Effect of Immobilization Stress on Spermatogenesis of Albino Rats

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Abstract. — As the stress is increasing in our life day by day, the aim of the present study was to establish the fact that stress is one of the causes of infertility. The present study was performed on 40 male albino rats, out of which 20 served as control and 20 were exposed to immobilization stress. After two months the histological study of testis revealed marked suppression of spermatogenesis.

Key words: — Stress, immobilization, restraint, spermatogenesis

Introduction:

The separation of psychology from the premises of biology is purely artificial because the human psyche lives in indissoluble union with the body. Cannon (1990) was one of the first research workers who postulated that events disrupting homeostasis are stressful and may result in disease.

There are evidences that environmental factors, whether chemical, physical or emotional may adversely affect the testicular functions (Steinberger, 1978). Immobilization (restraint) stress has been commonly used by other workers as stress inducers in rats (Bharihoke, Gupta & Gohil, 2000, Bajkova, 1988). In the present project we have studied the effect of immobilization stress, which acts as physical as well as psychological stressor, on the testis of albino rats.

As stress is increasing in our life day by day, the aim of the present study was to establish the fact that stress is one of the causes of infertility, and this was well proved by the histological study of testis.

Materials and Method:

The study was carried out on 40 male albino rats, weighing about 100-150 gm. The animals were divided into two groups of 20 each.

Group A: Served as control.

Group B: Rats of this group were immobilized by keeping them into transparent plastic jars with 5 holes for 4hrs. a day.

All the animals were sacrificed after two months. The testis was preserved in Bouin’s fluid and 10% Formal saline. H&E and Masson’s Trichrome staining was done.

Observations:

All the rats of control group showed normal fertility, whereas in stress group none of the rats showed fertility. Gross examination of the testis revealed reduction in the weight of the testis of Group B rats. The average weight of the testis in control group was 1025mg. In stressed group the testicular weight was between 700-725mg.

In control group, normal histology of testis was seen. The testis was enclosed by a dense fibrous capsule, the tunica albugenia, underneath it tunica vasculosa was present. The seminiferous tubules were closely packed. Internal to the basement membrane spermatogenic cells of increasing maturity formed a concentric band and circumscribed a discrete lumen. The lumen was fully occupied by the tails of sperms. (Fig.1)

The stress group revealed marked suppression of spermatogenesis. (Fig.2) The seminiferous tubules were markedly reduced in diameter and thus appeared to be loosely packed. The spermatogonia, sited upon the thickened basement membrane, were reduced in size and number. The light type spermatogonia were much reduced in number than dark type spermatogonia, so much so that light type cells were seldom seen. The spermatogonia were spherical in shape, with scanty cytoplasm and almost spherical, intensely hetrochromatic nuclei. (Fig.3)

Primary spermatocytes showed reduction in their number. Varying types of shapes of primary spermatocytes were noted viz. circular, pyriform, oval, and club shaped. (Fig.3) In some cells cytoplasmic vacuolation were present giving them ‘soap bubble’ like appearance. Nuclei were eccentrically placed and were mildly to moderately...
heterochromatic. (Fig.3) Marked reduction in size of the cell and the nucleus was also noted.

Some cells showed chromatolysis and in some after complete chromatolysis only cytoplasmic remnants remained detectable as 'ghost cells'.

Secondary spermatocytes were markedly reduced in number. The cytoplasm was scanty, nucleus was heterochromatic and nucleoli were not visible.

In accordance with marked suppression of spermatogenesis, the population of spermatids was markedly reduced. The cytoplasm was scanty, nucleus was heterochromatic and nucleoli were not visible.

The germ cells of different stages of maturity along with some abnormal cells were exfoliated into the lumen of the tubule. (Fig.4) Few almost empty seminiferous tubules were noted in which almost all the spermatogenic cells had degenerated and disappeared to large extent, except for few spermatogonia which escaped destruction. (Fig.2)

**Discussion:**

It is clear from the above observation that immobilization stress causes marked suppression of spermatogenesis. The reason behind it is that restraint is a potent stimulus which induces depression of hypothalamus-pituitary-testis axis (Norman & Smith, 1992), mediated by activated hypothalamus-pituitary-adrenocortical axis, resulting in fall in plasma LH and testosterone levels (Knol, 1991). Testosterone & FSH act directly upon germinal epithelium and are required for spermatogenesis. (West, 1990) Setchell et al (1965), Vandemark & Free (1970) studied the effect of various kinds of stress on the testis and found the similar results.

The seminiferous tubules were reduced in diameter and basement membrane was thickened. Outer to the basement membrane, fibroblasts were increased in number indicating the tendency towards fibrosis, which may be one of the possible explanation for the reduced diameter of seminiferous tubules. Marked reduction in the population of light type cells shows that the maturation of cells was impaired from the very beginning. The nuclei of these cells were heterochromatic, revealing that the activity of the cells was also decreased. We have noticed varying types of shapes of primary spermatocytes. The lipid vacuoles were present in the cytoplasm of primary spermatocytes. Similar intra cytoplasmic lipid vacuoles in the spermatogenic cells were also reported by Steinberger (1970). These lipid vacuoles indicate intratesticular lipid deposition during the period of stress. A few primary spermatocytes showed chromatolysis. We also observed exfoliation of germ cells into the lumen, which was in agreement with the finding of Setchel al (1965) and Aitken et al (1996). Most probably exfoliation of cells occurred because the number of germ cells was reduced and the cytoplasmic processes of the Sertoli cells, which were extending between the different layers of germ cells and supporting them, were retracted so the cells became loosely arranged and were easily sloughed out. At some places we observed almost empty tubules except for few spermatogonia revealing severe suppression of spermatogenesis. Dumontier & Burdick (1977), proposed that the preservation of stem cells might be responsible for reversing the sterilizing effect.

**Conclusion:**

From the above findings we have concluded that stress causes reduction in size and weight of testis as well as marked suppression of spermatogenesis. Spermatogenesis was suppressed at all the stages of cell division and maturity. Stress also causes increased intratesticular lipid accumulation. Where the effect on spermatogenesis was severe degenerative changes were also seen.

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References:


Fig-1
Photomicrograph of seminiferous tubules showing basement membrane, spermatogonia, spermatocytes, spermatids and sertoli cells.
Control. (Masson's trichrome x 600)

Fig-2
Photomicrograph showing almost empty seminiferous tubule in centre. Tubular diameter is reduced and it is lined by few spermatogonia. Stress group (H & E x 150)
Fig-3
Photomicrograph of seminiferous tubule showing degenerative changes, prominent dark type spermatogonia, vacuolated primary spermatocytes and ring shape nucleus of spermatids. Stress group. (H & E x 600)

Fig-4
Photomicrograph of seminiferous tubule showing exfoliation of different stages of germ cells with obvious degenerative changes in the germinal epithelium. Stress group. (H & E x 600)