CHAPTER V

EFFECT OF ESTRADIOL AND PROGESTERONE ON THE SPERMATOGENESIS OF UPERODON SYSTOMA

Introduction

Administration of exogenous estrogen produces testicular regression and atrophy of the sex accessories in several vertebrate groups (Emmens and Parker, 1947). It has long been known that estrogens are synthesised and secreted by the vertebrate testis and are present in general circulation (Chieffi, 1966 and Sharpe, 1982 for reviews). Effect of natural and synthetic estrogens are known in mammals, including in man (References in Albert, 1961; Burger, 1945) and they are known to cause arrest of spermatogenesis and atrophy of the testis. In mammals, studies on ram testis give evidence on seasonal variations of the intratesticular content of estradiol-17β (Barenton and Pelletier, 1983), and estrogen receptors have been found in rat testis (Sharpe, 1982). Testicular preparations from non-mammalian species (Mak et al., 1983a,b; Callard and Mak, 1985; Ho et al., 1987) also contain estrogen receptors.

Moore and price (1930) have experimentally proved that the effect of estrogen is exerted by depressing the gonadotropic factor of the pituitary. Cej et al. (1955) have reported suppressive effect of estrogen on the spermatogenesis of Leptodactylus Cheguensis. Similar observations are reported by

Recently in the frog, *Rana esculenta*, estradiol-17β has been found in plasma (Polzonetti-Magni et al., 1984) and testis concomitantly with a dramatic decrease of plasma androgens (Varriale et al., 1986).

In addition, in vitro incubation of minced testes revealed an inhibitory action of estradiol-17β on androgen output (Pierantoni et al., 1986).

In the light of the presence of estrogen-binding sites in frog testis (Fasano et al., 1986) it would be useful to investigate whether or not they undergo seasonal fluctuations, on the exogenous administration of estradiol 17-β on the testicular activity of *Uperodon systoma*.

**Materials and Methods**

Adult male frogs (*Uperodon systoma*) averaging 45gm. in body weight and 60mm snout to vent length were selected for the experiment.

Estradiol-17β were obtained from the sigma (U.S.A.) steroid preparation for injections were done as mentioned in (Vide Page No.22.). Ten animals were given a total of 10mg of Estradiol-17β for 15 days. Each injections with 0.06mg of estradiol mixed with 0.12ml of Amphibian Ringer solutions. Injections, were
given continuously for 15 days. As placebo control an equal number of normal frogs was injected with Amphibian Ringer solutions 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.

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

The available current information regarding the impact of progestational steroids on the spermatogenesis of amphibians is inadequate. In the testes of Bufo Vulgaris Chieffi and Lupo (1961,a) have reported the presence of progesterone and 17-B estradiol. Ericsson and Dutta (1965) have studied the effect of progesterone on the spermatogenesis of ram. In mammals its inhibitory effect has been observed by Selye (1940) and Kar et al., (1967). In progesterone treated specimens, testicular biopsies have revealed Leydig cell atrophy with sloughing of seminiferous tubules and disorganization of germinal elements. Relatively little work has been done in elucidating the effect of progestational steroids on the spermatogenesis in anurans. Houssay, (1954) Cei et al., (1955) and others have reported that progesterone treatment inhibits the dividing stages of the spermatogenetic cycle in several salientia.
Kasinathan and Basu (1977) have observed that progesterone at low doses inhibits testicular growth and brings about atrophy of seminiferous epithelium and Leydig cells in *Rana hexadactyla*. More over there is also significant inhibition of primary and secondary spermatogonial cell nests.

Recently Fasano et al., (1989) have reported the presence of progesterone at high level during spring in a seasonal breeder *Rana esculenta*.

In the light of above observation, the present study would have been taken to study the effect of progesterone on the spermatogenesis of *Uperodon systoma*.

**Materials and Methods**

Adult male *Uperodon systoma* of 45gm body weight and 60mm snout to vent length were selected for the experiment.

Progesterone hormone materials were donated by the JIPMER, Pathology Department, Pondicherry. Hormone preparation for injection have been done as the method prescribed previously. (vide page No:.22.). A group of ten animals have been selected as the experimental subjects. Each frog received a total of 7.5mg of progesterone for the entire period of experiment. Each injections having 0.5mg of progesterone, which is dissolved in 0.5ml of Amphibian Ringer solution. Injections were given subcutaneously, and continuously for 15 days. Placebo control frogs equally receive 0.5ml of Amphibian Ringer solution only. After the last injections, both the groups of frogs were autopsied and their body weight and the weight of the testes have
been taken individually for each animal.

The testes were fixed in Bouin's and paraffin sections of 7
μ thickness were taken and stained with haemalum eosin for
histological observations. Relative testicular weight and the
assessment of spermatogenetic activity can be done as per the
previous method.

Observation

Estradiol

Estradiol treatment at low dose level (Table 12 Fig 29) has
significantly (P<0.02) reduced the testicular weight. Almost all
the spermatogenetic cell nests had become pycnotic and there was
complete disorganisation. Only a few secondary spermatocytes and
spermatids were discernible in the tubular lumen. Spermatozoal
heads were seen somewhat distorted and at places appeared short,
stumpy and swollen. There was well marked dehison of
spermatozoa in good majority of the seminiferous tubules.
Conglomeration of the spermatogenetic cysts was well marked in
some of the tubules. (Fig 29). Interstitium was almost
completely reduced with shrunken Leydig cells. Further more a
thickening of the tubular walls and shrinkage of the lumina were
very remarkably seen.

Progesterone

In progesterone treated animals (Table 12 Fig 30) the
relative testicular weight is reduced significantly (P<0.1). In
treated animals, primary spermatogonia (stage 0) and secondary
spermatogonia stage I and II are reduced significantly (P<0.1).
There has been apparent rise in the cell nests of primary spermatocytes (Stage III). Interstitium is sparse with shrunken Leydig cells (Fig 30).

Results of "two way analysis of variance" summarised in table 13 show significant \( p<0.01 \) variation between different groups. Similarly, the interaction between the stages and treatment is also significant. \( P<0.001 \).

**DISCUSSION:**

**ESTRADIOL**

There seems to be some variation in the effects produced by estrogen administration on the testes of amphibians. In the present investigation, it has been noted that estradiol administration inhibits the spermatogonial and spermatogonial stages and there is almost degeneration of testicular follicles. Our results are in consonance with the earlier findings of Kasisnathan and Basu (1975) in frog *Rana hexadactyla*, Saidapur and Nadkarni (1975b) in frog *Rana cyanophlyctis* and *Rana tigrina* and of Srivastava (1986) in *Rana tigrina*. It is believed that the spermatogonial suppression may be due to the suppression of the secretion of gonadotropic hormones of the pituitary (Basu, 1968).

**Summary**

In the present investigation, it has been observed that estradiol, results in pycnosis of all spermatogonial cysts.
DISCUSSION

PROGESTERONE

In small doses, progesterone inhibits the testicular growth and spermatogenesis and brings about atrophy of seminiferous epithelium and Leydig cells. This is stated to be due to the ability of these steroids to inhibit the gonadotropic hormone secretion of the pituitary (Ericsson and Dutta, 1965). The present investigation also corroborates the earlier findings (Selye, 1940; Kar et al., 1967; Kasinathan and Basu 1975; Srivastava, 1986) that progesterone inhibits spermatogenesis. It is evident that progesterone inhibits the mitotic division of secondary spermatogonia. Interestingly, it has got a stimulatory effect on the meiotic divisions of primary spermatocytes. With regards to secondary spermatocytes this sex steroids apparently has inhibitory effect on the meiotic divisions of this stage.

Summary

In the present investigation, it has been noted that progesterone inhibits the primary spermatogonial and secondary spermatogonial stages considerably and retards the mitotic divisions of secondary spermatogonia as well as meiotic divisions of secondary spermatocytes.
### Table 12

**Effect of estradiol and progesterone on the spermatogenesis of**

*Uperodon 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>0</td>
<td>I</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3.7500</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1984</td>
<td>0.1322</td>
</tr>
<tr>
<td>Estradiol</td>
<td></td>
<td>3.5500</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2288</td>
<td>0.0000</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td>3.5000</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2500</td>
<td>0.1024</td>
</tr>
</tbody>
</table>

3) a) 3) 3) a) 3) 3) a) 21

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a) Hyaline cell nests

Values are Mean ± SEM

1) *P* < 0.02
2) *P* < 0.01
3) *P* < 0.1
**TABLE 13**

Two way analysis of variance data for the spermatogenetic stages of estradiol and progesterone treated frog *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>494.3250</td>
<td>5</td>
<td>98.8650100</td>
<td>196.752000</td>
<td>0.001</td>
</tr>
<tr>
<td>Between treatments</td>
<td>2.2166</td>
<td>2</td>
<td>1.1083370</td>
<td>2.205711</td>
<td>0.001</td>
</tr>
<tr>
<td>Interaction</td>
<td>169.3833</td>
<td>10</td>
<td>16.9383300</td>
<td>33.709100</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>171.8500</td>
<td>542</td>
<td>0.5024854</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>837.7750</td>
<td>559</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. 28. Section through the testis of control frog. (Haemalum and eosin X450)

Fig. 29. Section through the testis of frog after estradiol administration showing disorganizations of spermatogenic cysts and Pycnosis of germinal cysts. (Haemalum and eosin X450)

Fig. 30. Section through the testis of frog after progesterone administration showing a reduction in spermatogenetic stages. (Haemalum and eosin X450)
Bar diagram showing the Spermatogenetic stages of Control, Estradiol and Progesterone treated Frog *Uperodon systoma.*