

Effect of transient prepubertal hypothyroidism on serum testosterone level and seminal characteristics of chickens

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Summary

Effect of propylthiouracil (PTU)-induced hypothyroidism during the prepubertal period, at a dose of one g per kg of the diet, on serum testosterone level and seminal characteristics of Fars indigenous chickens was studied. PTU was fed between 7th to 13th weeks of age and semen was collected at weekly intervals, starting at 21st week of age and continued for seven weeks. The effect of PTU treatment on serum testosterone level and body weight was significant ($P < 0.05$). The effect of age on all parameters, including body weight, semen volume, sperm motility, percentage of live sperm, sperm concentration, total number of live sperm, and serum testosterone and thyroxine (T_4) levels was also significant ($P < 0.01$). The interaction effect of PTU treatment and age on semen volume, sperm motility, and percentage of live sperm was not significant ($P > 0.05$); but it was significant for body weight, sperm concentration, total number of live sperm, and serum testosterone and T_4 levels ($P < 0.01$). At weeks 11 and 13 of age, serum T_4 concentration in PTU birds was significantly lower but serum testosterone level was higher than in the control group. Sperm concentration in PTU birds was generally higher than in the control group, although the differences between the two groups were significant at weeks 20, 23 and 24. The total number of live sperm produced by PTU birds at week 21 of age was about 60% of the control group ($P < 0.01$). A positive correlation ($P < 0.01$) was found between testosterone level and body weight ($r = 0.54$ and 0.36 for the control and PTU groups, respectively). A small but significant positive correlation was found between T_4 levels and body weight in the PTU group ($r = 0.23$; $P < 0.01$), but not in the control group ($P > 0.05$). The correlation between testosterone and T_4 levels was not significant. Transient prepubertal PTU-induced hypothyroidism resulted in an increase in sperm concentration and production at certain stages after treatment, but the effect did not seem to last for a long period. The potential for increased efficiency associated with raising smaller birds which pass a neonatal hypothyroidism and eat less feed and produce normal semen, may be economical.

Key words: Hypothyroidism, Chicken, Testosterone, Seminal characteristics, Rooster

Introduction

Reproduction is under the influence of many factors one of them is optimal thyroid hormone level. Thyroid hormone seems to play an important role in testicular development in rats (Cooke, 1991) and studies have been done to investigate the relationship between gonads and thyroid in this species. Thyroidectomy brought about a decrease in testicular development, spermatogenic disorders, and sometimes reduced the libido in males (Jacquet *et al.*, 1993; Dickson, 1996). Thyroid hormone is said to be critical in triggering the onset of

mesenchymal precursor cell differentiation into Leydig progenitor cells, and to regulate the duration of Sertoli cell proliferation, affecting adult Sertoli cell number and hence the capacity of the testis to produce sperm in rats (Mendis-Handagama and Ariyaratne, 2001). Neonatal hypothyroidism in rats led to a prolongation of the period of Sertoli cell proliferation, giving rise to an increase in the number of Sertoli and germ cells, and concomitant increase in testis size, but opposite results were observed in hyperthyroidism (Buzzard *et al.*, 2000). Fallah-Rad *et al.* (2001) reported that transient neonatal hypothyroidism,

decreased serum FSH concentrations temporarily, increased testosterone pulses and sperm production, and advanced puberty in Suffolk ram lambs. In poultry, thyroidectomy resulted in gonadal aplasia and considerable diminution in egg production in hens and testicular atrophy in cocks (Falconer, 1971). Siopes (1997) showed that PTU-induced hypothyroidism terminated the photorefractoriness and reinitiated lay in turkey breeder hens. Jacquet *et al.* (1993) reported an inhibitory effect of large doses of thyroxine (T_4) (5 mg/kg diet) on reproductive function in broiler cockerels (*Gallus domesticus*) aged 96 weeks.

The study of Kirby *et al.* (1996) in broiler breeder male chicks seems to be the only report of the effect of transient prepubertal hypothyroidism on testis development and function. They administered propylthiouracil (PTU) between 6 and 12 weeks of age and measured the blood testosterone level, testis weight at slaughter, testis histology, and daily sperm production based on measurements made on the testes which had been removed at slaughter. No information is available on the effect of transient prepubertal hypothyroidism on the characteristics of the semen after puberty in cockerels. More studies are needed before a firm conclusion can be made with regard to the effect of thyroid gland on puberty in male poultry and on the seminal quality after puberty. Therefore, the present study was carried out to investigate the effect of transient prepubertal PTU treatment on serum testosterone level and seminal characteristics of Fars indigenous chickens. These are small birds which have not been genetically selected for any production traits.

Materials and Methods

Forty 6-week-old indigenous male chicks in Fars province, Iran were randomly assigned to two pens of 20 birds and covered with the litter materials. The lighting schedule was 14 hr-light:10 hr-darkness, and the house temperature was about 22°C during the experiment. One group received PTU and the other one served as the control.

PTU (Darou Pakhsh, Tehran) was administered at a dose of 1g per kg of the diet (as fed) for a 6-week period between weeks 7 and 13 of age. From 13th to 26th weeks of age, both groups were fed with the same diet without PTU. All birds received feed and water ad libitum throughout the experiment. Diets were formulated according to NRC (1994), using the UFFDA program for personal computers. Blood samples (1 ml) were drawn from the wing vein by using a 2.5 ml syringe, on seven occasions, starting at week 7 (before feeding PTU diet), and repeated once every two weeks until week 19.

Blood samples were centrifuged and the serum was separated and stored at -20°C until assayed for testosterone (T) and T_4 by ELISA (Sauer *et al.*, 1982). The intra- and inter-assay coefficients of variation (CV) were 5.32 and 5.1% for T_4 ($n = 80$), and 8.7 and 8.5% for testosterone ($n = 10$), respectively. Body weights were also measured before each blood sampling. Chicks were trained for semen collection for two weeks (weeks 19 to 20), by the massage method. Semen samples were collected from week 21 and repeated once a week for 7 weeks, and semen volume, sperm motility (0 to 5), sperm concentration and the percentage of live sperm (eosin-nigrosin staining) were determined (Donoghue and Wishart, 2000).

Statistical analysis

Statistical analysis was performed by using the Proc Mixed of SAS (1996) for repeated measure data. The effects of treatment, age and treat by age interaction were included in a model in which a covariance structure known as autoregressive-1 (AR-1) was fitted. Body weight was included in the model as a covariate for analysis of variance, and the mean separation was done by Student-Newman-Keuls (SNK test ($P < 0.05$)). Pearson's correlation coefficients were also calculated for some of the measurements.

Results

Body weight

Body weight was significantly affected

Table 1: Effect of propylthiouracil (PTU) treatment and age on body weight, testosterone and thyroxine levels, and seminal characteristics of Fars indigenous chickens (mean \pm SE)

	Group		P		
	Control	PTU	Treatment	Age	Interaction
Body weight (g)	1343.0 \pm 41.9	1161.3 \pm 36.5	0.035	0.0001	0.001
Thyroxine (nmol/L)	62.8 \pm 1.9	52.5 \pm 1.5	0.003	0.006	0.0008
Testosterone (nmol/L)	0.51 \pm 0.04	0.67 \pm 0.04	NS	0.0001	0.0001
Semen volume (ml)	0.56 \pm 0.03	0.51 \pm 0.02	NS	0.0004	NS
Sperm concentration ($\times 10^9$ /ml)	1.35 \pm 0.04	1.48 \pm 0.03	NS	0.0001	0.0004
Percentage of live sperm	86.45 \pm 1.73	84.02 \pm 1.87	NS	0.0002	NS
Sperm motility	4.10 \pm 0.10	4.21 \pm 0.08	NS	0.005	NS
Total live sperm ($\times 10^9$)	0.68 \pm 0.04	0.63 \pm 0.04	NS	0.0007	0.007

NS: Not significant ($P > 0.05$); P: probability of significance of treatment effect

by PTU treatment ($P = 0.035$) and age ($P = 0.0001$). There was also a significant interaction ($P = 0.001$) between PTU and age (Table 1). The overall mean of body weight in PTU-treated birds was 1161 vs 1343 g for the control group. There was a gradual increase in body weight of both groups from 7 to 19 weeks of age. From weeks 11 to 19, body weights of PTU birds were significantly lower than the control group; but body weights at weeks 7 and 9 were not significantly different between the two groups.

Serum thyroxine (T_4) and testosterone (T)

There was a significant effect of PTU ($P = 0.003$), age ($P = 0.006$) and PTU \times age interaction ($P = 0.0008$) on serum T_4 concentration (Table 1; Fig. 1). The overall mean of serum T_4 concentration in PTU group was significantly lower (52.5 nmol/L) than the control (62.8 nmol/L) group. In the control birds, the highest T_4 concentration was found at week 13 of age. Serum T_4 concentration in the PTU group was low at weeks 11 and 13 but increased gradually to values similar to those found at subsequent weeks. Serum T_4 concentration of the PTU group was significantly lower at weeks 11 (35.0 vs 59.5 nmol/L) and 13 (46.1 vs 74.5 nmol/L) as compared with the control group. PTU treatment did not significantly affect serum T concentration, but there was a significant effect of age ($P = 0.0001$) and PTU \times age interaction ($P = 0.0001$) on T level (Table 1; Fig. 2). Serum T concentrations were 0.51 and 0.67 nmol/L in the control and PTU groups, respectively.

Serum T levels in the control group increased gradually from week 7 (0.11

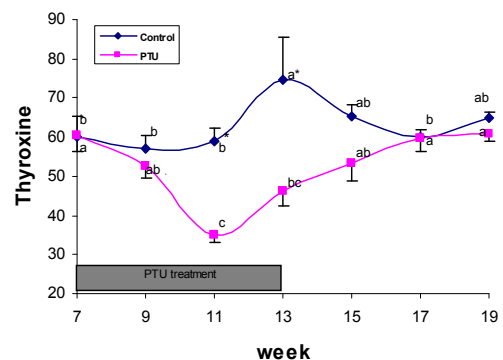


Fig. 1: Mean (\pm SE) serum thyroxine concentration (nmol/L) of the control and propylthiouracil (PTU)-treated male chicks. a, b: Within each curve, means with similar superscript(s) are not statistically different at $p < 0.05$ (SNK test) and *: Significant difference ($P < 0.05$) between the control and PTU-treated chicks for each week (t-test)

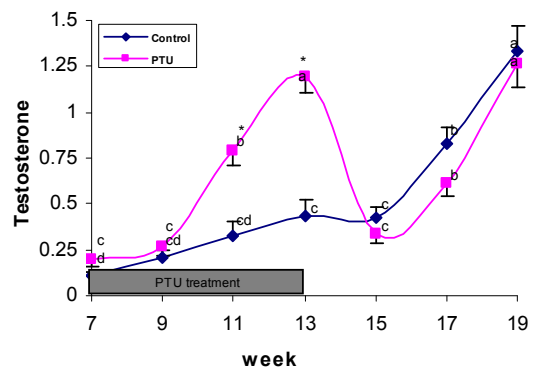


Fig. 2: Mean (\pm SE) serum testosterone concentration (nmol/L) of the control and propylthiouracil (PTU)-treated male chicks. a, b: Within each curve, means with similar superscript(s) are not statistically different at $P < 0.05$ (SNK test) and *: Significant difference ($P < 0.05$) between the control and PTU-treated chicks for each week (t-test)

nmol/L) to week 19 (1.33 nmol/L). In PTU birds, T levels increased from week 7 (0.20 nmol/L) to week 13 (1.19 nmol/L), decreased significantly at week 15 (0.34 nmol/L) and then increased to values (1.26 nmol/L) observed at week 19. Serum T concentrations were significantly higher in the PTU group at weeks 11 (0.79 vs 0.33 nmol/L) and 13 (1.19 vs 0.43 nmol/L) as compared with the control birds.

The correlation coefficients of testosterone concentration with body weight were 0.54 and 0.36 for the control and PTU groups, respectively ($P < 0.01$). In the PTU group a significant and positive correlation was found between T_4 levels and body weight ($r = 0.23$; $P < 0.01$), while there was no significant correlation between these measurements in the control group. Furthermore, there was no significant correlation between testosterone and T_4 levels.

Seminal characteristics

Semen volume was significantly affected ($P = 0.0004$) by age. The overall mean of semen volume in PTU birds was 0.51 vs 0.56 ml in the control group ($P > 0.05$). There was no interaction of age and PTU on semen volume (Table 1). Except for a small and significant decrease in semen volume at week 21, semen volume was between 0.5 and 0.6 ml at the other weeks of age.

There was no significant effect of PTU on sperm concentration, but the effects of age ($P = 0.0001$) and PTU \times age interaction ($P = 0.0004$) were significant. The overall mean of sperm concentration in the PTU group was 1.48 vs 1.35×10^9 /ml for the control group. In the control birds, the highest sperm concentration was observed at weeks 21 and 22 of age, but in PTU birds it was found at week 20. At weeks 20, 23 and 24, sperm concentrations of PTU birds were significantly greater than the control group ($P < 0.05$).

Percentage of live sperm was not significantly affected by PTU treatment and PTU \times age interaction, but varied significantly ($P = 0.0002$) according to the age. Similar trend was noted for sperm motility scores. The overall mean of percentage of live sperm in PTU birds was

84.02 vs 86.45 in the control group. The average motility scores were 4.2 and 4.1 for PTU and control birds, respectively. The mean percentage of live sperm at weeks 20 and 21 of age was about 80% as compared with 86 to 89% at weeks 22 to 26. Similar trend was observed for motility scores.

Total number of live sperm in the ejaculate was not significantly affected by PTU but there was a significant effect of age ($P = 0.0007$) and PTU \times age interaction ($P = 0.007$) for this measurement. The overall mean of total live sperm in the PTU group was 0.63 vs 0.68×10^9 in the control birds. There was a significant decrease in the number of live sperm at week 25 in the control group and at week 21 in the PTU group. At week 21, the number of live sperm was significantly lower in PTU (0.38×10^9) as compared with the control (0.67×10^9) birds.

Discussion

There was a significant decrease in body weight of the PTU group compared to the control birds. Either thyroidectomy or goitrogen feeding at the age of 9 weeks in growing chicks resulted in a very low growth rate in both sexes (Pandey and Bajaj, 1974). Weight loss due to PTU treatment was also reported in cockerels (Kumaran and Turner, 1949; Jacquet *et al.*, 1993), and rats (Mendis-Handagama and Ariyaratne, 2001). Jiang *et al.* (2000) reported that T_4 treatment of adult male hypothyroid *rdw* rats resulted in increased body weight. Thyroid hormones stimulate GH secretion in birds, reptiles, rats, and human (Rousseau *et al.*, 2002), and are required for normal body growth. Tsukada *et al.* (1996) reported that PTU treatment in chicken significantly reduced the mRNA expression of GH receptor and insulin-like growth factor (IGF-I) in the liver, so any deficiency in thyroid hormone can result in stunted growth.

As expected, treatment by PTU resulted in a sharp decrease in serum T_4 level between week 11 to 15 of age. PTU interferes with thyroid hormone biosynthesis by inhibiting thyroid peroxidase; PTU also inhibits 5'-deiodinase in peripheral tissues and prevents conversion of T_4 to T_3 (Ganong, 1993; Taurog, 1996).

Two weeks after PTU withdrawal, serum T_4 level was restored to levels similar to those of the control group. Similar findings were reported by Knowlton *et al.* (1999). de Costa *et al.* (2001) reported a negative correlation between age and serum T_4 levels in rat which is not in agreement with present study. This difference may be a result of different pattern of serum T_4 alterations during aging between rodents and chickens (Kirby *et al.*, 1996).

PTU feeding treatment between 7 to 13 weeks of age resulted in higher testosterone levels between 11 and 13 weeks of age along with a decline in T_4 concentration. Kirby *et al.* (1996) reported that prepubertal hypothyroidism induced by PTU treatment between 6 and 12, 8 and 14, or 10 and 16 weeks of age, resulted in an elevation in serum testosterone level in commercial broiler breeder male chicks. Similar results in male breeder turkeys were reported by Knowlton *et al.* (1999) who showed that PTU treatment between 8 to 16 weeks of age and at a level of 0.1% increased serum testosterone level. An inverse relationship between gonadal and thyroid hormones was found in layer pullets (Sechman *et al.*, 2000) and cockerels (Kirby *et al.*, 1996) during sexual maturation. Choudhury and Chaturvedi (1995) proposed that thyroid hormones may be a substitute for gonadal steroids for inducing feedback initiation of LH activity in castrated subtropical finch. Decreased levels of testosterone after T_3 treatment in rats, was shown by Teerds *et al.* (1998). Jacquet *et al.* (1993) indicated that T_4 treatment in cockerels caused a decline in LH and testosterone level. This decrease in serum LH levels occurred 3 weeks after the beginning of treatment; although treatment by T_4 could not exert any effect on pituitary sensitivity to LHRH. Chiasson *et al.* (1979) found that feeding rapeseed meal with goitrogenic properties and the goitrogen methimazole in growing broiler cockerels, caused higher level of LH in serum. They postulated that induced hypothyroidism may be a reason for this elevation in LH level. LH stimulates Leydig cells to produce testosterone (Hafez *et al.*, 2000). Although we did not measure LH concentration, it can be concluded that higher LH level during the period of PTU treatment may be a reason for

higher testosterone level in PTU birds.

Any alteration in Leydig cell numbers, can affect testosterone level. Transient neonatal thyroid hormone deficiency in rats resulted in an increase in the number of Leydig cells in testes at adulthood (Mendis-Handagama and Sharma, 1994; Hardy *et al.*, 1996). Indeed, transient neonatal hypothyroidism increased the number of mesenchymal cells which are precursors of Leydig cells (Mendis-Handagama *et al.*, 1998). To be effective, PTU treatment in rodents (Cooke *et al.*, 1992; Joyce *et al.*, 1993; Kirby *et al.*, 1995) and domestic fowls (Kirby *et al.*, 1996) must occur during a narrow and species-specific window of opportunity corresponding to the period of Sertoli cell proliferation (Hess *et al.*, 1993; Meisami *et al.*, 1994; Kirby *et al.*, 1995). The effective treatment window in domestic fowls appears to be between 6 and 12 weeks of age (Kirby *et al.*, 1996). In our study PTU was administered between 7 and 13 weeks of age, so it can be suggested that PTU treatment during this period, could increase Sertoli cell proliferation, although no histological study of testis was performed in the present investigation. Conversely, it is indicated that T_3 inhibits immature rat Sertoli cell proliferation (Pezzi *et al.*, 2001). PTU treatment in rat resulted in a delay in Sertoli cell differentiation and a prolongation of Sertoli cell proliferation led to an approximately 80% increase in Sertoli cell and germ cell number (Teerds *et al.*, 1998). PTU treatment at a time other than period of Sertoli cell proliferation, adversely, resulted in lower testosterone level in rats possibly by preventing mRNA expression of two important enzymes required for cholesterol conversion to testosterone in Leydig cells, namely, P450_{scc} and steroidogenic acute regulatory protein (Chiao *et al.*, 2002). It has been suggested that PTU may act through different mechanisms in rodents and domestic fowls (Kirby *et al.*, 1996), explaining different responses to PTU between these species.

Since androgen production in male birds is simultaneous to onset of spermatogenesis and testis maturation (Etches, 1996), sharp increases in testosterone level in both groups after the week 15, observed in the present study, correspond to the onset of puberty.

Methimazole- or PTU-induced hypothyroidism has been reported to result in precocious puberty in fowls when administered from hatch to 16 weeks of age (Kumaran and Turner, 1949; Maqsood, 1952). In this respect, there are some contradictory reports for rodents. Early hypothyroidism in rats inhibited testicular development during the treatment period (Palmero *et al.*, 1990; Francavilla *et al.*, 1991) and stimulatory effects of transient hypothyroidism on gonadal function were not apparent until adulthood (Cooke and Meisami, 1991; Cooke *et al.*, 1991). Transient neonatal hypothyroidism suppressed the pubertal increases in serum FSH and LH level and delayed the pubertal rise in serum testosterone level by 10-20 days in rats (Kirby *et al.*, 1992).

The results of this study showed that PTU treatment had no effect on semen characteristics; however, PTU \times age interaction was significant for sperm concentration and total live sperm per ejaculate. Sperm concentration at weeks 20, 23 and 24 was higher in the PTU group. Similar effects on sperm concentration due to PTU treatment were reported by Kirby *et al.* (1996). Knowlton *et al.* (1999) observed no differences in sperm concentration in the PTU and control groups of breeder turkeys and suggested that it might have been due to suboptimal dose (0.1%) of PTU blended with the diet.

Appropriate treatment with PTU and the resultant hypothyroidism resulted in dramatic and permanent hypertrophy (80%) of testis and increased sperm output (140%) in rats (Cooke, 1991; Cooke and Meisami, 1991) and mice (Joyce *et al.*, 1993). In chicks, 0.1% dietary PTU treatment from 10 to 16 weeks of age resulted in a substantial increase in adult testis size (96%) and sperm production (115%) (Kirby *et al.*, 1996). Increased testicular size at puberty in rats was shown to be a result of hypertrophy and hyperplasia of Sertoli and Leydig cells (Cooke *et al.*, 1992). Van Haaster *et al.* (1992), Hess *et al.* (1993) and Meisami *et al.* (1994) reported a 1.57 fold increase in Sertoli cell numbers as a result of PTU treatment in rats. Increase in the number of Sertoli cells, and the positive correlation between the number of Sertoli cells and

sperm production rate (Berndston and Thompson, 1990; Hess *et al.*, 1993; Meisami *et al.*, 1994), could be a reason for higher sperm concentration in PTU birds.

As testosterone is required to maintain spermatogenesis (Sharpe, 1994; Kirby *et al.*, 1996), elevated serum testosterone level in the PTU group may lead to accelerated seminiferous tubule maturation and a concomitant increase in the rate of survival of germ cells that have entered meiosis (Kirby *et al.*, 1996). It can be concluded that both higher testosterone level and increased number of Sertoli cells are involved in higher sperm concentration in the PTU group.

PTU treatment had no effect on sperm motility, percentage of live sperm, and total number of live sperm. No significant differences were found in sperm motility and percentage of live sperm. Lien and Siopes (1991) also did not observe any differences between thyroidectomized and the control group in terms of percentage of live sperm in male domestic turkeys; but this was observed only before photostimulation. In fact, after initiation of photostimulation, larger value for percentage of live sperm was noted in thyroidectomized group (Lien and Siopes, 1991). The lighting condition in the present study was constant (14 hr-light: 10 hr-darkness) throughout the experiment; therefore, no photostimulation existed to affect the percentage of live sperm. Since the total number of live sperm was calculated by multiplying three parameters including the percentage of live sperm, semen volume, and sperm concentration, it is clear that any alteration in each of these parameters can affect the total number of sperm. Higher, although not significant values noted for these parameters at week 21 in the control group, can be an explanation for significantly higher value in total number of sperm in this group compared to the PTU-treated chicks.

It can be concluded that transient prepubertal PTU treatment could result in an increase in the adult testis size and sperm production provided that the effective treatment window of time has been considered (Kirby *et al.*, 1996). Transient prepubertal PTU-induced hypothyroidism resulted in an increase in the sperm

concentration and production at certain stages after treatment, but the effect did not seem to last for a long period. The potential for increased efficiency associated with raising smaller birds which pass a neonatal hypothyroidism and eat less feed and produce normal semen, may be economical.

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References

- Berndston, WE and Thompson, TL (1990). Changing relationship between testis size, Sertoli cell number and spermatogenesis in Sprague Dawley rats. *J. Androl.*, 11: 429-435.
- Buzzard, JJ; Morrison, JR; O'Bryan, MK; Song, Q and Wreford, NG (2000). Developmental expression of thyroid hormone receptors in the rat testis. *Biol. Reprod.*, 62: 664-669.
- Chiao, YC; Cho, WL and Wang, PS (2002). Inhibition of testosterone production by propylthiouracil in rat Leydig cells. *Biol. Reprod.*, 67: 232-239.
- Chiasson, RB; Sharp, PJ; Klandorf, H; Scanes, CG and Harvey, S (1979). The effect of rapeseed meal and methimazole on levels of plasma hormones in growing broiler cockerels. *Poultry Sci.*, 58: 1575-1583.
- Choudhury, A and Chaturvedi, CM (1995). L-thyroxine regulates pituitary LH function in castrated subtropical finch *Estrilda amandava*. *Poult. Avian Biol. Rev.*, 6: 253 (abs).
- Cooke, PS (1991). Thyroid hormones and testis development: a model for increasing testis growth and sperm production. *Ann. NY Acad. Sci.*, 637: 122-132.
- Cooke, PS; Hess, RA; Porcelli, J and Meisami, E (1991). Increased sperm production in adult rats after transient neonatal hypothyroidism. *Endocrinology*. 129: 244-248.
- Cooke, PS and Meisami, E (1991). Early hypothyroidism in rats causes increased adult testis and reproductive size but does not change testosterone levels. *Endocrinology*. 129: 237-243.
- Cooke, PS; Porcelli, J and Hess, RA (1992). Induction of increased testis growth and sperm production in adult rat by neonatal administration of the goitrogen propylthiouracil (PTU): the critical period. *Biol. Reprod.*, 46: 146-154.
- de Costa, VMC; Moriera, DG and Rosenthal, D (2001). Thyroid function and aging: gender-related differences. *J. Endocrinol.*, 171: 193-198.
- Dickson, WM (1996). Endocrinology, reproduction, and lactation. In: Swenson, MJ and Reece, WO (Eds.), *Duke's physiology of domestic animals*. (11th. Edn.), London, Cornell University Press. PP: 642-644.
- Donoghue, AM and Wishart, GJ (2000). Storage of poultry semen. *Anim. Reprod. Sci.*, 62: 213-232.
- Etches, RJ (1996). *Reproduction in poultry*. 1st. Edn., Cambridge, CAB International. PP: 208-233.
- Falconer, IR (1971). The thyroid gland. In: Bell, DJ and Freeman, BM (Eds.), *Physiology and biochemistry of the domestic fowl*. (1st. Edn.), Vol. I. London, Academic Press. PP: 467-470.
- Fallah-Rad, AH; Conon, ML and Del Vecchio, RP (2001). Effect of transient early hyperthyroidism on onset of puberty in Suffolk ram lambs. *Reproduction*. 121: 639-646.
- Francavilla, S; Cordeschi, G; Properzi, G; Coco, L; Jannini, EA; Palmero, S; Fugassa, E; Loras, B and Armiento, D (1991). Effect of thyroid hormone on the pre- and post-natal development of the rat testis. *J. Endocrinol.*, 129: 35-42.
- Ganong, WF (1993). *Review of medical physiology*. 16th. Edn., San Francisco, Appleton and Lange. PP: 287-301.
- Hafez, ESE; Jainudeen, MR and Rosnina, Y (2000). Hormones, growth factors, and reproduction. In: Hafez, B and Hafez, ESE (Eds.), *Reproduction in farm animals*. (7th. Edn.), Philadelphia, USA, Lippincott Williams and Wilkins. PP: 33-54.
- Hardy, MP; Sharma, RS; Arambepola, NK; Sottas, CM; Russel, LD; Bunick, D; Hess, RA and Cooke, PS (1996). Increased proliferation of Leydig cells induced by neonatal hypothyroidism in the rat. *J. Androl.*, 17: 231-238.
- Hess, RA; Cooke, PS; Bunick, D and Kirby, JD (1993). Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers. *Endocrinology*. 132: 2607-2613.
- Jacquet, MJ; Seigneurin, F and de Reviers, M (1993). Effect of thyroxine on testicular function, circulating luteinising hormone and pituitary sensitivity to luteinising hormone-releasing hormone in the cockerel. *Brit. Poultry Sci.*, 34: 803-814.

- Jiang, JY; Umezu, M and Sato, E (2000). Improvement of follicular development rather than gonadotropin secretion by thyroxine treatment in infertile immature hypothyroid *rdw* rats. *J. Reprod. Fertil.*, 119: 193-199.
- Joyce, KL; Porcelli, J and Cooke, PS (1993). Neonatal goitrogen treatment increases adult testis size and sperm production in the mouse. *J. Androl.*, 14: 448-455.
- Kirby, JD; Cooke, PS; Bunick, D; Hess, RA; Kirby, YK and Turek, FW (1995). Does TSH potentiate the effect of neonatal PTU treatment on testis growth in the rat? *Assist. Reprod. Technol. Androl.*, 7: 171-189.
- Kirby, JD; Jetton, E; Cooke, PS; Hess, RA; Bunick, D; Ackland, JF; Turek, FW and Schwartz, NB (1992). Developmental hormonal profiles accompanying the neonatal hypothyroidism induced increases in adult testis size and sperm production in the rat. *Endocrinology*, 131: 559-565.
- Kirby, JD; Mankar, MV; Hardesty, D and Kreider, DL (1996). Effects of transient prepubertal 6-N-propyl-2-thiouracil treatment on testis development and function in the domestic fowl. *Biol. Reprod.*, 55: 910-916.
- Knowlton, JA; Siopes, TD; Roads, ML and Kirby, JD (1999). Effects of transient treatment with 6-N-propyl-2-thiouracil on testis development and function in breeder turkeys. *Poultry Sci.*, 78: 999-1005.
- Kumaran, JDS and Turner, CW (1949). The endocrinology of spermatogenesis in birds. III. Effect of hypo- and hyperthyroidism. *Poultry Sci.*, 28: 653-665.
- Lien, RJ and Siopes, TD (1991). Influence of thyroidectomy on reproductive responses of male domestic turkeys (*Meleagris gallopavo*). *Brit. Poultry Sci.*, 32: 405-415.
- Maqsood, M (1952). Thyroid function in relation to reproduction of mammals and birds. *Biol. Rev.*, 27: 281-319.
- Meisami, E; Najafi, A and Timiras, TS (1994). Enhancement of seminiferous tubular growth and spermatogenesis in testis of rats recovering from hypothyroidism: a quantitative study. *Cell Tissue Res.*, 275: 203-211.
- Mendis Handagama, SMLC and Ariyaratne, HBS (2001). Differentiation of the adult Leydig cell population in the postnatal testis. Mini-review. *Biol. Reprod.*, 65: 660-671.
- Mendis Handagama, SMLC; Ariyaratne, HBS; Van Menen, KRT and Haupt, RL (1998). Differentiation of adult Leydig cells in the neonatal rat testis is arrested by hypothyroidism. *Biol. Reprod.*, 59: 351-357.
- Mendis-Handagama, SMLC and Sharma, OP (1994). Effects of neonatal administration of the reversible goitrogen propylthiouracil on the testis interstitium in adult rats. *J. Reprod. Fertil.*, 100: 85-92.
- NRC (1994). *Nutrient requirements of poultry*. 9th. revised Edn, National Research Council, National Academy of Sciences, Washington, DC.
- Palmero, S; de Marchis, M; Gallo, G and Fugassa, E (1990). Thyroid hormone affects the development of Sertoli cell function in the rat. *J. Endocrinol.*, 123: 105-111.
- Pandey, JN and Bajaj, MR (1974). Effect of thiouracil administration on growth rate and carcass yield of Australorp chicks. *Haryana Agric. Univ. J. Res.*, 4: 153-157.
- Pezzi, V; Panno, ML; Sirianni, R; Forastier, P; Casaburi, I; Lanzino, M; Rago, V; Giordano, F; Giordano, C; Carpino, A and Ando, S (2001). Effect of tri-iodothyronine on alternative splicing events in the coding region of cytochrome P450 aromatase in immature rat Sertoli cells. *J. Endocrinol.*, 170: 381-393.
- Rousseau, K; Le Belle, N; Sabaihi, M; Marchelidon, J; Schmits, M and Dufour, S (2002). Evidence for a negative feedback in the control of eel growth hormone by thyroid hormone. *J. Endocrinol.*, 175: 603-613.
- SAS (1996). *SAS/STAT Software* changes and enhancement through release 6.12, SAS Institute Inc., Cary, NC.
- Sauer, MJ; Cookson, AD; McDonald, BJ and Foulkes, JA (1982). The use of enzyme immunoassay for the measurement of hormones with particular reference to the determination of progesterone in unextracted whole milk. In: Wardley, RC and Crowther, JR (Eds.), *The ELISA*. (1st. Edn.), The Netherlands, Martinus Nijhoff Publishers. PP: 271-296.
- Sechman, A; Rza, J and Paczoska-Elasiewicz, H (2000). Thyroid hormones (T₄, T₃ and rT₃) concentrations in chicken ovarian follicle during sexual maturation and egg-lay. 21st World's Poultry Congress. Montreal, Canada (No page numbers; Abstract on CD).
- Sharpe, RM (1994). Regulation of spermatogenesis. In: Knobil, E and Neill, JD (Eds.), *The physiology of reproduction*. (2nd Edn.), Vol. I., New York, Raven Press. PP: 1363-1434.
- Siopes, T (1997). Transient hypothyroidism reinitiate egg laying in turkey breeder hens: termination of photorefractoriness by propylthiouracil. *Poultry Sci.*, 76: 1776-1782.
- Taurog, A (1996). The mechanism of action of the thioureyline antithyroid drugs. *Endocrinology*, 98: 1031-1046.
- Teerds, KJ; de Rooij, DG; de Jong, FH and Van

- Haaster, LH (1998). Development of the adult type Leydig cell population in the rat is affected by neonatal thyroid hormone levels. *Biol. Reprod.*, 59: 344-350.
- Tsukada, A; Ohkubo, T; Sakaguchi, K; Tanaka, M; Nakashima, K; Hayashida, K; Wakita, M and Hoshino, S (1996). Thyroid hormones are involved in insulin-like growth factor (IGF)-I production by regulating growth hormone receptor (GHR) in the chicken. *Poult. Avian Biol. Rev.*, 8: 253 (abs).
- Van Haaster, LH; De Jong, FH; Docter, R and De Rooij, DG (1992). The effect of hypothyroidism on Sertoli cell proliferation and differentiation and hormone levels during testicular development in the rat. *Endocrinology*. 131: 1574-1576.