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Effect of Antiestrogen (Tamoxifen Citrate) on the Ovary of the Pigeon, *Columba livia* during Preincubation Phase with Reference to Follicular Growth

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Abstract : The effect of an antiestrogen, Tamoxifen Citrate on the differential follicular counts in the domestic pigeon Columba livia is investigated. 250µg/tamoxifen citrate/0.2 ml olive oil/ day (7 injections) were administered to female pigeons during preincubation phase. The weight of the ovary, oviduct and length of the oviduct were noted at autopsy. The number of follicles at different developmental stages was counted and classified depending on their diameter. There was a significant decrease in the length of the oviduct and weight of the ovary and oviduct in tamoxifen citrate treated pigeons. There was a significant increase in the total number of normal and atretic follicles and only Stage I to Stage III follicles were found in the tamoxifen citrate treated pigeons. This study suggests that, estrogen is needed for the maintenance of dominant yolky follicles. The growth of previtellogenic follicles, stage I does not seem to be dependent upon estrogen. A significant increase in the number of Stage I previtellogenic follicles in tamoxifen citrate treated pigeons suggests that the recruitment of these follicles might be under inhibitory/ regulatory influence of dominant yolky follicles in the ovary.

Key words : Follicles, Diameter, Tamoxifen Citrate, *Columba livia*.

Introduction

Estrogen is known to perform variety of functions in female reproduction. The estrogen secreted by the large developing follicle has local action in the control of follicular growth, needed for maintenance of structure and function of female reproductive tract and feedback action on LH release leading to ovulation in vertebrates. Among mammals, estrogen is reported to have stimulatory action on the growth of the ovary in hypophysectomised rats (Harman *et al*., 1975; Hillier and Ross, 1979). On the other hand, in infant mammals (rats, mare, hamster, monkeys and humans), estrogen is known to exhibit inhibitory function on the follicular growth and its function (Burns and Douglas, 1981; Dierschke *et al*., 1983; Archer *et al*., 1988; Hutz *et al*., 1987a & b, 1989). Active immunization of guinea pigs against estrogen (Peddie, 1977) and passive immunization with estrogen antibodies of monkeys (Zeleznik *et al*., 1985) increased the number of developing and antral follicles. It is observed that follicles other than dominant follicles are under inhibitory influence of estrogen (Hutz, 1989).

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Among birds, estrogen has stimulatory effect on the ovarian growth in 6 week old pullets and non-breeding pin tail ducks (Phillips and Van Tienhoven, 1960). On the contrary, estrogen has inhibitory effect on development of follicle and it will increase the incidence of atresia and reduce ovarian weight in yellow throated sparrow (Gupta and Maiti, 1987). Eventhough, estrogen will mobilize yolk precursor in birds, does not necessarily result in an increase in the number of yolky follicles (Schjeide, 1967; Lofts and Murton, 1973; Follett, 1984). The facillitatory role of estrogen in progesterone induced LH surge causing ovulation in hen is well known (Wilson and Sharp, 1976 a & b ; Wilson and Cunningham, 1981). By the above references it is clear that there is no work on the effect of estrogen on the growth of the follicle belonging to different development stages.

Tamoxifen, a potent antiestrogen is known to interfere with the uptake of estradiol at its receptor thus block the biological activity of the steroid (Sutherland *et al*., 1977 ; Scheib *et al* ; 1984). Hence the present study was undertaken to see the effect of Tamoxifen Citrate on the ovary of the pigeon, *Columba livia* during the preincubation phase, particularly on the growth of different size range developing follicles in order to know role of estrogen on growth of the follicle.

Materials and Methods

Adult female pigeons of preincubation phase were chosen from the pigeon colony maintained in the Zoology Department, Karnatak University, Dharwad. The pigeons were grouped and treated as follows:

Group I (5 pigeons)

250 µg of Tamoxifen citrate (Khandelwal Laboratories Ltd, Bombay, India) in 0.2 ml olive oil every day for 7 days.

Group II (5 pigeons)

0.2 ml olive oil to serve as control.

All the injections were administered intramuscularly at the pectoral region and pigeons were autopsied a day after the last injection. At autopsy, the weight of the body, ovary and that of the oviduct was recorded for each bird. The length of the oviduct was also recorded.

The ovaries were fixed in Bouin's fluid and dehydrated up to 70 % alcohol. The follicles were separated by spring scissor and diameter of the follicles were measured with the help of micrometer screw guage. The follicles were classified as follows:

Stage I - 0.5 mm to 1.00 mm.
Stage II - 1.1 mm to 3.00 mm.
Stage III - 3.1 mm to 5.0 mm.
Stage IV - 5.1 mm and above.

The total number of normal and atretic follicles was calculated for each group. Representative ovaries from each group were processed for histological studies.

The data were statistically analysed by using Mann-Whitney 'U' test and was considered significant when $P < 0.05$.

Observations

There was a significant decrease ($P<0.05$) in the length and weight of the oviduct and ovarian weight in Tamoxifen Citrate treated pigeons when compared to that in controls.

The ovaries in control group contained Stage I to Stage IV follicles while Tamoxifen treated pigeons contained Stage I to Stage III follicles. There was a significant increase in the number of Stage I follocles in the

Tamoxifen treated group when compared to that in control. Stage II and Stage III follicles were significantly decreased in Tamoxifen treated pigeons when compared to that in control. There was a significant increase in the total number of normal follicles in Tamoxifen Citrate treated group.

The number of atretic follicles was significantly more in Tamoxifen treated group when compared to that in control group.

Discussion

Since, Tamoxifen is antiestrogenic, a significant reduction in the length and weight of the oviduct in Tamoxifen treated group is in accordance with the report in chicken (Sutherland *et al*., 1977).

A few large yolky follicles in various stages of atresia in Tamoxifen treated group and absence of Stage IV follicles suggests the degeneration of large follicles, since Stage IV follicles were present in the control group. Tamoxifen is known to interfere with estrogen binding receptors in the target organs. The absence of dominant Stage IV vitellogenic follicles in the ovary of Tamoxifen treated group shows that maintenance and/ recruitment of Stage IV follicles is dependent upon estrogen. The significant reduction in the number of Stage II follicles (early vitellogenic) and Stage III vitellogenic in Tamoxifen treated group are in accordance with the concept that mobilization of yolk precursor in the liver and its incorporation in the developing follicles is known to be estrogen dependent.

It is evident that reduction in the number of Stage II and III follicles and absence of Stage IV follicles in Tamoxifen treated pigeons explains the incidence of increased atresia in this group. On the otherhand increase in the number of Stage I follicles forms a larger pool in the ovary of Tamoxifen treated group, suggest that recruitment of Stage I previtellogenic follicles may not be dependent upon estrogen.

In vitro studies on rats have shown that Tamoxifen interferes with the synthesis of estrogen by blocking stimulatory action of FSH (Watson and Howson, 1977). In hypophysectomised immature rats, treatment of gonadotrophins as well as estrogen is reported to decrease the number of atretic follicles (Peters and Mc Natty, 1980). However, gonadotrophins given with antiestrogen could not overcome the follicles undergoing atresia suggesting that anti-atretic effect of gonadotrophin is mediated through estrogen synthesis (Louvet *et al*., 1975). In the present study, increase in the number of Stage I follicles and decrease in the Stage II and III follicles in Tamoxifen treated groups suggests that inhibitory effect on the recruitment of Stage I follicles is abolished after Tamoxifen treatment. The inhibitory source may be of intraovarian origin, possibly from dominant follicles of the ovary. Whatever may be the inhibitory substance regulating the recruitment of Stage I follicles one thing is clear that estrogen is not needed for recruitment of Stage I follicles in *C. livia*.

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Table 1 : Effect of Tamoxifen citrate (250 µg) on the weight of the body and that of the ovary and oviduct and oviduct length in the preincubating pigeons, *Columba livia*.

Groups	Body weight (gm) ± S.E.	Ovary weight (gm)/ 100 gms	Oviduct weight (gm)/ 100 gms	Oviduct length (cm) ± S.E.
Control (olive oil treated)	341.20 ± 5.45	a 0.770 ± 0.071	b 1.456 ± 0.405	c 25.60 ± 3.018
Tamoxifen Citrate treated (250 µg)	299.00 ± 12.88	a 0.132 ± 0.020	b 0.820 ± 0.174	c 17.81 ± 2.17

Figures superscribed by the same alphabets are statistically significant at 0.05 level.

Table 2 : Effect of Tamoxifen citrate (250 µg) on the number of normal follicles of different size, and total number of normal and atretic follicles in pigeon, *Columba livia* during the preincubation phase

	Number of follicles ± S.E.				Total	
Groups	**Stage I (0.5-1.0 mm)**	**Stage II (1.1-3.0 mm)**	**Stage III (3.1 – 5.0 mm)**	**Stage IV (5.1 mm & above)**	**NF**	**AF**
Control (olive oil treated)	a 39.00 ± 2.00	b 34.88 ± 2.83	c 9.66 ± 0.88	3.00 ± 2.44	d 83.00 ± 9.35	e 39.20 ± 4.09
Tamoxifen Citrate treated (250 µg)	a 78.50 ± 1.80	b 25.25 ± 2.09	c 5.00 ± 1.10	–	d 115.50 ± 4.36	e 79.80 ± 2.85

Figures superscribed by the same alphabets are statistically significant at 0.05 level.
NF - Normal Follicle; AF - Atretic Follicle.

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