Summary and Conclusion
SUMMARY AND CONCLUSION

Male albino rats were studied from birth (D0) to day hundred (D100).

- The period of study was divided into three parts:
  a) Pre-weaning period – D0 to D20.
  b) Post-weaning period – D21 to D70.
  c) Quiescent period – D71 to D100.

- Modes of stress presented at above periods:
  a) Maternal deprivation (MD)
  b) Electrical foot shock (FS)
  c) Stress free (Quiescent)

- Environmental conditions – Room temperature with 55 to 70%, relative humidity and light : dark 12:12 h cycle. Food supplied – rodent food as per recommendations of NIN, Hyderabad, India.

- Litter with one dam till D21, thereafter only male rats.

- Stress regimen for MD and FS, progressively varied to avoid acclimatization or resistance to stress.
- Animal sacrifice:
  a) Decapitation during pre-weaning period.
  b) Sodium pentobarbitol during post-weaning period.

- Fixation of tissues:
  a) Perfusion and immersion in Bouins fluid.
  b) All organ weights were recorded after fixation.

- Tissue processing and preparation of histological slides were done as per the normal histological techniques.

- Morphometric procedures were carried out for estimation of volume, volume density, population, diameter of the respective tissues and cells. Crude counts were corrected by Abercrombie's formula. Tables presented are showing corrected counts.

Observations showed:

- Stress started affecting the BW soon after it was presented to pre-weaning rats. In post-weaning period, significant effect of stress was observed on BW. In the quiescence period young post-stressed rats were slowly regaining BW (Table 1).
- Weight of adrenals of pre-weaning as well as post-weaning stressed rats were greater than the controls from D21. Adrenal hypertrophy persisted over 30 days after withdrawal of stress (Table 2).

- The volumes of adrenal cortical zones showed maximum effect of stress on the Zona fasciculata. It did not recover from stressful effect even after withdrawal of stress. Zona glomerulosa and Zona reticularis were least affected by FS stress (Table 3).

- In consonance with the BW, the weight of the testis increase with age. In stressed rats testicular weight increase was slower. At any stage upto D100 significant differences between the testicular weight of controls and stress induced rats persisted. Maximum weight gain in rat testis takes place between D28 and D70 (Table 4).

- Between D7 and D100, the diameter of seminiferous tubules increase more than 5 folds. Stress reduced it significantly, it remains remarkably lesser in stressed rats even after a months quiescence (Table 5). Where as, the absolute volume of seminiferous tubules in healthy rat increases by 108.28 times but in stressed rats, it increases by 97.58 times only. Chronic stress, at any stage of PN life of rat significantly keeps the tubular volume low (Table 6).

- In the pre-weaning period though there were differences in the absolute volume of seminiferous epithelium, it was not significant. In the post-weaning period,
upto D70 the absolute volume of the seminiferous epithelium of normal rats increased 11.09 times but during the same period in stressed rats it increased only 8.86 times. Between 71 and 100 day period where as the volume of seminiferous epithelium increased 1.28 time in normal healthy rats, in the post-FS stressed rats it increased 1.58 times showing improvement in the seminiferous epithelial volume in the quiescence period (Table 7). Changes in the absolute epithelial volume of the seminiferous tubules were reflected on the absolute luminal volume of the seminiferous tubule (Table 8). Non-significant differences were seen till D28; later very significant changes were seen in the luminal volume upto D70 and thereafter, the lumen of the tubules were more defined.

- MD did not appreciably alter the testicular stroma. FS stress affected the stroma of testis, the effect was severe after D49 in sexually mature rats. Afterwards till D70 the stromal volume was significantly different between the groups. In the quiescence period it was progressively gaining normalcy (Table 9).

- In early PN life gonocytes were conspicuous in the seminiferous tubules, soon they were replaced by spermatogonial cell type A and B. The ratio between type A and B spermatogonia in control rats ranged between 1:6.8 to 1:9.4. In the stressed rats, the ratio was disturbed but at last, after withdrawal of stress it was restored (Table 10 and 11).
- The nuclear diameter of type A spermatogonia was affected by stress in post-weaning period. In the quiescence period again there was negligible difference between control and stressed group. The nuclear diameters of type B spermatogonia of stressed rats till D28 was slightly but not significantly more in stressed rats. Later, till D80 it was significantly lesser but the difference normalised during end part of quiescence period (Table 12).

- Due to chronic stress the population of preleptotene and pachytene spermatocytes per tubule of rat testis were very significantly affected and there was no improvement after total withdrawal of stress (Table 13 and 14). The nuclear diameters of spermatocytes were affected by stress after 4th and 5th week. After withdrawal of stress it normalised in preleptotene spermatocytes but not in pachytene spermatocytes (Table 15).

- In continuation with the severe effect of stress on the spermatocytes the population of round spermatids per tubule of rat testis were very significantly reduced (Table 16). The nuclear diameter of the round spermatids were late to show the effect of stress and showed signs of gaining normalcy during the quiescence period (Table 17).

- Till 4 weeks PN the population of Sertoli cells per tubule of rat testis were generally high in control as well as in stressed rats. It declined from D35 in both groups of rats. Interestingly, Sertoli cell number per tubule was found more in stressed rats (Table 18). Initially, till 5th week PN the nuclear volume of Sertoli
cells of stressed group of rats were nonsignificantly less than in control rats. From 6th week onwards till D100 the nuclear volume of Sertoli cells of stressed rats remained suppressed (Table 19).

- Leydig cells were studied in post-weaning period in normal and stressed rats. Leydig cell per unit volume of testicular tissue was fairly consistent in post-weaning rats. There was slight nonsignificant variability in number per unit volume in stressed rats (Table 20). Chronic stress caused significant variability in Leydig cell volume density but in quiescent period it showed signs of normalisation within three weeks (Table 21). Mean total cytoplasmic volume of the Leydig cells were always significantly greater in normal control rats as against the rats under stress (Table 22). The nuclear volume of the Leydig cells fluctuate within a limited range, the variation between the groups was found inconsistently nonsignificant or significant (Table 23).

In short, it may be concluded that MD stress is effective, its onset is quick, however, effect is milder. On the other hand FS stress generally brings about quicker and lasting effects. In majority of parameters, quiescence after chronic stress did not reverse the effect of stress within a period of one month. As some of the parameters have shown reversal trend towards the end of quiescence period it can be expected that chronic MD and FS stress may not be of long term detriment. Further studies on stress with different stressors may go a long way towards better understanding.