UTTAR PRADESH JOURNAL OF ZOOLOGY

43(13): 9-15, 2022
ISSN: 0256-971X (P)

EFFECT OF PRE-CONCEPTIONAL EMOTIONAL STRESS OF PARENTS ON THE HPG AXIS OF OFFSPRING

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i133075

Editor(s):
(1) Dr. Juan Carlos Troiano, University of Buenos Aires, Argentina.
Reviewers:
(1) Nigus Kassie Worku, Dire Dawa University, Ethiopia.
(2) Galal Lotfi, Suez Canal University, Egypt.
(3) Smitha Thadathil, Maharashtra University of Health Sciences, India.

Received: 10 April 2022
Accepted: 12 June 2022
Published: 30 June 2022

Original Research Article

ABSTRACT

Introduction: The most of studies have focused on the effects of different types of stressors on the health of the mother as well as on her offspring. But no study has investigated the emotional stress applied before gestation, and its effects on the HPG axis of offspring. Our experiment aims to elucidate the effects and the transmission of stress, experienced before pregnancy from mother to offspring and to see whether this stress persists into adulthood or not.

Materials and Methods: Chronic restraint stress (emotional stress), it was applied an hour's time each day, for four days a week, over a period of five weeks before gestation. Female rats were placed in perforated plastic cylinders. Our study was carried out on the offspring (males and females) at the age of 65 days, and then was decapitated, the blood was collected and the gonads were weighed.

Results: Steroid hormones and gonads: Female offspring; results show a highly significant increase of progesterone compared to controls offspring at the adulthood, on the other hand, a significant decrease of the relative weight of ovaries.

Male offspring; a highly significant decrease in the concentration of testosterone, and a highly significant decrease of the relative weight of testicles.

Glycemia: showed a very highly significant increase in the stressed offspring males and females compared to the control's offspring.

Conclusion: The hypothalamic-pituitary-adrenal HPA axis of stressed mothers was disrupted, leading to disruption of the Hypothalamic-pituitary- gonadal HPG axis of the offspring.

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Keywords: Emotional pre-gestational stress; female rats; offspring; hormonal perturbation.

1. INTRODUCTION

The stress response is an integral part of an adaptive biological system emotional and environmental stressors like restraint stress reportedly influence brain function.

It is known that the body's response to stressful stimuli leads to the release of several hormones and neurotransmitters, whose role is to adapt the body to the stressful situation [1,2]. The hypothalamic-pituitary-adrenal (HPA) axis is activated following stressful situations, which increases the secretion and release of corticotrophin releasing hormone (CRH) from the para-ventricular nucleus of the hypothalamus, causing the secretion of adrenocorticotropic hormone (ACTH) from of the anterior pituitary. This latter stimulates the secretion of corticosterone from the adrenal cortex [3,4].

Stress can suppress or impair reproductive functions [5]. This leads to decreased reproductive capacity or to infertility caused by disruption of gonadal hormones [6].

All these modifications are related to the adaptation of the The hypothalamic-pituitary-adrenal HPA axis, which leads to many changes such as; psychological, physiological and behavioral change [7]. Several studies have been conducted to study the factors that contribute to neuropsychological disorders such as emotional stress which affects brain function [8,9].

In this context, we are trying to explore the effects of chronic restraint stress (emotional or psychological stress) on the male and female offspring gonadal axis at the adulthood resulting from the pre-gestational stressed mothers. This assessment was based on the measurement of progesterone and testosterone concentration in offspring.

2. MATERIALS AND METHODS

2.1 Animals

Albino Rats coming from Pasteur Institute of Algiers were used for the experiment. The animals were housed in specific cages maintained in natural photoperiod temperature with standard conditions: an average temperature of 22 ± 4ºC and a relative humidity of 50-70%. After three weeks of adaptation, we divided the rats into two equal groups, the first group is controls and the second group exposed to chronic restraint stress. These rats were coupled and their offspring were used for neurodevelopmental assessment. After parturition, at the adulthood (65 days) [10] we randomly selected six males and six females of offspring from each group of rats.

2.2 Application of Restraint Stress

Our model of stress is according to the method of Bardin L [11]. This stress consists of placing the rats in a cylindrical plastic bottle perforated 1 hour in the morning at the same time for 4 days a week during 5 weeks in the same room. After application of the restraint stress, all the rats were returned to their rearing cages. At the end of the stress protocol, the rats of the two batches were mated in order to study the effects of this type of stress on the neurodevelopment of the offspring (gonadotropic axis).

2.3 Measurement of Hormonal and Biochemical Parameters (Plasma Progesterone, Testosterone, and Glycemia)

The taking away is done starting from the lachrymal vein. The blood samples are collected in the heparin tubes then centrifuged at 1500 rpm for 10 minutes, the plasma used for measurement of progesterone and testosterone assay and at 5000 rpm for 15 minutes for the biochemical parameters [12]. The progesterone is proportioned by the conventional ELISA method [13]. Measurement is done using a reader ELISA TECAN Magellan provided with data-processing software which calculates the range standard automatically and the value of the progesterone to the desired unit gives us directly.

2.4 Organ's Removal

At the end of the experimental period, the animals were sacrificed and some organs were removed and then weighed using a precision scale (SCALTEC SBC 51). The relative weight of the organs is calculated according to the formula:

Relative Weight (g/100 g PV) = (Body Weight / Individual Body Weight) x100

2.5 Statistical Analysis

Data are expressed as a Mean ± Standard Deviation (M ± SD) and were analyzed by Student's t-test with

the program Minitab (version 13). They are regarded as being significant $p < 0.05$.

3. RESULTS

3.1 Variation of the Body Weight

The results obtained in our study showed that the average of the body weight of stressed offspring females has increased very highly significant compared to controls offspring females (Stressed offspring females: 118 g ± 4.65 vs controls offspring females 94.2 g ± 6.77). Also, the average of the body weight of stressed offspring males has increased significantly compared to controls offspring males (Stressed offspring males: 107.6 g ± 7.02 vs control offspring males: 89.27 g ± 7.74). The difference is significant between the stressed offspring males and females.

3.2 Variation of Glycemia

Results concerning glycemic showed a very highly significant increase in the stressed offspring males and females compared to the controls offspring males and females (Stressed offspring females: 1.43 g ± 0.017 vs controls offspring females 1.24 g ± 0.01) and (Stressed offspring males: 1.59 g ± 0.056 vs controls offspring males 1.13g ± 0.005). The difference is significant between the stressed females and the males (Stressed females: 1.43 g ± 0.017 vs stressed males: 1.59 g ± 0.056).

3.3 Variation of Gonads Relative Weight

The significant decrease of the relative weight of ovaries was observed in the stressed offspring females compared to the control's offspring females at the adulthood (Stressed offspring females: 0.0223 ± 0.0028 vs controls offspring females: 0.0280 ± 0.0045).

Results concerning the relative weight of the testicles show that the pre-gestational stress caused a highly significant decrease in the relative weight of testicles in the male's rats resulting from the stressed mothers compared to male's rats from the controls mothers (Stressed offspring males: 1.048 ± 0.060 vs controls offspring males: 1.384 ± 0.125).

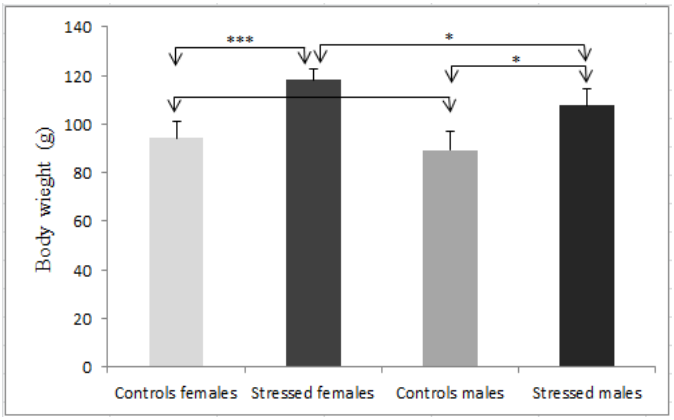

Fig. 1. Variation of body weight (g) of controls and stressed offspring at the adulthood (m ± s; n= 6)
*(*p < 0.05; **p <0.01; ***p < 0.001)*

Fig. 2. Variation of glycemia (g/l) of controls and stressed offspring at the adulthood (m ± s; n= 6)

*(*p < 0.05; **p < 0.01; ***p < 0.001)*

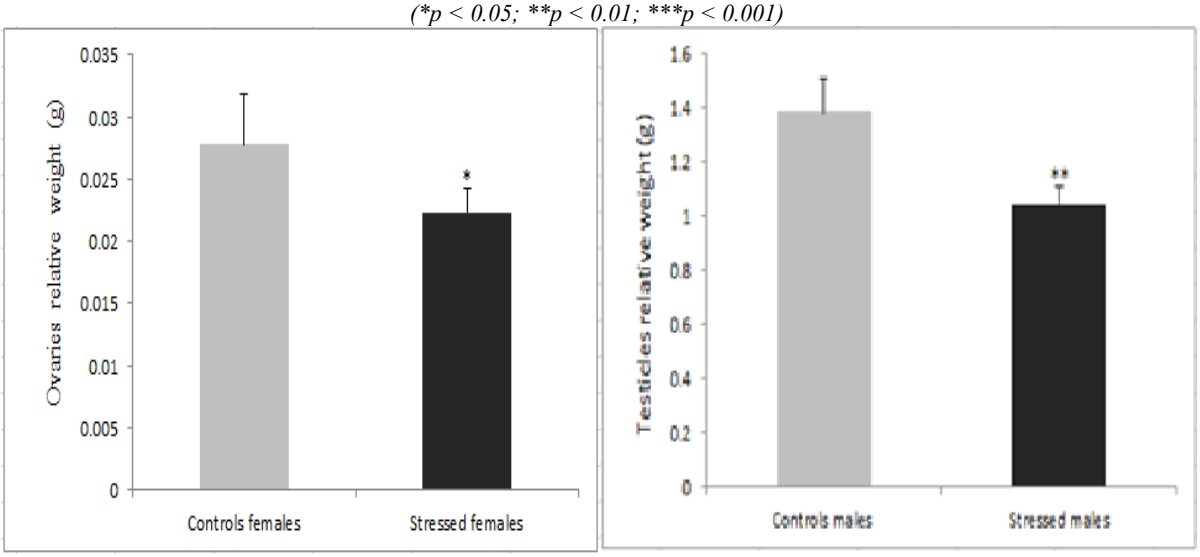

Fig. 3. Effects of the chronic restraint stress on the relative weight of gonads in the offspring at the adulthood Offspring (m ± s; n= 6)

*(*p < 0.05; **p < 0.01; ***p < 0.001)*

3.4 Variation of Progesteronemia (ng/ml)

The variation of progesteronemia of the stressed offspring females at the adulthood (Fig. 4) shows a highly significant increase compared to controls offspring females at the adulthood (Stressed offspring females: 8.56 ± 1.42 vs controls offspring females: 3.680 ± 0.669).

3.5 Variation of Testosteronemia (ng/ml)

The variation of the testosterone level (Fig. 5) in the offspring males seems affected by stress showed a highly significant decrease compared to controls offspring males (Stressed offspring males: 0.0529 ± 0.0199 vs control offspring males: 0.3038 ± 0.0844).

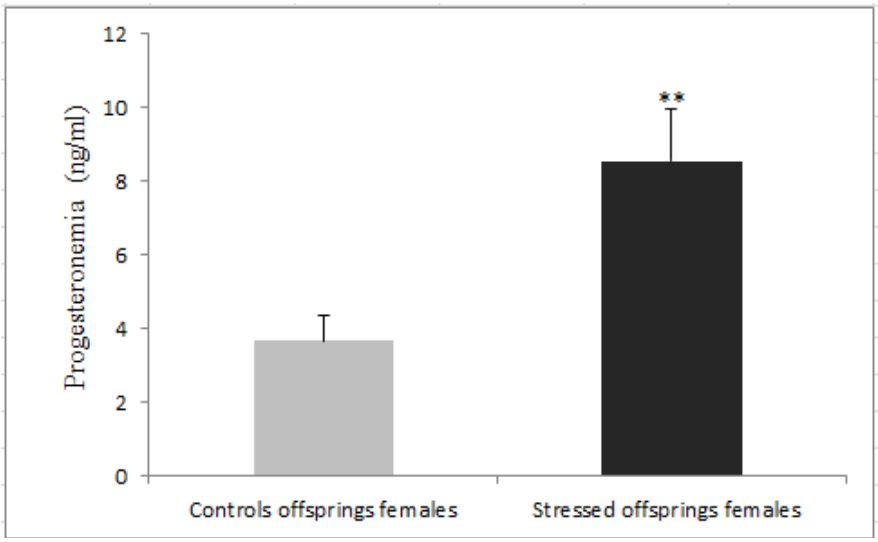

Fig. 4. Variation of progesteronemia (ng/ml) of the offspring females at the adulthood (m ± s; n= 6)

*(*p <0.05; **p <0.01; ***p <0.001)*

Fig. 5. Variation of testosteronemia (ng/ml) of the offspring males at the adulthood (m ± s; n= 6)
*(*p <0.05; **p <0.01; ***p <0.001)*

4. DISCUSSION

The relation between stress and the alteration of the reproduction axis is due to the direct neural connection between CRH and GnRH which can induce perturbation in reproductive functions [5]. Animal models of perturbations such as gestational stress have used to the programming of long-term offspring outcomes. As in humans, offspring outcomes from maternal stress studies in animals have varied depending upon stressors used, outcomes examined and timing of the stress during pregnancy [14,15].

In this experiment, we have been able to demonstrate that the chronic restraint stress CRS applied pre-gestational induces an increase in the concentration of plasma progesterone as well among the mothers than among their descendants which could be mediated slowly by the adrenal glands. Adding to this increase, the reduction of the ovaries weight (but without meaning) of stressed rats compared to the witnesses. The latter indicates the decrease in the activity of the stroma of the follicle and the luteal body in the ovaries.

This decrease is due to the disability or the absence of hormones gonadotrophins steroid, or both which are due to oxidative stress [16]. There is an interaction between The HPA axis and the steroid hormones of the ovaries [17] to release the ACTH, corticosterone, [18] and the adrenal progesterone [19] As well among the mothers that among their descendants to adulthood.

The highly significant reduction of testosterone level of male rats resulting from the stressed mothers accompanied with a significant reduction in the weight relative of the testis, are explained by a reduction of hypothalamic secretion GnRH, under the negative feed-back exerted by the initial increase in the plasmatic testosterone concentration [20], Or by the direct effect of cortisol [21].

In a stressful situation, proopiomelanocortin, which is a precursor peptide for the synthesis of GnRH and ACTH, will be redirected for the synthesis of large amounts of ACTH necessary to maintain the homeostasis [22]. This results in a decrease of the pulsatile release of LH. This process is unrelated to the level of cortisol [23] However, the frequency of GnRH pulses is suppressed by the elevated concentration of cortisol, but only in the presence of ovarian steroids (Oakley et al 2008) which can negatively affect the reproductive function [24]. This reduction contributes indirectly to the elevation of the plasma concentration of the progesterone, which is associated with a reduction of the GnRH gonadotropin [25,21].

Sexual steroids would have organizational effects during fetal and neonatal development that can organize neuronal substrates, resulting in lifelong alterations of endocrine function. Moreover, would have activational effects on the HPA axis in the adult with direct or indirect effects of circulating levels of these hormones. Sex differences in neurodevelopment are the result of both genetic sex and circulating gonadal hormones. The ovary, uterus and placental trophoblast present receptors for CRH, [2] which can lead to a perturbation in the development of the offspring, even if the stress is applied before pregnancy.

5. CONCLUSION

Pre-pregnancy stress can contribute to developmental disorders in offspring by affecting the Hypothalamo-pituitary axis that regulates the secretion of sexual hormones (Testosterone, Progesterone) by the gonads (Testicles, Ovaries).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), revised 1996.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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