CHAPTER: 5

Effect of short photoperiod and transient timed hypothyroidism in RIR pullets on histomorphology of ovary, ovarian and thyroid hormones and organ growth kinetics

Physical growth and physiological maturation leading to attainment of adult homeostasis occur primarily during the posthatched growth phase in birds. Adaptive alterations in the endocrine *milieu* can be of crucial significance in the process. Supportive evidence to this Supposition is provided by the reported decrease in body weight gained in ducks and fowls when the chicks are hypophysectomised, thyroidectomised or rendered hyper or hypo corticalic (Blivaiss, 1947; Winchester and Davis, 1952; Howard and Constable, 1958; Baum and Meyer, 1960; Nagra et al., 1963; Nagra and Meyer, 1963; Nagra et al., 1965; Raheja et al., 1971; King and King, 1973; Kallicharan and Hall, 1974; Carasia, 1987; Bartov, 1982; Kuhn et al., 1984; Akiba et al., 1992; Hayashi et al., 1994). Though many past studies have shown parallel or inverse thyroid – gonad and adrenal - gonad inter-relationships in birds (Riddle et al., 1924; Legait and Legait, 1959; Fromme-bouman, 1962; Thapliyal and Pandha, 1967a, b; Jallages and Assenmacher, 1973,1974; Oshi and Konishi, 1978; Patel et al., 1985; Ramachandran and Patel, 1988;

Ayyar *et al.*, 1992), a previous study from this laboratory has shown retarded growth and functional maturation of testes by hyper corticalism and stimulated growth and maturation by hypocorticalism in 30 day old white leghorn cockerels (Joseph and Ramachandran, 1993). It has also been shown that chronic mild hyper or hypocorticalism during the first 90 days in RIR pullets does exert some modulatory influences on the features of normal yield of egg lay and egg composition (Dandekar, 1998; Dandekar *et al.*, 2001). The same studies also attempted to relate the observed consequential effects with pattern of growth kinetics and histomorphology of adrenal, thyroid, ovary, oviduct and serum profiles of T₃, T₄, corticosterone and progesterone during the period of hypo or hypercorticalism (Dandekar, 1998).

In poultry practice, maintenance of birds in artificial photoperiod during the rearing period has been a *modus* operandi to improve laying performance (Dunn *et al.*, 1990; Lewis *et al.*, 1996a, b; Sandoval and Gernat, 1996; Etches, 1996). Rearing Indian RIR pullets under a short photoperiod of LD 6:18 or 8:16 (Short photoperiod) from the day of hatch till 90 days and then shifting to a normal photoperiod, amounting to a step- Up photoschedule has been shown to hasten sexual maturity and improve the laying performance significantly (Dandaker, 1998; Chapter-1). The effect of rearing under the short photoperiod

was also assessed in terms of growth kinetics, histological alterations in ovary, thyroid, adrenal and serum profiles of T_3 , T_4 , progesterone and corticosterone during the period of maintenance under short photoperiod so as to relate the alterations with the observed effects on laying performance. Accordingly organ weighs except that of ovary were decreased under short photoperiod (SP). The transition from small to big follicles was found to be slower but also with reduced follicular atresia. The levels of T_3 , T_4 and progesterone were also found to be lower (Dandekar, 1998). In the previous study, a favourable influence of hypothyroidism on activation of hypothalamohypophyseal axis as well as ovarian development was also observed (Chapter-4).

Since both short photoperiod (SP) and hypothyroidism were shown to affect various aspects of laying performance in RIR hens (chapter 1 and 2), an attempt was made to study the interactive effects of SP and hypothyroidism, which revealed dominant influence of hypothyroidism over that of Step-Up photoperiod and a significant effect on egg composition (Chapter-3). It is presumable that the interactive influences and modulatory effects of hypothyroidism on laying performance and egg composition could be a consequence of changes induced by the combination status of photoperiod and hypothyroidism on the histomorphology of endocrine profile

during the experimental period rear. Hence in the present study, the growth of ovary and oviduct and serum levels of T_3 , T_4 and progesterone have been assessed along with histomorphology of the ovary during the 90 day of rear of hypothyroid pullets under SP.

<u>Results:</u>

Body, Ovary and Oviduct weights:

The body weight of chicks maintained under LD 8:16 showed steady Increase from the day of hatch till 190 days. The hypothyroid groups of chicks also showed a steady increase though with a significantly reduced weights during and in the post-hypothyroid periods. On a comparative basis, whereas the 15-45and 30-60 days hypothyroid group of chicks showed almost similar body weight as the controls, the 45-75 and 60-90 days hypothyroid groups showed significant reduction in body weight at 120 days.

In general, both the absolute and relative weights of ovary and oviduct show a reduction in all the hypothyroid groups, though less obvious in 15-45 days and more pronounced in 60-90 day group.

Both the absolute and relative weight of thyroid gland were significantly increased in hypothyroid groups except for the 15-45 day group. (Table: 5.1, 5.2; Fig: 5.1 to 5.6)

Fig. 15

Ovary of 30-60 days SP chicks showing more number of small follicles with growing medullary cells (Arrowed)

Fig. 16

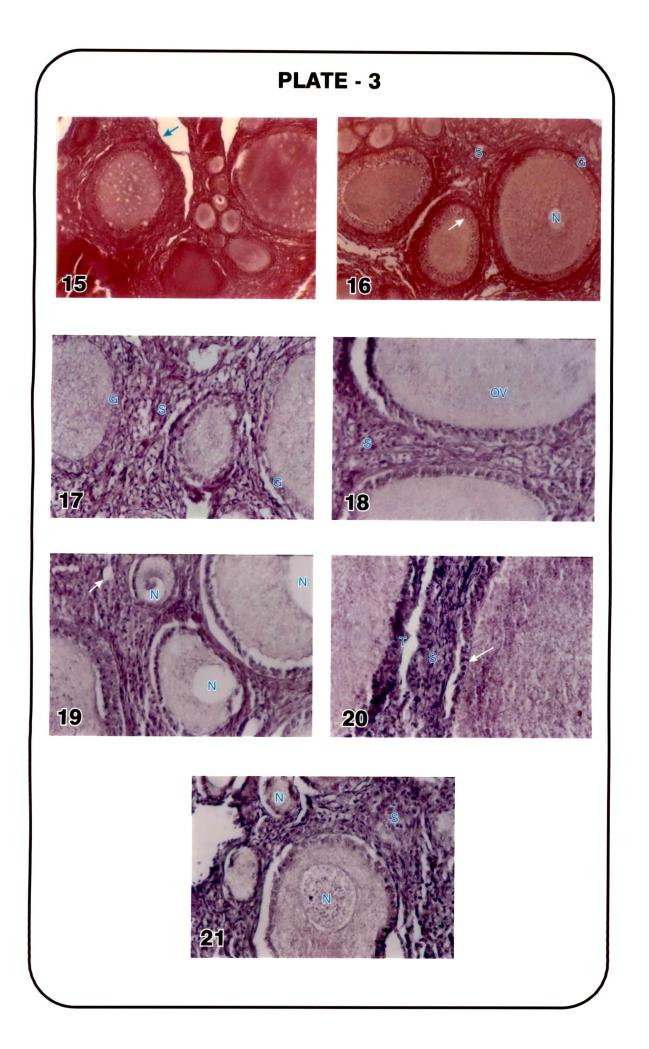
Ovary of 30-60 days chicks showing higher number of follicles with marked medium to large sized follicles

Fig. 18& 20

Ovary of 45-75 and 60-90days SP chicks showing relatively less number of follicles

Fig. 17, (19 & 21)

Ovary of 45-75 and 60-90days HPOT+SP chicks showing higher number of follicles. Large sized follicles are evident in 60-90 days HPOT pullets.



Histological observations and follicular count:

In the ovary of 15-45 days hypothyroid group showed increased number of small follicles with more compactly packed stromal cells and follicles. All the other hypothyroid group also showed hyperplastic compactly packed stroma with more prominent larger follicles with advancing age. Atretic changes were quite similar both control and hypothyroidic groups.

The total follicular count and the number of follicles were both increased with age in the ovary of both control and hypothyroid groups of pullets. However the number was significantly higher in the experimental groups, more pronounced in the 45-75 and 60-90 days hypothyroid groups. Though there was a gradual progression of folliculogenesis marked by increasing number of greater sized follicles, with age, in the control ovary, the ovary of hypothyroid pullets showed a faster progression and relatively greater number of larger sized follicles, more pronouncedly in the 45-75 and 60-90 days hypothyroid pullets. (Table: 5.4; Plate No. 2 and 3)

Hormone Profile:

In general, T₃ and T₄ were decreased significantly in hypothyroid groups while progesterone was significantly increased in all the experimental groups.

Table 5.1: Body weight change under the effect of hypothyroidism and step-up photoschedule

Body wt. of			We	ight cho	Weight change on successive days (gm)	successi	ve days	(mg)	
day- chick (gm)	Treatme nt period	15	30	45	60	75	60	105	120
	امطسمت	80.3	177.66	326.8	418.3	590	913	1300	1383.33
	51110	±3.58	±6.00	±10.45	±12.02	±6.66	±20.03	±3.48	±84.52
<u> </u>	15 45	73.1	160	270 a	375	540	860	1210℃	1320
	04-01	±5.3	±5.70	±5.00	±8.33	±7.36	±10.11	±13.11	±16.33
29.93			173	237 b	318 c	500 b	813c	1200 ℃	1373.3
±3.33	00-00	1	±3.03	±4.08	±9.38	±11.54	±20.38	±15.11	±48.07
	45 75			324.9	380.1 a	473	890	1190 ℃	1250c
	67-04	ţ	1	±5.3	±9.27	±9.36	±13.03	±30.84	±15.13
<u>L</u>					420.4	430.1ª	780.8 c	0001 ∘	1126°
	04-00	1	1	1	±5.93	±5.33	±10.34	±9.83	±65.6

Control: NLD, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT, a: $p \ \square \ 0.05, \ b: p \ \square \ 0.02, \ c: p \ \square \ 0.001$ of 6 animals

hotoschedule
Step-up p
of HPOT in
the effect
ng) under
in weight (i
le5.2: Orga
Tabl

			Ö	Control			T	HPOT	
Tissue	Weight		Experime	Experiment Groups			Experim	Experiment Groups	Š
	ŀ	Group- I	Group- II	Group- III	Group- IV	Group -1	Group- II	Group- III	Group- IV
		118.66	155.33	215.6	288.0	₀00.66	122.0 c	193.66 c	261.0℃
	Apsolute	±2.027	±2.906	±2.848	±2.309	±2.309	±2.645	±2.027	±1.527
	Deletino	36.30	37.13	36.54	31.54	36.66	31.44 c	33.79 c	33.4 °
	veidilve	±0.526	±0.454	±0.356	±0.24	±0.641	±0.456	±0.274	±0.253
		57.33	92.33	135.00	243.0	45.66 ^b	75.66°	122.3℃	148.6 c
toutoinO	ADSOIUTE	±2.33	±2.603	±2.004	±2.645	±2.027	±2.333	±1.762	±1.73
	ميالدام	17.54	22.08	22.88	26.61	16.91	19.50 ^b	21.34°	19.03 c
	Keldlive	±0.606	±0.406	±0.190	±0.275	±0.563	±0.402	±0.238	±0.150
		48.0	44.33	58.00	87.0	57.33°	78.0 c	129.33 c	176.0°
Thurdid	ADSOIUTE	±1.15	±2.185	±2.081	±3.21	±0.577	±3.844	±2.333	±3 055
		14.68	10.59	9.83	9.52	21.20℃	20.12℃	22.57 c	22.54 c
		±0.299	±0.341	±0.330	±0.334	±0.667	±0.662	±0.206	±0.381
Control	Control: NLD, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT, a: p 🗆 0.05, b: p 🗆 0.02, c: p 🗆 0.001 of 6 animal	5-45day H a: 1	POT, Gr-ll: p 🗆 0.05, b	30-60day F : p □ 0.02, ¢	/ HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day H a: p 🗆 0.05, b: p 🗆 0.02, c: p 🗆 0.001 of 6 animal	: 45-75da) of 6 anim	/ HPOT, Gr nal	-1V: 60-90d	ay HPOT,

Pullets
in RIR I
period
photo
tep-up
under s
ained u
maint
evels ma
hormone
Serum
Table5.3:

	Treatme		Experime	Experimental Groups	
	ħ	Group-I	Group-II	Group-III	Group-IV
		2.37	1.85	1.77	1.55
T3	Conirol	±0.210	±0.125	±0.163	±0.141
	TCau	1.73	0.98c	0.81c	0.73c
	БЪ Б	±0.17	±0.06	±0.041	±0.1
		18.46	21.56	22.61	24.49
11		±1.154	±1.94	±1.01	±1.24
t	тОал	13.41ª	16.41	19.12	21.94
		±1.201	±2.04	±1.164	±1.551
	Ictuci	4.61	3.209	3.043	3 953
Bronesterone		±0.89	±0.997	±1.014	±0.887
	тСап	10.649 ^b	4.474	5.170	4.614
	5	±1.13	±0.784	±1.48	±1.332

Control: NLD, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT,

a: $p \ \square \ 0.05, \ b: \ p \ \square \ 0.02, \ c: \ p \ \square \ 0.001$ of 6 animals

Table 5.4: Follicular count, under the effect of HPOT, in Step up photoperiod (SuP)

					Follici	Follicle Size			
Groups	Groups Treatment	Follicle Type	S1 (6- وراس)	52 (91- 120µm)	B1 (121- מווש)	82 (241- 00im)	ل (301- 00الس)	12 (>400µm	Total
	C.	PoF		5	12	-	-		56
Group		AF	ł	m	Fact	1		ł	4
		PoF	43	20	11	8	1	1	76
		AF	have	-	1	1	1	8	2
	2	PoF	27	δ	_	F	1	1	51
Group	L D C	AF		m	I	1	J	t	4
	TCall area	РоF	18	24	15	2	,	ł	60
	SULT-TICOL	AF	i	3		1	g	ſ	Э

Continue.....

Groups	Groups Treatment	Follicle Type	(mц09 -6- 12	S2 (91- 120µm)	B1 (121- 40µm)	B2 (241- 00µm)	L1 (301- 00µm)	L2 (>400µm)	Total
r	Ç	PoF	65	ε	2	2	-	ł	72
Group		AF	l	J	1	I	I	1	10
		PoF	78	18	15			7	105
	DE	AF	ł	2	3	8	-	1	13
	Ĺ	PoF	20 ⁻	L	18	3	4	ł	86
Group		AF	5	4	4		.	1	15
≥	LGU	PoF	62	15	20	5	6	3	6 mm
	DE	AF	6	5	Ż	ſ	P	ţ	18

Control: NLD, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT, a: p 🗆 0.05, b: p 🗆 0.02, c: p 🖾 0.001 of 6 animals

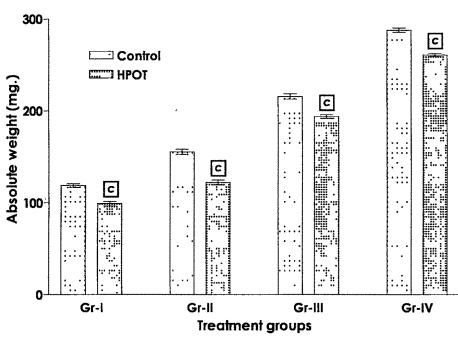
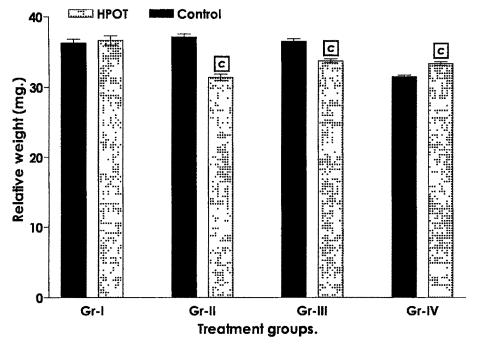


Fig. 5.1: Absolute weight of ovary in HPOT hens at short photoperiod.

Fig. 5.2: Relative weight of ovary in HPOT hens at short photoperiod.



Control: Short Photoperiod, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT, a: $p \le 0.05$, b: $p \le 0.02$, c: $p \le 0.001$ of 6 animals

÷

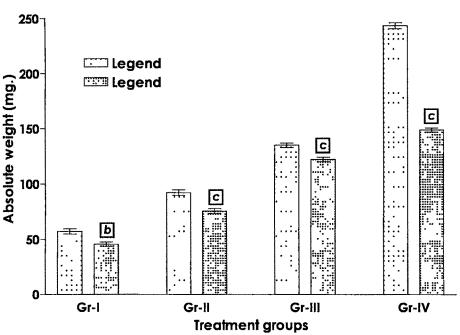
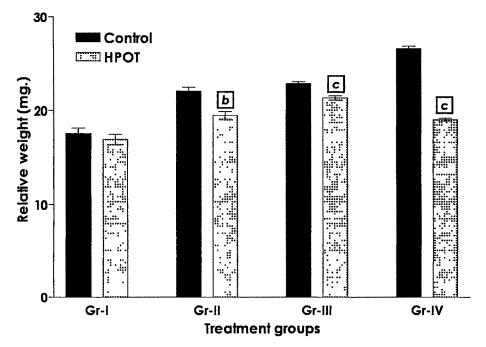


Fig .5.3: Absolute weight of oviduct in HPOT hens at short photoperiod.

Fig. 5.4: Relative weight of oviduct in HPOT hens at short photoperiod.



Control: Short Photoperiod, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT, a: $p \le 0.05$, b: $p \le 0.02$, c: $p \le 0.001$ of 6 animals

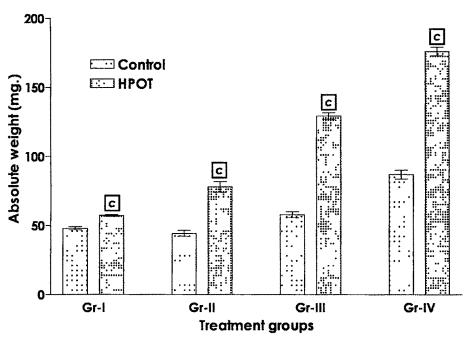
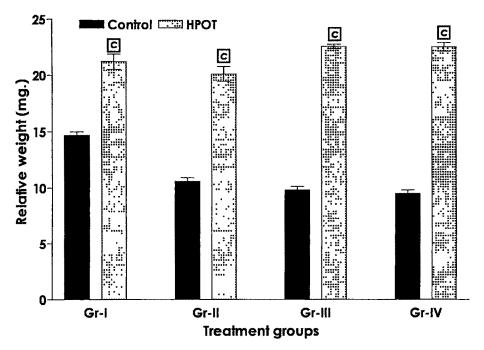


Fig. 5.5: Absolute weight of thyroid in HPOT hens at short photoperiod.

Fig. 5.6: relative weight of thyroid in HPOT hens at short photoperiod.



Control: Short Photoperiod, Gr-I: 15-45day HPOT, Gr-II: 30-60day HPOT, Gr-III: 45-75day HPOT, Gr-IV: 60-90day HPOT, a: $p \le 0.05$, b: $p \le 0.02$, c: $p \le 0.001$ of 6 animals

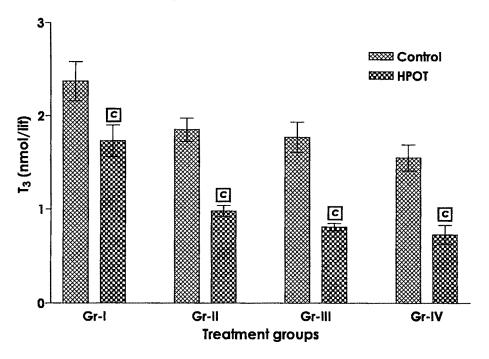
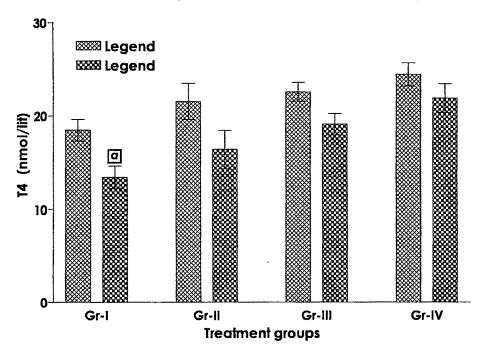


Fig. 5.7: Serum T_3 levels of HPOT hens at short photoperiod.





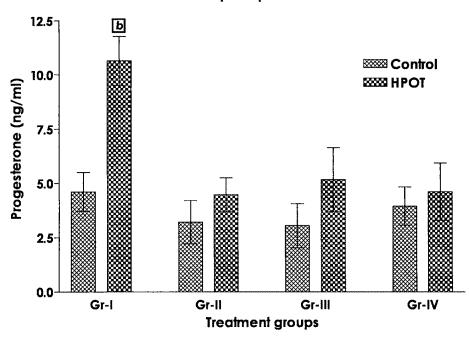


Fig. 5.9: Serum progesterone levels of HPOT hens at short photoperiod

Discussion:

The dampening effect of hypothyroidism is clearly evident during and in the immediate post-hypothyroid periods in all the experiments groups of pullets. This is indicated by the percentage difference in body weight of experimental pullets with the age matched controls both during the Methimazole feeding period as well as post-withdrawal period. In a previous study on hypothyroidism, induced under a normal light and dark schedule of LD 12:12, had shown a greater sensitivity in the earlier periods (i.e. 15-45 and 30-60 days) as marked by greater body weight difference at 120 days compared to controls and, the later hypothyroid groups (i.e. 45-75 and 60-90 days) almost equaling the control weight at 120 days (Chapter-4). However in the present study, the 45-75 and 60-90 days of hypothyroid pullets show greater weight reduction at 120 days compared to 15-45 and 30-60 days pullets under SP. Obviously, SP seems to have differential effect on hypothyroidism induced retardation in body weight with hypothyroidism induced at the mid phase between hatching and attainment of sexual maturity being more effective in the expression of the degree and extent of body weight reduction. Body weight changes with photoperiodic manipulations involving duration of light exposure or intensity of light have been reported in various strains of domestic fowls (Renema et al., 2001; Renema and Robinson, 2001; Renema et

al., 2001). Decrease in body weight due to hypothyroidism or aoitrogen treatment has been effectively shown in the domestic hen of various breeds by different workers (Singh and Parshad, 1978; Decuypyre et al., 1987; Peebles et al., 1994; 1997). It has been shown that hypothyroidism induced during earlier ages is more effective and that birds of higher ages may have to be fed high doses of goitrogen to maintain suppression of plasma T₄ concentration (Peebles et al., 1994). In the present study however, thyroid Suppression does not seem to be much different irrespective of the period of Suppression during the rearing age of 15-19days as seen by the T₃ and T₄ levels which are both reduced almost to the same extent in all groups. This is confirmed by the observed degree of thyroid hypertrophy, a marker of thyroid Suppression, which is almost to the same extent in all hypothyroid groups. Interestingly, short photoperiod itself seems to have a goitrogenic effect and as thyroid weights of control SP(short photoperiod) pullets are significantly greater than those reported for birds reared under a normal photoperiod (Chapter 4). This effect of SP seems to be more pronouncedly marked in the 15-45 and 30-60 days hypothyroid group. Decuypere et al. (1987) had shown that Methimazole induced hypothyroidism results in decreased Somatomedin C production without effecting GH level and which was related with the observed decrement in body weight. Apparently Somatomedin

C production may be more sensitive to thyroid hormone levels. Curiously, SP pullets show significantly greater T₃ levels and almost similar T₄ levels compared to birds reared under a normal photoperiod and further, the hypothyroid short photoperiod reared pullets also show relatively higher levels of T₃ and similar levels of T₄ compared to hypothyroid normal photoperiod reared pullets (Chapter 4). Looked in the above perspective of reduced Somatomedin C production under hypothyroidism, the decreased body weight of SP birds as well as hypothyroid SP birds compared to normal photoperiod and hypothyroid normal photoperiod birds despite the higher levels of thyroid hormones cannot be related with reduced Somatomedin C production. Since Decuypere et al. (1987) had shown no change in GH levels simultaneous to a reduction in Somatomedin C levels in the hypothyroid birds, it is likely that the presently observed decrease in body weight in SP pullets is due to reduced GH levels and the further reduction in body weight shown by hypothyroid SP pullets should be a consequence of lowered Somatomedin C as well as GH levels.

Based on the weight and histoarchitechture of ovary, an early maturation of the hypothalamo hypophyseal gonadal axis by rearing chicks under a short photoperiod from 0-90 days was inferred earlier (Dandekar, 1998). In the above study on a rearing photoperiod of LD 6:18, a favorable influence was seen in the

form of increased number of follicles in the ovary. However, the transition in terms of follicular size hierarchy from small to big follicles was found to be slowed down. This slower initial phase of follicular progression was Supposed to provide more time for maturational changes and, ultimately result in an augmented stimulatory response to the increased hypothalamo hypophyseal output due to shift to an LD 12:12 photoperiod (amounting to a step up photoschedule) resulting in faster progression through white and yellow follicular hierarchy as characteristics of hens closer to sexual maturity and oviposition (Sharp, 1993; Dandekar, 1998; Etches, 1996). Even the present study involving a rearing photoperiod of LD 8:16 also shows increased number of follicles compared to pullets reared under normal photoperiod (Chapter-4). The increased relative weights of ovary and oviduct of both SP as well as hypothyroid +SP pullets recorded in the present study is guite similar to the increased weight observed in the hypothyroid pullets reared under a normal photoperiod (Chapter-4). Obviously the hypothyroidic effect is dominant over that of photoperiod and in the hypothyroid + SP pullets, apart from an increase in the number of follicles, there is also an earlier progression to larger follicles. From the present observations on the histoarchitecture of the ovary and the previous observations on initiation of egg lay and the number of eggs laid in the first month (Chapter- 3), it becomes clear that hypothyroidism induced during the second to third month of rearing is more favorable for initiation of egg lay as well as egg yield. A precautious gonadal development as marked by the weight of gonads has also been reported by Singh and Parshad (1978) in their Methimazole treated 9 week old chicken. The weight of oviduct is almost identically high in both SP and hypothyroid +SP pullets compared to pullets exposed to a normal photoperiod. Neverthe less, the weight of oviduct recorded herein is quite similar to the weight recorded in pullets reared under a normal photoperiod and subjected to hypothyroidism (Chapter 4). In the above study, the relatively less prominent effect on oviducal weight compared to the ovarian weight was suggested to be due to a possible reduced estrogen stimulation under a hypothyroid state. However the currently observed higher oviducal weights suggest a favorable influence of estrogen. The above suggested reduced estrogenic effect may not be relevant as the thyroid hormone levels under both SP and hypothyroid + SP conditions are relatively higher than the normal photoperiod + hypothyroid condition. Apparently, despite the reduced thyroid hormone levels, the levels are still quite optimal for their permissive influence on estrogen action. A higher estrogen level unlike in the pullets reared under normal photoperiod and rendered hypothyroidic, is suggested in the present case as the progesterone levels in SP as well as SP +

hypothyroid are lower than those of NLD and NLD + hypothyroid pullets. Increased fertility during egg lay due to induced hypothyroidism prior to sexual maturity has been shown by Marks (1969). He has suggested this increase in fertility to be due to a physiological hyper secretion of T₄ after the removal of goitrogen from the diet. Precocious puberty in white leghorn pullets has also been shown by induced hypothyroidism attempted earlier prior to attainment of normal sexual maturity has also been reported by Williams (1994). Kuenzel *et al.* (1988) have observed significant increase in oviduct weight and length during the first eight weeks of lay in single comb white leghorn pullets rendered hypothyroidic from 2-12 weeks of age.

The herein observed increase in the number of large follicles in the ovary and the advancement in the age at first egg of both SP and SP + hypothyroid pullets suggests a possible early activation of the hypothalamic pituitary axis. Apparently a regulated and synchronized hierarchical transition in terms of follicular size on a temporal basis could be the determining factor in the attainment of sexual maturity and total egg yield. An elevated serum LH level has been reported in broiler breed of birds rendered hypothyroidic (Chaisson *et al.*, 1979). Since hypothyroidism has been reported to stimulate LH secretion in juvenile and adult rats (Larochelle and Freeman, 1974; Bruni *et*

al., 1975; Umezu, 1976) and as TRH has also been shown to stimulate LH release in Turkeys (Wentworth et al., 1976), it is surmisable that the hypothyroidic pullets in the present study release more LH in response to hypothyroidism and/or hypothyroidism induced elevation in TRH. A possible role of reduced metabolic clearance of LH under hypothyroidism could also be considered as a possible contributory factor (Shaisson et al., 1979). Other favorable observations in the context of activated hypothalamo hypophyseal axis is reported the increase in the number of gonadotrophs in the pituitaries of thyroidectomised white leghorn cockerels (Snapir et al., 1982). It is from these that hypothyroidism induced in growing pullets could lead to a precocious activation of hypothalamo hypophyseal axis with increase in gonadotropin hormone and augmented ovarian development. From the present results it appears that hypothyroidism induced towards the end of exposure to short photoperiods (60-90 days) followed by a Step-Up photoperiods is more potent in manifesting the favorable influence on reproduction functions like initiation of egg lay and egg yield (See Chapter -3).

Finally it can be concluded that a rearing pullets under a photoperiod from day of hatch to 90 days followed by a Step-Up photoperiod has a favorable influence in the number of follicles while rendering the pullets hypothyroidic just prior to a shift to a

Step-Up photoperiod has a further favorable influence on progression of follicular growth.

<u>Summary:</u>

In the present study, the growth of ovary and oviduct and serum levels of T₃, T₄ and progesterone have been assessed along with histomorphology of the ovary during the 90 day of rear of hypothyroid pullets under SP. Pullets fed with MMI at different age groups were maintained under step-up photic schedule. Birds were sacrificed after each treatment termination with their respective control animals. Group 1: induced HPOT on 15th day till 45th day of age. Group 2: induced HPOT on 30th day till 60th day of age. Group 3: induced HPOT on 45th day till 75th day of age. The results obtained are, the body weight of chicks maintained under LD 8:16 showed steady Increase from the day of hatch till 190 days. The hypothyroid groups of chicks also showed a steady increase though with a significantly reduced weights during and in the post-hypothyroid periods on a comparative basis. The ovary of 15-45 days hypothyroid group showed increased number of small follicles with more compactly packed stromal cells and follicles. The total follicular count and the number of follicles are significantly higher in the experimental groups, more pronounced in the 45-75 and 60-90 days hypothyroid groups. It can be concluded that rearing pullets

under a short photoperiod from day of hatch to 90 days followed by a Step-Up photoperiod has a favorable influence in the number of follicles while, rendering the pullets hypothyroidic just prior to a shift to a Step-Up photoperiod has a further favorable influence on progression of follicular growth.

-