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RESEARCH ARTICLE
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COMPARISON OF LUTEINIZING HORMONE LEVELS IN CHRONIC STRESS RAT MODEL AND ITS OFFSPRING

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ABSTRACT

Background: Stress has become a part of our everyday life**.** Stress has an adverse effect on all systems of the body including the reproductive system. This project was designed to study the transgenerational effect of stress on rats by comparing the reproductive hormones.
Objective: To find the effect of chronic alternating stress on parents and offsprings by comparing Corticosterone and Luteinizing Hormone levels.
Methods: This was an experimental case-control trial. We took 136 healthy wistar albino rats. They were 11 weeks old and we assayed them at the start of the experiment. Then we divided them into two groups. The case parents and the control parents. To the case parents we gave 3 weeks of chronic alternating stress and to the controls we did not give any stress. The genders were kept separate before and during the stress after which they were allowed to mate. The offsprings of case and control parents were divided into groups. One group was assayed at 5 weeks before any stress. One group was given early life stress starting at 5 weeks and ending at 8 weeks. One group was given late-life stress starting at 11 weeks and ending at 14 weeks. One group received both early and late life stress. Then there were controls that did not receive any stress. The protocol of stress was the same. We conducted behavioral tests and assayed for hormones.
Results: The early life stressed rats had increased Corticosterone levels (p-value <0.05) while the Luteinizing Hormone levels were decreased in the early life stressed rats.
Conclusion: The rats given both early and late life stress had somewhat better results than the rats given only early life stress.

Key Words: Corticosterone, Luteinizing Hormone, Chronic Alternating Stress.

1. INTRODUCTION

The term “stress” was used for the first time in medical literature by Hans Selye who emphasized that stress was the body’s nonspecific response to any demand.[1]
A stress reaction is triggered by a stressor that can be physical or psychological that disrupts

homeostasis. These stressors behave as stimuli and the reaction of the person which can be physiological or psychological is the stress response. A combination of complex neurological, endocrine and immunological systems encompassing the stimulation of the sympathetic-adreno-medullar (SAM) axis, the hypothalamus-pituitary-adrenal (HPA) axis and the immune system mediates a stress response.[2] The stress response at first tries to adapt the body to the interference, it gets the body ready to face the problems posed by an environmental stressor which may be internal or external. For example, the body's natural reactions to damage or surgery can aid in preventing further tissue destruction. However, the stress response turns maladaptive and harmful to physiology if the stressor whether real or is believed to be intense like recurrent acute stress, chronic stress of prolonged duration, or both. For example, facing stressors of chronic origin can result in maladaptation in the form of diseases like depression, anxiety, cognitive decline and heart disease. Selye also noted that genetics, personality and coping methods affect stress response. Some people are more resilient to stress as compared to others and become habituated to stress.[3]Effect of stress on human body includes reduced fertility[4] menstrual problems[5]erectile dysfunction[6]reduced libido[7] and pregnancy complications.[8]

Luteinizing Hormone (LH) is also called Lutrophin or Lutropin. It is secreted by the anterior pituitary cells under the influence of GnRH. In females there is a surge of LH which causes ovulation and it also cause the development of corpus luteum. In males it acts on the interstitial cells of Leydig and cause the secretion of testosterone.[9]

Chronic stress can alter the function of Hypothalamo Pituitary Gonadal axis, contributing to reduced estrogen and testosterone production in rats.[10] Chronic activation of the stress axis due to repeated stress inhibits the release of gonadal steroids. Stress inhibits the release of GnRH from the hypothalamus. Corticosterone inhibit the release of LH from the pituitary and estradiol and progesterone from the ovary.[11]

Stress impacts reproductive hormones in both sexes. In females, it disrupts the hypothalamic-pituitary-ovarian (HPO) axis through elevated CRH, ACTH, glucocorticoids, and endorphins. These inhibit GnRH pulses and reduce LH/FSH responsiveness, leading to anovulation, impaired steroid production, amenorrhea, and adverse pregnancy outcomes. CRH also directly affects the ovary, suppressing ovarian steroidogenesis, while glucocorticoids reduce LH receptor activity and aromatase function, contributing to estrogen deficiency and follicular atresia. Estrogen shortages can hinder implantation and delay labor.[12] In animal models social stress, high altitude stress, restraint stress and surgery can affect the body weight, levels of testosterone and testicular morphology and male fertility including sexual behaviour and reproduction.[13]

Rationale: Stress to the parents can alter the expression of genes. The offsprings of such parents may have altered physiology. These offsprings might be more vulnerable if they are exposed to the same stress as their parents or they can also be more fit for the same stress compared to control group who is not exposed to stress. Since this study could not be done on humans so we devised a rat model of chronic stress to study this trans- generational effect.

2. MATERIAL AND METHODS:

Study Setting: Animal house, Peshawar Medical College. Animal research lab, Agriculture University, Peshawar. Khyber Medical University, Peshawar.

Study Design: Experimental Case Control Study.

Inclusion Criteria: Healthy Wistar albino rats of required age.

Exclusion Criteria: Unhealthy rats, not of the required age group or having any observable disease or were pregnant were excluded.

Sampling Technique: Lottery Method.

Study Duration: Two years from November 2019 to December 2021.

Ethical Approval: Ethical approval was taken from Ethical Committee of Khyber Medical University (Reference No = DIR-KMU-EB/HS/000675) and from Peshawar Medical College (Reference No=Prime/IRB/2023-207).

Sample Size Calculation:
There were around 136 rats in the parents' group.
Sample size of offspring was calculated by resource equation, E=Total number of rats – Total number of groups E is degree of freedom of analysis of variance, should lie between 10 and 20, Assume 14 rats in a group and there are 12 groups.
E= (14x12) – 12 =168 – 12= 156
In each group, number of rats=156/12=13 which is rounded off to 14.
In each group, 12 rats were required for behavioural tests due to high variability in results of behavioural tests, 10 for blood sampling and 4 for histology. For histology of gonads, 4 male and 4 female samples were taken. Keeping an attrition rate of 10 percent so we took 14 rats in each group.[14]

Experimental Procedure:
A total of 136 adult healthy Wistar rats were taken weighing, 280-300 g; aged 11 weeks that were raised at the Peshawar Medical College Animal House. The rats were housed in cages with 6 rats per cage at 25±2℃temperature in a humidity of 40-60% under a 12-hour light/dark cycle. There was free availability of food (standard laboratory diet and water). Preliminary behavioural tests were carried out on all rats. The rats that were already stressed were removed. 10 out of remaining rats were sacrificed after giving isoflurane anaesthesia through open drop method [15]for baseline values for control parents, P1B group. Blood specimens were collected for Corticosterone, and a few other hormones through intracardiac puncture.[16] Also, gonadal tissue sections were made for histological evaluation from 4 males and 4 females. The remaining rats in the parent generation were divided into case parents; P1A (exposed to chronic stress) and control parents; P1B (unexposed to chronic stress). The case parents group were exposed to chronic stress at 11 weeks of age for three weeks i.e, from 11 to 14 weeks of age. The chronic stressors applied were alteration in circadian rhythm, cold water immersion stress, followed by restraint stress on the third day. We gave one stressor per day and the cycle was repeated for 21 days. The stress protocol is explained in another paper by Khattak et al.,[17] and the behavioural tests are explained by Usman et al.,[18] in another research article. We alternated the stressors to avoid adaptation. Behavioural tests (open field and hole board) were carried out to ensure induction of stress and the rats that were not stressed were removed. In the remaining rats, 10 were sacrificed for the baseline values of case parents, P1A group. The remaining case parents left were allowed to mate. The control parents group were also allowed to mate. We took 14 offsprings in each group. There were 6 case and 6 control offsprings groups respectively so required number of rats in case and control offsprings groups were 14X6=84 respectively and the total number of offsprings required were 84+84=168.
This research work is part of the PhD thesis of the first author and the rest of the hormones and histology results will be published later.

Statistical Analysis:
The data was analysed by SPSS software version 25. Normality of the data was checked using tests of normality, Kolmogorov- Smirnov and Shapiro-Wilk test and came out to be a non-normal distribution. Kruskal Wallis test was applied for comparison between all the groups and Mann Whitney U test was applied for comparison between two groups. $P \leq 0.05$ was taken as a cutoff point for significance. Graphs were made through Graph Pad Prism version 9.1.0 for all the blood markers.

Hormone Levels:
We used ELISA kits for measuring the hormone levels. The graphs for the hormone levels as well as

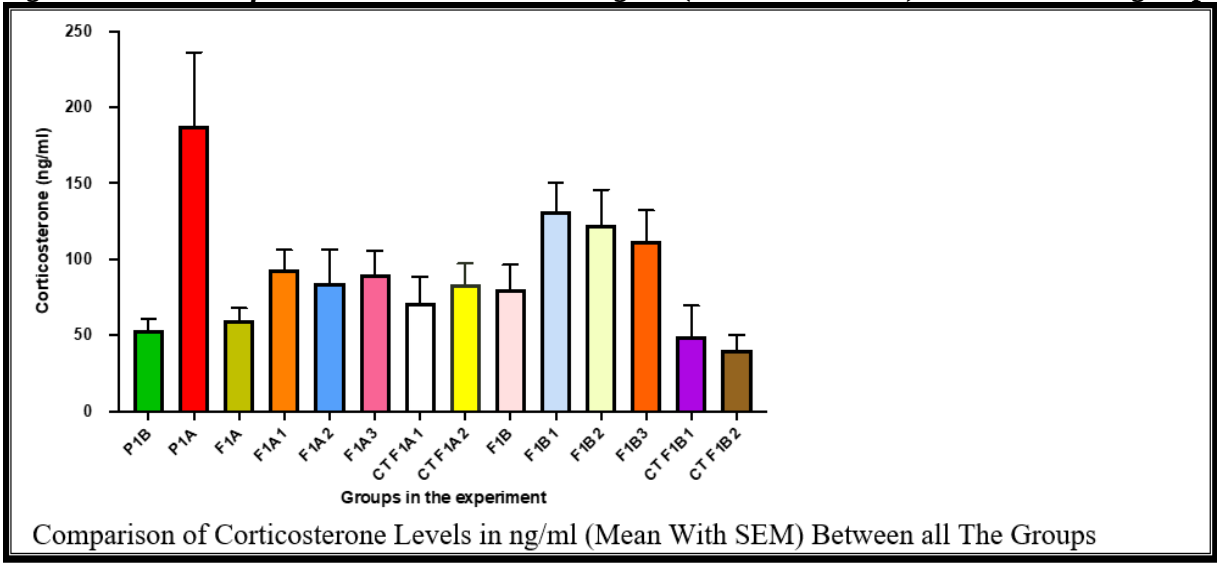
histology parameters measured in the research project were made by Graph Pad Prism and follows the following color coding for each group. P1 stands for first generation of parents, P1A represents the parent generation given stress and P1B is the parent generation not given any stress. F1 stands for the first filial generation of offsprings. The A group of offsprings are the offsprings of stressed parents while the B group are the offsprings of control parents.

Table 1: Color Coding for various Groups for Hormone Levels.

Table: Color Coding for Various Groups for Graphs of Hormone Levels		
	P1B	Negative Control Group or parents not given stress
	P1A	Parents given stress
	F1A	Preliminary tests of 5-week offsprings of case parents
	F1A1	Early life stress given offsprings of case parents
	F1A2	Both early and late life stress given offsprings of case parents
	F1A3	Late life stress given offsprings of case parents
	CtF1A1	Control for early life stress given offsprings of case parents. They were themselves the offsprings of case parents not given any stress.
	CtF1A2	Control for late life stress given offsprings of case parents. They were themselves the offsprings of case parents not given any stress.
	F1B	Preliminary tests of 5-week offsprings of control parents
	F1B1	Early life stress given offsprings of control parents
	F1B2	Both early and late life stress given offsprings of control parents
	F1B3	Late life stress given offsprings of control parents
	CtF1B1	Control for early life stress given offspring of control parents. They themselves were the offsprings of control parents but they were not given any stress.
	CtF1B2	Control for late life stress offsprings of control parents. They themselves were the offsprings of control parents but they were not given stress.

Corticosterone Levels

Figure 1: Results of Corticosterone Levels in ng/ml (Mean with SEM) between all the groups



Summary: P1A had increased corticosterone levels as compared to P1B. Among the offsprings of case parents F1A1 had significantly increased corticosterone level. Among the offsprings of control parents F1B1 was the most stressed as shown by increased corticosterone levels followed by F1B2 and F1B3 while both the controls had significantly decreased corticosterone levels. Thus, the early life stressed offsprings had the worst results.

Table 2: Significant Differences (p-value) among Groups in Corticosterone Levels

Groups	P1A	P1B	F1A	F1A1	F1A2	F1A3	CtF1A1	CtF1A2	F1B	F1B1	F1B2	F1B3	CtF1B1	CtF1B2
P1A		**0.000**	**0.001**	**0.028**	**0.05**	**0.016**	**0.005**	**0.006**	**0.019**	0.326	0.496	0.089	**0.002**	**0.000**
P1B			0.597	**0.019**	0.705	0.082	0.496	0.082	0.290	**0.000**	0.082	**0.023**	0.070	0.199
F1A				**0.034**	0.880	0.140	0.821	0.130	0.734	**0.010**	0.112	**0.041**	**0.034**	0.112
F1A1					0.597	0.623	0.131	0.734	0.290	0.130	0.257	0.496	**0.013**	**0.003**
F1A2						0.650	0.821	0.762	0.705	0.082	0.226	0.364	0.199	0.174
F1A3							0.226	0.880	0.406	0.173	0.326	0.364	**0.023**	**0.016**
CtF1A1								0.130	0.880	**0.041**	0.290	0.151	**0.028**	**0.05**
CtF1A2									0.496	0.082	0.326	0.257	**0.019**	**0.013**
F1B										**0.059**	0.290	0.174	**0.028**	0.070
F1B1											0.820	0.520	**0.013**	**0.002**
F1B2												0.571	0.820	**0.034**
F1B3													**0.049**	**0.016**
CtF1B1														0.597
CtF1B2														

Table 3: Corticosterone Levels in various Experimental Groups	
Groups	**Corticosterone (ng/ml)**
P1B	53.57±22.55
P1A	187.82 ± 152.53
F1A	60.06 ± 24.21
F1A1	93.16 ±40.55
F1A2	84.58 ± 67.53
F1A3	89.67 ± 50.36
Ct F1A1	71.33 ± 53.16
Ct F1A2	83.25 ± 43.97
F1B	80.11 ±51.16
F1B1	131.70 ± 58.42
F1B2	122.57 ±73.25
F1B3	111.90 ± 63.46
Ct F1B1	49.33 ± 63.17
CtF1B2	40.20 ± 31.86

Values are Mean ± Standard Deviation

Luteinizing Hormone In both Male and Female Rats

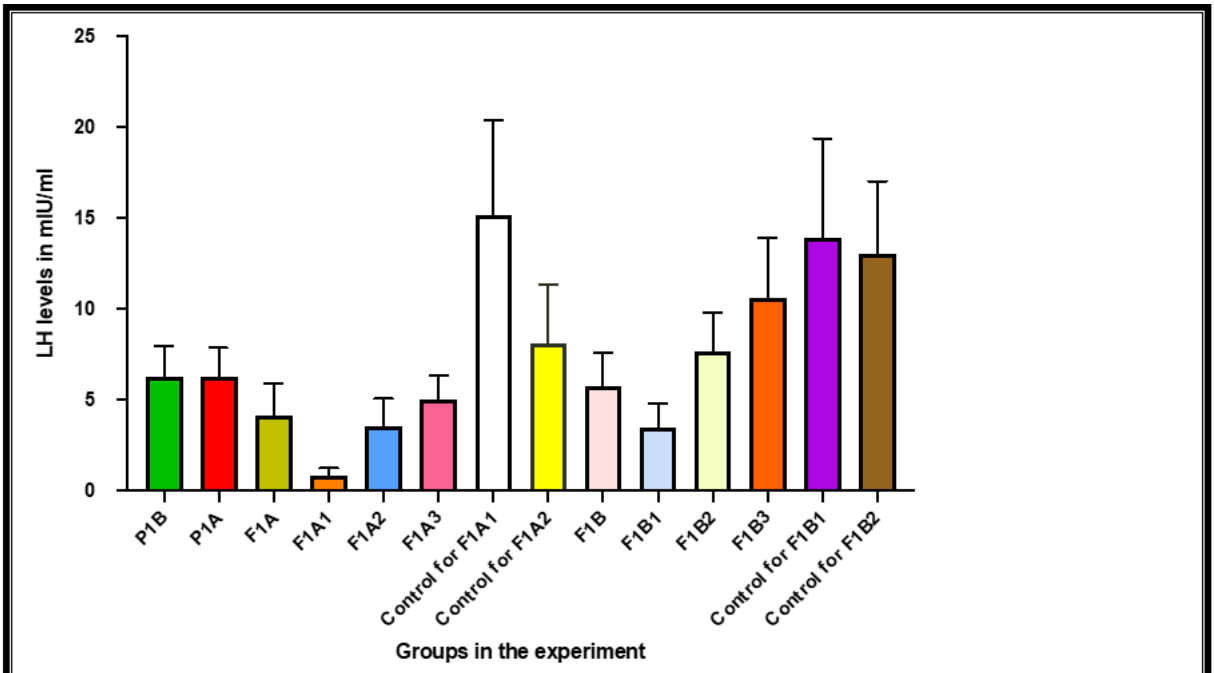


Figure 2: Comparison of LH Levels (Mean with SEM) in mIU/ml Between all The Groups (Both Gender)

Luteinizing Hormone Levels In both Males and Females Summary:

The F1A1 had the least amount of LH as compared to its other offsprings and similarly F1B1 also had the least amount of LH as compared to the control and the late life stressed offsprings. Thus early life stressed offsprings fared the worst when given stress in early life.

Table 4: Significant Differences (p-value) among Groups in LH Levels (Both Gender)														
Groups	P1A	P1 B	F1 A	F1 A1	F1 A2	F1 A3	CtF1 A1	CtF1 A2	F1 B	F1 B1	F1B 2	F1B 3	CtF1 B1	CtF1 B2
P1A		.821	0.131	**0.001**	**0.059**	0.650	0.326	0.762	0.705	0.096	0.597	0.131	0.082	0.762
P1B			0.112	**0.001**	**0.028**	0.406	0.226	0.821	0.364	0.070	0.705	0.257	0.174	0.290
F1A				0.762	0.290	0.406	**0.034**	0.384	0.212	0.257	0.131	0.064	**0.041**	**0.028**
F1A 1					0.089	0.007	**0.002**	**0.034**	**0.002**	**0.041**	**<0.001**	**<0.001**	**<0.001**	**0.034**
F1A 2						0.199	**0.049**	0.364	**0.070**	0.623	**0.049**	**0.026**	**0.019**	**<0.001**
F1A 3							0.151	0.821	0.762	0.257	0.326	0.151	**0.028**	**0.023**
CtF1 A1								0.257	0.199	**0.059**	0.450	0.821	0.821	0.762
CtF1 A2									1.000	**0.406**	0.450	0.273	0.112	0.096
F1B										0.173	**0.406**	0.130	**0.023**	**0.023**
F1B 1											**0.041**	**0.016**	**0.013**	**0.005**
F1B 2												0.597	0.104	0.112
F1B 3													0.545	0.406
CtF1 B1														0.545
CtF1 B2														

Groups	LH (mIU/ml) Both Gender
P1B	6.3 ± 5.17
P1A	6.28 ± 5.01
F1A	4.18 ± 5.49
F1A1	0.88 ± 1.17
F1A2	3.57 ± 4.79
F1A3	5.01 ± 4.26
CtF1A1	15.14 ± 16.66
CtF1A2	8.06 ± 10.36
F1B	5.78 ± 5.87
F1B1	3.48 ± 4.27
F1B2	7.69 ± 6.57
F1B3	10.60 ± 10.52
CtF1B1	13.90 ± 17.33
CtF1B2	13.05 ± 12.62

Values are Mean ± Standard Deviation

DISCUSSION

Corticosterone Levels

P1A had significantly increased corticosterone levels as compared to P1B. Among the offspring of case parents F1A1 had significantly increased corticosterone level and among the offspring of control parents F1B1 was the most stressed as shown by increased corticosterone levels. Thus, here the early life stressed offspring had the worst results. Our research was similar to another study which took 6-7 week rats and subjected them to chronic unpredictable mild stress and reported raised corticosterone levels.[19]In contrast to our finding of significantly raised corticosterone in younger age group exposed to early life stress, Toth et al., did not observe any significant difference between the effects of chronic mild stress (CMS) on circadian corticosterone levels in the different age groups suggesting the development of resilience.[20]

The same age group was researched in a different study by Henry et al., but in that study, stress was given to mothers during pregnancy, and once the pups were born, they were also exposed to stress alongside pups from control rats. According to the study, maternal stress during pregnancy affects the offspring HPA axis reactivity in both the short- and long-term. Male 3- and 21-day-old rats showed that prenatal stress substantially increased plasma corticosterone levels in response to stress. Corticosterone levels in adult control rats started to fall after 120 minutes, while they remained high in prenatally stressed rats at the same time. Corticosterone released by the mother under stress may be a key factor in this behaviour. This hormone could communicate with the growing fetus's central nervous system since it can penetrate the placental and hematoencephalic barriers.[21]

Luteinizing Hormone Levels

The F1A1 had the least amount of LH as compared to its other offsprings and similarly F1B1 also had the least amount of LH as compared to the control and the late life stressed offsprings. Thus early life stressed offsprings fared the worst when given stress in early life. There are a lot of studies which state that stress affected the HPA axis and this axis in turn inhibited the Hypothalamo Pituitary Gonadal (HPG) axis and affected the GnRH pulses leading to decreased secretion of LH in male rats.[22] Contrary to our study, it was noted that when stress was given to the female animal and estrogen levels are sufficient and the HPA axis is activated due to stress, a substantial increase in LH release occurs.[23]However our findings of reduced LH secretion in response to stress are also supported by McCosh et al., who emphasized that different types of stress, such as metabolic stress (hypoglycemia), immune stress, and psychosocial stress, seem to suppress LH secretion through partially distinct neural and endocrine pathways. The suppressive effects of many components of the HPA axis on intermittent LH production have been widely investigated and seem to contribute to some, but not all, stress-induced inhibitory effects.[24]

The early life stressed offsprings had increased corticosterone and reduced LH as compared to the offsprings given late life stress especially the offsprings given both early and late life stress. Thus, some stress within physiological levels is beneficial during the growing age to develop resilience in adult hood.

CONCLUSION

The hormonal assays show that the early life stressed offspring had elevated levels of corticosterone along with decreased levels of LH. 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

Future research should focus on elucidating the molecular mechanisms underlying the observed changes in this study. Additionally, replicating this study in other species such as guinea pigs, rabbits, or more intelligent species like primates could further validate and extend these findings.

Limitations of the Study
One limitation of this study is that it did not investigate the molecular mechanisms underlying the stress responses observed.

Authors Contributions
1. Dr Madiha Khattak
Study Design, lab work, data collection and analysis, manuscript writeup.

2. Dr Omar Malik
Manuscript writeup, idea of study, statistics, critical reading, correspondence.

3. Dr. Robina Usman
Study design, Research work. Statistics, critical thinking.

Conflict of interest: The authors declare that there is no conflict of interests.

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REFERENCES
1. Tan SY, Yip A. Hans Selye (1907–1982): Founder of the stress theory. Singapore Med J. 2018 Apr 1;59(4):170.
2. Mifsud KR, Reul JMHM. Mineralocorticoid and glucocorticoid receptor-mediated control of genomic responses to stress in the brain. Stress Amst Neth. 2018 Sep;21(5):389–402.
3. Chu B, Marwaha K, Sanvictores T, Ayers D. Physiology, Stress Reaction. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2023 Sep 6]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK541120/
4. Chung TKH, Lau TK, Yip ASK, Chiu HFK, Lee DTS. Antepartum Depressive Symptomatology Is Associated With Adverse Obstetric and Neonatal Outcomes: Psychosom Med. 2001 Sep;63(5):830–4.
5. Khattak M, Sultana N, Khattak AF, Usman R, Khattak A. Comparison of Estradiol levels in Polycystic Ovary Syndrome and non-Polycystic Ovary Syndrome Infertile Patients. Adv Basic Med Sci. 2022;6(1):20–3.
6. Kalaitzidou I, Venetikou MS, Konstadinidis K, Artemiadis AK, Chrousos G, Darviri C. Stress management and erectile dysfunction: a pilot comparative study. Andrologia. 2014 Aug;46(6):698– 702.
7. Hamilton LD, Julian AM. The relationship between daily hassles and sexual function in men and women. J Sex Marital Ther. 2014;40(5):379–95.
8. Vrekoussis T, Kalantaridou SN, Mastorakos G, Zoumakis E, Makrigiannakis A, Syrrou M, et al. The role of stress in female reproduction and pregnancy: An update. Ann N Y Acad Sci. 2010;1205:69–75.
9. Ezcurra D, Humaidan P. A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. Reprod Biol Endocrinol RBE. 2014 Oct 3;12:95.
10. Rivier C, Rivest S. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol Reprod. 1991;45(4):523–32.
11. Chrousos GP, Torpy DJ, Gold PW. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med. 1998 Aug 1;129(3):229– 40.
12. Chatterjee A, Chatterjee R. How stress affects female reproduction: An overview. Biomed Res [Internet]. 2009 [cited 2023 Aug 6];20(2). Available from: https://www.alliedacademies.org/abstract/how-stress-affects-female-reproduction-an-overview-1583.html

13. McGrady AV. Effects of psychological stress on male reproduction: a review. Arch Androl. 1984;13(1):1–7.
14. Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. Malays J Med Sci MJMS. 2017 Oct;24(5):101–5.
15. Open-Drop or Nose Cone Method of Isoflurane Anesthesia in Mice and Rats | University of Kentucky Research [Internet]. [cited 2023 Dec 8]. Available from: https://www.research.uky.edu/division-laboratory-animal-resources/open-drop-or-nose-cone- method-isoflurane-anesthesia-mice-and
16. Beeton C, Garcia A, Chandy KG. Drawing blood from rats through the saphenous vein and by cardiac puncture. J Vis Exp JoVE. 2007;(7).
17. Khattak DM, Malik DMO, Usman DR, Habib DSH, Saddique DU. DEVELOPING CHRONIC UNPREDICTABLE/ALTERNATING STRESS MODEL IN WISTAR ALBINO RATS. J Popul Ther Clin Pharmacol. 2023 Dec 7;30(19):223–32.
18. Usman DR, Malik DMO, Khattak DM, Habib DSH, Khan RU. DEVELOPMENT OF PROTOCOL FOR TRANSGENERATIONAL STRESS IN WISTAR RATS. J Popul Ther Clin Pharmacol. 2023 Dec 6;30(19):151–70.
19. Zhou XM, Liu CY, Liu YY, Ma QY, Zhao X, Jiang YM, et al. Xiaoyaosan Alleviates Hippocampal Glutamate-Induced Toxicity in the CUMS Rats via NR2B and PI3K/Akt Signaling Pathway. Front Pharmacol. 2021 Apr 12;12:586788.
20. Toth E, Gersner R, Wilf-Yarkoni A, Raizel H, Dar DE, Richter-Levin G, et al. Age-dependent effects of chronic stress on brain plasticity and depressive behavior. J Neurochem. 2008;107(2):522– 32.
21. Henry C, Kabbaj M, Simon H, Moal M, Maccari S. Prenatal Stress Increases the Hypothalamo-Pituitary-Adrenal Axis Response in Young and Adult Rats. J Neuroendocrinol. 1994 Jun;6(3):341–5.
22. Kirby ED, Geraghty AC, Ubuka T, Bentley GE, Kaufer D. Stress increases putative gonadotropin inhibitory hormone and decreases luteinizing hormone in male rats. Proc Natl Acad Sci U S A. 2009 Jul 7;106(27):11324–9.
23. Minkowicz C. By What Mechanism Does Stress Affect Ovulation?
24. McCosh RB, Breen KM, Kauffman AS. Neural and Endocrine Mechanisms Underlying Stress-Induced Suppression of Pulsatile LH Secretion. Mol Cell Endocrinol. 2019 Dec 1;498:110579.