Effect of hypokinesia on myocardium structure. Probl Health Ecol. **2007**:114.doi.org/10.51523/2708-6011.2008-5-2-25

[11] Nuwayhid, B. Hemodynamic changes during pregnancy in the rabbit. Am J Obstet Gynecol. **1979**;135(5):590–596. doi: 10.1016/S0002-9378(16)32982-9

[12] Shao, T.; Verma, H.K.; Pande, B.; Costanzo, V.; Cai, Y. Physical activity and nutritional influence on immune function: an important strategy to improve immunity and health status. Front Physiol. **2021**;12:751374. doi:10.3389/fphys.2021.751374