Summary
The effects of single dose, 4 consecutive days, 4 and 8 weekly doses of MTX treatment with and without LCN supplementation on body weight, testicular, accesssory sex organ weights and serum hormonal titres like FSH, LH, testosterone and estradiol were studied in adult albino rats. Besides, histological and biochemical parameters like nucleic acids, protein, enzymes involved in steroidogenesis, HMP-shunt pathway, pyruvate-malate cycle and various lipid classes were studied in testis.

1. A marked reduction in the body weight was recorded in rats received 4 days, 4 and 8 weekly doses of MTX administration. LCN did not bring the body weights back to normalcy in weekly dose treatment groups.

2. A marked duration dependent reduction in the wet weights of testis, caput, corpus and cauda epididymides, vas deferens, seminal vesicle was evident. In testis and vas deferens, while the effects were more pronounced in 4 days, 4 and 8 weekly dose treatment groups, in seminal vesicle and caput epididymidis, the effects were more striking only in 8 weekly dose MTX treated group. Corpus and cauda epididymidal weights were reduced only in rats that have received 4 consecutive doses of MTX. In coagulating glands, dorsolateral and ventral prostatic lobes, although, such an inhibitory response was seen, it was not time-dependent. LCN supplementation did not restore the weights to normalcy.

3. MTX treatment caused a marked reduction in serum FSH, LH, testosterone and estradiol titres in all the treatment groups and this effect on testosterone level alone was duration dependent. LCN
supplementation did not maintain any of the hormone titres at normal range.

4. Caput and cauda epididymal sperm counts were markedly decreased in weekly dose MTX treated groups. The sperm motility was impaired in rats that have received 4 days, 4 and 8 weekly doses of MTX. LCN supplementation could bring back only the sperm count to normalcy.

5. MTX caused spermatogenic arrest and this effect was time-related. In 4 days, 4 and 8 weekly dose drug treated groups, the histopathological changes were manifested by sloughing of primary spermatocytes and spermatids followed by pronounced exfoliation and clumping in the seminiferous tubular lumen with prominent decrease in mature spermatozoa, late spermatids and even early spermatids. The germ cells showed pycnotic and cytolytic changes. In addition, in 4 weekly dose drug treated rats, the appearance of multinucleated giant cells, lipid droplets and large vacuoles were common features. Interstitium was oedematous and the Leydig cells were smaller in size. LCN supplementation could not restore the histoarchitecture in these animals.

6. A marked diminution in RNA, DNA and total protein concentrations was evident in 4 consecutive days, 4 and 8 weekly dose MTX treated groups. This inhibitory effect was not reverted in the latter two groups even after LCN supplementation.
7. The specific activities of steroidogenic enzymes, viz. 3β- and 17β-hydroxy steroid dehydrogenase were markedly diminished in both MTX and MTX + LCN treated groups.

8. HMP-shunt dehydrogenases viz. glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenases showed a stimulatory response to MTX and MTX + LCN treatment groups.

9. A similar stimulatory effect on the specific activities of key enzymes of the pyruvate-malate cycle, viz. NADP⁺-ICDH, ATP-citrate lyase, MDH and malic enzyme was evidenced following MTX treatment and this effect was not reverted even after LCN supplementation.

10. Total and free cholesterol concentrations remained unaltered in both MTX and MTX + LCN treated groups. On the other hand, a marked increase in esterified cholesterol concentration was evident only in weekly dose treatment groups.

12. Total glyceride glycerol and monoacyl glycerol concentrations were unaffected by MTX treatment with and without LCN supplementation. Triacyl glycerol concentration alone showed accumulation in weekly dose treatment groups.

13. Total phospholipid, phosphatidyl ethanolamine and sphingomyelin were resistant to single dose of MTX and MTX + LCN administration. However, 4 days, 4 and 8 weekly doses of MTX treatment exerted an inhibitory influence on total lipid, total phospholipid, phosphatidyl
choline, phosphatidyl serine and phosphatidyl ethanolamine in a time-
dependent manner. This response was persistent even after LCN 
supplementation.

14. The effects of MTX on phosphatidyl inositol were biphasic. While this 
phospholipid class was depressed following single dose treatment, the 
same was elevated following 4 and 8 weekly doses. This response was 
persistent even after LCN supplementation.

15. While 4 days MTX treatment caused a perceptible increase in 
sphingomyelin concentration, 4 and 8 weekly dose MTX administration 
lowered the same. LCN supplementation, on the other hand, brought 
about a marked increase in sphingomyelin concentration at all durations 
of treatments.

16. While phosphatidic acid registered a marked increase following 4 
weekly doses of MTX treatment, the same being depleted following 
single dose MTX treatment. LCN supplement caused a further lowering 
of phosphatidic acid in these two groups.

17. Cardiolipin was markedly increased only in rats subjected to 4 
consecutive days treatment of MTX. Such a stimulatory response to 
LCN supplementation was evident in 4 and 8 weekly dose treated 
groups. In contrast, single dose MTX + LCN treatment depleted the 
cardiolipin concentration appreciably as compared to control and MTX 
treated groups.
In conclusion, MTX exerted an adverse effect on body weight, primary and accessory sex organ weights, serum gonadotropins, testosterone and estradiol levels. In addition, it arrested spermatogenesis and reduced epididymal sperm count and motility. The drug further decreased RNA, DNA, protein, total lipid, total phospholipid, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine concentrations and steroidogenic enzymes. On the other hand, lipogenic enzymes, HMP-shunt dehydrogenases, esterified cholesterol and triacyl glycerol concentrations exhibited a stimulatory response to MTX treatment. The other phospholipid classes exhibited a differential effect to MTX treatment. LCN, a known supplement to MTX was ineffective in restoring the serum hormonal levels, body and organ weights, sperm motility and spermatogenesis. Biochemical parameters were only partially restored.