Original Article

Stress Alters Hematologic Indices in Pregnant Wistar Rats and Their Offspring: Pathologic Processes and Health Implications

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ABSTRACT

Background: Pregnancy is accompanied by substantial physiological and hematological adaptations that may be influenced by maternal stress. However, the hematological responses to varying types and intensities of stress during pregnancy, as well as the extent to which these maternal changes are reflected in the offspring, remain poorly understood.

Objectives: This study examined stress-induced alterations in hematologic indices in pregnant female Wistar rats and their offspring to unravel the pathological process involved in hematologic diseases.

Methods: Sixty (60) healthy female rats between 12 and 14 weeks old, weighing between 160 and 200 grams, were used for phase 1. At the end of each week, following exposure to the different stress model, the rats were euthanized and bled in the first, second, and third weeks, respectively, to estimate total white blood cell (TWBC), red blood cell (RBC), and platelet counts, packed cell volume (PCV), and hemoglobin (Hb) concentration. For the phase 2 of the experiment, twelve offspring were used, six from the control group of stressed parents and six from the intruder-stressed parents. Blood samples were collected from offspring of stressed parents 4 weeks after gestation to estimate hematologic indices. The study adopted restraint chamber, mirror chamber, and resident intruder stress models for stress induction.

Results: The different stressors studied caused a significant ($P<0.05$) reduction in TWBC and RBC counts, while PCV, Hb concentration and platelet counts increased. Apart from the TWBC count, where the value increased significantly ($P<0.05$), exposure to intruder stressor decreased PCV values and RBC and platelet counts. The mean values of the hematologic indices of the offspring of parents exposed to intruder stressor increased; however, the increase was significant ($P<0.05$) only with respect to PCV, Hb concentration, and platelet counts. Perhaps, in terms of the degree of intensity of the stressors, intruder stressors induced the highest intensity of stress in rats compared to the other stressors. The restraint stressor was the next, followed by mirror stressors.

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Conclusion: In conclusion, the effect of stress on hematologic indices varies during different trimesters of pregnancy and this dependent on the nature, intensity, and duration of the stressor applied. However, the effects of stress during pregnancy on the blood parameters of pregnant mothers cannot be transmitted to their infants, especially in the case of the intruder stressor studied.

Keywords: In vivo, Hematological indices, Offspring, Pregnancy, Stress

Introduction

Blood acts as a pathological indicator of the health status of humans exposed to toxicants and other possible conditions; as such, humans with good blood compositions are likely to show good performance and/or resistance to diseases (Fielder, 2012; Mmereole, 2008). Hematological indices are good indicators of the physiological state of animals and are related to blood and blood-forming organs (Bamishaiye et al., 2009; Khan & Zafar, 2005). Clinicians and physiologists are interested in hematologic research because they can better understand how blood properties relate to the environment (Ovuru & Ekweozor, 2004). Therefore, hematological indices could be useful for selecting humans who are genetically resistant to certain diseases under different environmental and/or stress conditions (Isaac et al., 2013). Stress is a phenomenon that denotes the general non-specific response of the human body to changes or any demands caused by pleasant or unpleasant stimuli, and it is also believed to contribute or trigger various disease conditions (Nwogueze et al., 2022). Stress is a compromising occasion that every living being experiences throughout life. The term 'stress' has various definitions in the previously reviewed literature; however, for this review, 'stress' is described as any threatening situation that provokes an anxious response—that is, the impact of an organism's experience and its response to it (Merwe, 2010). For a clearer understanding of 'stress', pioneering research by Walter Cannon and Hans Selye in 1929 emphasized that all organisms respond to changes within their environment (Cannon, 1929). They believed that this physiological response is responsible for the survival and maintenance of a relatively stable equilibrium among different physiological processes (homeostasis) and for the 'fight' or 'flight' response in living organisms (Shahsavarani et al., 2015).

Given the preceding information, stress was seen in a previous study as an erratic illustration of psychological evaluation, physiological responses, and social tendencies that arise in reaction to an apparent imbalance between the demands of the situation and the resources expected to meet them (Akter, 2008). Stress has obviously become part of our daily lives, especially in developing nations. Each living organism encounters one type of stress or another during its lifetime (Nwogueze et al., 2021). In reality, stress has been defined as an external stimulus or event that is inflicted on a person. Examples of external stressors include hunger and environmental stressors, such as natural disasters. Unusual and uncontrollable stress will generally have more impact than exposure to a similar stressor in an anticipated or controllable way (Nancy et al., 2013). The impact caused by stress on an organism can depend to a large extent on the stressor involved. There is a general impression that chronic stressors pose serious health challenges in life. One of the ways stress can interfere with health is by altering both the quantity and quality of white blood cells. In a report by Michael et al. (1999), it was indicated that academic examination stress induces changes in the distribution of peripheral blood mononuclear cells. Although stress may induce an increase in total white blood cell (TWBC) counts (National Heart, Lung, and Blood Institute, 2011), it causes the redistribution of different white blood cells, which enhances activation of the immune system.

Stress during pregnancy is a common feeling characterized by profound changes in almost every organ and system to meet the demands of the fetoplacental unit. Elevated stress levels have been associated with the pathological processes involved in hematologic diseases related to complications in reproductive success (Stanton et al., 1992). Hematologic indices during pregnancy reflect, to a large extent, the general health of the individual. Exposure to stressors within the environment leads to maladaptation and changes in various human pluripotent hematopeotic systems during pregnancy (Karamala et al., 2011). The purpose of this study was to clearly understand the ability of stressors of varying types to induce fear, anxiety, and physical stress in pregnant female Wistar rats and to determine whether this responsiveness is transmitted from parents to offspring hematological indices, especially in females and their offspring. Thus, the study provides a reliable basis for policy formulations about stress and peripheral blood cell counts in the circulation, which is one of the homeostatic components of the animal's internal environment. Therefore, this paper examined stress-induced alterations in hematologic indices among pregnant female Wistar rats and their offspring.

Materials and Methods

Materials

Some of the materials used were wire mesh cages, mirror chambers, restraint tubes, standard rat chaw and clean water, 5 mL syringes and needles, sample collection containers, resident intruder cages, laboratory coats, hand gloves, cotton wool, a sensitive digital weighing balance, a disserting kit, and hematology analyzers.

Study site

This experiment was carried out at the Animal House of the Department of Human Physiology under the supervision of the Faculty of Basic Medical Sciences (FBMS) in the College of Health Sciences (CHS) of Delta State University, Abraka, Nigeria.

Experimental protocol

The study design was experimental and invasive. Sixty healthy female Wistar rats between 12 and 14 weeks of age and weighing between 160 and 200 g were used for phase 1. Based on the result of a pilot study conducted in a previous study by Nwogueze et al. (2020b), the experimental rats were stressed for a period of 3 h per day and for a period of 1, 2, and 3 weeks in phase 1 of the study. Meanwhile, in the phase 2 study, twelve female offspring from the control pregnant rats and the intruder-stressed parents, respectively, were used based on the outcome of the phase 1. Variations in peripheral blood counts of the offspring were measured after 4 weeks of life. The offspring of intruder-stressed rats were considered for a period of 3 h per day for 3 weeks.

Animal handling and grouping

The animals were obtained from the Department of Animal and Cell Biology of Delta State University, Abraka, and transported in plastic cages to the Animal House of the Faculty of Basic Medical Sciences of the same institution. The animals were acclimatized for 2 weeks prior to the study and were allowed free access to rat chow and ad libitum water. They were kept in wire-mesh cages in the animal house under clean environmental conditions. The study covered a 14-week period. The experimental animals were randomly distributed into four groups of six rats (n=6) each, with each group composed of three subgroups, as described in Table 1, while the offspring of the control pregnant rats and the stressed intruder parents (n=6) are presented in Table 2.

Stress induction

For stress induction, the study adopted the following three different types of stress models: the restraint chamber test was used to induce physical stress; the mirror chamber test was used to induce anxiety stress; and the resident intruder test was used to induce psychosocial stress (i.e. the presence of cat in a separate compartment in the model), as described in a previous study (Nwogueze et al., 2021).

Mating arrangement and confirmation of pregnancy

Female rats were mated using a 1:1 combination ratio. The rats remained in the same cages (although not for more than 5 days) until pregnancy was established, as described by Nwogueze et al. (2020b). To confirm the

Table 1. Animal grouping (phase 1)

Groups	Description	No. of Rats
Group 1	Control pregnant Wistar rats	6
Group 2[a]	Pregnant Wistar rats exposed to varying types of stressor for 3 h for 1 week	6×3=18
Group 3[b]	Pregnant Wistar rats exposed to varying types of stressor for 3 h for 2 weeks	6×3=18
Group 4[c]	Pregnant Wistar rats exposed to varying types of stressor for 3 h for 3 weeks	6×3=18
	Total	60

[*]Male rats used only for mating, [a] Rats exposed to stress within the first trimester. [b]Rats exposed to stress.

Table 2. Animal grouping (phase 2)

Groups	Description	No. of Rats
Group 1	Offspring of control pregnant Wistar rats	6
Group 2	Offspring of intruder-stressed parents for 3 h for 3 weeks	6
	Total	12

Note: Blood samples were collected from offspring of stressed parents 4 weeks after gestation.

evidence of pregnancy, a vaginal smear was carefully performed on each of the mated females, and then the presence of spermatozoa was observed under the microscope (Nwogueze et al., 2021).

Sample collection

At the end of each week following exposure to the different stress models, the rats were euthanized and bled in the first, second and third weeks, respectively. Blood samples were collected by cardiac puncture into sterile EDTA containers to estimate hematologic indices.

Biochemical investigation

The blood collected from each rat was subjected to biochemical investigations for TWBC count, red blood cell (RBC) count, platelet counts, packed cell volume (PCV), and hemoglobin (Hb) concentration using blood cell counting devices (hematological analyzers).

Statistical analysis

Data collected from the study were statistically analyzed for Mean±SEM, while one-way analysis of variance (ANOVA) was used to compare means between groups using SPSS software, version 22. The least squares difference (LSD) was used for the post hoc test, and a P<0.05 was considered significant.

Results

Figure 1 shows the mean variation in TWBC counts of pregnant Wistar rats after weekly exposure to stressors of varying types at a rate of 3 h per day. The results obtained revealed that prolonged exposure to the same stressor for 2 weeks caused a significant decrease (P<0.05) in TWBC counts. However, further exposure of rats to the restraint stressor for 3 weeks inversely caused a significant increase (P<0.05) in TWBC counts compared to control levels. TWBC counts were elevated after exposure to the mirror stressor for 1, 2, and 3 weeks, respectively, compared to control levels. The observed

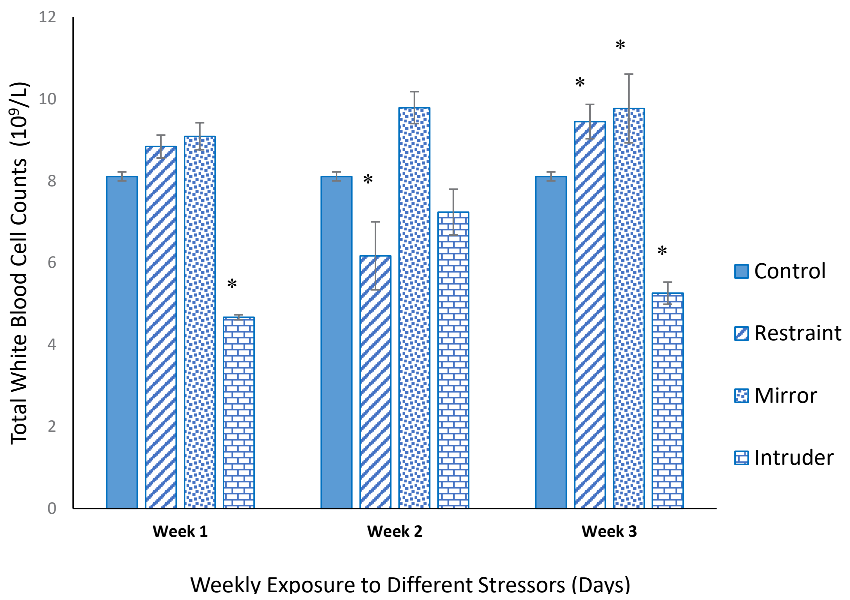

Figure 1. Changes in TWBC counts after exposure to different stressors for 3 h per day for 1, 2, and 3 weeks, respectively

*Significant difference (P<0.05) compared to the control group.

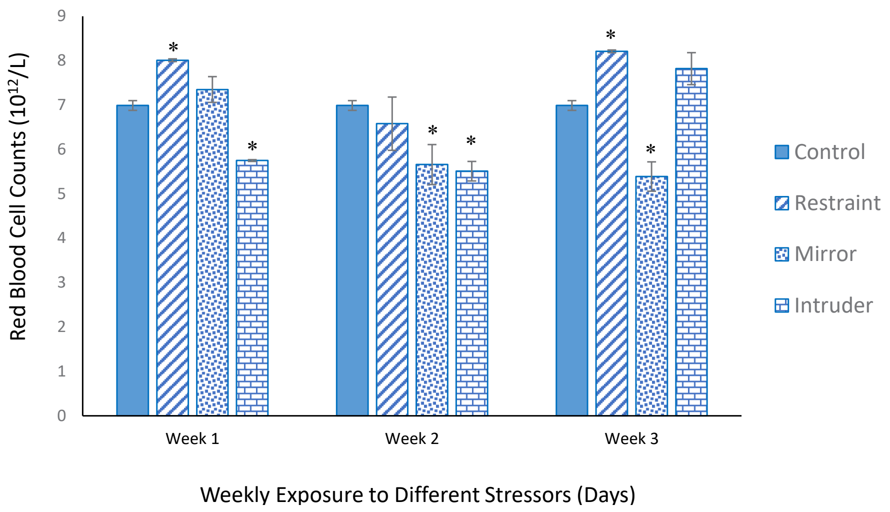

Figure 2. Changes in RBC after exposure to different stressors for 3 h per day for 1, 2, and 3 weeks, respectively

*Significant difference (P<0.05) compared to the control group.

increase was only significantly (P<0.05) different from the control levels in rats exposed for 3 weeks. Meanwhile, the application of an intruder stressor to pregnant Wistar rats for 1 and 3 weeks, respectively, significantly decreased (P<0.05) TWBC counts. However, exposure of rats to an intruder stressor for 2 weeks caused a significant increase (P<0.05) in TWBC counts compared to control levels.

Figure 2 shows the mean variation in the RBC counts of pregnant Wistar rats after weekly exposure to stressors of varying types at a rate of 3 h per day. RBC counts of pregnant Wistar rats increased significantly (P<0.05) after exposure to a restraint stressor for 1 and 3 weeks, respectively, compared to control levels. RBC counts were elevated after exposure to the mirror stressor for 1 week compared to control levels. However, a significant reduction (P<0.05) in RBC counts was observed in pregnant rats after prolonged exposure to the mirror stressors for 2 and 3 weeks, respectively, compared to control levels. Meanwhile, RBC counts of pregnant rats significantly reduced (P<0.05) after exposure to an intruder stressor for 1 and 2 weeks, respectively.

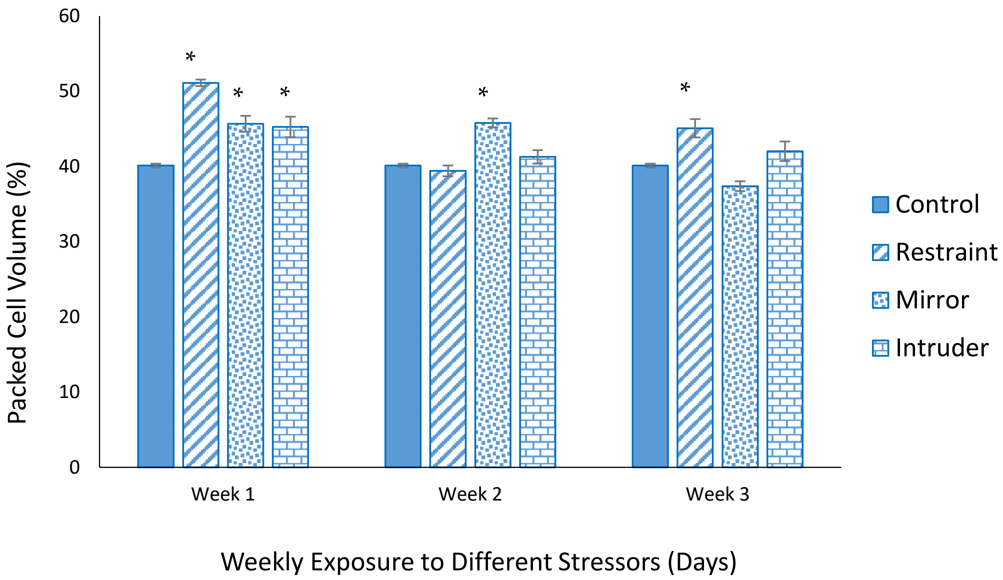

Figure 3. Changes in PCV values after exposure to different stressors for 3 h per day for 1, 2, and 3 weeks, respectively

*Significant difference (P<0.05) compared to the control group.

Figure 3 shows changes in the mean PCV values of pregnant Wistar rats after weekly exposure to stressors of varying types at a rate of 3 h per day. PCV values significantly (P<0.05) increased in pregnant Wistar rats, regardless of the stressor applied for 1 week compared to the control values. However, PCV values increased after exposure to the mirror or intruder stressor for 2 weeks. The observed increase in PCV values was only significant (P<0.05) in rats exposed to the mirror stressor compared to the control values. PCV values increased after exposure to the restraint or intruder stressor for 3 weeks. The observed increase was significant (P<0.05) in rats exposed to the restraint stressor compared to control values.

Figure 4 shows the changes in the Hb concentration of pregnant Wistar rats after weekly exposure to stressors of varying types at a rate of 3 h per day. As was observed for PCV, the Hb concentrations increased significantly (P<0.05) in pregnant Wistar rats, irrespective of the stressor applied for 1 week compared to the control levels. Exposure of rats to the restraint or intruder stressor for 2 weeks resulted in a reduction in the Hb concentration of pregnant Wistar rats. The observed decrease in Hb concentration was only significant (P<0.05) in rats exposed to the intruder stressor compared to control levels. The Hb concentration of rats was equally significantly reduced (P<0.05) after prolonged exposure to the intruder stressor for 3 weeks compared to control levels.

Figure 5 presents the mean variation in platelet counts of pregnant Wistar rats after weekly exposure to stressors of varying types at a rate of 3 h per day. Similar to RBC counts, platelet counts of pregnant Wistar rats significantly (P<0.05) increased after exposure to the restraint stressor for 1 and 3 weeks, respectively, compared to control levels. Platelet counts increased after exposure to the mirror stressor for 1 and 2 weeks, respectively, compared to control levels. The observed increase was significant (P<0.05) following exposure to the stressor for 1 week. However, platelet counts were found to decrease in pregnant rats after prolonged exposure to mirror stressors for 3 weeks compared to control levels. Meanwhile, platelet counts in pregnant rats reduced after exposure to intruder stressors for 1 and 2 weeks, respectively. However, prolonged exposure of rats to the intruder stressor for 3 weeks caused a slight increase in platelet counts compared to control levels.

Table 3 shows the mean values of hematological indices of the female offspring of intruder-stressed rats compared to the Wistar rats of the intruder parents. It was observed that, except for RBC counts, the hematological indices of the offspring of intruder-stressed parents increased compared to the values of the offspring of control pregnant rats, but the increase was significant (P<0.05) only with respect to PCV, Hb concentration, and platelet counts. For intruder-stressed parents, except for the total WBC count, which was significantly (P<0.05) greater than the control, the values of other indices studied decreased. However, the decrease was significant (P<0.05) only with respect to RBC counts and Hb concentration. The PCV, Hb concentration, and platelet counts of the offspring of intruder-stressed rats were significantly higher (P<0.05) than the corresponding indices of the stressed parent rats.

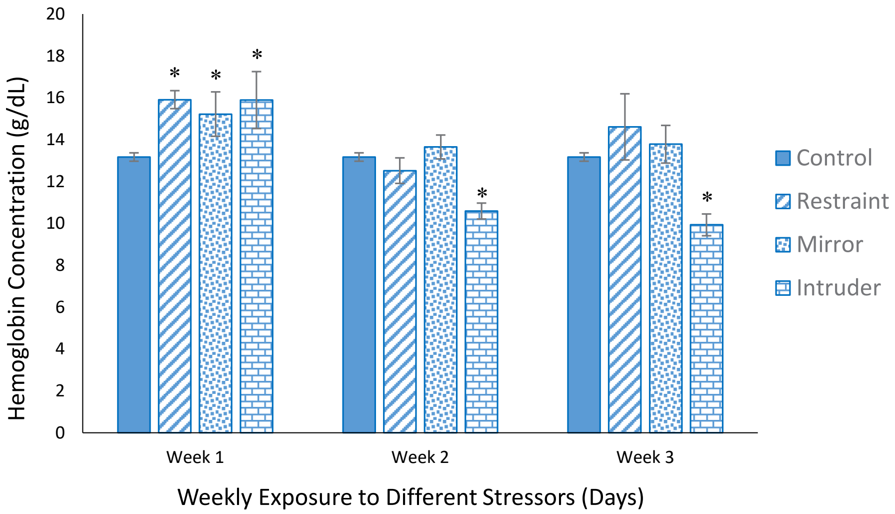

Figure 4. Changes in Hb concentration after exposure to different stressors for 3 h per day for 1, 2, and 3 weeks, respectively

*Significant difference (P<0.05) compared to the control group.

Table 3. Hematological indices of female offspring of Wistar rats stressed with intruder stressor for 3 h per day and for 3 weeks

Variables	Hematological Indices				
	TWBC (10^9/L)	RBC (10^{12}/L)	PCV (%)	Hb (g/dL)	Platelets (10^9/L)
Offspring of control pregnant rats	7.79±0.21	6.97±0.09	41.41±0.75	13.86±0.34	5.16±0.17
Offspring of intruder-stressed rats	8.57±0.37	6.94±0.54	50.62±0.62^{*+}	15.49±0.25^{*+}	8.46±0.37^{*+}
Intruder-stressed Parents	9.77±0.84*	5.39±0.33*	37.38±0.65	9.93±0.52*	4.93±0.24

*Significant difference (P<0.05) in stress-induced groups compared to the control group, $^{+}$Significant (P<0.05) compared to parental values.

Discussion

Knowledge of the variation in hematological indices during pregnancy, fetal life, and infancy is critical to avert the possible adverse effect of exposure to unpredictable maternal stress, which can result in maternal and infant morbidity and mortality linked to perinatal, antepartum, and postpartum hemorrhage, unsafe abortion, eclampsia, obstructed labor, and infection (Chandra et al., 2012). The results of this study revealed that the TWBC count responded in various ways after Wistar rats were exposed to different stressors for 3 h per day for 1, 2, and 3 weeks, respectively. The observed variability of TWBCs, regardless of the stressor applied, could be related to immuno-suppression, fetal immunomodulation, selective immune tolerance, and bone marrow hyperplasia (Osonuga et al., 2011; Verma & Chaudhary, 2013). However, of greater significance are the health implications of these observation, for which the data from this study provide no clue. However, whether exposure to the studied stressors caused an increase or decrease in TWBCs or not, the possibility of an alteration in differential counts cannot be ruled out. Redistribution usually favors the activation of the immune system in rats (Herbert, 1994; Junqueira et al., 2008; Siems et al., 2000; Wintrobe & Greer, 2009).

In our study, exposure to the restraint stressor for 1 week and 3 h per day for 3 weeks resulted in a significant increase in RBC counts compared to the control values, but exposure to the mirror stressor for 2 and 3 weeks, respectively, caused a significant reduction in RBC counts compared to control values. Exposure of rats to the intruder stressor for 1 and 2 weeks, respectively, caused

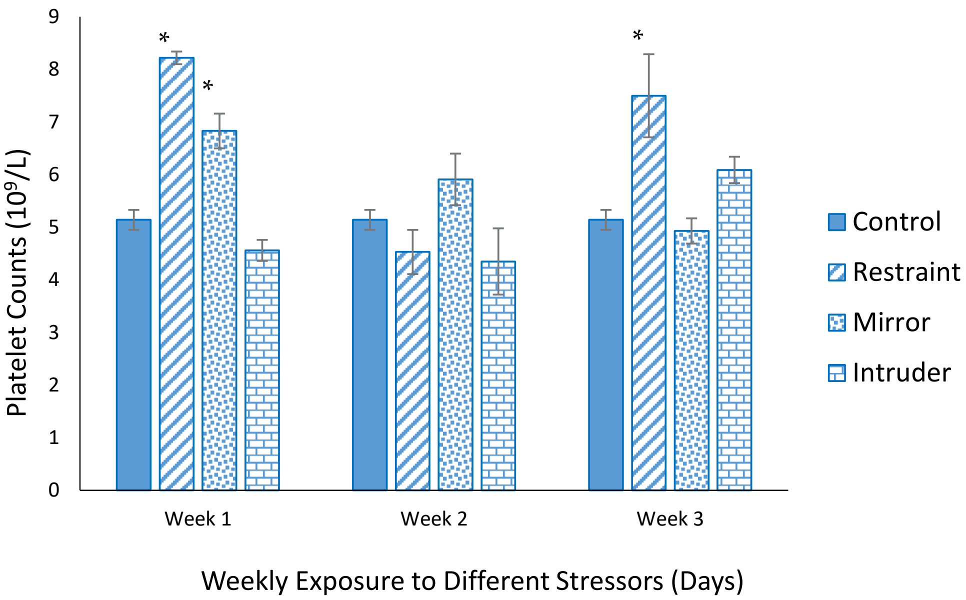

Figure 5. Changes in platelet counts after exposure to different stressors for 3 h per day for 1, 2, and 3 weeks, respectively

*Significant difference (P<0.05) compared to the control group.

a significant decrease in RBC counts compared to the control values. The observed increase in RBC counts of offspring from intruder-stressed parents was not statistically significant compared to the control values. Such changes support the fact that oxidative stress readily alters the deformability of RBCs and contributes to cell aging and elimination from the circulation (DeOliveira & Saldanha, 2010; Esterbauer & Cheeseman, 1990; Nwogueze et al., 2020a). Oxidative stress achieves this by damaging the RBC membrane (Mohanty et al., 2008; Mohanty et al., 2014), resulting in altered brain oxidative status, anorexigenic or orexigenic hormones (Anachuna et al., 2020), and the cellular integrity of tissues (Enebeli et al., 2022; Nwogueze et al., 2023; Ofili et al., 2015). Therefore, it is reasonable to expect a decrease in RBC counts after exposure of rats to stressors, except if the stressors are not powerful enough to further the oxidative state of the RBCs and overwhelm the cells' antioxidant system. Based on the earlier speculation that the intruder stressor is far more potent than the others used, the above result (from exposure to the intruder stressor) suggests a rapid clearance of RBCs from the circulation without a commensurate increase in the rate of cell production (Ofili & Ncama, 2014; Onyesom et al., 2013).

In this study, the exposure of Wistar rats to the different stressors studied significantly altered PCV values of the respective rats. For example, PCV values increased significantly after exposure to the restraint stressor compared to the control values. Similarly, exposure of rats to the mirror stressor for 1 and 2 weeks, respectively, caused a significant increase in PCV values compared to the control values. RBC counts, PCV, and Hb concentration are hematologic indices critical in determining the level of transport in blood. They are related in the sense that the PCV represents the percentage of blood volume occupied by the cellular component of blood, of which RBCs constitute the majority (Heidt et al., 2014; Kaur et al., 2014; Nwogueze et al., 2024). Hb is contained in RBCs, and the Hb concentration is usually about one-third of the PCV value (Almis & Aksoy, 2018; Herbert, 1994). From this narrative, it seems obvious that a factor that affects any one of these indices is likely to have an impact on the others. There was an observed increase in PCV in stressed pregnant rats, and this increase in their offspring could be associated with pregnancy-induced hormonal changes and systemic vasodilatation, increased vascular capacitance and plasma renin activity, as well as reduced levels of atrial natriuretic peptide (Bernstein et al., 2001; Herbert, 1994).

Hb concentrations were altered in the same direction as the RBC count and PCV values. Hb concentration increased significantly after 1 week of exposure to the restraint and mirror stressors, respectively, compared to the control values. However, exposure of rats to the intruder stressor for 2 and 3 weeks, respectively, caused a significant decrease in Hb concentration compared to the control values. However, there was a significant increase in the Hb concentration of the offspring compared to the rats exposed to the intruder stressor and compared to the control values. Karamala et al. (2011), in a study involving lead poisoning, observed a significant reduction in Hb concentration, which was associated with oxidative stress induced by lead poisoning. An abnormally low level of Hb concentration can be induced by stress and will lead to iron deficiency and, consequently, to anemia (Herbert, 1994).

In this study, stressors were applied exogenously, unlike in the case where the stressor was an endogenous anxiety disorder. It is possible that the different observations made in our study may be due to the differential nature of the stressors involved in the two studies. Exposure of rats to the restraint stressor for 1 and 3 weeks, respectively, was found to significantly increase the platelet count of rats compared to the control values, whereas platelet count only significantly increased after exposure to the mirror stressor compared to the control values. Our result showed that there was a significant increase in platelet counts of the offspring compared to their stressed intruder parents and compared to the control values. Taking into account the varied nature of the stressors, the quality of stress induced in the rats may have determined the variable platelet count responses observed. It is possible that exposure of rats to stressors (in the case of varying types) may cause platelet responses that are not captured by the procedure adopted in this study. It has been suggested that stress improves platelet activity, cell reactivity, lipid profile variability, and immune modulatory capacities (Koudouovoh-Tripp & Sperner-Unterweger, 2012; Ossai et al., 2022; Verma & Chaudhary, 2013).

Conclusion

Hematological indices are vital routine investigations performed during maternal gestation and after delivery. The variability of the hematologic profile studied after exposure to different types of stress suggests that stressors do not have a uniform impact on rats and that the intensity of stress induced by the different stressors applied varied considerably depending on the duration of exposure. Exposure to an intruder stressor up to the third week was more harmful—especially to the values of PCV, RBC counts, and Hb concentration—in stressed pregnant Wistar rats, compared with the restraint or mirror stressors. This was not the case for offspring from intruder-stressed parents. In conclusion, the effects of stress during pregnancy on the blood parameters of pregnant mothers cannot be transmitted to their infants, especially in the case of the intruder stressor studied.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research, Ethics, and Grants Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka and the study was conducted according to the Helsinki guidelines with the experimental procedures of ARRIVE (Code: REC/FBMS/DELSU/19/58).

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Authors' contributions

Conceptualization, supervision, funding acquisition and resources: Bartholomew Chukwuebuka Nwogueze; Methodology: Tochukwu Nnamdi Nnama; Investigation, Data collection: Ufuoma Jemima Mukoro and Alphonsus Okafor Mbah; Data analysis: Mary Isioma Ofili; Writing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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