Research Paper

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Effect of Progestin (Depot Medroxy Progesterone Acetate) on Testis and Pituitary Gland : Ultrastructural and Biochemical studies

Zoology

KEYWORDS : Alkaline phosphatases, Acid phosphatases, Depo Provera, RER, Funambulus pennanti, DMPA

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ABSTRACT

The effect of Depot Medroxy Progesterone Acetate (Depo Provera- DMPA) at the dose of 1mg/animal/day for 30 on testis and pituitary gland in Indian palm squirrel Funambulus pennanti has been studied. The treatment caused significant changes in ultra structural and Biochemical parameters. The changes seen in testis were damage to Golgi, early cap and late cap phase spermatid as well as spermatogonial cells. The treatment also revealed pronounced effects on two Gonadotrophs; FSH and LH as well as Lactotrophs (PRL). The changes in anterior Pituitary include significant reduction in number and size of gonadotrophic cells, secretory granules, regression of RER and Golgi vesicles. DMPA also caused decrease in Acid and alkaline phosphatases, slight decrease in Citric acid content, significant decrease in total proteins and increase in fructose content. The ultra structural and Biochemical changes were correlated as the cellular degeneration is responsible for decrease in the activity of marker enzymes. Thus overall result suggested a strong anti-androgenic effect of DMPA.

INTRODUCTION

Depot Medroxy Progesterone Acetate is an anti-androgenic and anti-gonadotrophic drug, arrests spermatogenesis and can be used as an effective male contraceptive. It is a synthetic steroidal hormone and it has a close structural similarity to natural progesterone. Depot Medroxy Progesterone (Provera, Upjohn) was administered to adult male squirrels, *Funambulus pennanti* for 30 days(1mg/animal/day) and its effect on testis and pituitary gland was studied at ultrastructural and Biochemical levels. Long term treatment of males with progesterone and synthetic progestin has been shown to inhibit spermatogenesis.(Ericsson and Dutt, 1965; Coutinho et.al, 1966; Kar et. al,1967; Rao et al. 1995) and lower the testosterone levels (Gordan,1970). However, correlative studies on ultrastructure of pituitary and bio-chemical values have not yet been studied hence we have examined in male squirrels the effect of progesterone, DMPA on spermatogenesis by light microscopy and Electron microscopy as well on its marker enzymes through biochemical estimation.

The specific objective of the study is to determine which stages of spermatogenesis are affected by the treatment and the nature of possible cytological changes in germ cells, Sertoli cells and Gonadotrophic cells.

MATERIAL AND METHODS

Drugs

Depot Medroxy Progesteron Acetate (DMPA) injection (Upjohn USA). Olive oil was used as dissolving agent and also used for control animals in equal volume.

Animals

A set of 12 adult male squirrels weighing between 100 to 150gms were trapped alive in and around Nagpur City during the breeding period from January to July, 2011 (Reddi and Prasad, 1968). They were fed in the morning and in the evening daily with the soaked grams, chappatis, breads, cooked rice, dal, fruits, vegetables, ground nuts and water. The animals were housed at constant temperature (28±2°C) and relative humidity (60±10%) with a 12h light: 12h dark cycle.

Treatment

One week after arrival, male squirrels were assigned to DMPA treatment. The control animal received same amount of olive oil (Table-1).

TABLE-1: Experimental Design

Number of animals and sex	Treatment	Dose mg/ Kg BW	Route	Duration
6 males (control)	Olive oil	E.V.	I.M.	30 days
6 males (Experimental)	DMPA	1mg daily	I.M .	30 days

Abbreviations: E.V. = Equal volume, I.P. = Intramuscular, B.W. = Body weight

The animals were sacrificed using chloroform 24 hrs after the last day of experiment . Immediately, testes were excised. The left testes was used for Ultrastructural testes and right testes was used for biochemical parameters. Similarly pituitary gland was fixed for Ultrastructural studies.

Table-2 Effect of 1mg daily for 30 days treatment on biochemical parameters of Indian Palm Squirrel (Values are mean ± SE. Figure in parentheses are number of animals used).

Treatment	Acid Phosphatase	Alkaline phosphatase	Proteins
Control (6)	0.83± 0.09	0.74±0.19	6.41±0.18
1mgDMPA daily 30 days(6)	0.74±0.05	0.54±0.04**	5.26±o.32*

*P< 0.05. **p< 0.01

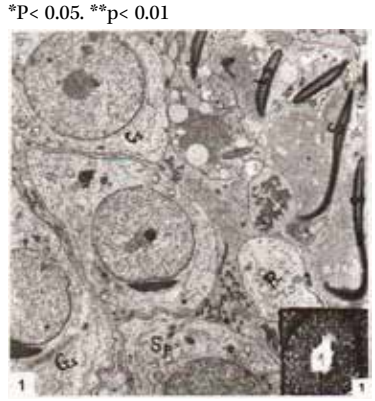

Fig.1: Seminiferous epithelium from vehicle-treated control. Note spermatocytes (Sp), Golgi phase spermatid (Gs), cap phase spermatid (Cs), elongated spermatid (Ls), differentiated spermatozoa (S) and residual bodies (R) X 2500.

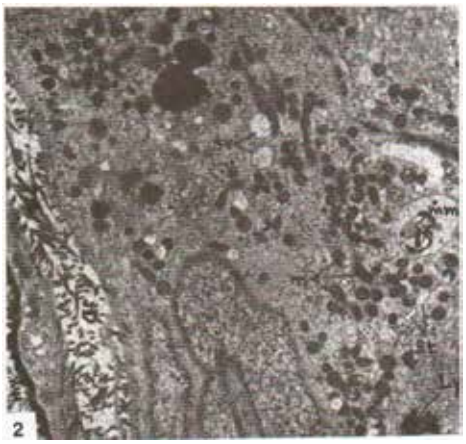

Fig 2: Basal portion of seminiferous tubule from the vehicle-treated control. Note myoid cell (my) and the fine interlacing filaments (If) arranged in parallel lamellae. The basal Sertoli cell cytoplasm shows presence of all normal cellular organelles – mitochondria (m), smooth coated vesicles (sv), smooth surfaced tubules (st), mylinated mitochondria (mm), lipid droplets (L) and membrane limited lysosome (Ly) X 6000.

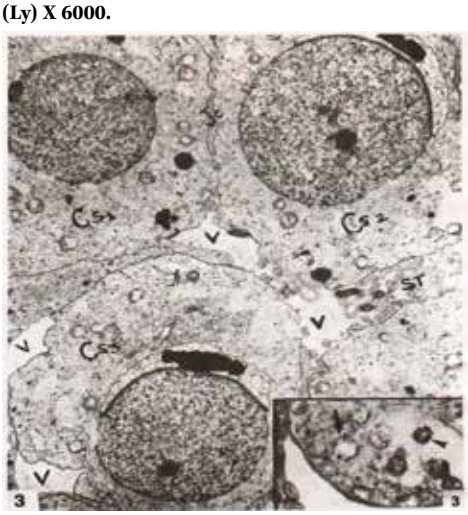

Fig 3: (Inset) Seminiferous tubule from animals treated with 1 mg DMPA for 30 days. Note thick lamina propria, reduced number of spermatogonia, round vacuolated spermatocyte with peripheral fragmented nuclear material (arrow) and darkly stained spermatid present in hallow spaces (arrow head) X 8000.

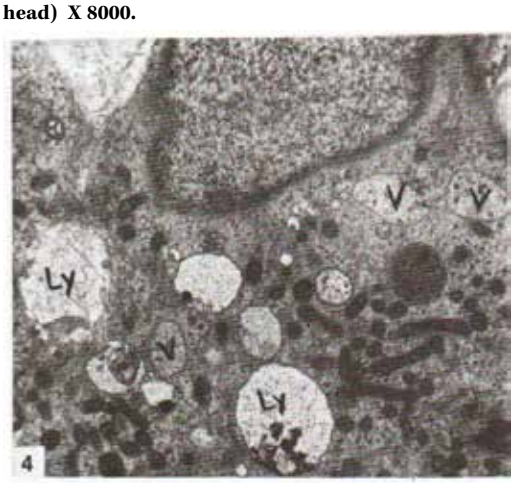

Fig. 4: Sertoli cells from 1mg DMPA daily treatment for 30 days. Note an intense lysosomal activity (Ly) with many vacuoles (V) X 8000.

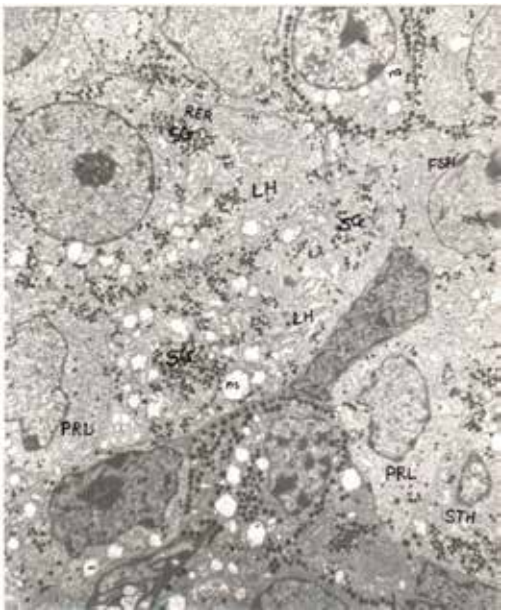

Fig.5: - A low power electron micrograph of pars distalis after 1mg DMPA treatment daily for 30 days showing LH, FSH and STH cells. Prevalence of hypertrophied mitochondria (m) as well as depletion in the secretory granules (SG) is a common sight. PRL cells shows indented nuclei and concentrically arranged rough endoplasmic reticulum (RER)X2500.

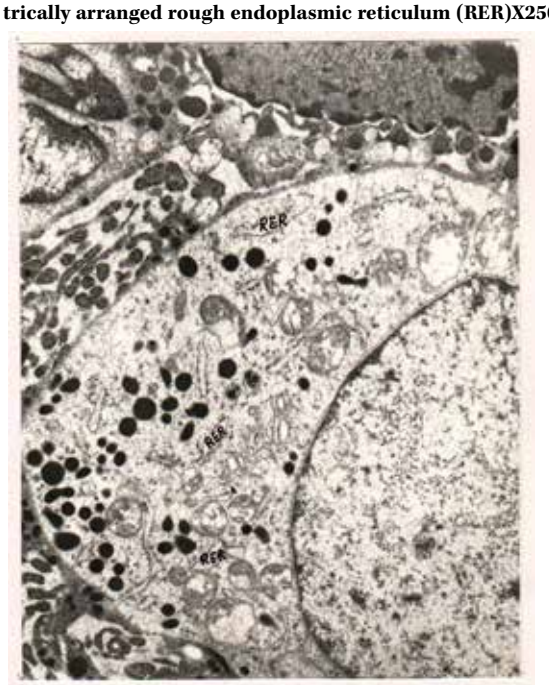

Fig 6:- Electron micrograph highlighting part of the prolactin cell following 30 days treatment. The cisternal membranes of the rough endoplasmic reticulum (RER) appear diffuse, dilated and engorged with secretion. Degenerative changes in the enlarged in the mitochondria are visible X8000.

DISCUSSION

DMPA treatment for 30 days daily has resulted into arrest of spermatogenesis at round spermatid stage revealing the disorganization and disintegration of seminiferous epithelium, sloughing off of apoptotic cells into testicular lumen as described by earlier workers like Ericsson et al, 1964; Ericsson

and Dutt, 1965; Russel and Grisworld, 1993; Meistrich and Van Beek, 1993; Volkmann et al. 2011; Nurmio et al. 2012 after the progestagen treatment and appearance of large vacuoles due to cytolysis in the germinal epithelium, an indication of androgen deprivation (Russel and Clermont, 1977 ; Jeff et al. 1988; Kerr et al. 1993).

Ultrastructural studies similarly showed damage to Golgi phase spermatids, late Cap phase spermatids and spermatogonial cells as well as prominent changes in the organelles responsible for energy generation from the mitochondria. The evidence of impaired FSH secretion is provided by the Sertoli cells The degenerative changes like hypertrophied mitochondria and absence of smooth endoplasmic reticulum were observed in the apex of Sertoli cell, which is the seat of ABP secretion. Similiar changes were also seen in biochemical parameter (Hansson et al. 1976; Gunsalus et al. 1980; Clermont et al. 1993; George and Wilson, 1994; Guraya, 2001; Felig and Frohman, 2001; Bolander Jr. 2004). The most important lysosomal enzyme in mammalian testis is acid phosphatases and alkaline phosphatases, as they have a role in differentiation of spermatogenic stages. We have found slight or significant decrease in acid phosphatases and alkaline phosphatases activities. This may be due to the inhibitory action of DMPA on enzyme activities (Neimi and Kormano, 1965). Thus the present findings provide evidence that DMPA acts directly on testes through pituitary gland altering their histology, ultrastructurally and biochemically.

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REFERENCE

Bolander Jr (2004) Molecular endocrinology. Third edition, Department of Biological Science. University of south Carolina, Columbia. | Clermont Y, Oko R and Hermo L (1993) Cell biology of mammalian spermatogenesis. In: Cell and molecular biology of the testis. Desjardins C and L. Ewing L (eds). New York, Oxford University Press. | Coutinho EM and Desouza JC and Caspo AI (1966) Reversible sterility induced by medroxy progesterone injection. Fertil. Steril. 17: 261-264. | Ericsson RJ and Dutt RH (1965) Projesterone and 6α-methyl-17α-hydroxy progesterone acetate as inhibitors of spermatogenesis and accessory gland function in the ram. Endocrinol. 77:203-205. | Ericsson RJ, Dutt RH and Archdeacon JW (1964) Progesterone and 6-chloro-17 acetoxy progesterone as inhibitors of spermatogenesis in the rabbit. Nature 204:261-265. | | Ericsson RJ, Dutt RH (1965) Progesterone and 6 α-methyl-17 α-hydroxy progesterone acetate as inhibitors of spermatogenesis and accessory gland function in the ram. Endocrinology 77:203-204. | | Felig P and Frohman LA (2001) The testis In: Endocrinology and Metabolism, 4th edition McGraw Hill Inc. Medical publishing Division. 635-705. | George FW and Wilson J (1994) Gonads and ducts in mammals. In: The physiology of reproduction. 2nd ed. Knobil E and Neill JD eds. New York: Raven Press. 3–27. | Gordon GG, Southern Al, Tochimoto S and Altman K (1970) Effect of medroxy progesterone acetate (Provera) on the metabolism and biological activity of testosterone. J.Clin. Endocrinol. Metab. 30:449-451. | Gunsalus GL, Musto NA and Bardin CW (1980) Bidirectional release of Sertoli cell project androgen binding protein into the blood and seminiferous tubules. In Testicular Development, Structure and Functions Eds. A Steinberger and E. Steinberger. Raven Press., New York 291. | Guraya SS (2001) Comparative cellular and molecular biology of testis in vertebrates. Trends in Endocrine, Paracrine and Autocrine Regulation of Structure and Functions. Oxford and IBH Publishing Co. Pvt. Ltd. 1-91. | Hansson CG, Weddington SC, French FS, McLean W, Smiths AA, Nayfeh SN, Ritzen EM and Hegenas, L (1976) Secretion and Role of androgen binding proteins in the testis and epididymis. J. Reprod. Fertil. 24:17-19. | | Jeff E, Fegan and Larry I and Lipshultz MD (1988) Abnormalities of the testes leading to male infertility: Evaluation and diagnosis. Hospimedica. 6(1): 33-40. | | Kar AB, Kamboj VP and Shetty BS (1967) Antispermatogenic effect of some progestational steroids in rats. Ind. J. Expt. Boil. 5:45-46. | | Kerr JB, Miller M, Maddocks S and Sharpe RM (1993) Stage dependant changes in spermatogenesis and Sertoli cells in relation to the onset of spermatogenic failure following withdrawl of testosterone. Anat. Rec. 235:547-250. | | Meistrich ML and van Beek M (1993) Spermatogonial stem cells. In: Cell and Molecular Biology of the Testis. Desjardins C and Ewing L (eds.) New York: Oxford University Press 288. | | Neimi M and Kormano M (1965) Cyclic changes and significance of lipids and acid phosphatases activity in the seminiferous tubules of rat testis. Anat. Rec.151:159160. | | Nurmio M, Kallio J, Adam M, Mayerhofer A, Toppari J, Jahnukainen K (2012) Peritubular myoid cells have a role in postnatal testicular growth. Spermatogenesis. 2(2):56-67. | Paulson CA, Brenner WS and Leonard JM (1985) Male contraception: Clinical trials. In: Advance in fertility research Ed. By D.R.Mishell New York Raven Press. 157. | Rao MV, Shah KD, Darji A and Postwala I (1995) Effects of medroxy progesterone acetate and progesterone acetate and progestin-androgen combination on reproductive organs of the male rat. Geo. Biosc. 22:217-220. | | Reddi AH and Prasad MRN (1968) The reproductive cycle of the male Indian palm squirrel, Funambulus pennanti (Wroughton). J. Reprod. Fertil. 17(2):2-3. | | Russell LD and Clermont Y (1977) Degeneration of germ cells in normal, hypophysectomized and hormone treated hypophysectomized rats. Anat. Rec. 187: 347-366. | Russell LD and Griswold MD (1993) The Sertoli cells, (eds) Clear water, fla Cache River Press. | Volkmann J, Müller D, Feuerstacke C, Kliesch S, Bergmann M, Mühlfeld C and Middendorff R (2011) Disturbed spermatogenesis associated with thickened lamina propria of seminiferous tubules is not caused by dedifferentiation of myofibroblasts. Hum. Reprod. 26(6):1450–146. | | |