SUMMARY, CONCLUSIONS AND SIGNIFICANCE OF THE
STUDY

Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone originally designed to provide enhanced anabolic (tissue-building) potency with negligible androgenic (masculinizing) effects. To date, almost 100 different AAS compounds that vary in their chemical structure, physiological effects and in their metabolic fate have been designed and synthesized.

In fact AAS compounds were originally developed for the treatment of (usually in small doses) aplastic anemia, endometriosis, hypogonadal dysfunction in men, initiation of delayed puberty, and growth promotion. They continue to be used today for these treatments, as well as for therapy in chronic conditions including HIV/AIDS, debilitating illness, trauma, severe burns, anemia, hepatic and kidney failure, inoperable breast cancer, and hereditary angioedema, radiation therapy, anemia associated with leukemia and osteoporosis. In addition to legitimate therapeutic applications of these AAS compounds are being abused (without proper awareness of physiological effects of AAS) by many recreational and professional athletes to improve physique, athletic performance, to enhance muscle mass, strength and endurance. However, the beneficial potential of AAS can come at the cost of variety of secondary adverse effects.

Recent studies have shown that there are no pure anabolic steroids that are devoid of androgenic actions and they have androgen receptor binding properties. Further, the use of AAS is becoming increasingly popular among the adolescent girls and women athletes, yet the actual influence of these compounds on ovulation, folliculogenesis, fertility, pregnancy and puberty, endocrine and immune response etc.
is yet to be established clearly. Since many of these AASs are used therapeutically the better understanding of the physiological mechanisms of these steroids is essential. One of the AASs compounds the Stanozolol (17β-hydroxy-17α-methyl-androstano (3, 2-c) pyrazole) chosen for this study is commonly used either clinically or abused by athletes and teenagers. Hence, the present study is under taken to evaluate the effect/efficacy of this AASs compound on female reproduction (gametogenesis) in mice, *Mus musculus*.

*Mus musculus* is a poly-ovulatory and polyestrous mammal. Studies using mice as a model are directly applicable to human beings, shorter reproductive lifespan and generation times, short estrous cycles. The laboratory mouse, *Mus musculus* is a complete genome sequenced mammal. Hence the present work we have utilized mice as model system.

**CHAPTER-I**

The Chapter-I aims to evaluate the short-term effect of AAS compound, stanozolol, on lipoprotein profile, granulopoiesis and immune response in adult female mice *Mus musculus*.

1. The mice were assigned to five experimental groups and different doses of stanozolol were administered s.c. for 15 days.

2. A decrease in high density lipoprotein cholesterol (HDL-c) as well as total cholesterol (TC) in all the stanozolol treated groups and an increase in low density lipoprotein (LDL-c) in high and the highest dose treated groups indicate that stanozolol alters serum lipoprotein profile which may reflect
upon the universal adverse effects of AAS on liver enzymes such as hepatic triglyceride lipase and hepatic lipase.

3. A significant increase in the percentage of myelocytes, metamyelocytes and neutrophils in all the treated mice unveils the stimulation of granulopoiesis through the acceleration of neutrophil precursors’ maturation in the bone marrow of mice.

4. The stimulation of erythropoiesis was also noted in all the treated groups. Stimulation of erythropoiesis may indicate a greater androgenic to anabolic efficacy of this compound.

5. The flow cytometry analysis of lymphocyte subpopulations (CD3+ and CD4+) revealed immunoenhancing response of stanozolol at optimum physiological dose, however, it is immunosuppressive at supraphysiologic level.

6. We conclude that stanozolol accelerates granulopoiesis and stimulates immune response (at physiologic level only), though it alters the lipoprotein profile in mice.

CHAPTER-II

Use of AAS by women is less established than in men and little information exists on the effects of these AAS compounds on female reproduction and its endocrine control, from a morphologic point of view. Because AAS are derivatives of T they act via negative feedback to the hypothalamus, short-term effects of AAS consumption, associated on the female reproduction is still an unsolved issue.

Hence, in the second chapter we wish to know the role of stanozolol on the follicular growth, estrous cycle and steroid hormone profile in adult female mice Mus musculus.
1. Different doses of stanozolol administered by s.c. injection in 1% alcohol daily for 15 days.

2. Estrous cycle was determined by examination of cell types recovered from a vaginal lavage collected daily. Steroid hormone concentrations of T and E2 were measured by competitive binding immunoenzymatic determination (ELISA) using antibodies specific for each hormone. Differential follicular count was made based on the methodology opted by Pedersen and Peters (1968) and Hirshfield and Midgley (1978).

3. The results of the present study revealed an irregular pattern of vaginal estrous and stretched estrous, metestrous and diestrous duration in all treated groups leading to the disruption of estrous cycle. It could be suggested that the low levels of circulating estrogen produced the diestrous pattern observed in the treated group. It is evidenced by a decrease in the plasma estradiol level and stanozolol may break up female neuroendocrine function.

4. Stanozolol treatment resulted in significant increases in the number of small growing primary and secondary follicles which reveals that short-term stanozolol treatment might stimulate early, presumably gonadotropin-independent, stages of follicular development. In conclusion our study divulges that short-term treatment of stanozolol might stimulate early stages mice ovarian follicular development.

5. Cystic follicles were increased significantly in all the treated groups except low dose treated group. An insignificant increase in atretic follicles in high and highest dose treated groups when compared to control. These are the consequences of endocrine imbalances, often impairments in the release of pituitary gonadotrophins.
2. The results revealed that treatment of stanozolol is not able to maintain gestation to full term, resulting in partial foetal resorption with many placentomas, placental scars and no viable fetuses indicate the functional failure of corpus luteum.

3. In the current experiment a significant reduction in the number of corpora lutea, secondary and antral follicles was observed when compared to control. The reduction in the said follicles, presence of placental scars and absence of viable fetuses, suggest that treatment of stanozolol (though it is of therapeutic dose) may affect the secretion and release of FSH, LH causing an imbalance in progesterone and estrogen secretions by the ovary leading to the interruption of pregnancy.

4. Histological indices especially of CL reveal reduction in diameter of the CL and luteal cell size in both the treated groups. These morphological changes in the luteal cells reveal regression of this gland by the androgenic effect of stanozolol. It also reflects the nonfunctioning of CL leading to an imbalance in the progesterone and estrogen secretions causing the interruption of pregnancy.

5. The progesterone, estradiol and testosterone levels in the plasma could be an important tool in evaluation of CL function. A noticeable decrease in serum estradiol and testosterone concentration in both treatment groups (day 8 to 14 and day 8 to 19 of pregnancy) suggest that treatment of stanozolol (though it is of therapeutic dose) may affect the secretion and release of FSH, LH. This warrants further study on the quantification of serum FSH, LH and Progesterone.

6. An increase in adrenal gland diameter and hypertrophy of adrenal cortex suggest that stanozolol might have lead to more production of adrenocorticosteroids (via ACTH signalling) and it might have also inhibited progesterone production.
number of atretic follicles (cystic follicles excluded) in the ovaries of mice treated was also evident.

4. The occurrence of three distinct types of cystic follicles was a common phenomenon with reference to the follicular composition in contrast to the ovaries from control mice. These are the consequences of endocrine imbalances, often impairments in the release of pituitary gonadotrophins. Besides, in the ovarian sections the stromal component consisted of cords of interstitial gland cells which are luteinised.

5. In the present study chronic administration of different doses of stanozolol induces a noticeable increase in the serum concentration of testosterone an insignificant decrease in the concentration of serum estradiol in mice when compared to control suggests a greater anabolic to androgenic effect of stanozolol and might have altered the hypothalamic-pituitary-gonadal axis. The observed results also suggest the alteration in ovarian steroidogenesis leading to the negative steroid feedback signaling to the hypothalamus.

6. The androgen excess may possibly be due to the increased amount of stroma, stromal components and the formation of cystic follicles (rather the defect in the enzymes) resulting in hyperadrogenism. It is suggested that unlike many of the androgen induced models, alterations in follicular dynamics, a key feature of human PCOS.

7. The observed results of the present study on the recovery efficacy of this compound it is proposed that although pregnancy is maintained to full term, the number of pups reduced considerably which may be because of maternal endocrine disruption due to postnatal treatment. This also suggests that the 45 days of recovery period may be in adequate.
8. A future study is needed at the molecular level for better understanding of the role of AASs in female reproductive physiology i.e. androgen receptor (AR)-mediated mechanisms governing the rate of follicular development. Such studies are helpful in the treatment of age-related infertility as well as androgen-associated disorders, such as polycystic ovary syndrome (PCOS). These findings will help us to understand the influence of AAS use during postnatal life on female reproductive health and fertility. In this context, rodents model provide a versatile tool for deciphering the precise biological mechanism(s) associated with the development of PCOS.

9. In conclusion, the results of the present provide new information about the consequences of administration of AASs on the maturing neuroendocrine system and point to a role for multiple biochemical and neuroendocrine substrates.