CHAPTER III

EFFECT OF STILBESTROL ON THE SPERMATOGENESIS OF UPERODON SYSTOMA

INTRODUCTION

Several investigators have studied the effect of exogenous stilbestrol on males of various vertebrates. It is well known that in mammals, prolonged administration of stilbestrol to males produces testicular regression and atrophy of the sex accessories (Emmens and Parker, 1947). But little work has been done on the male salientia. Van Oordt and Klomp (1946) noted a distinct influence of estrone on the Mullerian duct of only Bufo bufo. Cei et al., (1955) observed in Leptodactylus chaquensis that injection of estradiol suppressed spermatogenetic progression. Similarly spermatogenetic suppression and Mullerian duct proliferation by estradiol implants were also observed in Bufo melanostictus (Basu and Mondal, 1960). Recently, Kasinathan (1974) made an observation on Rana hexadactyla, that exogenous stilbestrol, almost reduces all the spermatogenetic stages in the experimental animals. From these findings, it appears that exogenous estrogen (stilbestrol) administration produces different effects in various species of the Amphibia. These differences may probably also be due to different methods and doses of estrogen administration and also to physiological variations. For the present investigation, Uperodon systoma Schneider from Thanjavur, where temperature fluctuation is very
meagre, was selected to analyse quantitatively the effect of booster and split dose of stilbestrol administration.

**Materials and Methods**

Male frogs (*Hyla passerina*) were collected locally and maintained under uniform husbandry conditions, throughout the experimental period and fed twice weekly with ants and termites. Adult frogs averaging 45gm in body weight and 60mm snout to vent length were selected for the purpose.

The animals for the experiments were divided into two groups. Ten animals as one group was injected with the microcrystals of stilbestrol weighing 1.25mg dissolved in 1ml of Amphibian Ringer in a single dose. As a placebo control an equal number of normal frogs were injected with Amphibian Ringer only. Both the experimental animals and controls were sacrificed at the end of two weeks. After autopsy the total body weight and weight of testes were determined for each animal.

In a second group of animals 1.25mg of stilbestrol was administered in five doses of 0.25ml, each, on every alternate day. Control animals were maintained by injecting normal Amphibian Ringer. All the animals were sacrificed at the end of two weeks.

The testes were fixed in Bouin's fluid, embedded in paraffin, sectioned at 7μ, and stained haemalum eosin for histological observations. Relative testicular weights were calculated, in order to avoid individual variations. The
spermatogonetic activity was quantitatively assessed as per previous method.

OBSERVATION

Both booster and split dose administration of stilbestrol resulted in a marked decrease in testicular weight in comparison with control animals.

It is seen that the split dose administration of stilbestrol affects all spermatogonetic stages Table 8 Fig 22 and it was statistically significant (p<0.001). Secondary spermatogonia of stages I and II are inhibited drastically.

Booster administration affects mostly the cell nest of the stage 0, I and II (Table 8 Fig 21). The P value between experimental and control in stages 0 and is (P<0.02). Administration of stilbestrol in general results in pycnosis (Table 8) of the spermatogonetic stages and disorganisation of the seminiferous tubule. The thickening of the tubular walls and shrinkage of lumina are quite remarkable. But the spermatids in bundles are abundant throughout the tubules in all individual. Similar degenerative changes are noticed in both the experimental groups, split dose has sustained action and hence the effect is more pronounced than booster dose.

Data on the experimental and control animals were analysed by using "Two way analysis of variance" technique Table 9 to determine the differences of the spermatogonetic stages in the treated and control groups. It may be seen that the results of
the spermatogonetic degeneration in all the experiments are significant at 0.001 percent level.

Discussion

From the Table 8 it may be observed that all the spermatogenetic stages are significantly reduced in experimental animals. Exogenous estrogen acts as a spermatogenetic inhibitor (Emmens and Parkes 1947). Moore and Price (1930) experimentally proved that the effect of estrogen is exerted by depressing the gonadotropic factor of the pituitary. This opinion was supported by later investigators. Cei et al., (1955) reported the suppressing effect of estrogen on the spermatogenesis of Leptodactylus chaquensis.

Inhibitory effect of stilbestrol on the spermatogenesis of Rana hexadactyla has been observed. (Kasinathan and Basu, 1974). Similarly spermatogenetic suppression by estradiol was observed in many salientia (vide Table 1) we corroborate the findings of earlier investigators. The reduction of spermatogonial stages suggests the blocking effects of estradiol on initial stages of spermatogenesis. It may also be probably that prolonged treatment produces a complete degeneration of the testicular follicle. In the split dose administration of stilbestrol, it is seen that estrogen affects all the spermatogenetic stages, whereas in booster dose administration, the changes are marked in stage only. Complete inhibition of the stage I and II in both split and booster dose administrations indicate that stilbestrol
regresses the spermatogenetic progress from secondary spermatogonial stages, leading to the blockade of the divisional potency of spermatocytes. Further experiments are necessary to prove whether the inhibitory effect of stilbestrol is due to suppression of gonadotropin secretion at the hypothalamo-hypophyseal axis or directly on the target organ or both.

**SUMMARY**

From the findings, it can be inferred that, the exogenous administration of stilbestrol suppresses spermatogenesis in frog, but the inhibitory effect is more pronounced and suppression of all stages noticed when split dose treatment is allowed.
TABLE B

Effect of stilbestrol administration (Booster and split dose) on the spermatogenesis of *Upereodon systoma* (n=10)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Spermatogenetic stages</th>
<th>Testicular weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O I II III IV V</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.7500 0.7500 1.0000 1.9500 1.2000 2.0500 21.4000</td>
<td>+ + + + + + + + + +</td>
</tr>
<tr>
<td></td>
<td>0.2817 0.1391 0.1224 0.1495 0.1816 0.2497 0.4500</td>
<td></td>
</tr>
<tr>
<td>Booster dose</td>
<td>1.9000 0.0000 0.0000 1.0500 0.8000 1.2500 15.9000</td>
<td>+ + + + + + + + + +</td>
</tr>
<tr>
<td>Stilbestrol</td>
<td>0.1987 0.0000 0.0000 0.1495 0.1816 0.1854 0.5700</td>
<td></td>
</tr>
<tr>
<td>Split dose</td>
<td>0.9000 0.0000 0.0000 0.4000 0.6000 0.7000 19.8000</td>
<td>+ + + + + + + + + +</td>
</tr>
<tr>
<td>Stilbestrol</td>
<td>0.1857 0.0000 0.0000 0.1095 0.1483 0.1746 0.2700</td>
<td></td>
</tr>
</tbody>
</table>

a) Pycnotic cell nests  
Values are Mean ± SEM

1) P<0.02
2) P<0.001
3) P<0.01
4) P<0.05
TABLE 9

Two way analysis of variance of data on the effect of stilbestrol on the spermatogenesis of *Uperodon systoma*.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Stages</td>
<td>153.1333</td>
<td>5</td>
<td>30.626660</td>
<td>56.95659</td>
<td>0.001</td>
</tr>
<tr>
<td>Between treatments</td>
<td>115.3999</td>
<td>2</td>
<td>57.699950</td>
<td>107.30500</td>
<td>0.001</td>
</tr>
<tr>
<td>Interaction</td>
<td>35.4667</td>
<td>10</td>
<td>3.546674</td>
<td>6.59577</td>
<td>0.001</td>
</tr>
<tr>
<td>(Stages x treatments)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>189.9000</td>
<td>342</td>
<td>0.537719</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>487.9000</td>
<td>359</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = Sum of Square
DF = Degree of freedom
MS = Mean square
LEGENDS FOR FIGURES

Fig.20. Section through the testis of control frog (Haemalum and eosin X450)

Fig.21. Section through the testis of frog after booster administration of stilbestrol. Note the pycnosis and disorganisation of the cell nests. It is less compared to split dose administration. (Haemalum and eosin X450)

Fig.22. Section through the testis of frog after split dose administration of stilbestrol. Note the overall disorganised condition. (Haemalum and eosin X450)
Fig. 23

Bar diagram showing the Spermatogenetic stages of Control, Booster and Split dose administration of Stilbestrol.