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# Deranged Spermatogenesis of Adult Swiss Albino Mice as Effect of Immobilization Stress -Histological Study.

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***Abstract :*** *As the stress is increasing in our life day by day, so we decided to study weather increased stress is one of the causes of infertility. We used immobilisation as stress inducer in our study. Immobilization is mixed type of stress. It acts as physical and psychological stress. The aim of study was to study effect of immobilization stress on spermatogenesis of mice. The present study was performed on 40 adult male Swiss albino mice, out of which 20 served as control and 20 were exposed to immobilization stress .Experimental mice were immobilized by keeping in white transparent plastic jar with 5 holes for 4 hrs a day for 60 days .Food and water provided ad libitum during period of immobilization stress. After two months of immobilization stress mice of both control and experimental group were sacrificed by giving thiopentone anaeshthesia. Testes were removed and labelled. Histological processing of testis tissues was done. Morphological, histological and histomorphometric study of testes revealed suppression of spermatogenesis.*

***Keywords*** *– Stress, mice, immobilization, spermatogenesis, testis*

## I. INTRODUCTION

In modern era of urbanisation stress has became inevitable part of human life. It affects all age groups and both sexes .Stress affects all systems of body including reproductive system. Cannon (1990) was one of the first research workers who postulated that events disrupting homeostasis are stressful and may result in disease. Reproductive failure (infertility) in human newly married couples is becoming an ever rising problem now days. Increased stress because of urbanisation has been pointed out as the causative factor but there is very little documentation on this account. Immobilisation is most commonly used by other workers as stress inducer in (Bajkova 1988 Bharihoke et al 2000) and is less harmful to animals and it is mixed type of stress. So in order to study whether stress of urbanisation is one of the causes affecting process spermatogenesis and to find out structural and functional changes in testis by this stress, we decided to study effect of immobilisation stress on spermatogenesis of mice.

## II. Materials And Method:

The study was carried out on 40 male Swiss albino mice. The animals were divided into two groups of 20 each Group A: Served as control.Group B: Mice of this group were immobilized by keeping them into transparent plastic jars with 5holes for 4hrs a day for 60 days as shown in figure 1All the animals were sacrificed after two months. The testes were preserved in 10% Formal saline. Histological processing of tissues was done as described by Drury RA and Wallington EA (1980). H&E staining was done. Morphological, histological and histomorphometric study was carried out.

## III. Observations:

Reduction in weight of mice of experimental group was observed as compared with control as shown in Table1.Reduction in weight of testes of experimental group of mice were observed as shown in Table-2. Disorganised germinal epithelium in seminiferous tubules of experimental group compared with control Increase in spaces between adjacent seminiferous tubules and deformed seminiferous tubules. Reduced spermatids & spermatozoon in lumen of most of seminiferous tubules compared with control, reduced pachytene stage of primary spermatocytes, reduced height of germinal epithelium of seminiferous tubules (Table 3, 4)and reduced number of leydig cells in testes of experimental mice compare to control as shown in fig. 2,3,4,5

## IV. Discussion

There were numerous studies showing effect of stress on human health. It also affects reproductive system. We used immobilization as stress inducer in our study. Immobilization is mixed type of stress. It acts as physical and psychological stress. Initial weights of mice in control and experimental groups were in range of (40.000±2) gm.After 60 days of immobilisation stress we measured weight of mice and weight of testes after dissection of mice. Control group of mice showed gain in weight but in experimental mice we found reduction in weight. The reduction in weight of experimental mice may be due to reduced food intake or anorexia as an effect of immobilisation stress. Similarly we found reduction in weight of testes in experimental mice compare to control. Rai J et al (2003) found reduction in weight of mice and of testes in experimental group of rat which is similar to our study. In H & E sections of testes were observed. We found lumen of seminiferous tubules of testes of experimental mice shows reduced density of spermatozoa as compare to control group as shown in figure2,3 .Germ cells at different stages of division and maturity were arranged regularly in seminiferous tubules of control group mice with no spaces between different stages of maturating germ cell as seen in compare to experimental mice. The cells of germinal epithelium also show disorganised arrangement in experimental group. This suggest disturbed milieu in interior of tubules and suppression of process of spermatogenesis in experimental mice. The primary spermatocytes were very few in experimental group of testes as compare to control group. Pachytene stages of primary spermatocytes were also reduced in experimental group compare to control group. We counted pachytene spermatocytes in experimental and control group. On histological observation we found reduction in pachytene spermatocytes in experimental group as compare to control group. After statistical test we found significant difference in pachytene spermatocytes count of experimental group as compare to control group. In experimental group we observed that secondary spermatocytes, spermatids were markedly reduced in number .The elongated spermatids were much less in number. This suggests arrest in division of primary spermatocytes in experimental group as compare to control group. We also found reduced leydig cells in experimental group compare to control on histological observations.

Rai J et al (2003) reported that stress causes reduction in size and weight of testes as well as marked suppression of spermatogenesis and spermatogenesis suppression was observed at all the stages of cell division and maturity. They said that reason behind suppression of spermatogenesis is the restraint which is a potent stimulus inducing depression of hypothalamus-pituitary-testis axis as also and this depression of hypothalamus-pituitary-testis axis mediated by activated hypothalamus pituitary-adrenocortical axis, results in fall in plasma LH and testosterone levels *(*Norman &Smith, 1992*)* and we are also of same opinion. Parisa Tavakoli et al (2012) observed deformed seminiferous tubules, reduced cellular concentration, and decreased number of spermatocytes, spermatids and spermatozoa in restrained rats compared to control animals similar to our study.
Knol (1991)) proposed that stressors generally induce depression of hypothalamus-pituitary-testis system, mediated by activated hypothalamic-pituitary-adrenocortical system, resulting in fall in plasma LH and testosterone levels. CRH induces the release of endogenous opioids from hypothalamus, which along with corticosteroids suppresses the secretion of hypothalamic gonadotropin releasing hormone (GNRH). Suppression in secretion of GNRH causes reduced secretion of LH & FSH from pituitary, which in turn causes decrease in testosterone level and spermatogenesis.We found heights of germinal epithelium lining seminiferous tubules of experimental group were less than control group on histological observation and also on histomorphometricaly we found significant difference in mean heights of germinal epithelium of experimental group compare to control group of mice. Reduction in height of germinal epithelium produced due to reduced number of primary spermatocytes, spermatids and spermatozoa as observed by histology and histomorphometric study of testes.

## V. Conclusion

Although we have not studied the hormonal level directly in our study, disorganised germinal epithelium of seminiferous tubule, reduction in height of germinal epithelium, reduced leydig cell as seen on histological observations, decreased in number of pachytene spermatocytes count, suggest that there is suppression of hypothalamic pituitary testicular system in albino mice if exposed to immobilisation stress, affecting process of spermatogenesis in mice. So from our above observations we concluded that immobilisation stress for 60 days period (4hr/day) caused deranged spermatogenesis in Swiss albino mice.

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**Table 1: showing observed values of weights of mice in control and experimental group after 60 days of immobilisation stress (in gm)**

| S.No | Control | Experiment |
|---|---|---|
| Mean | 44.084 | 36.039 |
| SD | 1.4000 | 1.2661 |
| t(19.06042) , p<0.05 | | |

**Table 2: Mean weight of testes in control and experimental group of mice (in mg)**

| S.No | control | experiment |
|---|---|---|
| Mean value | 79.12 | 64.22 |
| SD | 3.6189 | 2.0522 |
| t value(16.01689) P <0.05 | | |

**Table 3: showing observed values of heights of germinal epithelium in control and experimental group of mice.**

| S.No | Control | Experimental |
|---|---|---|
| Mean | 15.78x3.33=52.54µm | 12.27x3.33=40.85µm |
| SD | 2.8980 | 1.6112 |
| df-38, t value(15.76683),p<0.05 | | |

**Table 4: showing observed values of pachytene stage of primary spermatocytes count per unit area testis of control and experimental group of mice**.

| S.No | Control | Experiment |
|---|---|---|
| Mean | 86.55 | 64.7 |
| SD | 4.5444 | 4.2907 |
| t value(15.63583) P<0.05 | | |

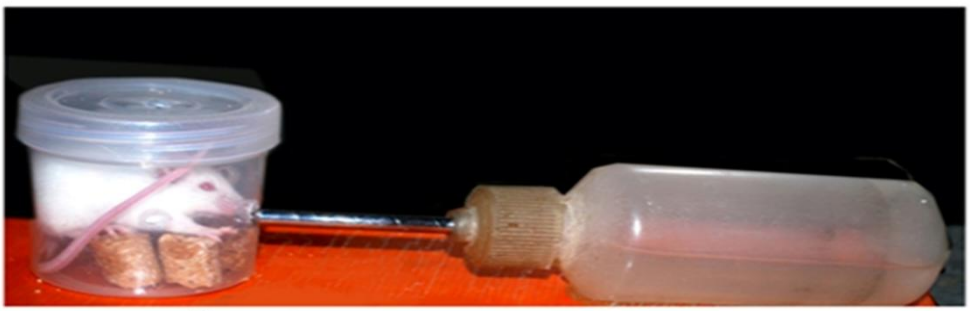

**Figure 1 Experimental mice in Immobilisation stress in white transparent box provided with food & water**

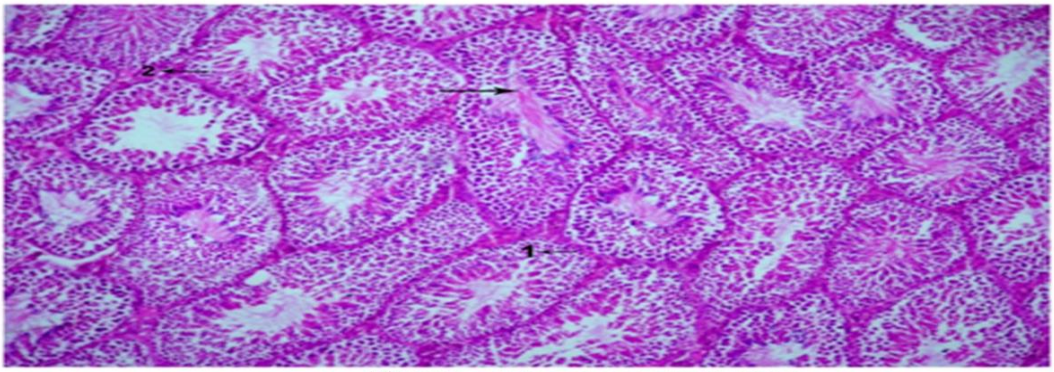


**Figure - 2 (Control,H & E 100X)**
**Control testis showing 1) Regularly arranged germinal epithelium of seminiferous tubule. Nuclear density in luminal aspect (arrowed) representing densely packed spermatozoa., 2) Leydig cell in stroma.**

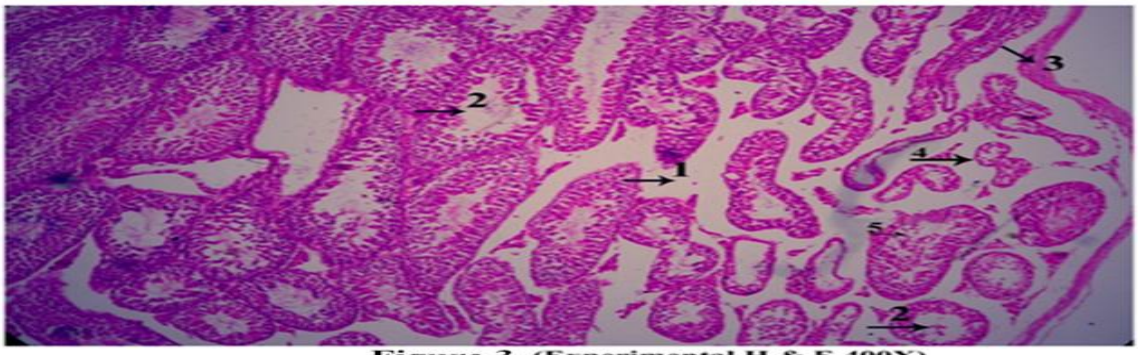


**Figure 3 (Experimental H & E 400X)**
**1) Increase space between seminiferous tubules (Interstitial odema), 2) Lumen of tubule empty, 3) Tunica albugenia 4) Disorganised tubule, 5) Disorganised epithelium.**

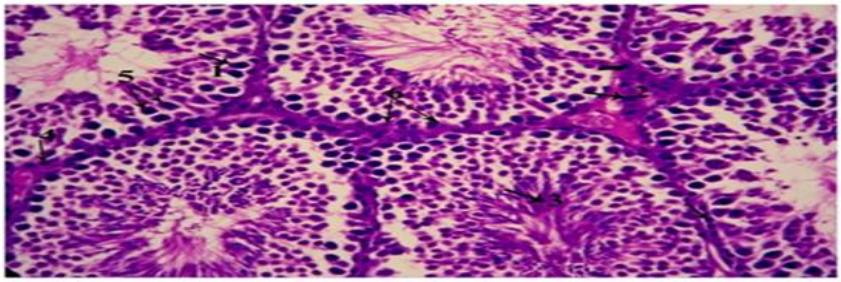


**Figure 4 (Control H & E 400 X)**
**1)Seminiferous tubules showing germinal epithelium regularly arranged lining inside tubules. 2)Leydig cells with blood capillary, 3) Densly packed spermatozoa in lumen. 4)Spermatogonia 5) Primary spermatocytes**

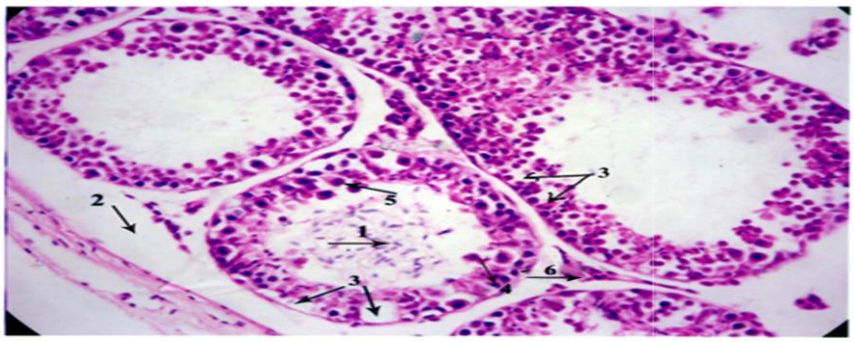


**Figure 5 (Experimental H & E 400X)**
**1) Lumen of seminiferous tubules showing very few spermatozoa 2)Empty spaces between seminiferous tubules 3) Disorgnized germinal epithelium with spaces in between cells 4)Spermatogonia 5)Reduced primary spermatocytes 6) Few leydig cells**