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Effect of Gonadotrophins and Gonadotrophin Releasing Hormone (GnRH) on folliculogenesis in the domestic pigeon, *Columba livia*.

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Abstracts : Effect of Gonadotraphon FSH (5 IU/day/D.Water/10 injections and 10 IU /day/ D.Water/10 injections) Gonadotraphon L H (5 IU/day/D.Water/10 injections) and GnRH (50 ng/day/D.Water/10 injections) on folliculogenesis in 5 day hatchlings of the domestic pigeon *Columba livia* was investigated. The ovaries in initial control (5 day old Hatchlings) contained only oocytes but not primordial follicles. The diameter of the follicles present in other groups were measured and classified. Follicles above 75 µm appeared in 5 IU Gonadotraphon FSH treated, 10 IU Gonadotraphon FSH treated and GnRH treated birds. The follicles above 125 µm were found only in 10 IU Gonadotraphon FSH treated birds. Also, there was a significant increase in the diameter of the largest follicle of 10 IU Gonadotraphon FSH treated group when compared to that in final control group. The present study suggests that in *C. livia* gonadotrophins may not be required for folliculogenesis.

Key word : Gonadotrophins, GnRH, Folliculogenesis, *Columba livia*

Introduction

Patterns of oogonial proliferation, oogenesis and folliculogenesis are described in some species of vertebrates (Gondos, 1978; Peters, 1978; Tokarz, 1978 ; Licht 1984; Guraya 1989; Saidapur, 1989; Byskov and Hoyer, 1994). Later study by Motta et al (1995) on lizard *Podarcis sicula* suggested that FSH in addition to regulating oogonial proliferation and oogenesis it also influenced folliculogenesis.

The process of folliculogenesis (formation of primordial follicles) is similar in all vertebrates. There is no definite information on the endocrine events , if any, controlling folliculogenesis in teleosts. In amphibians (anurans) available reports suggest that early primary follicular growth is independent of hormonal regulation (Saidapur, 1989). On the other hand, some studies on reptiles point out that follicle stimulating hormone (FSH) is needed for the growth of primary follicle (Jones *et al*., 1975, 1976) while others suggest that early follicular growth is gonadotrophin independent (Eyeson, 1971; Ortiz and Marales, 1974). To the best of our knowledge, nothing is known about the hormonal regulation of folliculogenesis in birds.

The present study was undertaken to find out the effect of gonadotrophins (Gonadotraphon-FSH and gonadotraphon-LH) and GnRH on the folliculogenesis in newly hatched domestic pigeon, *Columba livia.*

Material and Methods

Five day old female hatchlings were selected from the pigeon colony maintained in the Zoology Department, Karnatak University, Dharwad, India. A group of five hatchlings was autopsied on the day of commencement of the experiment to serve as the initial control. At autopsy, weight of the body and that of the left ovaries were taken

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and fixed in Bouin's fluid. (Since there is no sexual dimorphism in hatchlings of pigeon, the number of hatchlings used in each group was more and at autopsy, only the female hatchlings were taken for further analysis).

The remaining hatchlings were divided intyo five groups (5 hatchlings/group) and treatment protocol was as follows:

Group 1 - Final control - 0.1 ml of distilled water.

Group 2 - LH treated - 5 IU of gonadotraphon –LH (Batch No. 782007/1 Paines and Byrne Ltd., England).

Group 3 - FSH treated - 5 IU of gonadotraphon –FSH (Batch No. 720052, Paines and Byrne Ltd.,England)

Group 4 - FSH treated -10 IU of gonadotraphon –FSH (Batch No. 720052, Paines and Byrne Ltd.,England)

Group 5 - GnRH treated - 50 ng of GnRH / 0.1 ml distilled water. (LH-FSH-RH (Chloride form) Batch No. 2 NIAMDD, NIH, Bethesda, Maryland).

Since avian gonadotrophins are not commercially available the mammalian gonadotrophins were used. All the injections were made through the intramuscular route in the wing web every alternate day till day 23 after hatching (total 10 injections per bird). The hatchlings were autopsied a day after the last injection.

At autopsy, the weight of the body and that of the ovary was recorded. The ovaries were fixed in Bouin's fluid and processed for histological studies. 5 µm thick serial sections were cut and stained with Harris haematoxylin and eosin. The following histometric data were collected from these sections.

1. The number of normal and atretic follicles was counted from every 10th section of each ovary and the mean number of normal and atretic follicles for each groups was calculated.

2. The follicles were classified into four groups based on their diameter :

(i) Follicles less than 25 µm diameter.

(ii) Follicles between 26 µm to 50 µm diameter.

(iii) Follicles between 51 µm to 75 µm diameter.

(iv) Follicles greater than 75 µm diameter.

3. Diameters of 15 largest follicles were recorded using an ocular micrometer from each bird in every group and the mean of largest follicular diameter for every group was calculated.

The data obtained were analysed by Kruskal-Wallis one way of analysis of variance followed by Scheffe simultaneous confidence interval test (Miller, 1981).

Results

There was no significant change in the weight of the ovary in different groups. The ovaries of initial controls contained oocytes and no primordial follicles.

In all treatment groups, the ovaries contained follicles of different sizes. Some follicles were also seen to undergo atresia in all the groups. The ovaries in 5 IU gonadotrophin LH treated group were similar to that present in the final control. There was no significant change in either the total number of follicles or the diameter of the largest follicle in LH-treated group when compared to that in final controls. (Table A). In both the final control and LH-treated groups, follicles above 75 µ m diameter were not found.

In 5 IU and 10 IU gonadotraphon-FSH and GnRH treated groups, follicles above 75 µm diameter appeared whereas in the group treated with 10 IU gonadotraphon-FSH, the follicles ranged up to 120 µm. and there was a significant increase in diameter of the largest follicles when compared to that in the final control group (Table 1). However, there was no significant change in the total number of follicles in Gonadotraphon-FSH and GnRH treated birds when compared to that in the final control (Table 1).

Discussion

The fecundity of females of each vertebrate species is primarily determined by the number of oogonia produced, their differentiation in to primary oocyte and the number of primordial follicles produced by the ovary (Tokarz, 1978). Consequently, a number of studies on mammals and some non-mammalian vertebrates were directed to understand the endocrine mechanism (if any) controlling the oogonial proliferation, oogenesis and folliculogenesis.

There is sufficient evidence suggesting that the early follicular development is influenced by gonadotrophins in mammals (Greenwald and Terranova, 1988; Byskov and hoyer, 1994). In nonmammalian vertebrates whether early follicular growth is gonadotrophin dependent or not, is not resolved with certainity. In immature intact and adult hypophysectomised lizard *Anolis. Carolinensis* and *Podarcis sicula* mammalian

FSH increased the number of primordial follicles (Jones *et al.*, 1975, 1976; Motta *et al*, 1995). On the otherhand, in lizard *Agama agama* early follicular growth is shown to be gonadotrophin independent (Eyeson, 1971; Ortiz and Marales, 1974). In anuran amphibians early follicular growth is considered to be gonadotrophin independent (Tokarz, 1978; Saidapur, 1989).

To the best of our knowledge, the present study is the first report on the effect of gonadotrophins and GnRH on the follicular growth in newly hatched birds. Since there was no significant change in the number of follicles below 25 µm diameter and the total number of follicles in gonadotraphon-LH, gonadotraphon-FSH and GnRH treated birds, it seems that folliculogenesis is independent of gonadotrophins in *Columba livia.* However, the appearance of follicles above 75 µm diameter in gonadotraphon-FSH treated hatchlings suggest that FSH has a stimulatory role in follicular growth. GnRH treated birds also had follicles above 75 µm diameter. Since, in the present study, gonadotraphon-LH had no stimulatory role in the growth of the follicles, the stimulatory effect of GnRH on follicular growth seems to be due to its action via FSH of bird's own pituitary.

The present study infers that FSH type of gonadotrophin is needed for growth of the follicles, whereas folliculogenesis may not be dependent on gonadotrophins in *C. livia.*

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Table 1 :Effect of Gonadotraphon -LH, Gonadotraphon-FSH and GnRH on the ovary with respect to number of different size normal follicles and the mean diameter (µm) of the largest follicles in hatchlings of the pigeon, *Columba livia*.

Groups	<0.25 µm diameter	Number of follicles ± S.E.		>0.76 µm diameter	Total No. of Normal follicles	Total No.l of Atretic follicles	Mean Diameter of largest follicle (µm)
		0.26-0.50 µm diameter	0.51-0.76 µm diameter				
Control (Dist. Water Treated 0.1 ml./Alternate day)	3130.20 ± 546.07	1289.80 ± 217.00	727.00 ± 152.60	-	5147.00 ± 784.90	702.80 ± 110.10	76.65 ± 2.40
Gonadotraphon-LH Treated (5 IU/Alternate day)	4038.40 ± 716.90	1024.60 ± 186.20	551.40 ± 113.90	-	5614.00 ± 390.60	863.00 ± 51.80	66.80 ± 4.40
Gonadotraphon-FSH Treated (5 IU/Alternate day)	3745.80 ± 257.80	1151.00 ± 130.70	462.20 ± 69.30	138.20 ± 16.30	5498.00 ± 175.50	632.20 ± 29.80	86.70 ± 1.90
Gonadotraphon-FSH Treated (10 IU/Alternate day)	4098.00 ± 401.00	1319.20 ± 238.30	822.20 ± 113.10	653.20 ± 77.20	6251.00 ± 600.30	774.20 ± 136.60	120.30 ± 11.00
GnRH Treated (50 ng/Alternate day)	4028.00 ± 702.60	1101.00 ± 201.80	879.60 ± 122.20	721.40 ± 39.00	6700.00 ± 800.20	621.60 ± 28.00	93.30 ± 5.80

1. Total number of injections ten.
2. Figures superscribed by the same alphabets are statistically significant at 0.05 level.

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