Chapter V

INFLUENCE OF CORTICOSTERONE ON TESTICULAR FUNCTIONS AND THYROID ACTIVITY IN PIGEONS TREATED WITH GONADOTROPHIC HORMONES IN THE QUIESCENT-PHASE

Though the temperate species of birds tune their reproductive activities in relation to photoperiodic changes (see Murton and Westwood, 1977; Follett and Robinson, 1980; Follett, 1984; Nicholls, 1988), the tropical species of birds relate their breeding activities with many environmental factors like temperature, rainfall, humidity and even light (see Thapliyal and Gupta, 1989; Kumar and Kumar, 1991). The tropical feral pigeons enter into breeding phase in the summer months (March-May) followed by rapid gonadal regression (May-June) and then remain in the quiescent phase till December (Patel, 1993). By January, gonadal recrudescence commences and attains full testicular size by February. The phases of gonadal recrudescence and regression correspond roughly to winter and summer solstice respectively. Previous studies have shown a parallel adrenal testes relationship and an inverse thyroid-testes relationship (Patel et al., 1985; Ramachandran and Patel, 1986; Ayyar, 1987; Ayyar et al., 1992). Confirmation to this earlier inference was provided by the observation of increased adrenocortical activity with higher serum

corticosterone (CORT) level coupled with reduced thyroid activity and lower serum T_4 level in the recrudescence phase and vice-versa in the regression phase (Patel et al., 1993; Patel, 1993). An interrelationship between adrenal and thyroid activity in regulating testicular functions was revealed by the observation of testicular regression and increased thyroid activity with higher serum T_4 level by experimental adrenocortical suppression in the breeding season (chapter I). As a corollary to this observation, CORT administration either in the regression phase or quiescent phase was seen to reduce thyroid activity and lower serum T_4 level without however, activating the gonad (chapter I and II). The ability of CORT to bring about testicular activation in the quiescent phase, despite the fact that a favourable situation in the form of reduced serum T_4 level, was inferred to be due to the inactivity of the HHG axis. Hence in the present study, the influence of short term administration of FSH or a combination of FSH and LH in either normal or CORT treated birds on testicular histology and activity of adrenals and thyroid has been assessed.

MATERIALS AND METHODS

Procurement and maintenance of pigeons and preparation of CORT are as outlined in chapter I.

Follicle Stimulating Hormone (FSH)

Metrodin, manufactured by Serono laboratories, Switzerland was procured from the local market and was used for the present study.

FSH. LH

Pergonal, (a combination of FSH and LH) manufactured by Serono laboratories, Switzerland, was procured from the local market and was used for the present study.

Experimental setups :

In the non-breeding phase (October), a total of 42 male pigeons were randomly divided into seven groups. Two female birds were kept per group.

Group I (Control). These birds were given daily (ip) injections of 0.9% saline.

Group II (Corticosterone : CORT). These birds were given daily (ip) injections of 2µg CORT in 0.1 ml vehicle.

Group III (FSH). These birds were given daily (im) injections of 50 µg FSH in 0.1 ml saline.

Group IV (FSH.LH). These birds were given daily (im) injections of 50 µg FSH.LH mixture (25 µg of each) in 0.1 ml saline.

Group V CORT primed(P) + FSH. These birds were first primed with daily (ip) injections of 2 µg CORT for 15 days, followed by daily 50µg (im) injections of FSH in 0.1 ml saline for the next 15 days.

Group VI CORT + FSH (Simultaneous : S). These birds were given daily injections of 2 µg CORT (ip) and 50 µg FSH (im) in 0.1 ml saline.

Group VII CORT + FSH.LH. These birds were given daily injections of 2 µg CORT (ip) and 50 µg FSH.LH mixture (im) in 0.1 ml saline.

All the above injections were given at 09.00h for 15 days.

Parameters and Methodology of evaluation.

Morphometry and histological evaluations are as outlined in chapter I.

Measurement of Seminiferous tubular and leydig cell nuclear diameter.

Diameters of 20 more or less round seminiferous tubular sections and of at least 50 leydig cell nuclei from the sections of the testis were measured (magnification 10x100) using caliberated oculometer. (Table V a)

Serum hormone level :

Measurement of T_4, T_3 and CORT are as outlined in chapter I. Circulating levels of testosterone (T) was analysed using RIA kit provided by WHO and was expressed as ng/ml.

RESULTS :

Gravimetric changes :

The relative weight of testes increased significantly in birds treated with only FSH or FSH.LH. Both FSH and FSH.LH in combination with CORT also increased the weight of testes. However, the increase was more pronounced in those birds administered with CORT + FSH (S). The relative weight of adrenals increased following administration of FSH or FSH.LH in combination with CORT. Thyroid weight was

Treatments	Control	CORT	FSH	FSH.LH	CORT+FSH(P)	CORT+FSH (S)	CORT+FSH.LH
Testes	14.28 ± 0.43	26.42* ± 1.81	24.91* ± 0.89	28.48* ± 1.20	24.13* ± 1.17	46.80* ± 2.85	26.73* ± 1.62
Adrenals	9.09 ± 0.26	8.74 ± 0.26	9.50 ± 0.54	12.74* ± 0.54	5.75* ± 0.40	7.86* ± 0.22	8.31* ± 0.21
Thyroid	9.16 ± 0.24	8.32 ± 0.39	6.72 ± 0.25	10.90 ± 1.59	8.18 ± 0.19	7.81 ± 0.57	5.46* ± 0.53

Table Va : Changes in relative weights of testes, adrenals and thyroid of pigeons treated with gonadotrophic hormones alone or with cortcosterone in the quiescent phase.
(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

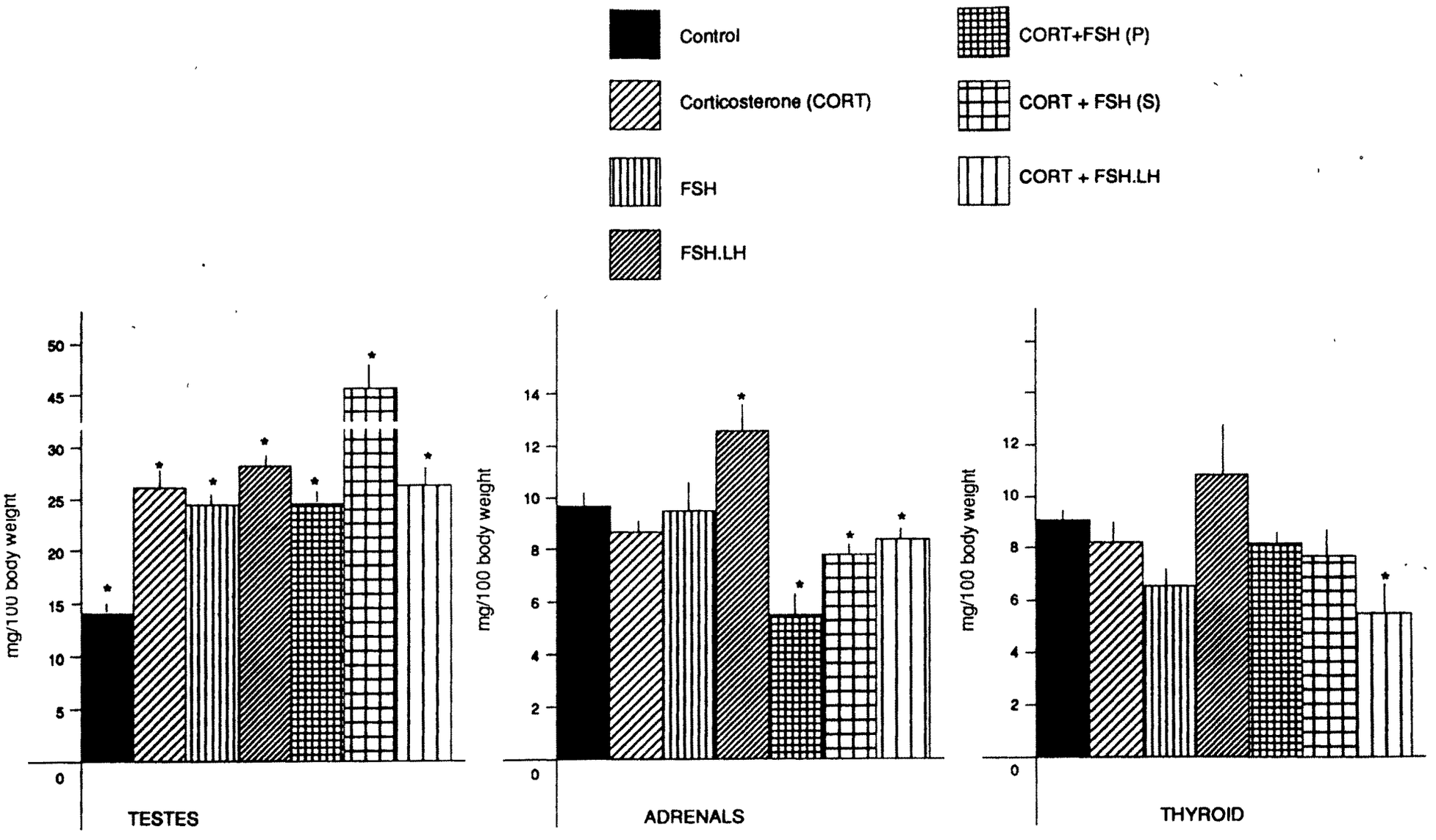

Fig. : 5. Changes in relative weights of testes, adrenals and thyroid of pigeons treated with gonadotropins alone or with corticosterone (CORT) in the quiescent phase.
(* Significant at $P < 0.05$, values are $\bar{X} \pm$ SEM).

Treatments	Mean tubular diameter (µm)	Nuclear diameter of interstitial (µm)	No.of Spermato-gonia	Stages of primary spermatocytes			Total No.
				Leptotene	Zygotene	Pachytene	
Control	33.33±4.5	0.99±0.17	13±4	-	-	-	13
CORT	59.20±7.40	2.44±0.98	33±4	4±1	-	-	37
FSH	68.88±4.91	4.80±1.14	36±6	7±2	3±1	-	46
FSH.LH	66.10±4.17	3.65±1.09	40±5	5±1	3±1	-	48
CORT+FSH(P)	105.81±9.11	2.94±0.57	20±4	5±2	27±6	4±1	56
CORT+FSH(S)	97.83±8.06	3.80±1.12	49±2	3±1	4±1	7±2	63
CORT+FSHLH	97.28±6.77	4.48±1.59	23±3	7±2	11±2	10±3	51

Table V b Changes in tubular diameter, Nuclear diameter of interstitial cells and number of primary spermatocytes in the testes of pigeons treated with gonadotrophins alone or with corticosterone in the quiescent phase.
(* Significant at $P<0.05$; values are $\bar{x} \pm SD$)

decreased in all treatment schedules except in FSH.LH group of birds, the thyroid of which registered increased weight (Table V a; Fig.5)

Histology :

Testis : The testis of control birds showed totally regressed tubules with a single layer of gonial cells. The interstitial cells were also regressed. The testis of CORT treated birds showed slightly enlarged seminiferous tubules with active gonial proliferation. Many of the tubules showed two layers of germ cells and occasionally leptotene spermatocytes could be seen in some tubules. The interstitial cells however did not show any change though the nuclear diameter was significantly increased as compared to the controls (Table-V b), The testis of birds treated with FSH also showed enlarged tubules with spermatogonial proliferation. There were mainly two layers of germ cells and some of the tubules showed leptotene and zygotene spermatocytes. Interstitial cells were hypertrophied with significantly greater nuclear diameter (Table-V b). The peritubular myoid cells depicted some degree of hypertrophy. The testis of birds treated with FSH.LH also showed enlarged seminiferous tubules with spermatogonial proliferation. In many of the tubules primary spermatocytes upto zygotene stage could be visualized. There were generally 2-3 layers

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PLATE I

Figures 1-6 Photomicrographs of sections of testis of control and experimental birds in the quiescent phase (200 X)

Fig.1 Testis of control bird showing regressed inactive seminiferous tubule and interstitial cells.

Fig.2 Testis of corticosterone (CORT) treated bird. Note the spermatogonial proliferation.

Fig.3 Testis of FSH treated bird showing germ cell activation in the seminiferous tubule.

Fig.4 Testis of CORT primed birds treated with FSH showing spermatogenic activation in the tubule.

Fig.5 Testis of birds treated with FSH.LH combination showing spermatogenic activation in the tubule.

Fig.6 Testis of bird treated with CORT and FSH.LH. Note the active state of the tubules with the initiation of spermatogenesis.

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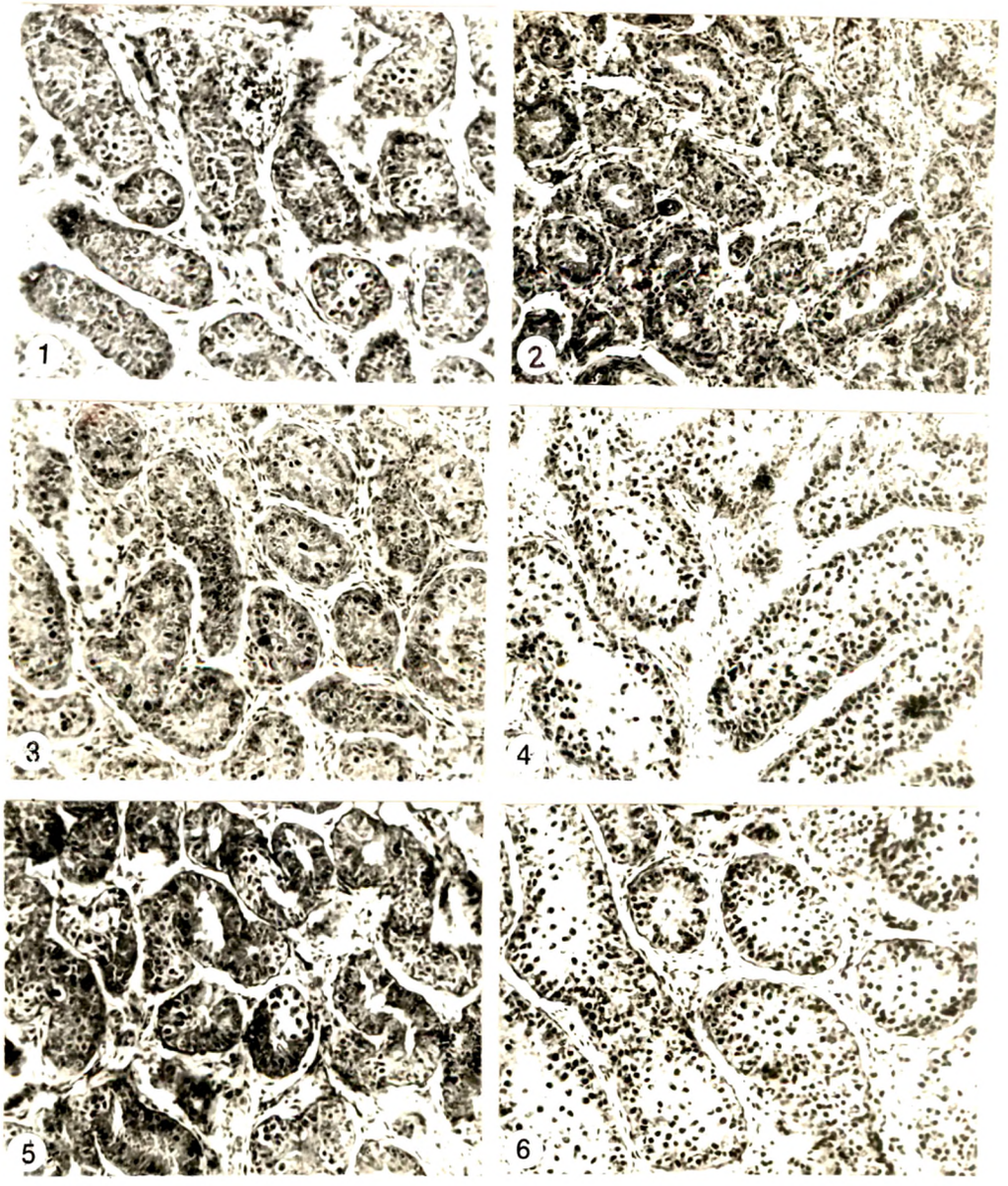

PLATE II

Figures 7-12 Photomicrographs of sections of testis of control and experimental birds in the quiescent phase (400 X)

Fig.7 Testis of control bird showing regressed inactive seminiferous tubule (ST)

Fig.8 Testis of CORT treated birds showing spermatogonial proliferation and active germ cells.

Fig.9 Testis of FSH treated birds showing enlarged tubules with proliferating germ cells.

Fig.10 Testis of CORT primed bird treated with FSH. Note the enlarged ST containing active and proliferating germ cells.

Fig.11 Testis of birds treated with FSH.LH combination showing enlarged ST with active spermatogenesis.

Fig.12 Testis of birds treated with CORT and FSH.LH. Note the enlarged ST with active spermatogenesis.

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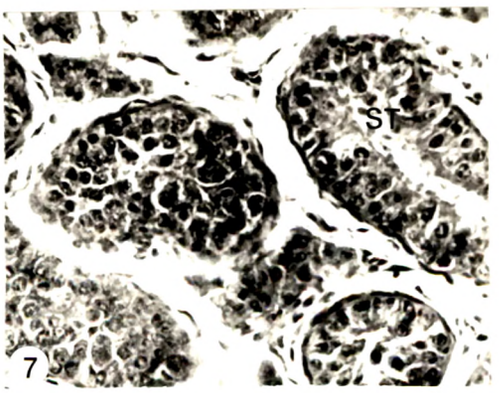

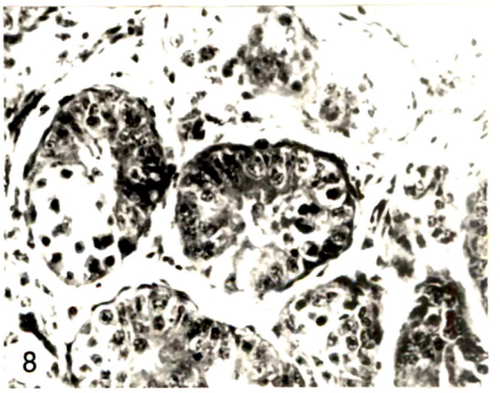

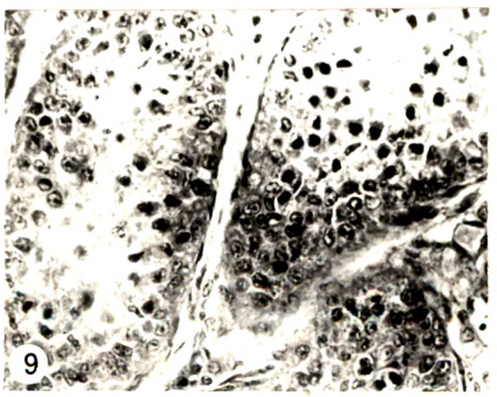

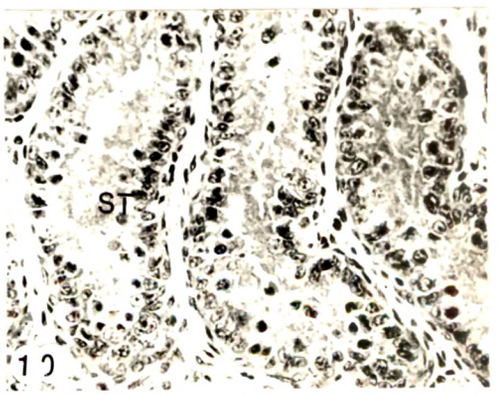

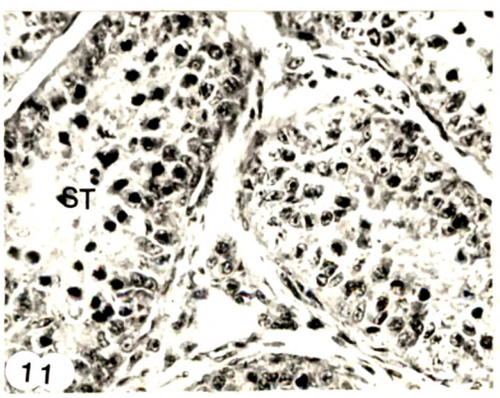

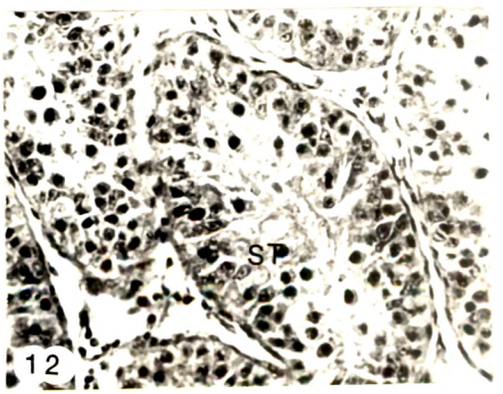

PLATE III

Figures 13-20 Photomicrographs of section of testis of control and experimental birds in the quiescent phase. (640 X)

Fig.13 A single inactive tubule of control bird showing many degenerating germ cells (DG)

Fig.15 A part of a tubule of FSH treated bird showing spermatogonial proliferation.

Fig.16 Testis of FSH treated birds showing prominent hypertrophied interstitial cells (IC)

Fig.17 Part of a tubule of CORT primed pigeon treated with FSH showing many zyotene (Z) spermatocytes.

Fig.18 Part of a tubule of pigeons treated with CORT + FSH. Note the appearance of pachytene (P) spermatocytes.

Fig.19 Testis of CORT primed pigeons treated with FSH, showing a mixture of prominent hypertrophied and small interstitial cells (IC).

Fig.20 Testis of pigeons treated with CORT and FSH simultaneously showing hypertrophied interstitium (IC).

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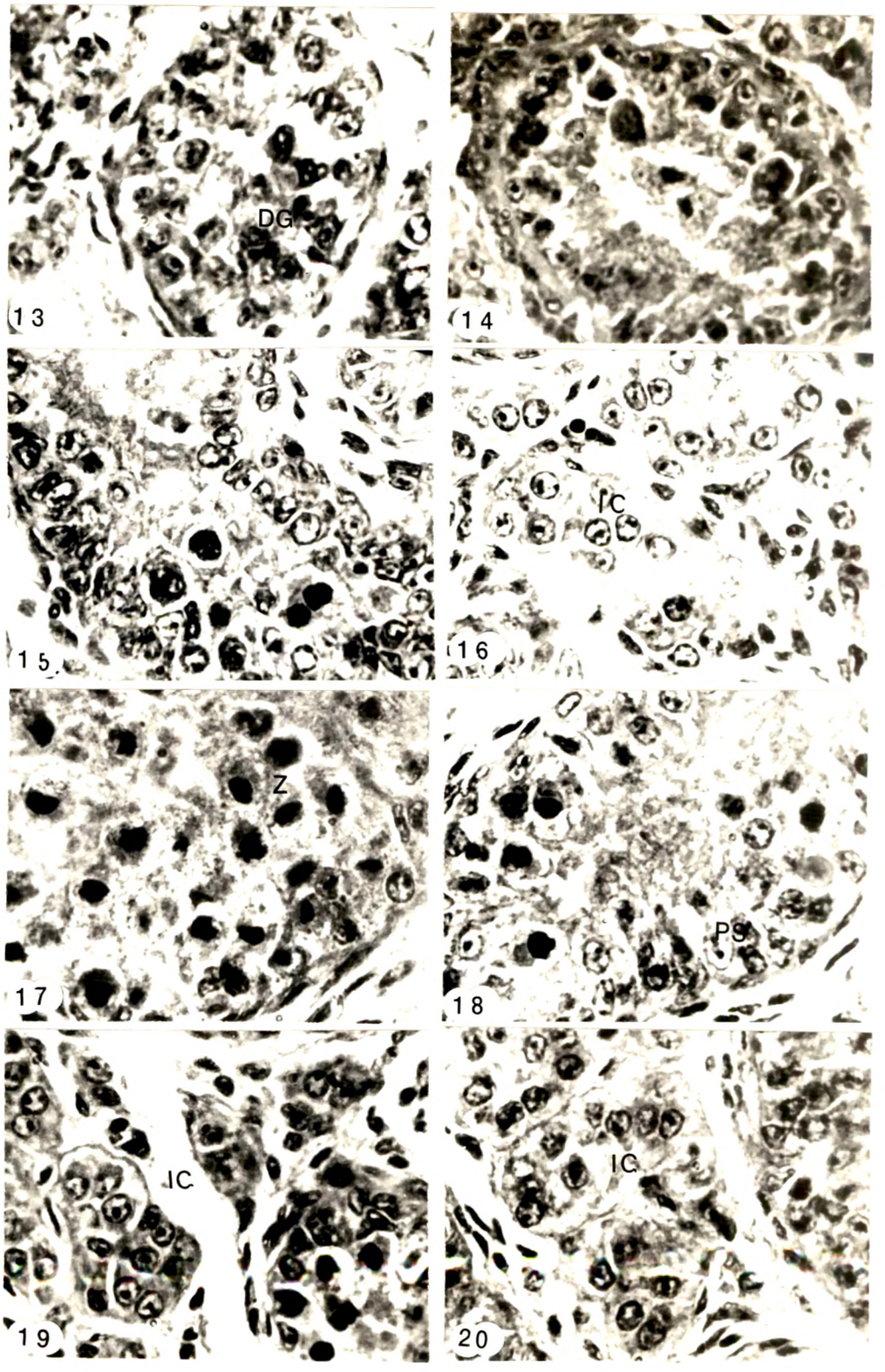

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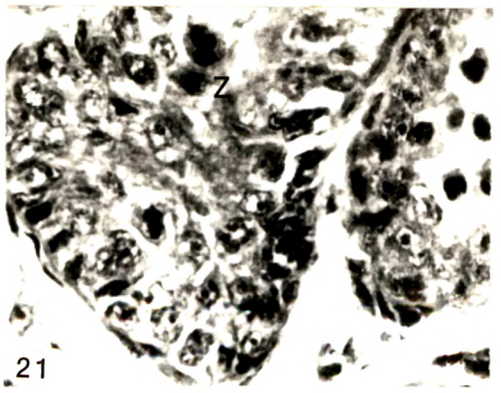
PLATE IV

Figures 21-25	Photomicrographs of sections of testis of experimental birds in the quiescent phase (640 X)
Figs 21 & 23	Testis of FSH.LH treated birds showing proliferating gonial cells and zyotene (Z) spermatocyltes (fig.21), and prominent interstitial (IC) cells. (fig.23)
Figs 22, 24 and 25	Testis of pigeons treated with CORT and FSH.LH showing primary (PS) and secondary spermatocytes (SS), (figs 22 and 24), and prominent interstitial cells (fig.25)


Z
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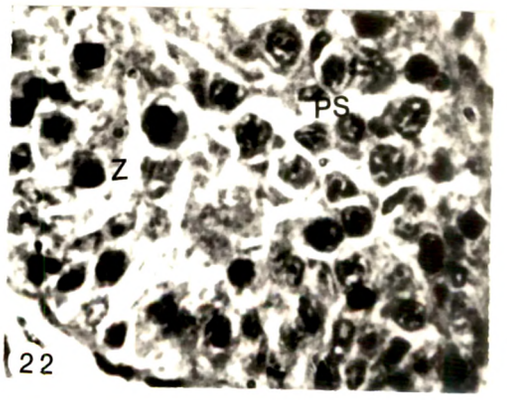
PS
Z
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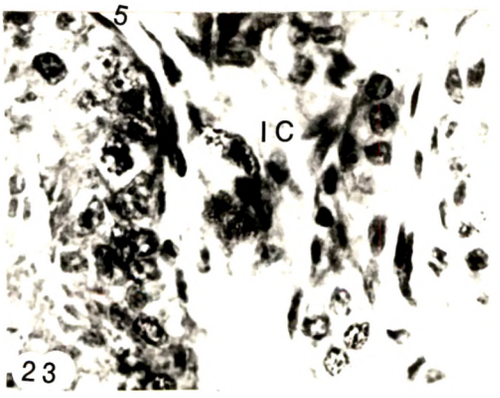
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IC
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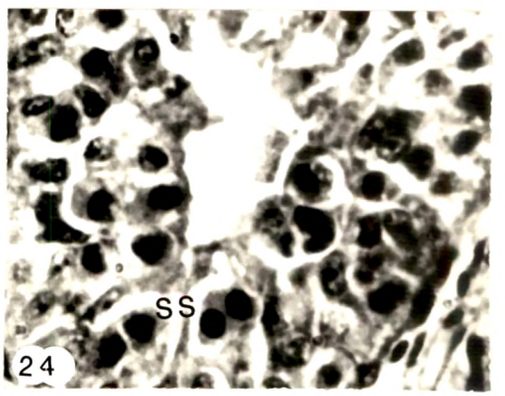
SS
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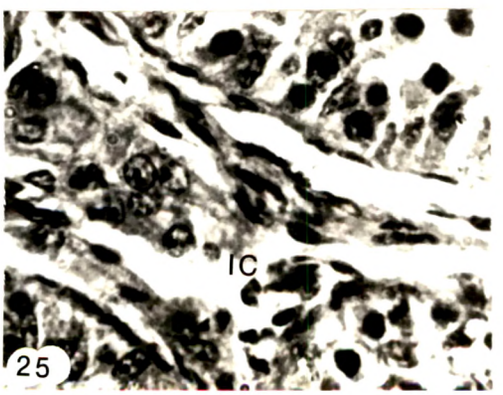
IC
25

of germ cells in each tubule. The diameter of the interstitial cell nuclei was increased and many dividing interstitial cells also could be seen. The testis of CORT + FSH (P) showed further increase in tubular diameter with signs of spermatogenic activation. Proliferating gonial cells and primary spermatocytes could be seen. There was significantly greater number of zygotene spermatocytes. The interstitial cells appeared normal with increased nuclear diameter, though lesser than FSH and FSH.LH treated birds (Table V b). Similarly, birds treated with CORT + FSH (S) also showed increased tubular diameter with stimulated spermatogenesis. However, there appeared to be greater number of pachytene spermatocytes. Interstitial cells were active and hypertrophied with significantly greater nuclear diameter. Few dividing interstitial cells could also be seen. The testis of birds treated with CORT + FSH.LH also showed increased tubular diameter like the previous two groups with spermatogenic activation. There were three to four layers of germ cells and greater number of leptotene, zygotene and pachytene spermatocytes as compared to any of the treated groups. In some of the tubules appearance of few secondary spermatocytes were also evident. The interstitial cells were prominent and active with significantly increased nuclear diameter more comparable to that of FSH treated birds (Plates I-IV).

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PLATE V

Figures 26-31 Photomicrographs of section of thyroid of control and experimental birds during the quiescent phase. (260 X)

Fig.26 Thyroid of control bird showing hypertrophied epithelium and depleted colloid content.

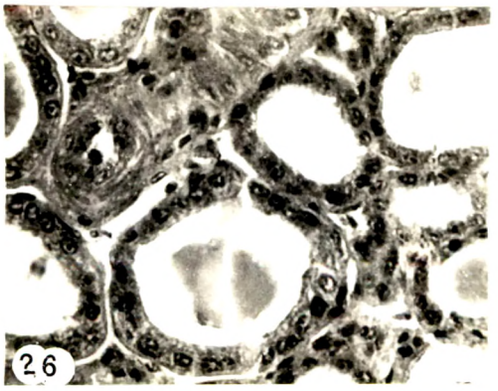
Fig.27 Thyroid of CORT treated birds showing colloid filled follicles and narrow follicular epithelium.

Fig.28 Thyroid of FSH treated birds showing a mixture of colloid filled and empty follicles and increased cell height.

Fig.29 Thyroid of CORT primed birds treated with FSH. Note the hypertrophied epithelium and colloid retention.

Fig.30 Thyroid of pigeons treated with FSH.LH. Note the follicles with hypertrophied epithelium and narrow lumen and depleted colloid content.

Fig.31 Thyroid of birds treated with CORT and FSH.LH. Note the follicles with depleted colloid content and reduced cell height.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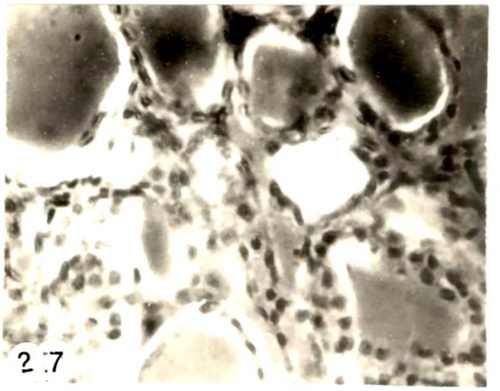
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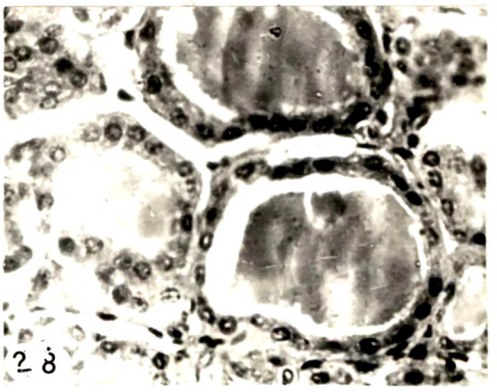
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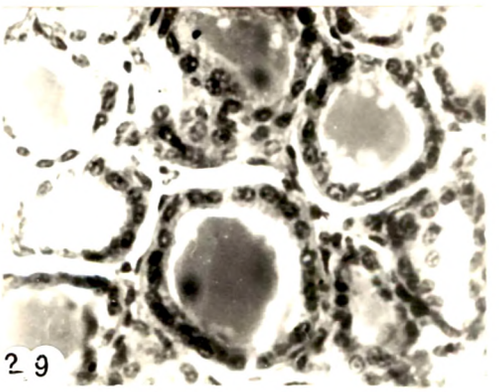
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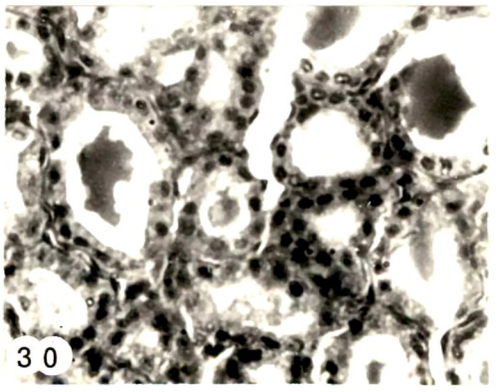
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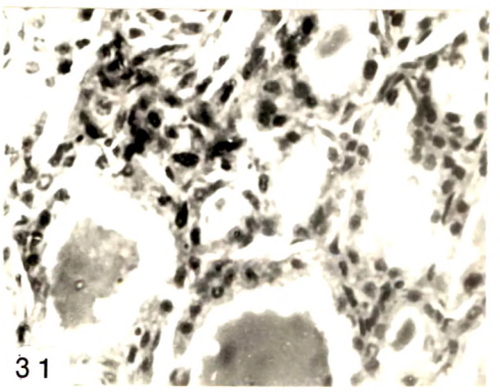
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Thyroid. The thyroid of control birds showed follicles lined by cuboidal epithelium and with varying degrees of colloidal depletion. The epithelial cells tended to be hyperplastic. Treatment with CORT reduced the cell height and brought about retention of colloid within the follicles, with the result, most of the follicles were filled with colloid. Epithelial cell hyperplasia was also clearly evident. Birds treated with FSH showed histological features quite similar to those of control birds though there was a tendency for colloid retention in many of the follicles and a decrease in cell height. In contrast, treatment with FSH.LH made the follicular epithelium more hypertrophied leading to a near obliteration of the lumen of many follicles and many of the follicles appeared empty. Both CORT + FSH (P) and CORT + FSH(S) showed hypertrophied epithelium as in FSH treated birds and increased colloid retention as in CORT treated birds. Epithelial cell hyperplasia also could be seen. Treatment with CORT + FSH.LH showed mixed population of follicles with depleted and full colloid content. The epithelial cell height was more like CORT + FSH birds and less than FSH.LH treated ones (Plate V).

Adrenal . The adrenal of control birds showed regressed cortical and medullary cords with a cortico-medullary ratio of 1:1. Treatment with CORT induced cortical activation. The cortical cords were enlarged with cells depicting prominent

PLATE VI

Figures 32-37 Photomicrographs of sections of adrenal of control and experimental birds in the quiescent phase. (200 X)

Fig.32 Adrenal of control birds showing narrow cortical cords (C) with many inactive cells.

Fig.33 Adrenal of CORT treated birds showing distinct cortical cords (C) with active cortical cells.

Fig.34 Adrenal of FSH treated birds showing enlarged cortical cords. (C)

Fig.35 Adrenal of corticosterone primed birds treated with FSH. Note the enlarged cortical cords with active state of the cortical cells.

Fig.36 Adrenal of FSH.LH treated birds showing activated cortical cords.

Fig.37 Adrenal of birds treated with CORT + FSH.LH. Note the activation of cortical cords. (C).

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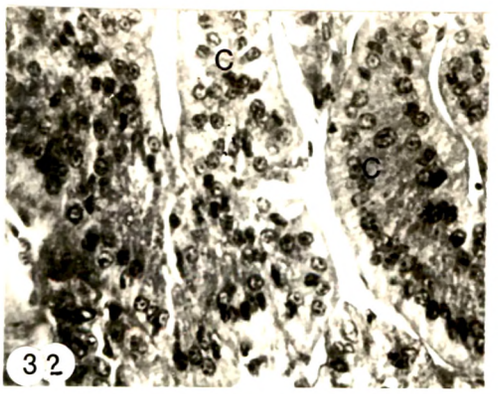

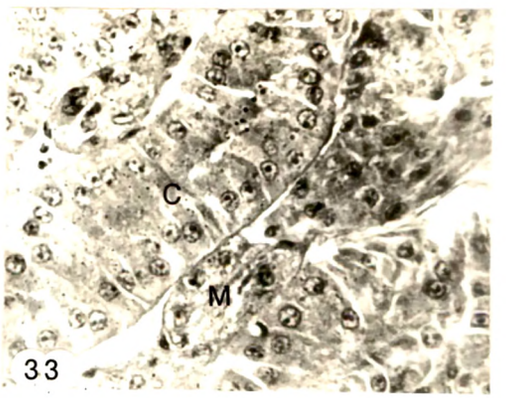

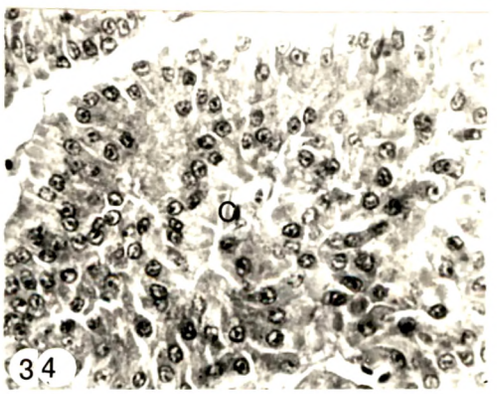

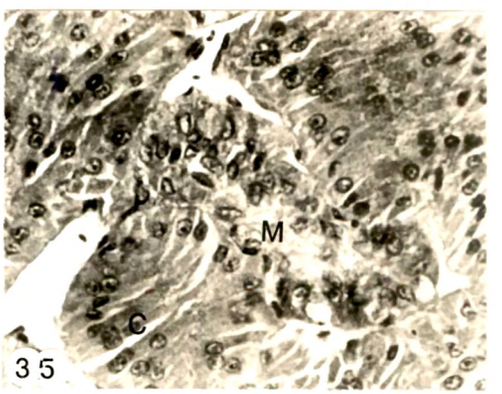

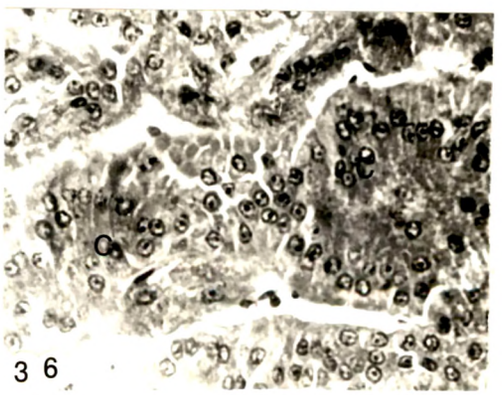

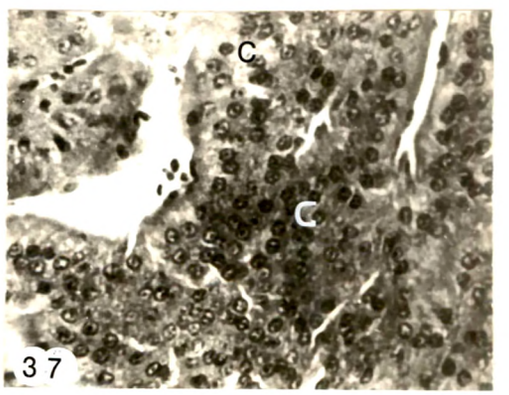

nucleus and nucleolus. FSH treatment induced prominent cortical enlargement with hypertrophied nucleus and nucleolus indicating increased activity. Birds treated with CORT + FSH (P) as well as CORT + FSH (S) also showed cortical enlargement. Both FSH.LH as well as CORT + FSH.LH also depicted cortical enlargement as well as hypertrophy of cells much like that of CORT + FSH treated birds (Plate VI)

Serum Hormone profile :

Serum T_4 level was decreased under all treatment schedules and maximum decrement occurred with CORT treatment and the least with FSH. Combinations of FSH or FSH.LH with CORT tended to have significantly decreased serum T_4 level as compared to FSH.LH alone. Except for treatment with FSH.LH alone or in combination with CORT (which showed significant increase), all other treatments involving CORT, FSH or CORT + FSH showed significantly increased CORT level. Serum testosterone level was also significantly elevated under all experimental schedules. Interestingly either CORT or FSH treatment also increased serum testosterone (T) level (Table V c)

Discussion :

The classic concept of gonadotrophic hormones regulating

Serum Hormone levels

Treatments	T_4 (ng/ml)	T_3 (ng/ml)	Testosterone(T) (ng/ml)	CORT (ug/dl)
Control	22.64 ± 2.18	2.98 ± 0.17	1.48 ± 0.05	7.20 ± 0.63
CORT	17.61 ± 1.18	2.42 ± 0.32	3.32 ± 0.06	8.93 ± 0.98
FSH	20.39 ± 1.64	2.19 ± 0.19	2.92 ±0.36	8.81 ± 0.79
FSH.LH	21.74 ± 0.95	2.44 ± 0.35	4.52 ± 1.02	7.99 ± 0.35
CORT+FSH(S)	19.80 ± 1.21	2.52 ± 0.41	3.61 ± 0.50	8.27 ± 0.49
CORT+FSH(P)	19.13 ± 1.10	2.30 ± 0.71	3.35 ± 0.14	8.10 ± 0.42
CORT+FSH.LH	19.62 ± 0.97	2.37 ± 0.66	3.93 ± 0.23	7.96 ± 0.81

Table V c :Changes in serum levels of T_4, T_3, T and CORT of pigeons treated with gonadotrophins alone or with CORT in the quiescent phase.
(* Significant at $P < 0.05$; values are x ± SEM)

testicular functions has been validated in birds by the many observations showing FSH induced spermatogenesis and LH induced steroidogenesis (Jones, 1970; Brown et al., 1975; Murton and Westowood, 1977; Follett, 1978; Follett and Robinson, 1980; Sakai and Ishii, 1986;Peczely, 1989; Bluhm et al., 1991). Parallel changes in gonadotrophic hormones and testosterone during the annual gonadal cycle of birds have been well established (Follett, 1975; Murton and Westwood, 1977; Sakai and Ishii, 1986; Follett and Robinson, 1980). Besides, a parallel adrenal-testis relationship has also been recorded (Patel et al.,1985; Ramachandran and Patel, 1986). This was supported by the recent observations of increased CORT level during the breeding phase and reduced CORT level in the non-breeding phase (Patel, 1993; chapters I and II). Moreover, exposures of pigeons to a long photoperiod towards the end of the non-breeding phase was shown to increase adreno-cortical activity in association with testicular recrudescence (Patel, 1993). These above observations strongly suggested a positive role for corticosteroids in modulating testicular function in the pigeon. The present investigations intended to test the response of the quiescent testes to gonadotrophic hormones as well as the role of CORT, have revealed hitherto unknown neuroendocrine regulation of testicular functions in birds.

The histologically observable activation of tubules and

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interstitial cells coupled with the increased serum testosterone (T) level in response to treatment with FSH.LH clearly indicate the sensitivity of the quiescent testes to gonadotrophins. Obviously, in feral pigeons as in many other birds, absence of gonadotrophins and not the reduced sensitivity of the gonads is responsible for testicular quiescence in the non-breeding phase. Treatment with FSH alone increased spermatogonial proliferation and formation of primary spermatocytes. Interestingly, FSH also induced interstitial cell hypertrophy and activation which was reflected in the elevated serum T level. Infact, the hypertophic response of the interstitial cells was greater than in any of the treatment schedules employed. Since the leydig cell response was coupled with hypertrophy of the myoid cells as well, it might bespeak of a paracrine interaction between the tubular epithelium and the interstitial cells. The increased serum T level in response to FSH, though paradoxical, finds some support from an earlier report of Sakaii and Ishii (1986) showing a definite correlation between T and FSH as both these hormones depicted parallelism during the annual testicular cycle of the Japanese common pheasant, Phasianus colchicus versicolor. Based on this disperate observation, they suggested that the hormonal control mechanism of androgen secretion in seasonally breeding feral species might be more complex and quite different from domestic species such as

the japanaese quail and domestic fowl and emphasised on the need for more studies to elucidate the mechanism of hormonal control of testicular functions in wild avian species. Since no studies have addressed to this problem, the present investigation gains added credence. An additional link available in the present investigations on feral pigeons is the concomitant adrenocortical activation and an increased serum CORT level. Though the effect of this increased CORT level could be to potentiate the responsiveness of the leydig cells in synergism with LH, the cause for increased adrenocortical activation is more likely to be due to the increased hypothalamic CRH activity induced somehow by FSH. Apparently, the increased CRH activity or CORT, could possibly induce some LH release through opioid pathways as a relationship between CRH and opioid peptides and LH release, though of a negative nature, has been shown in rats (Nikolarkis et al., 1990). A literature scan as well as studies from this laboratory on both domestic and wild birds indicate a similarity of neuroendocrine functions between the domestic avian species and mammals and an opposite functional features in the wild species. In this respect, ongoing preliminary studies have revealed a positive relationship between opioid actvity and testicular recrudescence in the feral pigeons. Apparently, even a small increase in LH induced by these mechanisms could have a profound effect on the leydig cells already rendered

potentially sensitive. Compelling support to this concept is further provided by the current observation of increased leydig cell activity and serum T level even in CORT treated pigeons. However, neither FSH nor CORT could advance spermatogenic functions beyond spermatogonial proliferation and appearance of a few primary spermatocytes. The importance of FSH and CORT in testicular activation is emphasised by the elevated serum T level and advancement of the spermatogenic process upto pachytene spermatocytes in pigeons treated with CORT + FSH (P or S), though qualilatively better in the latter schedule. Obviously, FSH in combination with CORT seems to have a more favourable influence in testicular functions as can be inferred from the germ cell population which is both quantitatively and qualitatively better in FSH + CORT treated birds than even in FSH.LH treated birds. However, CORT + FSH.LH showed a much better response than FSH.LH, again indicating the positive influence of CORT. Though there was no qualitative difference in terms of germ cell population between CORT + FSH.LH and CORT + FSH, there was nevertheless a quantitative influence due to the presence of LH in the former schedule. A negative thyroid-testes relationship has been inferred by the many observations made previously (Patel et al., 1985; Ramachandran and Patel, 1986) and reduced T_4 level coupled with increased CORT level was considered favourable for gonadal recrudescence in the Indian feral pigeons (Patel,

1993; chapter II). In this respect, CORT was found to inhibit the HHT axis leading to colloidal retention in the thyroid follicles and decreased serum T_4 level (Patel, 1993; chapter I and II). Hence, a synergistic action between CORT and gonadotrophic hormones is presumable during gonadal recrudescence. This is well reflected in the presently observed decreased ability of gonadotrophic hormones to stimulate spermatogenic functions in the absence of CORT. The relatively higher T_4 level in these birds is likely to antagonise the action of T as, such a negative influence of T_4 on T is well known (Silverin, 1980). In this respect the quantitatively and qualitatively better response of testis of pigeons treated with both gonadotrophins and CORT need no explanation.

Overall, it can be concluded from the present results that, FSH and CORT alone are capable of inducing testicular activation and that, FSH is able to mediate activation of the HHA axis which in turn could elicit some degree of LH release through opioid pathways.