Evaluations of the physiological parameters of pregnant mice under chronic stress conditions

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ABSTRACT

Chronic stress can cause significant changes in the behavior and organ functions of organisms exposed to stressful conditions. Currently, the concept of stress is defined as a disturbance of homeostasis resulting from environmental, physiological, and psychological influences. In this context, this study aimed to investigate the physiological effects on pregnant females subjected to stressful conditions. The research was conducted at the Laboratory of Neuropsychopharmacology at the Central Vivarium of the University of Feira de Santana, Bahia. A total of 12 adult Mus musculus mice were used, 8 females and 4 males. After mating, females were divided into four distinct groups, with 2 females in each: light cycle, social isolation, overcrowding, and control. For 19 days, 02 females were kept under the same stress conditions previously established, so weight, waist circumference, heart and respiratory rates were monitored. The data were recorded in Excel and the graphs were generated using Sigma Plot. The results indicated that the stressful conditions influenced the physiological aspects of the groups, including affecting weight. It was observed that the groups subjected to stress presented more physiological variations compared to the control group. Studies that aim to understand the effects of stress on the physiological aspects of animal species are of paramount importance, as they can provide relevant insights into the possible impacts of a stressful pregnancy on children and adolescents. Although this study is preliminary, the results are promising in showing that stress during pregnancy underscores the importance of providing a quiet environment for fetal development. It is expected that these results will contribute significantly to the advancement of knowledge in this area and may have important implications, especially when investigating the behavioral and physiological aspects of offspring born to mothers exposed to stress during pregnancy.

Keywords: Pregnancy, Physiology, Chronic Stress, Mice, Vivarium.

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INTRODUCTION

Stress is a complex reaction of the body to stimuli that disturb it. When repeatedly exposed to stressful situations, the body consumes more energy to deal with them. This response can be understood as an interaction between physiological, psychological and behavioral aspects, in an attempt to adapt to internal and external demands (Margis et al., 2003).

Normally, the stress response is designed to be temporary, with hormonal and neurotransmitter changes returning to pre-stress levels quickly. However, when stress persists chronically and the individual is unable to adapt, it can trigger other disorders, such as anxiety and depression. It is believed that, in the gestational phase, there is a greater sensitivity in response to the state of stress (Santos et al., 1998).

The pregnancy-puerperal cycle is characterized by a series of emotional changes, which are influenced by several social and psychological factors, playing a crucial role in the development of pregnancy, as well as in maternal and child well-being and health (Faisal-Cury et al., 2009). Among the psychological factors that are often associated with complications during pregnancy, childbirth, and the postpartum period, the stressors experienced throughout this period stand out. Stress during pregnancy is commonly related to specific events such as nausea, unplanned pregnancies, concerns about excessive weight gain in early pregnancy, and anxiety about childbirth during the second trimester (Esper et al., 2010).

On average, pregnant women experience about five stressful events during pregnancy, and this condition can be aggravated in family contexts marked by economic difficulties, domestic violence, use of psychoactive substances, depression, panic disorder, and prenatal complications (Segato et al., 2009; Woods et al., 2010). Studies indicate that more than 75% of pregnant women manifest significant signs of stress at some level during this sensitive period (Segato et al., 2009; Woods et al., 2010).

Maternal stress during pregnancy has been closely associated with a variety of changes at different stages of fetal development, which can have a strong impact on the neuronal formation of individuals (Bronson, Bale, 2016). Studies correlate that the higher the level of stress during pregnancy, the more likely the child is to develop temperamental and behavioral problems (Charil et al., 2010; Gutteling et al., 2005), which, in turn, may contribute to depressive symptoms in the mother (Britton et al., 2011). It is worth noting that stress can also result in harmful consequences for the health of the newborn, including prematurity and low birth weight, as well as obstetric complications for the pregnant woman (Diego et al., 2015). Additionally, maternal stress during pregnancy may be a risk factor for the development of postpartum depression (Brummelte et al., 2010; Gao et al., 2009).

Stress is associated with the release of hormones that, in addition to altering various aspects of physiology, also have a modulating effect on the body's defenses. In humans, the main hormone with these functions is cortisol (glucocorticoid). The constant high levels of cortisol can impact the individual through cognitive, behavioral, and emotional changes. Therefore, the study of the effects of maternal stress on the neural development of the fetus is complex, since there are other factors that can contribute to this correlation, for example, genetic factors that can influence the development of adverse behavioral patterns (Ventura, Neto, Simões, 2009).

Although many studies indicate that mental health can be affected as a result of traumas experienced throughout life, including during the gestational period, it is not common in the literature to find studies that aim to subject pregnant female mice to conditions of chronic stress. This is due to the fact that it is more common for studies to look for the least stressful conditions possible in order to preserve the fetal development of the animals. However, the present study sought to obtain animals born from chronic stress, since the intention of this research is to understand, throughout life, the various effects that stress can cause in the physiological and behavioral aspects of animals born from a stressful fetal period, since it is notable that this period is crucial for several aspects of animals. Thus, the results of this study may serve as a basis for a better understanding of the effect of stress on human beings.

Stressful conditions can be an aggravating factor in physical and mental health for several species, including humans. The primary intention of this study was to evaluate the physiological aspects of pregnant female mice subjected to chronic stress. Studies that seek to understand the effects of stress on the physiological aspects of animal species are of paramount importance, as they can be used for a better understanding of the effects that stressful pregnancy can cause in children and adolescents.

In summary, the proposed study aims to fill an important gap in scientific knowledge about the effects of chronic stress on pregnant females by providing valuable insights into the underlying mechanisms and consequences for maternal and fetal health. This information is essential to inform clinical practices and public health policies aimed at improving pregnancy outcomes and maternal and child well-being.

MATERIAL AND METHOD

Previously, the project was appreciated and approved by the Animal Use Ethics Committee (CEUA/UEFS), through Ordinance/UEFS 534/2022. After the declaration of approval of the project, it was presented to the Central Vivarium of UEFS, which produced the animals for the present study.

Twelve mice of the species *Mus musculus* Linnaeus, 1758, comprising four breeding males and eight females were used. The animals were acquired from the Central Vivarium of the State University of Feira de Santana (UEFS) and selected due to their greater sensitivity to behavioral variations, attributed to hormonal physiological issues (OECD, 2002). It is worth noting that males were used exclusively for reproduction, since the main objective of this study was to subject pregnant females to some stressful conditions. The central purpose of the research is to obtain a generation born from a prenatal development under chronic stress, and then to evaluate this generation. Although the focus of this article was to present the results only of pregnant females.

All guinea pigs are primiparous born from the colony of the central vivarium. Before the confirmation of gestation, they were kept under the same environmental conditions, that is, under the conventional conditions of rearing and housing of the animals in the UEFS vivarium, (*Open Caging System*), and the microenvironment was maintained under controlled conditions: temperature, water supply and feed. The temperature was maintained at 22 ± 2 °C, the relative humidity (RH) of the air was maintained at 50 ± 10% and the lighting was maintained with cycles, this during their conditioning.

The experimental stage began after the confirmation of the primiparous women's pregnancy. They were then randomly divided into pairs and subjected to specific stress conditions throughout the gestational period until the end of the gestational period. To induce stress in the females, they were selected and conditioned to one of three adverse conditions: maintenance in a clear cycle for 24 hours, exposure to overcrowding in nurseries, and social isolation of the animals. After defining the conditions that would trigger stress, the females were divided into 4 distinct groups as shown in figure 01:

Figure 01 – Experimental design of the stress conditions that the female mice are maintained in the experiment (n=8)

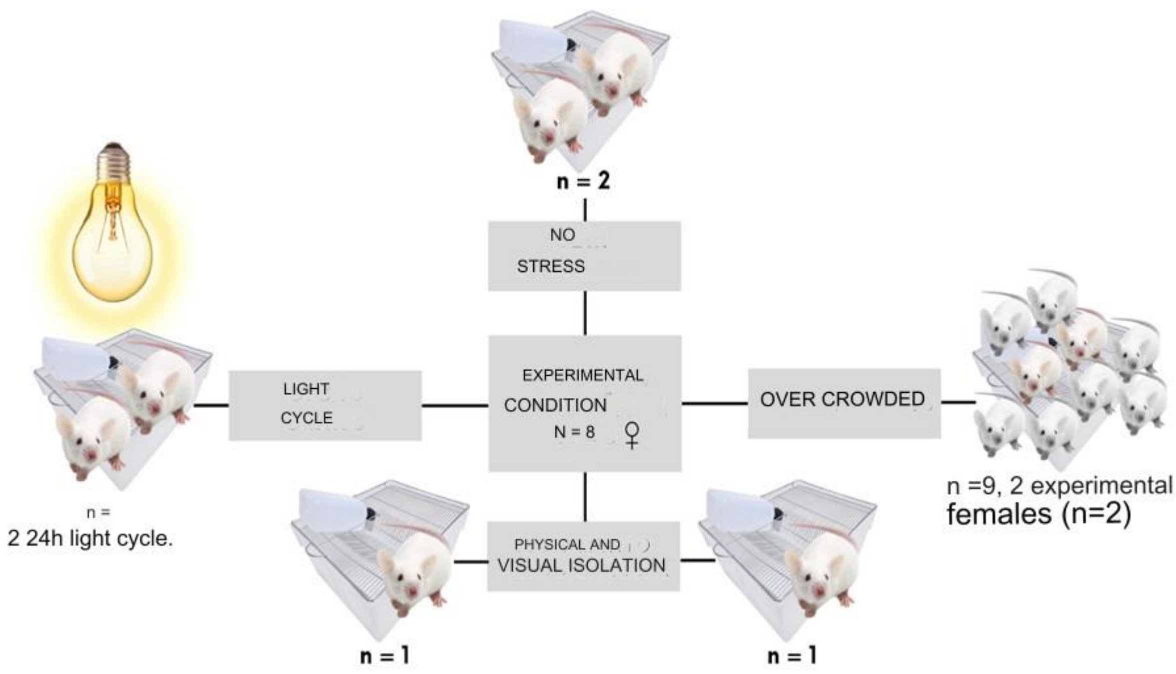


Figure 01 illustrates the conditions of the groups during the study. It is possible to observe that the control group was not subjected to stress conditions. In this case, the females were kept in the least stressful conditions possible, configuring themselves as the control group of the study. On the other hand, the females of the experimental groups were subjected to specific conditions. In the overcrowding group, females were kept in a nursery with nine animals. The light cycle group was kept in a room where there was no light restriction, i.e., the light conditions were maintained for 24 hours. Meanwhile, the females in the social isolation group were kept solitary, deprived of physical and visual contact with other animals. All females were subjected to these conditions for 19 days, after which they were removed from the stress conditions to await the birth of the offspring.

During all study periods, the vital physiological parameters (respiratory and heart rate) and non-vital physiological parameters (weight, behavior and abdominal circumference) of all females were recorded. The animals were weighed using an SF-400 digital scale with a precision of 1 gram. After weighing, each animal was placed inside a 1000 mL beaker to record the weight in grams. Then, the waist circumference was measured with the aid of a tape measure. Heart rate (HR) was measured by restraint of the animal, and with index finger positioned in the rodent's femoral artery for 15 seconds.)

For the analysis of physiological data, the *SigmaPlot software*, version 14.5., was used to calculate the mean, standard error of the mean and construct the graphs. Respiratory rate (RR), on the other hand, is a parameter that was obtained through the observation of the movements of inspiration and expiration of each animal, for this measurement the animal was placed inside a 1000 mL beaker for 15 seconds – the results found were multiplied by 4, because 15 seconds is equivalent to 60 seconds. On the other hand, the heart rate (HR) was measured by restraining the animal, and with the index finger positioned in the femoral artery of the rodent, for 15 seconds.$\frac{1}{4}$

RESULTS

Table 01 shows the results of the respiratory rate of females under different experimental conditions: overcrowding, social isolation, absence of stress and clear cycle. A progressive increase in respiratory rate is observed over the weeks. It is noteworthy that the group submitted to overcrowding exhibited the highest value in the third week of observation, recording an average of 218 respiratory movements per minute. On the other hand, the lowest values were observed at Baseline (LB), with an average of 161 respiratory movements per minute.

Table 01 - Respiratory rate of pregnant mice under or without stress conditions (n=8). *LB (Baseline)

Group	LB	Week 1	Week 2	Week 3
Overcrowding	161 (1,41)	190,5 (4,94)	195,5 (3,53)	218 (2,82)
Social isolation	194 (5,65)	216 (8,48)	216 (9,90)	212 (5,65)
No stress	175 (4,24)	198,5 (9,19)	207 (1,41)	215 (12,72)
Clear Cycle	163 (7,07)	198,5 (3,53)	194 (1,41)	210 (8,48)

According to Chumbinho (2012), mice usually have a respiratory rate that varies from approximately 163 respiratory movements per minute and a heart rate between 500 and 750 beats per minute when they are active. However, the respiratory rate results, as shown in Table 01, contradict the data provided by Chumbinho (2012), since a value higher than that mentioned by the author in this study is observed. It is plausible to consider that this study evaluated these vital parameters in at least two adverse conditions: the stress induced by the experimental conditions and the gestational stage of the animals.

It is plausible to consider that the increase in respiratory rate observed in the weeks in which the females were subjected to the stress condition may be a physiological response to the environmental demands faced daily by the mice. Stress can trigger a number of physiological responses in the body, including changes in breathing.

When an organism faces stressful situations, such as perceived threats to its well-being, thus, the stressor triggers a series of physiological responses, for example, increased basal metabolism, increased respiratory and cardiovascular rate, inhibition of digestion, growth, the reproductive system, and the immune system. This response occurs by the activation of the sympathetic nervous system, with the direct activation of target tissues by noradrenaline, or by the stimulation of the medullary region of the adrenal gland, increasing systemic levels of adrenaline (De Kloet, Joëls and Holsboer, 2005).

Thus, it is possible to suggest that, in the present study, the increase in respiratory rate observed in female mice submitted to stressors may be an adaptive response to the environmental demands faced by the guinea pigs, possibly related to the release of neurotransmitters associated with the body's defense. Therefore, the variation in heart rate is due to the stressful situation that the females were subjected to throughout the study phases.

Other factors that can also explain changes in breathing patterns are gestation conditions, since females go through this period. In humans, it is common for there to be changes in breathing patterns during pregnancy, as these changes are necessary to provide the embryo and fetus with the oxygen and nutrients necessary for their proper development. According to Reis (1993), the main changes in maternal physiology during pregnancy occur in the cardiocirculatory, respiratory, and gastrointestinal systems, as well as metabolic and hematological changes. The results of this study indicate that the changes resulting from pregnancy in this animal model are similar to those observed in women, since physiological changes in respiratory and cardiac vital measures are also noted in the experimental models.

Based on the information presented, an increase in heart rate was observed over the weeks of the experiment, as detailed in Table 02. However, there was a reduction in heart rate in the last week, resulting in an average of 203 bpm (beats per minute) for all four groups. The highest value recorded in the last week belonged to the social isolation group (209 bpm). It is noteworthy that, not only in the third week, but in all the previous weeks, the group of animals subjected to social isolation exhibited a higher heart rate than the other groups.

The analysis of Table 02 also reveals that the group kept under a constant clear cycle condition had a lower mean heartbeat compared to the other groups. However, a significant increase of 28.8% in heart rate was observed when comparing baseline (LB) values with those of the third week. In addition, this increase was not restricted to heart rate (HR), but was also reflected in respiratory rate (RR), with a 26% increase between LB and the third week.

The stress resulting from disturbances in the circadian cycle of animals can trigger serious physiological repercussions, since the oscillations of cortisol and other hormones are intrinsically linked to the cycle of light (wakefulness) and the cycle of darkness (sleep). However, in rodents, this cycle is reversed due to their nocturnal habits (REFERENCE?). Despite this, the aim of the study was to induce changes in wake and sleep patterns in these models. Consequently, it is observed that keeping the animals in permanent cycles of light or darkness may have affected hormone production, which contributed to the results found in the measurements of physiological parameters.

Table 02 – Heart rate of pregnant mice subjected or not to stress conditions (n=8). LB (baseline)

Group	LB	Week 1	Week 2	Week 3
Overcrowding	174 (16,97)	195,5 (2,12)	178 (2,82)	197 (1,41)
Social isolation	207 (7,07)	213 (11,31)	231 (1,41)	209 (15,55)
No stress	189 (7,07)	215 (1,41)	218 (11,31)	206 (11,31)
Clear Cycle	158 (14,14)	189 (2,82)	194 (0)	201 (7,07)

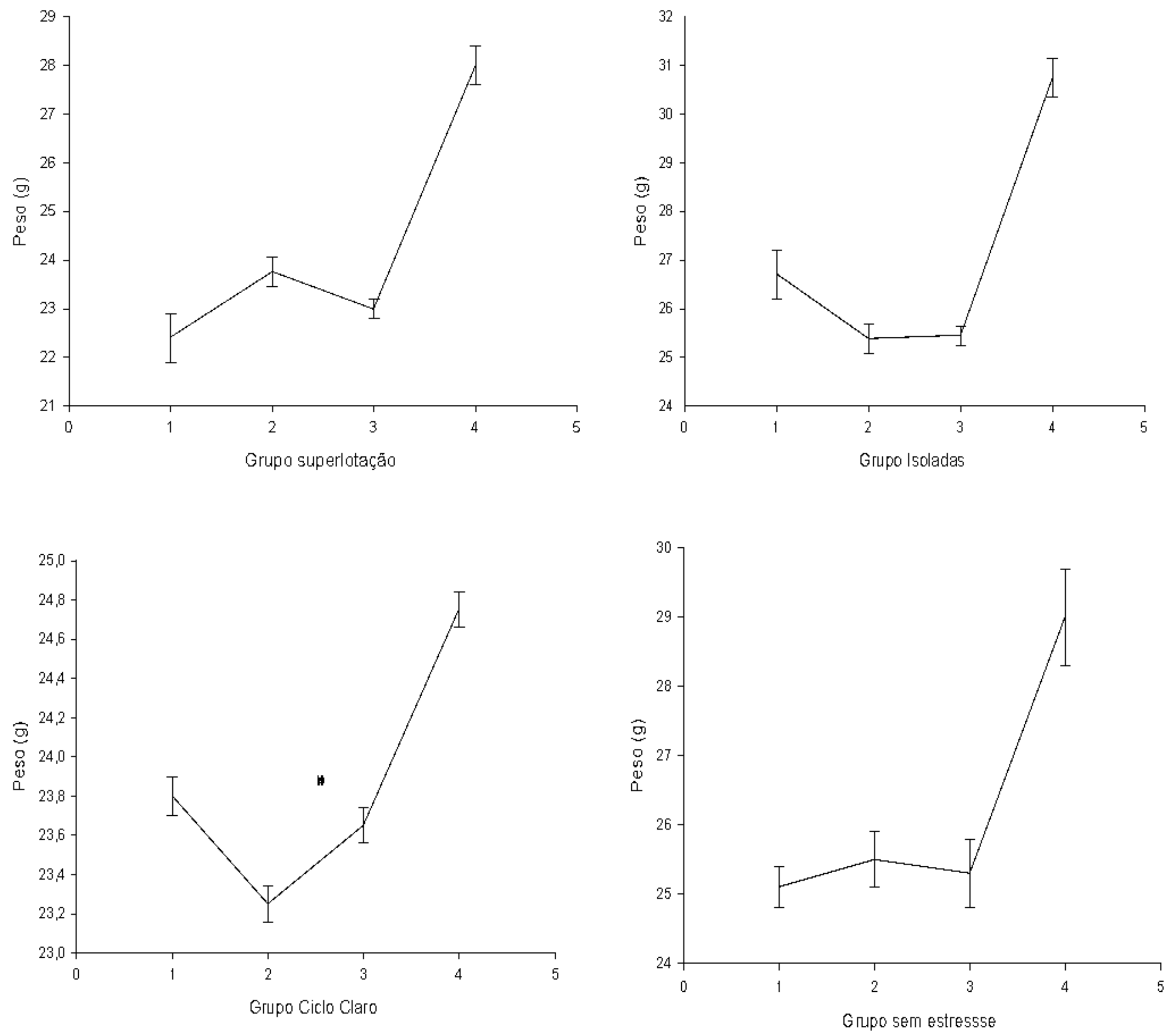
The desynchronization of the circadian cycle, which represents the interruption of the natural rhythm of activities and rest over approximately 24 hours, can cause serious damage to several species of mammals. According to studies conducted by Morris et al. (2017) and Pilorz et al. (2018), the health of the cardiovascular system is affected by sleep and wake cycle deprivation. The results of these studies pointed to an increase in blood pressure and a higher incidence of myocardial infarction in animals submitted to interruption of the light and dark cycle. Thus, the changes observed in the present study may corroborate the conclusions of these studies, since the animal models also demonstrated changes in the heart rate measured during the experimental phases.

Although there is much evidence suggesting that environmental factors, by disrupting physiological rhythms, may contribute to cardiovascular events and increase other risk factors commonly associated with cardiovascular disease, the impact of chronic circadian desynchronization on cardiovascular risk factors still needs to be further investigated. However, it is well known that the circadian cycle is a fundamental component of the biological functioning of many organisms, including mammals, and plays a crucial role in regulating a variety of physiological, behavioral, and metabolic processes.

In the present study, weight and waist circumference variables were also examined. Weight gain was observed in all groups between the third and fourth week of the study. We cannot say whether this increase is statistically significant, as the data presented here represent only the mean of the groups. However, we will perform statistical tests to determine in which phases there is a reduction and/or increase that present statistically significant differences, as shown in figure 02.

It is interesting to mention that the groups exposed to stress conditions, such as overcrowding (Figure 2.a), social isolation (Figure 2.b) and light cycle (Figure 2.c), showed a weight reduction between the week of LB (represented as week 1 in the graphs) and the second week of investigation. This shows how stress influences the physiological patterns of the animal, resulting in the impairment of its physical state. Studies indicate that chronic stress can impact the physiological factors and patterns of animals, especially in relation to hormone levels, and can lead to an increase in blood glucose in the body and, consequently, to the development of diabetes (Penteado et al., 2009).

Figure 02 – Presentation in weeks of the weight results of female mice in groups a) overcrowding; b) social isolation; c) light cycle; and, d) no stress (n=8) (Authorial image).



Females in the light cycle group (Figure 02.c) exhibit the lowest weight recorded among all groups. In addition, in this group, a decrease in weight is observed between weeks 1 and 2. It is plausible to consider that, in the same way as evidenced in CF, chronic stress induced by the abrupt alteration of the circadian cycle may have triggered the physiological changes observed, such as, for example, in metabolism, which is reflected in body mass.

Desynchronization can result in the breakdown of the body's internal balance, disrupting physiological rhythms and metabolic functioning, which can lead to adverse health impacts, including the development of chronic, neurological, and immunological diseases (Papakonstantinou et al., 2022). According to Challet (2019), food is one of the functions regulated by the brain throughout the circadian cycle. Therefore, depending on the phase of this cycle, the timing of when calories are consumed can influence weight gain or loss and the onset of diseases. In addition, studies indicate that circadian desynchronization is intrinsically linked to the development of chronic diseases, impairing homeostasis and triggering a series of physiological disorders (Costa et al., 2023).

Chronic stress affects the production of cortisol, which is directly linked to metabolism and other physiological patterns, but caution is needed to assume that cortisol has caused physiological changes, since no measurements of this hormone have been made. However, it is known in the literature that chronic stress generates dysregulation of the negative feedback of the Hypothalamus-Pituitary and Adrenal axis. According to Figueiredo *et al*. (2003) and Hu *et al*. (2020) rats and mice that are subjected to chronic stress show an exaggerated axis response, with increased corticosterone and CRH. Thus, it is reasonable to consider that the stressful conditions of the present study cause an increase in cortisol levels in the guinea pigs, which is a necessary measure of evaluation for the other phases of the study.

The animals in the non-stress group showed a remarkable stability in relation to weight, without major fluctuations over the observed period, unlike the other groups, figure 02. These females showed more consistent values during the first three weeks, with a noticeable increase only between the third and fourth weeks, due to gestation. This result corroborates those of Zhang et al. (2017) who demonstrated that chronic stressors cause significant weight loss, due to an increase in corticosterone levels.

Importantly, pregnancy is closely associated with significant physiological changes, and fetal adaptation plays a crucial role in its proper development; stress can interfere with this adaptation (Lazinski et al., 2008). Thus, it is plausible to infer that the stress experienced by the females in this study may impact the development of the offspring. To investigate this issue, the abdominal circumference of females was measured daily, as shown in Figure 03 below.

Figure 03 – Presentation in weeks of the results of the abdominal circumference of female mice in groups (a) overcrowding; (b) isolated; (c) light cycle; and (d) without stress (n=8) (Authorial image).

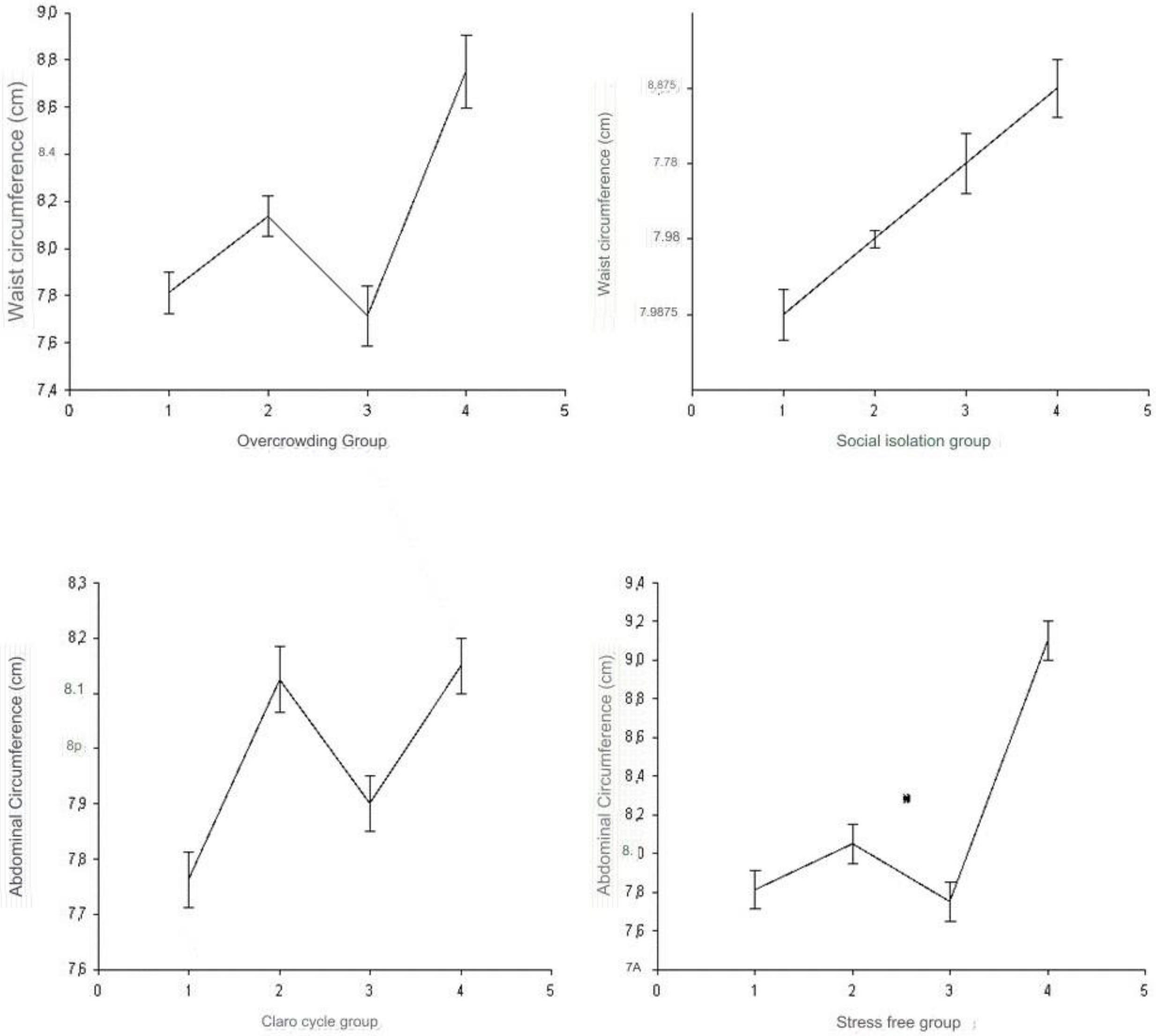


Figure 03 shows that females in the overcrowding (Figure 3.a) and non-stressed (Figure 3.d) groups have a similar pattern of abdominal circumference, as there is an increase in abdominal measurement between the 1st and 2nd week of study, followed by a reduction in the 3rd week and a marked increase between the 3rd and 4th week of study. In the group of females in the light cycle, a reduction in circumference was also observed in the 3rd week of the study, followed by an increase between the 3rd and 4th weeks, but this was less pronounced than in the overcrowded and non-stressed groups. On the other hand, in the social isolation group there was a constant increase between all weeks, as it is not possible to verify pronounced reductions and increases between the weeks of observations, but only a linear growth curve (Figure 3.b).

In Figure 03, it is possible to infer that there is fetal development by measuring the abdominal circumference. An average increase in this measurement is observed between weeks 1 and 2, followed by a reduction, except for the social isolation group, where the circumference remains stable, and then increases again in the final phase of pregnancy. It is evident that pregnancy induces profound changes in the maternal organism, in order to adapt it to the needs of the maternal-fetal complex and childbirth. It is noteworthy that all females studied gave birth, with the exception of one female in the light cycle. It is known that stressful conditions can affect the reproductive cycle of animals and fetal development of individuals Hamaué et al. (2011).

The hypothesis of this study is that the stress condition was extremely impactful for the female of the clear cycle that did not reproduce, even after being subjected to mating again, without success in pregnancy. It is important to note that the female of the clear cycle condition that has not given birth is being subjected to new stresses, and the intention is to study her specific condition. However, these results are still in the analysis phase.

In addition, another hypothesis underlying this study suggests that animals exposed to stress during the gestation period may manifest variations in physiological and behavioral patterns that persist throughout their development until the senescent phase. This assumption is being thoroughly investigated by the project team at the Laboratory of Neuropsychopharmacology of UEFS, under the coordination of Profa. Dr. Rosângela C. Rodrigues. Although preliminary results are in the analysis phase, there are significant indications of both behavioral and physiological changes in the offspring subjected to stress conditions. The data is currently in the process of being prepared for later release.

CONCLUSIONS

The study in question brings an interesting perspective on the effects of stress on the condition of pregnant female mice. It was observed that the groups of females under stress conditions present changes in the body weight and heart rate of the females compared to those under normal conditions.

One of the interesting aspects of this study was related to the light cycle, as it seems that the stress caused by this condition has significant impacts not only on the physical state, but also on the physiological and reproductive aspects. In addition, the study points to one perspective: the understanding of prenatal traumas and their consequences in the following generations. Exploring the effects of these traumas on the cognitive, physiological, and behavioral aspects of generations born to females born during gestation is a crucial step in better understanding the development and health of future populations.

Therefore, this study not only sheds light on the effects of stress during pregnancy, but also opens doors for deeper investigations into the long-term impacts of these experiences on future generations. This deeper understanding has the potential to inform public health policies and preventive interventions that aim to protect the well-being of mothers and future generations.

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