

## Maturation Assessment of Graffian Follicle in Female Mice under Immobilization, Hunger and Noise Stress

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### ABSTRACT

**Objective:** To evaluate the effects of immobilization, noise and hunger stress on the graffian follicle diameter.

**Study Design:** Laboratory based experimental study.

**Duration and Place of Study:** Department of Anatomy & Pathology, Army Medical College Rawalpindi Pakistan, with the collaboration of National Institute of Health (NIH), Islamabad Pakistan, from Dec 2013 to Dec 2014.

**Methodology:** Forty (n=40) adult BALBc female mice, having an average weight of 25-27gms. They were all in the pro-estrous phase. All were equally divided into four groups each containing 10mice. Group-A, was marked as control and kept for one month in standard atmosphere of animal house. Group-B was under immobilization stress for 06hrs/day by restricting them in locally made restrainers having 10 compartments each. Group-C was exposed to 100 decibels of noise stress of for 06hrs daily. Group-D was given hunger stress by diet control to average 3g of commercially pelleted food daily, which is around 50% of ad libitum food intake. Dissection of Animals was done after one month. For histological study ovaries were treated, fixed and stained. Graffian follicle diameter was measured. SPSS version 20 was used for statistical analysis.

**Results:** mean values of graffian follicle diameters. Group-A showed statistically significant difference when compared with groups B ( $p$ -value <0.001) and D ( $p$  <0.005), however Group-C was insignificant ( $p$ =0.649). The comparison of Groups B with C ( $p$ -value <0.001) and D ( $p$ =0.039) showed statistically significant and Group-C and Group-D also had statistically significant difference as  $p$ -value <0.001.

**Conclusion:** Diameter of graffian follicle was reduced by stress.

**Keywords:** Graffian follicle, Hunger, Immobilization, Mice, Noise, Ovary, Stress.

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### INTRODUCTION

Stress encompasses the biological events which are not precise and are basically the result of the procedures which overturn the homeostasis and rigorously undermine the physical and mental health of individual. The processes of Homeostasis in the entire period were faced by inner and outer forces which are adversative and called as stressful stimuli.<sup>1</sup>

Stress is involved in the communication of brain as a major organ with other body systems through endocrine and neural mechanisms.<sup>2</sup> The major contributors of physiological stress response are ANS and HPA axis activation. ANS is liable for more acute responses and HPA axis is slow in activation and has prolonged period of action. The stress has two phases, charge phase and discharge phase. Both are of same magnitude and charge phase is followed by discharge phase. Pre-activation homeostasis is then again estab-

lished and stress is resolved. Gathering of chronic stress with harmful consequences is the result due to failure of acute stress response.<sup>3</sup>

The response of stress turns to be negative when the exposure to stressors becomes too extended or serious. In response hypothalamic-pituitary-adrenal (HPA) axis and symptho-adreno-medullary system triggers and, the amount of activation be determined by its intensity, form and duration.<sup>4</sup> Long-lasting stress is a chief reason of anovulation that will ultimately leads to infertility due to decreased levels of gonadotrophic hormones. High level of stress perception is the leading cause of early menopause, delivery of preterm and low birth weight babies, severe premenstrual pain and postpartum phycosis.<sup>5</sup> It has been observed with great concern that anxiety, stress, aggression and worry have been increased manifolds in recent years. Major shift from infectious diseases to neuropsychological disorders are due to rapid speed of urbanization and unavailability of sources specially in emerging countries. Clinical results prove that stress causes health issues and reproductive compromise.

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World Health Organization (WHO) has recognized that stress is the major causative factor that caused maximum global disease burden by 2020.<sup>6</sup>

Immobilization is a blend of physical and psychological stressors that is not only responsible for constraint of mobility but also leads to isolation of the individual and earlier researches used all these types of stresses.<sup>7</sup> This stress causes non-maturation of follicles by prolonging the pro-estrous phase leading to irregular reproductive cycle which causes various morphological, physiological and histological changes in the ovary.<sup>8</sup>

Noise is one of the most common and intoxicating environmental pollutant. Apart from its effects on the auditory efficiency, with time it also reduces the multiplicative capability and had negative effect in mice embryos that are subjected continuous noise stress. Perturbs endocrine and cardiovascular system.<sup>9</sup>

Nutritional status has marked effects on the growth and development of follicles.<sup>10</sup> All living species have to cope up with problem of prolonged hunger. It has many metabolic and structural impacts. In the episode of hunger, cell division decreases causing lengthy cycles of cell multiplication and capturing few cells at G1 stage.<sup>11</sup> So stressors like immobilization, noise and hunger stress compromise reproductive health and organs through the involvement of hypothalamic, pituitary, ovarian and axis.<sup>12</sup>

## METHODOLOGY

This experimental study was performed at the Department of Anatomy & Pathology, Army Medical College, Rawalpindi Pakistan, in collaboration with National Institute of Health (NIH), Islamabad Pakistan, from December 2013 to December 2014. The experiment was carried out with the permission of ethical committee, Center for Research in Experimental and Applied Medicine (CREAM) of the Army Medical College, Rawalpindi (Ltr no: 02/CREAM-A).

**Inclusion Criteria:** Forty, non-pregnant female BALB /c mice 5-7 weeks old, with an average of 25-27 grams in the pro-estrous phase of estrous cycle were included.

**Exclusion Criteria:** Pregnant mice and with obvious deformity were excluded from the study.

Animals were divided into four groups, Group-A was labeled as control and were kept in Standardized conditions of animal house. The experimental Group-B was under immobilization stress by placing them in market made restrainer having 10 compartments for

6hrs/day for 1-month.<sup>13</sup> The dimension of each compartment was 1.5" (breadth) x 3" (length) x 2" (height).<sup>14</sup> Animals of Group-B were exposed to 100dB of pure tone noise (purchased from local market) for 6hr/day for 1-month. Power supply was maintained with DC adopter and standby battery of nine volts for nonstop power supply. To measure and maintain the intensity of noise sound level decibel meter was used. Sound intensity was measured with sound level meter placed outside the cages (Radioshack analogue model 33-4050) and it was recorded both at the start and end of the experiment. Group-C was exposed to hunger stress by restricting the commercially pelleted food to average 3g (about 50% of ad libitum food intake) per day for 1-month. Animals were sacrificed and dissected by the end of 1-month. To maintain uniformity right ovary of each specimen was weighed. Ovaries were placed in duly labeled tissue tek cassettes in 10% formalin. Paraffin wax with melting point 58°C was used for infiltration and embedding. The blocks were permitted to harden on cold plate. Rotary microtome was used to obtain cross sections of 5µm thickness. For routine histological study staining of section was done with hematoxylin and eosin (H&E) and diameter of graafian follicle was measured. Three slides per specimen was observed. Diameter (µm) of graafian follicles was measured using X10 objective. Maximum transverse and vertical diameters were measured for each graafian follicle. Vertical diameter at maximum was taken parallel to long axis and transverse diameter at maximum was taken right angle to the vertical one. Mean value of both diameters was taken in coronal section which results in transversal diameter of the graafian follicle. Largest and smallest diameters of each graafian follicle was measured then mean of all graafian follicles were calculated.

Data was analyzed by using Statistical Package for the social sciences (SPSS) version 20 Quantitative variable was said as mean and standard deviation. One way analysis of variance (ANOVA) followed by post Hoc Tuckey was used to determine significant difference. Results were considered as statistically significant when  $p$ -value was  $\leq 0.05$ .

## RESULTS

Forty (n=40) adult BALBc female mice, having an average weight of 25-27gms were included in the study. In all the stressors Group-B (immobilization stress), C (noise stress) and D (hunger stress) decreased the mean diameter of graafian follicles as compared to control Group-A. Microscopically, graafian follicles are

labeled as having a large antrum with cummulus cells surrounding peripheral oocyte and several layers of granulosa cells. Group-B average diameter of graafian follicle was 278.11±8.78 as shown in Table-I. Average diameter of graafian follicle was 320.56±6.02 in Group-C and Group-D 291.44±5.50 which are reduced as compared to Group-A.

**Table-I: Mean values of diameter of graafian follicles in control group A, experimental groups B, C and D (n=40)**

	Group-A	Group-B	Group-C	Group-D	p-value
	Mean±SD (n=10)				
Diameter of graafian follicle (µm)	326.11±116.11	278.11±8.78	320.56±6.02	291.44±5.50	<0.001

Table-II shows an intergroup comparison of mean values of graafian follicle diameters. Group-A showed statistically significant difference when compared with Groups-B (*p*-value <0.001) and D (*p* <0.005), however Group-C was insignificant (*p*=0.649). The comparison of groups-B with C (*p*-value <0.001) and D (*p*=0.039) showed statistically significant and Group-C and Group-D also had statistically significant difference as *p*-value <0.001.

**Table-II: Shows comparison of diameter of graafian follicles among groups A, B, C and D (n=40)**

Parameter	Group Comparison					
	Group A vs. B	Group A vs. C	Group A vs. D	Group B vs. C	Group B vs. D	Group C vs. D
Diameter of graafian follicles	<0.001	0.647	<0.001	<0.001	0.039	<0.001

## DISCUSSION

The stressful circumstances lead to various physiological and psychological changes in maintaining optimum balance in internal environment of the body and are a constant risk to the normal health and happiness. It is likely to continue increasing in magnitude and severity. This is fortified by demanding civilization, change in life style, advancement in industrialization increase in population and urbanization. Numbers of pathologies like high blood pressure/sugar, reproductive impairment, psychosis, low immunity, peptic ulcer are due to involvement of the endocrine and central nervous system.<sup>10,11</sup> Over the past, experimentation is suggestive of the fact that, stressors interfere follicular phase of estrous cycle resulting in reduced fertility rate. Hypothalamo pituitary adrenal axis is activated by acute stressors like immobilization, electric shock, heat, transport, and

hypoglycemia. It was expected that, the pulsatility of GnRHrLH was sluggish and insufficient for the development of growing follicles which required quicker pulse rates. As a result, estrous cycles and anestrus phase was not maintained.<sup>12</sup>

Regarding graafian follicles, the results were statistically significant in all the groups. There was significant reduction in the count of graafian follicles when exposed to Immobilization stress and same when compared with control and experimental groups C & D. The results showed that the histological picture of ovary of Group-C appeared nearly like control. A study on the effect of restraint stress on the oocyte developmental potential suggested the compensatory activation of HPA axis which may lead to impairment of oocyte development and its competence.<sup>15</sup>

LH and FSH receptors are present on Pre-antral and antral follicles and any variation of gonadotrophin levels as a result of stress causes deterioration of growing follicles. Since FSH is vital hormone for the wellbeing of healthy graafian follicles.<sup>16</sup> The percentage of primordial follicles as well as increase in number of growing follicles was observed due to increase uptake of food in cattle. The percentage of graafian follicles were found lower in a study exposed to 6 hr/day of maternal separation to 15 days old female rat for 07 days. In line with the previous literature, it was suggested that events related to stress during the follicular stage of the estrous cycle disturb the ovarian function and as a result reduce the rate of ovulation.<sup>17</sup>

Diameter of graafian follicle was significantly different in all the groups. Healthy diet has a positive effect on growth and size of preantral and antral follicles. Variety of hormones within the hypothalamus-pituitary-ovarian axis are influenced by food intake.<sup>18</sup> The relationship of oocyte developmental competence and dietary intake has been emphasized in recent studies.

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## CONCLUSION

Immobilization, noise and hunger stress in female mice reduces the diameter of graafian follicle.

**Conflict of Interest:** None.

**Author's Contribution:**

Following authors have made substantial contributions to the manuscript as under:

MY & AA: Study design, drafting the manuscript, concept, approval of the final version to be published.

KN & HA: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

HK & SM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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