COUNT VARIATION OF OVARIAN FOLLICLES AFTER IMMOBILIZATION

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ABSTRACT

Objective: To observe the effects of immobilization stress on the ovarian follicle count.

Study Design: It was laboratory based randomized control trial.

Place and Duration of Study: Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from April to May 2014.

Material and Method: Study comprised of 20 adult female mice (BALBc strain) weighing 25-27 gms on first day of estrous cycle. They were divided into two equal groups of 10 mice each. Group A, taken as control, was kept in normal environment of animal house for one month. Group B was given immobilization stress by keeping them in wire mesh restrainers locally fabricated consisting of 10 compartments. Animals were dissected after one month. Size, shape, color and weight of the right ovary were observed. Ovary was processed, embedded and stained for histological study. Primary, secondary and tertiary follicles were counted. SPSS 20 was used for statistical analysis. Independent sample's t test and chi square test was applied.

Results: The primary, secondary and tertiary follicular count was significantly decreased in the ovary of experimental Group B.

Conclusion: Immobilization stress reduces the number of ovarian follicles.

Keywords: Immobilization, Ovarian follicles, Ovary.

INTRODUCTION

According to Advanced Learners Dictionary of English stress is a physical or mental state which suppresses the homeostasis and severely undermines the individual mental or physical health[1]. Homeostasis process is all the time being opposed by external and internal forces which are adverse and are known as stressful stimuli[2]. Acute stress is the result of physical, emotional or psychological threat which is of immediate nature. The threat can be either real or unreal. Acute stress is mainly triggered by the activation of autonomic nervous system (ANS) which constitutes parasympathetic and sympathetic nervous system. Both the divisions work in opposite direction. Stress is resolved if normal homeostatic condition is restored due the stimulation of ANS and stress is considered chronic if body is unable to fall back to the pre stress level[3]. Nowadays it has been turned into an essential part of the human condition all over the world and all individuals are likely to face stressful conditions in daily routine life which acutely agitates the psychological and physiological homeostasis[4]. It produces significant endocrinological, social, immunological and neurobiological effects[5].

The body initially endeavors to cope up by all possible ways including the triggering of autonomic nervous system in order to maintain the homeostasis primarily by the release of different hormones. Ultimately the stress response turns to maladaptive when the exposure to different types of stress becomes too prolonged or critical. Stress triggers sympatho-adrenomedullary system and hypothalamic-pituitary-adrenal (HPA) axis, the degree of triggering depends on its type, intensity and duration[6]. Chronic stress is a major reason for anovulation which finally results in infertility because of down regulation of gonadotrophic hormones. High stress perception is causative factor for early onset of menopause, severe premenstrual pain, preterm delivery and low birth weight, as well as postpartum depression[7].

Immobilization stress is a combination of psychological and physical stressors that causes

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Received: 05 Nov 2014; revised received: 05 Dec 2014; accepted: 31 Dec 2014

restriction of mobility and segregation of the individual[8].Such kind of stress inducers have been previously used by researches[9].In mice this stress increases the pro-esterous phase that causes the non-maturation of follicles leading to irregularities in the estrous cycle which involve physiological, morphological and histological changes within the ovary[4]. The rationale of this study was to observe the effects of immobilization stress on the number of ovarian follicles count.

Table-1: Comparison of tertiary follicles between the groups.

Tertiary Follicles	Control Group (A)	Experimental Group (B)
0	1 (10%)	1 (10%)
1	1 (10%)	9 (90%)
2	4 (40%)	0 (0%)
3	4 (40%)	0 (0%)

p<0.001

MATERIAL AND METHODS

The experimental trial was conducted at the Anatomy Department, Army Medical College Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from April to May 2014. Twenty adult, non-pregnant female mice (BALB/c strain) 5-7 weeks old weighing 25-27grams were used for the study. They were kept in the NIH animal house. Animals were divided into two groups and were fed with NIH laboratory diet for one month. Animals in Group A served as control and were kept in normal environment of animal house for one month. The experimental Group B was given immobilization stress by keeping them in locally fabricated wire mesh restrainer consisting of 10 compartments. Each compartment had 3" (length) x 1.5" (breadth) x 2" (height) dimension[10]. Mice were stressed individually by placing within the restrainer for 6 hours/day for 1 month[11]. At the end of one month, the animals were sacrificed and dissected. The right ovary of each specimen was weighed. Its shape, color and size was noted. Ovaries were placed in 10 percent formalin. For infiltration and embedding paraffin wax with melting point 58ºC was used. The blocks were allowed to solidify on cold plate.Cross sections of 5µm thickness were obtained by using rotary microtome[12].The staining of section was done with hematoxylin and eosin (H&E) for routine histological study of ovary.Primary, secondary and tertiary follicles were counted and noted. Three slides per specimen was observed[13]. 10X objective was used for the counting of follicles. The morphology of follicle was noted according to the following classification[14]. Primary follicle (FI) as an oocyte completely surrounded by a single/double layer of cuboidal epithelium with zona pellucida in between. Secondary follicle (FII) (an oocyte surrounded by more than one layer of cuboidal cells but with antrum andzonapellucida in between). Graafian follicle (FIII) (an oocyte surrounded by zonapellucida, stratified layers of granulosa cells and a large antrum).

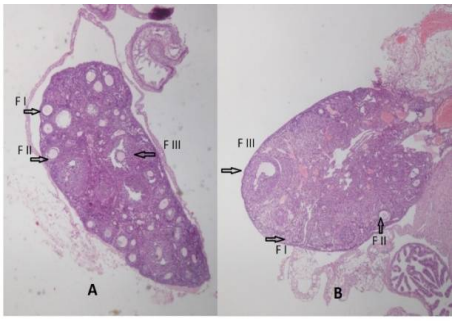

Figure-1: Photomicrograph at (H&E x 100) showing difference in number of ovarian follicles between control group A and experimental group B. Arrow heads showing FI (Primary follicle), FII (Secondary Follicle) and FIII (Tertiary follicle).

Data wasanalysed by using SPSS 20. Descriptive statistics was used to describe the results. Independent sample's t test was applied for comparisons of primary and secondary follicles. Results were considered significant at $p<0.05$. Chi square test was applied for comparision of tertiary follicles and represented by frequency and percentage.

RESULTS

The ovaries of control group A were ellipsoid, pale whitish incolor and firm in consistency with a beaded appearance.Mean weight of the ovary in control group A was 0.0185 gm (SD=0.000527) and in experimental group B it was 0.0177 gm (SD=0.000483). Control group A had significantly higher ovary weight (p=0.002). Histologically,the ovarian surface was covered with simple cuboidal epithelium. Underneath the epithelium there was tunica albuginea which consisted of collagen fibers. Cortex contained many follicles at various stages of development and medulla consisted of dense fibrous connective tissue stroma with vessels. Most of the ovaries of experimental group B were oval and smaller in size than the control group A. There was slight disruption of germinal epithelium and vacuoles were observed in tunica albuginea. Reducednumber of ovarian follicles was noted in the cortex and the dense fibrous stromal tissue of the medulla was filled with the congested vessels. No adhesion and inflammation was observed.

Mean number of primary follicles in control group A was 12.5 (SD=2.37), in experimental group B it was 8.9 (SD = 1.20) with significant difference ($p < 0.001$). Mean number of secondary follicles in control group A was 6.70 (SD=1.42), in experimental group B it was 4.60 (SD=0.843) and the difference was significant (p= 0.001) (fig-1). The number of tertiary follicles was less in experimental group B when compared with control group A as shown in table-1.

DISCUSSION

Stress has been claimed to be involved in the pathogenesis of number of diseases which covers peptic ulcers, anxiety, diabetes, hypertension, immunosuppression and impairment of reproductive system due to involvement of the endocrine and central nervous system. The present study showed decrease in size of ovaries of experimental group B. Shape of ovary was same as in control group A and were firm in consistency. Decrease

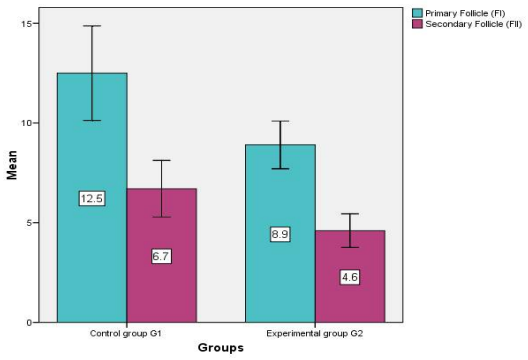

Figure-2: Comparison of primary & secondary follicles between the groups.

in the number of primary, secondary and tertiary follicles in group B was noted when compared with the control group A. Previous experimental studies revealed suppression of follicular development in ovary due to effect of various types of stress. Marked variations in the number of growing follicles in the ovary were seen which indicate compromised ovarian function, as follicle count gives a reliable information about the function of the ovary[15]. Shimiz observed that due to stress, function of follicular granulosa cells is inhibited and there is suppressed follicular development[16]. Studies conducted on rodents, cattle and sheep have also shown the resulting decrease in number of healthy follicles and increase in atretic follicles. Mechanism involved in altered follicular count due to stress is best understood by the fact that follicular recruitment and growth depends on gonadotrophins, Leutinizing hormone (LH) and Follicle stimulating hormone (FSH) from the

anterior pitutary[17]. Any change in the secretion of these hormones signifies the final sequence in neuroendocrine pathway leading to reduced reproductive ability.

CONCLUSION

Immobilization stressreduces the number of primary, secondary and tertiary ovarian follicles.

CONFLICT OF INTEREST

Study was partially funded by National University of Sciences And Technology Islamabad.

ACKNOWLEDGEMENT

Special thanks to Miss Irum Abid for assistance in statistical analysis.

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