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# COMPARISON OF FOLLICLE STIMULATING HORMONE LEVELS IN CHRONIC STRESS RAT MODEL AND THEIR OFFSPRING

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## ABSTRACT

**Background:** Stress is an integral aspect of modern life and has been shown to negatively impact various physiological systems, including the reproductive system. This study was conducted to investigate the transgenerational effects of stress in rats by analyzing and comparing reproductive hormone levels.

**Objective:** To find the effect of chronic alternating stress on rat parents and offsprings by comparing Corticosterone and Follicle Stimulating Hormone Levels.

**Methods:** This study employed an experimental case-control design involving 136 healthy Wistar albino rats, aged 11 weeks in the parent generation at the outset. All animals were subjected to behavioural tests at the start of the experiment to ensure that they did not show stressed behaviour prior to the intervention and subsequently divided into two groups: case parents and control parents. The case group was subjected to a three-week regimen of chronic alternating stress, while the control group was not exposed to any stress. Male and female rats were housed separately before and during the stress period and were allowed to mate only afterward. The offspring of both groups were further divided into multiple experimental groups: one group was assayed at 5 weeks of age without exposure to stress; a second group received early-life stress from weeks 5 to 8; a third group experienced late-life stress from weeks 11 to 14; and a fourth group was subjected to both early- and late-life stress. A control group of offspring was maintained without any stress exposure. The stress protocol administered to the offspring was identical to that used for the parent generation. Behavioural

assessments were performed, and hormonal assays were conducted to evaluate the effect of stress on behaviour and hormones.
**Results:** Rats exposed to early life stress exhibited significantly elevated corticosterone levels ($P < 0.05$) and reduced follicle-stimulating hormone (FSH) levels compared to the rest.
**Conclusion:** Rats subjected solely to early life stress demonstrated comparatively less favourable hormonal profiles than those exposed to both early and late life stress.

**Key Words:** Corticosterone, Follicle Stimulating Hormone, Chronic Alternating Stress.

## INTRODUCTION

Stress has become an omnipresent feature of contemporary life, exerting detrimental effects on multiple physiological systems, including reproductive function. The rising incidence of infertility may, in part, be attributed to increasingly stressful lifestyles. Investigating the transgenerational consequences of stress remains a complex undertaking. Accordingly, this study was designed to evaluate the effects of chronic stress across two successive generations in a rat model.

A stress reaction is initiated by a stressor either physical or psychological that disrupts the body's homeostasis. These stressors act as stimuli, eliciting a stress response that may manifest physiologically or psychologically. This response is mediated through a complex interplay of neurological, endocrine, and immunological systems, primarily involving activation of the sympathetic-adreno-medullary (SAM) axis, the hypothalamic-pituitary-adrenal (HPA) axis, and components of the immune system.[1]The primary function of the stress response is to help the body adapt to disturbances by preparing it to cope with environmental stressors, whether internal or external. For instance, physiological responses to injury or surgery can serve protective roles by minimizing further tissue damage. However, when stressors are perceived as intense or become persistent such as recurrent acute stress or prolonged chronic stress—the stress response may become maladaptive. This maladaptation can adversely affect physiological health and contribute to the development of conditions such as depression, anxiety, cognitive impairment, and cardiovascular disease.[2]

Stress can significantly disrupt hormonal and biochemical balance, pushing levels beyond the normal range required for homeostasis. A common consequence is the elevation of inflammatory mediators and oxidative stress, which are implicated in the development of conditions such as diabetes, polycystic ovary syndrome (PCOS), metabolic syndrome, and morbid obesity. Oxidative stress arises when environmental stressors disrupt the body’s antioxidant defences, allowing reactive oxygen species (ROS) to accumulate unchecked. This imbalance promotes inflammation, thereby contributing to disease progression and increased morbidity. Moreover, chronic stress can impair the hypothalamic-pituitary-gonadal (HPG) axis, resulting in reduced production of sex hormones such as estrogen and testosterone in rats.[3]Chronic activation of the stress axis resulting from repeated stress exposure suppresses the secretion of gonadal steroid hormones. Specifically, stress inhibits the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Elevated corticosterone levels further inhibit the secretion of luteinizing hormone (LH) from the pituitary gland, as well as estradiol and progesterone from the ovaries.[4] Testosterone release from testis is also inhibited.[5] As there is increased sympathetic activity during stress there is release of norepinephrine into the ovary which disrupts the normal process of folliculogenesis and ovulation.[6] Follicle Stimulating Hormone (FSH) is also a gonadotropin secreted by the anterior pituitary gland under the influence of GnRH from the hypothalamus. In females it causes follicle maturation and ovulation while in males it causes the development and maturation of the sperms. It also affects the growth of the reproductive system and pubertal maturation.[7]

Thus, we conducted this research study to find out the transgenerational effect of stress on reproductive hormones like Follicle Stimulating Hormone.

## METHODOLOGY

### Ethical Approval

The study received ethical approval from the Ethical Committee of Khyber Medical University (Reference No: DIR-KMU-EB/HS/000675). Additional ethical approval was obtained from the Institutional Review Board of Peshawar Medical College (Reference No: Prime/IRB/2023-207).

### Study Duration

Two years after the approval of research proposal by Advanced Studies and Research Board (ASRB).

### Study Design

Experimental case control study.

### Study Setting

This study was conducted at the Animal House and Pathology Laboratory of Peshawar Medical College, Peshawar, in collaboration with the Khyber Medical University Laboratory and the Animal Research Laboratory at the University of Agriculture, Peshawar.

### Sampling Technique

Lottery method.

### Sample Size

A total of 136 healthy Wistar rats, aged 11 weeks, were used in the parent generation. The sample size for the offspring was determined using the resource equation method. Each group was allocated 13 rats (156/12), but to account for potential attrition, 14 rats were included per group. Of these, 12 rats per group were recorded for behavioural testing due to the high variability typically observed in such assessments, while 10 rats were designated for blood sampling.[8]

**Experimental Procedure**: The parent generation was divided into two groups: case parents and control parents. The case parents were subjected to a three-week regimen of chronic alternating stress, comprising light-dark cycle reversal, cold water immersion, and restraint stress each stressor applied on separate days in rotation over the three-week period. In contrast, control parents were not exposed to any stress. Following behavioural assessments to confirm stress induction, ten case parents were euthanized for blood sampling and 4 for histological analysis.

Subsequently, both case and control parents were allowed to breed within their respective groups. The resulting offspring were assigned to different experimental conditions based on developmental stage stress exposure: early life stress (initiated at 5 weeks of age), late life stress (initiated at 11 weeks), or a combination of both. Control offspring from both groups were not exposed to any stress. The offspring underwent behavioural testing, hormonal assays, and histological examination of gonadal tissue.

At each experimental endpoint following behavioural testing, blood samples were collected from anesthetized rats via intracardiac puncture[9] which also served as the method of euthanasia. The hormones were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Commercially available ELISA kits were procured from Elabscience, including the corticosterone kit (Catalog No. E-EL-R0269) and the follicle-stimulating hormone (FSH) kit (Catalog No. E-EL-R0391).

Subsequently, the gonads were carefully dissected for analysis. The chronic alternating stress protocol applied in this study has been previously detailed by Khattak et al.,[10], while the behavioural testing procedures are described by Usman et al.,[11] These findings form part of the PhD research project of the first author, with additional publications anticipated from the broader dataset.

The following table shows the color coding, the abbreviations and the explanation of various groups. P stands for parent generation while F1 stands for first filial generation. A stand for case parent group to whom stress was given and the offsprings of case parents. B stands for the control parent to whom no stress was given or the offsprings of control parents. Weeks are represented by wks.

**Table 1: Color coding and explanation of various experimental groups**

| | | |
|---|---|---|
| | P1B | This is the control group of parents to whom no stress was given. |
| | P1A | Case parent group to which stress was given. |
| | F1A | 5 wks old rat who were the offspring of case parents and from whom preliminary parameters were recorded. |
| | F1A1 | Offspring of case parents given early life stress from 5 wks to 8 wks after which they were assayed. |
| | F1A2 | Offspring of case parents to whom stress was given two times once at 5 wks and once at 11 wks. Stress duration was 3 wks each time. They were assayed at 14 wks. |
| | F1A3 | Offspring of case parents to whom stress was given in late life at 11 wks and sacrificed at 14 wks. |
| | CtF1A1 | Case parent offsprings not given any stress and sacrificed at 8 wks. They served as controls. |
| | CtF1A2 | Case parent offspring not given any stress and sacrificed at 14wks. They served as controls. |
| | F1B | 5 wks old rat offspring of control parents from whom preliminary parameters were recorded. |
| | F1B1 | Offsprings of control parents given stress at 5 wks and sacrificed at 8 wks. |
| | F1B2 | Offsprings of control parents given stress twice once at 5 wks and the other at 11 wks. Each stress episode lasted for 3 wks and they were assayed at 14 wks of age. |
| | F1B3 | Offsprings given of control parents given only late life stress at 11 wks and were sacrificed at 14 wks. |
| | CtF1B1 | Offspring of control parents who were not given any stress and served as controls and assayed at 8 wks. |
| | CtF1B2 | Offspring of control parents who were not given any stress and served as controls and assayed at 14 wks. |

**Statistical Analysis**

The data was analyzed by SPSS software version 25. Normality of the data was checked using tests of normality, Kolmogorov-Smirnov and Shapiro-Wilk test. The data showed a non-normal distribution. Kruskal Wallis test was applied for comparison between the groups and $P \leq 0.05$ was taken as a cutoff point for significance. For comparison between two groups Mann-Whitney U test was used.

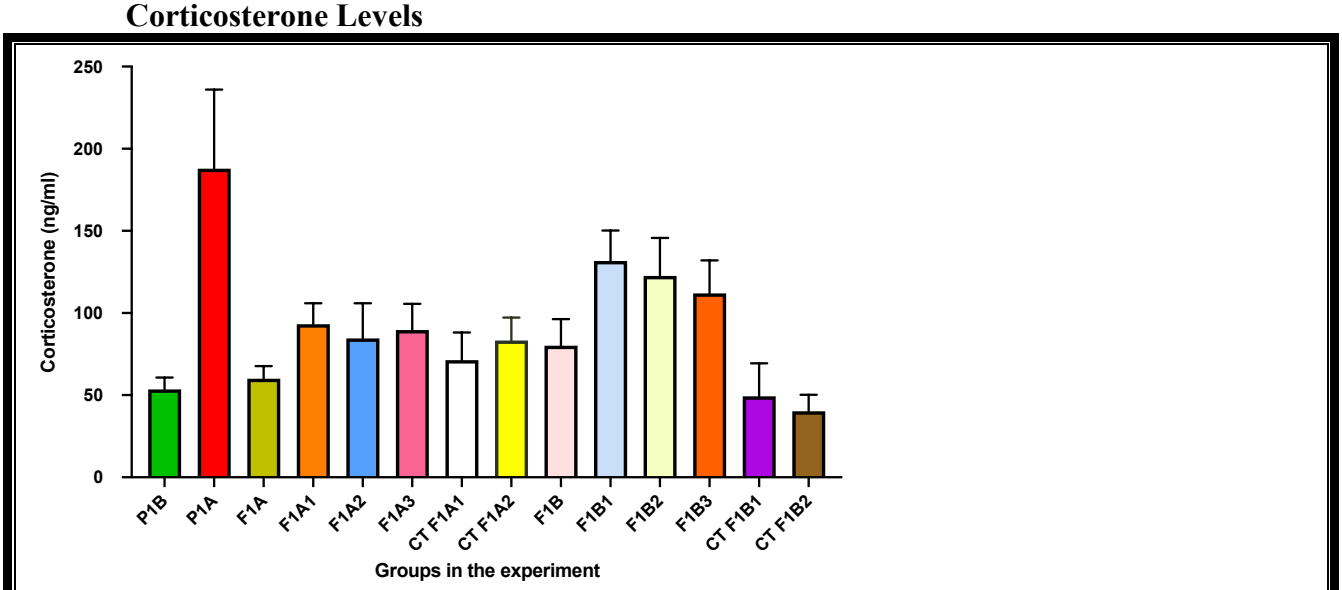


*Figure 1: Comparison of Corticosterone Levels in ng/ml (Mean With SEM) Between all The Groups*

Increased Corticosterone means more stressed. P1A had increased corticosterone levels as compared to P1B (P<0.001) and was more stressed as compared to all the offsprings. Among the offsprings of case parents F1A1 had significantly increased corticosterone level as compared to F1A (P= 0.034)) showing that early life stressed offsprings fared worst against stress. P1B the control parents were significantly less stressed as compared to their stressed offsprings F1B1(P<0.001) and F1B3(P= 0.023) Among the offsprings of control parents F1B1 was the most stressed as shown by increased corticosterone levels as compared to CtF1B1(P= 0.013) and CtF1B2(P= 0.002).When comparing the A group with the B group offsprings F1A1 and F1B1 had significantly increased Corticosterone levels as compared to the rest showing more stress levels. Refer to Figure 1 and Table 2.

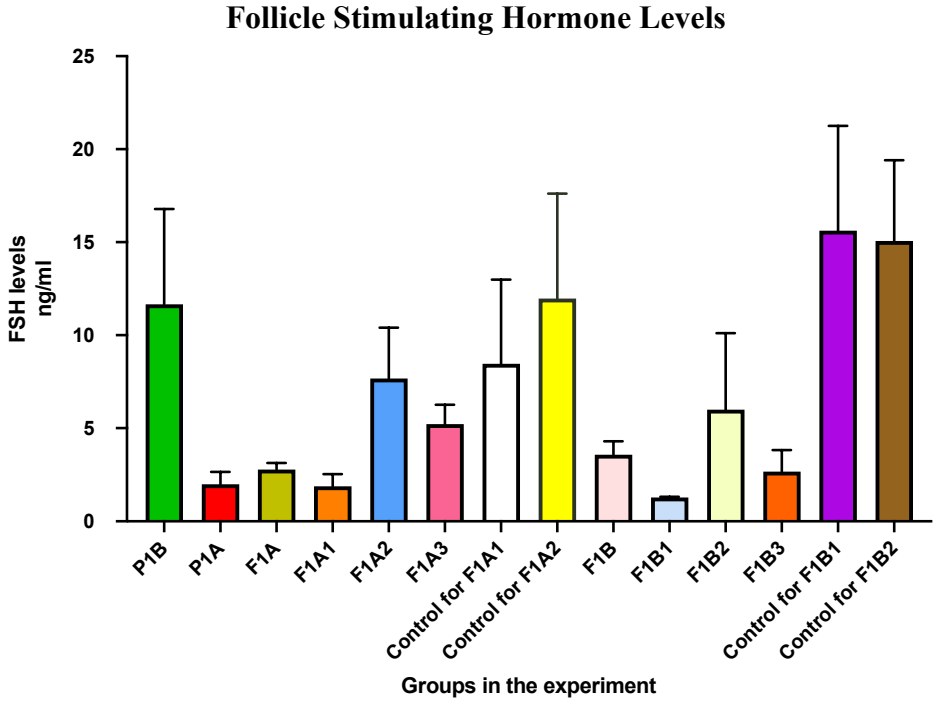


*Figure 2: Comparison of FSH Levels (Mean with SEM) in ng/ml Between all The Groups (Both Gender)*

The stressed parents had decreased FSH levels as compared to its own offspring F1A (P=0.007), F1A2 (P=0.049), F1A3 (P=0.007), CtF1A1 (P=0.010), CtF1A2 (P=0.041). and had decreased FSH levels as compared to all of offsprings of control parents F1B (P=0.016), CtF1B1 (P=0.008), CtF1B2 (P=0.004). P1B has increased levels of FSH as compared to F1B1 (P=0.028) and as compared to F1A1 (P=0.014).

Among the offsprings of case parents the early life stressed offspring i.e F1A1 had decreased FSH levels as compared to Ct F1A2 (P=0.016), F1A3 (P=0.002), F1A2 (P=0.049) and CtF1A1(P=0.004) and F1A (P=0.003) and also as compared to the late life stress given offspring of control parents F1B3 (P=0.023).

Among the offsprings of control parents the early life stressed offspring i.e F1B1 had decreased FSH levels as compared to CtF1B1 (P=0.001), Ct F1B2 (P=0.002), F1B (P=0.001). F1B1 also had decreased FSH levels as compared to the offsprings of case parents F1A2 (P=0.034), F1A3(P=0.001), CtF1A1 (P=0.001) and CtF1A2 (P=0.016).

In both the groups of offsprings the early life stressed offsprings had significantly decreased FSH levels as compared to the controls and as compared to both early and late life stressed offsprings which are F1A2 and F1B2. Refer to Figure 2 and Table 2.

**Table 2: Corticosterone levels and Follicle Stimulating Hormone levels in Experimental groups in Rats.**

| Groups | Corticosterone (ng/ml) | FSH (ng/ml) |
|---|---|---|
| P1B | 53.57±22.55 | 11.67 ± 16.20 |
| P1A | 187.82 ± 152.53 | 1.98 ± 2.10 |
| F1A | 60.06 ± 24.21 | 2.78 ± 1.11 |
| F1A1 | 93.16 ±40.55 | 1.88 ± 2.06 |
| F1A2 | 84.58 ± 67.53 | 7.68 ± 8.61 |
| F1A3 | 89.67 ± 50.36 | 5.23 ± 3.27 |
| Ct F1A1 | 71.33 ± 53.16 | 8.46 ± 14.33 |
| Ct F1A2 | 83.25 ± 43.97 | 11.98 ± 17.84 |
| F1B | 80.11 ±51.16 | 3.58 ± 2.28 |
| F1B1 | 131.70 ± 58.42 | 1.28 ± 0.12 |
| F1B2 | 122.57 ±73.25 | 6.00 ± 13.01 |
| F1B3 | 111.90 ± 63.46 | 2.67 ± 3.69 |
| Ct F1B1 | 49.33 ± 63.17 | 15.63 ± 17.79 |
| CtF1B2 | 40.20 ± 31.86 | 15.07 ± 13.71 |

Values are in Mean ± Standard Deviation

## DISCUSSION

### Corticosterone Levels

The case parent group (P1A) exhibited significantly elevated corticosterone levels compared to the control parent group (P1B). Among the offspring of case parents, the early life stress group (F1A1) showed a markedly higher corticosterone level than the 5 week old offspring before stress induction (F1A), indicating that offspring exposed to early life stress demonstrated the greatest vulnerability. Similarly, among the offspring of control parents, F1B1 (early life stress) had the highest corticosterone levels, followed by F1B2 (late life stress) and F1B3 (combined early and late life stress), whereas both control subgroups had significantly lower corticosterone concentrations Refer to figure 1 and table 2. These findings collectively suggest that early life stress exerts the most detrimental impact on stress response. Our results are consistent with a previous study that exposed 6–7-week-old rats to chronic unpredictable mild stress (CUMS), reporting elevated corticosterone levels as a result.[12]

Another investigation assessed the effect of an antidepressant on rat groups exposed to CUMS and found significantly increased corticosterone levels in the untreated CUMS group.[13]Similarly, Zhang et al. also observed elevated corticosterone levels in rats subjected to chronic unpredictable mild stress.[14]

Contrastingly, a study by Toth et al. did not observe significant differences in circadian corticosterone levels across age groups exposed to chronic mild stress (CMS), suggesting the possibility of resilience development.[15]Additionally, another study reported that social isolation during adolescence (postnatal day 21 to 70) in male mice resulted in reduced basal corticosterone levels but heightened reactivity to novel environmental stressors.[16]

**Follicle Stimulating Hormone Levels**

In our study, the group F1A2 and F1B2 which were given both early and late life stress had increased FSH levels probably because they were given stress two times and when added it was for a total of 6 weeks which was longer than the other offsprings given stress and thus the negative feedback had started working resulting in an increase in FSH. Our results were similar to a study by Fu et al., who gave chronic unpredictable mild stress to rats for 35 days. There was a drop in serum estradiol (E2), anti-Mullerian hormone (AMH), and gonadotropin-releasing hormone (GnRH) levels, accompanied by a rise in follicle-stimulating hormone (FSH) levels. This was similar to our results where the rats given stress twice (F1A2 and F1B2) had increased FSH levels as compared to the rats given stress once in early life.[17]

The levels of FSH were decreased in the parent generation given stress and also in the early life stressed offspring of both groups. There is a lot of evidence suggesting that stress can affect the GnRH hormone that in turn decrease the FSH secretion from the anterior pituitary gland.[18]

In a study of stress on albino rats it was found that giving 10 day stress of combination of restraint and nicotine the FSH levels decreased which were similar to the early life stressed rats of our group which showed decreased levels of FSH in our study.[19]

In another study male rats were immobilized for 4 days ranging from 20 minutes to 6 hours. There was a decrease in FSH and in LH in these rats. Thus short term stress decreased the FSH levels in rats.[20] Our findings were similar to another research which stated that psychological stress decreased FSH levels in men.[21]In another study it was reported that noise stress for 8 weeks given to male rats caused decreased testosterone, LH and FSH in rats.[22]

The results of another study contradicted our results in which immobilization stress of up to 6 hours in rat groups for 2 weeks caused a significant increase in serum FSH levels.[23] The immobilization stress was given for up to 6 hours a day and as it was not alternating with any other stressor so that might be the reason why the rat had developed resilience and the negative feedback pathway had kicked in leading to leading to increased FSH levels due to decreased sex steroid secretion from gonads due to stress.

## CONCLUSION

The hormonal assays show that the early life stressed offspring had elevated levels of corticosterone along with decreased levels of FSH. These hormonal imbalances are less pronounced in the offspring subjected to late life stress, especially those from control parents exposed to both early and late life stress. This suggests that exposure to manageable levels of stress during developmental stages can enhance resilience in adulthood.

**Recommendations**

Further research should aim to elucidate the molecular pathways responsible for the stress-induced changes observed in this study. In addition, conducting similar studies in other species such as guinea pigs, rabbits, or cognitively advanced animals like non-human primates could help confirm and expand upon these findings.

**Limitations of the Study**

A key limitation of this study is the absence of an investigation into the molecular mechanisms responsible for the observed stress responses.

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