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VARIATION IN THYROID ACTIVITY WITH RESPECT TO ANNUAL SEASONAL CYCLE OF REPRODUCTION IN TURTLE MELANOCHELYS TRIJUGA

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ABSTRACT

The present investigation was carried out to find out the relationship between annual cycle of reproduction and thyroid activity in *Melanochelys trijuga*. The animals were studied in the annual seasonal cycle of reproduction which is distinguished into three separate periods namely regenerative, reproductive and recrudescent. Thyroid is an unpaired, oval shaped gland. Paraffin embedded thyroid sections were studied by bromophenol blue method for thyroglobulin. Liver sections were localized for glycogen. Plasma glucose was estimated in different periods of reproductive cycle. Thyroid showed seasonal variation in its activity. The present investigation demonstrates increased thyroid activity from regenerative to reproductive period which decreased to minimum in recrudescent period. The active thyroids appeared spongy and translucent with maximum weight and size. Weight of the gland is positively correlated with weight of the animal. Liver glycogen and abdominal fat was utilized during winter months of recrudescent period to maintain basal metabolic rate. Environmental temperature appeared to play effective role on thyroid activity. Cyclic changes in the thyroid may have indirect effect on reproductive cycle, sexual behaviour as well as in gonad maturation.

Key words: Thyroid; Turtle; Season

1. INTRODUCTION

Many animals that breed seasonally measure the day length (photoperiod) and use these measurements as predictive information to prepare themselves for annual breeding. For several decades, thyroid hormones have been known to be involved in this biological process (Nakao N et al. 2008). *In vitro* culture was used to examine the direct actions of temperature at the levels of pituitary hormone responses to thyroxine and testicular androgen secretion in a turtle *Pseudemys scripta* (Licht P et al. 1989). Chow SY et al. (1990) reported relation between pH regulation and iodide transport in turtle thyroid glands. The ability of hormones to bind to their functional receptors on endocrine target tissue in cold and warm temperatures was studied by administration of thyrotropin releasing hormone, corticotrophin releasing hormone and growth hormone releasing hormone to pituitaries in *P. scripta* (Licht P et al. 1990). Machado VL et al. (1991) reported probable action of insulin on the uptake of radioactive glucose and alpha-aminoisobutyric acid in *in vitro* thyroid gland of *Chrysemys dorbigni*. Variations in plasma thyroxine binding activity induced by experimental or environmental manipulations were demonstrated in *Trachemys scripta* in relation to difference in thyroxine binding protein concentrations (Pavigi S and Licht P, 1992).

Thyroid hormone metabolism by 5'-monodeiodinase was characterized in peripheral tissues of turtle *T. scripta* and compared with activity measured in the rat (Hugenberger JL and Licht P, 1999). Seasonal changes in thyroid function in the desert tortoise *Gopherus agassizii,* exhibited distinct cycles in both sexes. Thyroxine was lowest during hibernation and rose at the time of emergence in *Gopherus agassizii*. Elevated thyroxine is associated with periods of increased feeding and reproductive activity (Kohel KA et al. 2001). Seasonal factors like temperature play an important role in influencing thyroid activity in Soft shelled Turtle *Lissemys p. punctata* (Sengupta A et al. 2003). The present investigation was carried out to find out whether there is any relationship between annual cycle of reproduction, atmospheric temperature and thyroid function in *Melanochelys trijuga***.**

2. MATERIALS AND METHODS

Indian Black Turtle/ Indian Pond Terrapin *Melanochelys trijuga* is a species of turtle found in South Asia. They are terrestrial or freshwater, found in slow flowing or sedentary water. Young are more aquatic than the adults which are semi-terrestrial. These are often found basking on rocks and logs during winter. *M. trijuga,* belong to family Bataguridae of order Chelonia (Das I. 1995). They remain active throughout the year and rarely found in the loose soil.

Twelve adult animals of both sexes of *M. trijuga* were collected from their natural habitat, Kabini reservoir (Latitude 11-0-30" N; Longitude 76-21-12"E; Catchment area 2142 Sq. km, about 69 km away from Mysore) and were maintained in an outdoor cement tank containing water and sand. The cistern measured 7X5X5 ft with a raised bottom at one end for basking. The animals in the present investigation weighed between 1100 to 2400 g. The animals were fed *ad-libitum* with fresh carrot and spinach, but were not fed 48 h prior to autopsy. The animals were injected with sodium pentobarbital (50 mg/kg b.w.) intra-peritoneal for recording their body length and weight and were sacrificed. "Guidelines for Care and Use of Animals in Scientific Research" were followed (Anonymous. 2000). During experimentation the temperature of Mysore varied with season (11^oC-37^oC).

2.1 *Thyroid histochemisrty*

The thyroid glands from twelve animals of four each in all the periods were removed, weighed and fixed in Bouin's fluid. Paraffin embedded tissues were sectioned at 10 µ. Bromophenol blue staining method was followed for histochemical localization of thyroglobulin/colloid.

2.2 *Liver histochemisrty*

Liver samples from the respective animals of all the periods were fixed in Rossman's fixative. Paraffin embedded tissues were processed, sectioned at 9-10 µ for Periodic Acid Schiff (PAS) histochemical technique of Hotchkiss RD. (1968) for localisation of glycogen.

2.3 *Plasma glucose*

At autopsy, blood samples from carotid artery were collected centrifuged and separated serum was used for estimation of plasma glucose by enzyme glucose oxidase method of Trinder P. (1969). Plasma glucose was determined by averaging the values of multiple (4X10) samples in every season. The averaged values represent order of magnitude rather than fixed levels, as the range of variability is great between individuals of this population.

2.4 *Measurements*

Ocular micrometer was fixed in Olympus Bx60 during measurement. The serial sections of thyroid were observed at regular intervals in the entire gland. The thyroid follicle diameter, follicular epithelial height and gap between the colloid and epithelium measurements were done at their longest axis by software Image pro express, version 5.1. The size was calculated by random selection of at least 4X25 observations in each period. Photographs were taken using Olympus B x 60.

2.5 *Statistical analysis*

The measurements were expressed as mean ± SD for follicular diameter; epithelial height; gap between colloid and follicular epithelium in microns (µ) and plasma glucose level in mg/dl during different periods was carried out using analysis of variance (ANOVA). Wherever the ANOVA values

(*F*) were found to be significant, Duncan's Multiple Range Test (DMRT) was applied. The P value < 0.05 was considered significant.

3. RESULTS

3.1 *Reproductive cycle*

The animals were studied in the annual seasonal cycle of reproduction which is distinguished in to three separate periods namely regenerative, reproductive and recrudescent. Different periods of reproductive cycles were assigned by careful observation of the status of the gonad during two successive cycles of reproduction. Initiation of gonad activity occurred in both the sexes during regenerative period. This period fall between March to June and corresponded with summer season, the temperature varied from maximum 37^{o}C to minimum 19^{o}C with longer days. The animals were active and voracious eaters.

The peak of gonad activity occurred in reproductive period in both the sexes, which fall between July to August. The temperature ranged from 28^{o}C maximum to minimum of 19^{o}C, these are the months of monsoon with longer days. In males the testis attained its largest size only between July and August after which it regressed very soon. Mating occurred during these months. The females showed the presence of eggs in the oviduct. This condition prevailed much earlier than July. They started laying eggs in clutches by the end of August and continued till October. The animals on an average weighed maximum of all periods (Table 1).

Table 1
Morphometric measurements of M. trijuga during annual cycle of reproduction

	Regenerative	Reproductive	Recrudescent
Months	March-June	July-Aug	Sept-Feb
Animal weight (g)	1550±474.3^{a}	2150±263.5^{b}	1750±263.5^{a}
Thyroid weight (g)	0.159±0.01^{a}	0.298±0.08^{c}	0.200±0.05^{b}
Follicle diameter (μ)	184±45^{a}	244±39^{b}	310±23^{c}
Epithelial height (μ)	13±3.5^{b}	15±0.7^{c}	10±5.5^{a}
Gap (μ)	35±1.2^{c}	26±4.2^{b}	12±3.7^{a}
Plasma glucose (mg/dl)	69.97±2.56^{a}	97.96±2.51^{b}	113.52±2.71^{c}
Liver glycogen	+++	++	+
Abdominal fat	+++	++	+
Note: Mean with same letters is not significantly different from each other;			
+++ = Maximum; ++ = Moderate; + = Minimum			

Recrudescent period fall between September to February and corresponded with winter when nights are longer with shorter days. During this period gonads showed degeneration in both sexes. The change ultimately resulted in loss of reproductive ability. At autopsy females showed the presence of eggs in the oviduct. The live animals even laid eggs until the end of October. The maximum and minimum temperatures were 28 ^{o}C and 11^{o}C respectively. *Melanochelys trijuga* usually bask under sun during this period or duck in water, very few animals were seen hiding in the loose soil for a short period in a day.

3.2 *Thyroid*

The thyroid in *M. trijuga* is a single, oval shaped gland present ventral to the trachea and just anterior to the heart on the right side of the animal. The gland is covered by a thin connective tissue capsule, lightly reddish-brown and of lenticular profile. On an average it measured 7-9 mm in length and 3-5 mm in width. The gland consists of many follicles that are surrounded by a single layer of epithelial

cells placed on basement membrane. The thyroid gland on an average weighed minimum in regenerative period while in reproductive period the gland weighed maximum and moderate in recrudescent period (Table 1).

3.3 ***Thyroid Follicle***

Follicles were polyhedral in shape. The diameter of the thyroid follicles at their longest axis was minimum during regenerative period which increased to maximum from reproductive period to recrudescent period (Table 1).

3.4 ***Follicular epithelium***

During regenerative period and reproductive period the follicular epithelium was continuous around all the follicles of the gland. Follicular epithelium measured moderate in regenerative and maximum in reproductive period. Epithelial cells have basally situated nuclei with distinct nucleoli. The epithelial cell cytoplasm contained granules in them which appeared as the droplets of secretion. In reproductive period they are found to be released into the lumen of the follicle at large numbers than in regenerative period. The epithelium of the thyroid follicles measured least (Table 1) being hazy in recrudescent period. Taller the epithelial cells are higher is the secretory activity.

3.5 ***Colloid***

Nature of colloid varied between periods. The gap between the colloid and epithelium measured highest in regenerative period, moderate in reproductive period being least in recrudescent period (Table 1).The colloid content appeared homogeneous in regenerative (Figure1 and 1a) and reproductive periods (Figure 2 and 2a) but very brittle during recrudescent period (Figure 3 and 3a).

Thyroid sections of *M. trijuga* showing variation in follicle size, epithelial height and nature of colloid in annual seasonal cycle of reproduction.

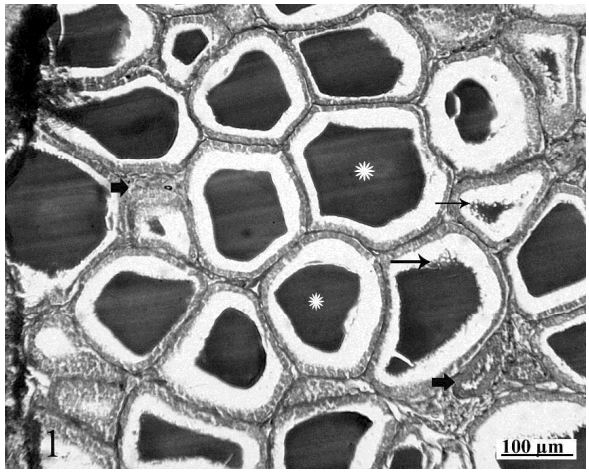

Figure 1. ***Regenerative period: Note uniformly stained colloid (white *) with chromophobe droplets at peripheries (arrows), a wide gap between colloid and follicular epithelium is seen, and vascularization at the corners of the follicles (broad arrows). X 10.***

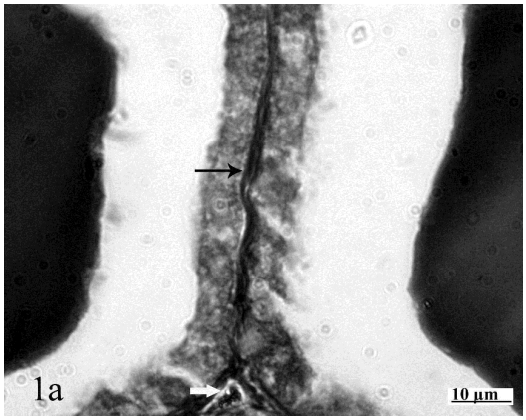

Figure 1a. ***Partial section of regenerative period: Showing columnar epithelial cells on basement membrane (arrow) releasing their contents in to the gap and vascularization at the corners of the follicles (white arrow). X 100***

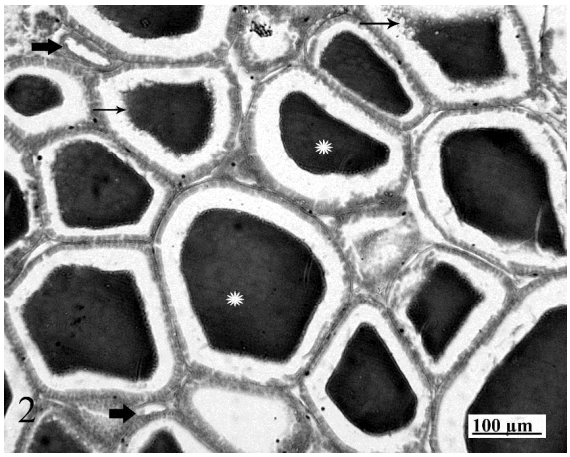

Figure 2. ***Reproductive period: Note uniformly dark stained colloid (white *) with many droplets at periphery (arrows), the gap between colloid and follicular epithelium as well as vascularisation at the corners of the follicles (broad arrows) are seen. X 10.***

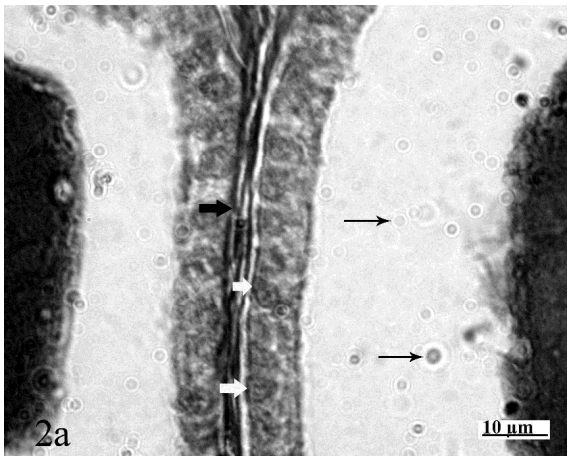

Figure 2a. ***Partial section of reproductive period: Note distinct follicular epithelium with nucleus (white arrow) on basement membrane (black arrow) and many chromophobe secretory droplets (thin arrow) in the gap between colloid and epithelium. X 100***

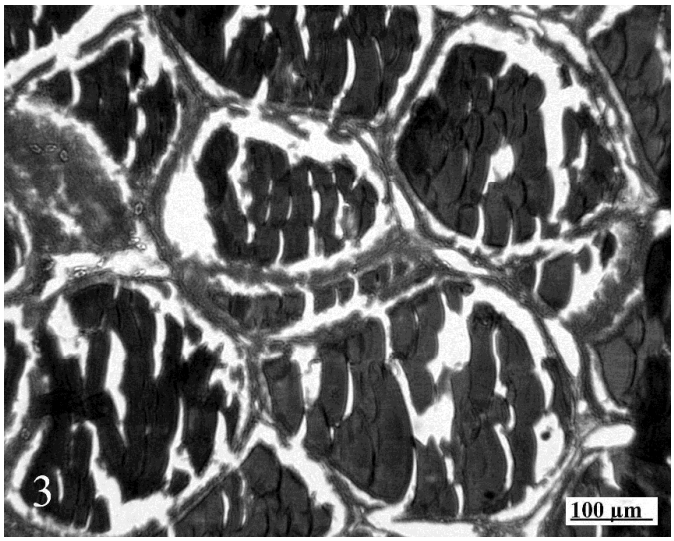

Figure 3. ***Recrudescent period. Note increased follicle size with brittle colloid, flat and discontinuous follicular epithelium. X 10.***

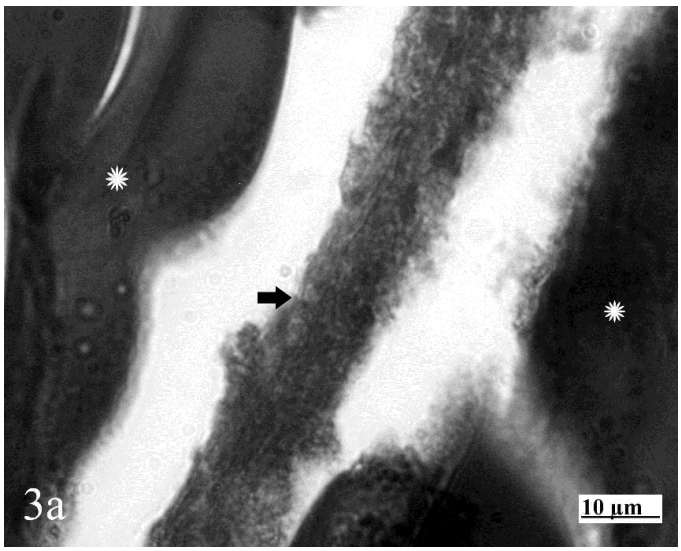

Figure 3a. ***Partial section of recrudescent period: Showing indistinct epithelium (arrow) with minimized gap between colloid (white *) and epithelium. X 100***

Note: The photographs are the representatives of each period.

3.6 ***Plasma glucose and liver glycogen***

The plasma glucose on an average measured minimum in regenerative period. Liver samples showed maximum glycogen mass. Both white and brown fat was abundant in the abdomen. Plasma glucose measured moderate in reproductive period, white and brown fat in abdominal cavity as well as liver glycogen was also moderate. Plasma glucose was maximum in recrudescent period; liver glycogen and abdominal fat were at minimum (Table 1).

4. DISCUSSION

The thyroid gland of *M. trijuga* weighed minimum during regenerative period. Follicle diameter was also measured minimum with tall columnar epithelium releasing contents into the lumen (Figure1&1a) indicating its active stage for synthesis of thyroglobulin. The colloid was uniformly dark stained. This condition continued even in reproductive period (Figure 2 & 2a) with maximum follicular diameter and higher epithelial height with moderate gap between the colloid and

follicular epithelium (Table. 1). This was due to continuous release of secretory droplets of colloid. The thyroid of reproductive period appeared more spongy and translucent due to accumulation of colloid because of increased activity. The length of the day may also influence thyroid activity in *Testudo horsfieldii* (Lynn WG. 1970). Thyroid hormone plays a key role in signalling day-length changes to the brain and thus triggers seasonal breeding (Nakao N et al. 2008). *Melanochelys trijuga* exhibits minimum value for plasma glucose during regenerative period. Initiation of thyroid activity might have utilized the glucose. Machado VL et al. (1991) demonstrated the uptake of glucose by thyroid fragments during *in vitro* studies. Part of glucose might have been stored in the form of glycogen as a reserve food material as indicated by maximum amount of glycogen masses in liver sections of regenerative period. White and brown fat was stored in the abdominal cavity to meet the demands of the subsequent reproductive period. The plasma glucose, liver glycogen and abdominal fat measured moderate during reproductive period. These changes may be due to utilization of energy for the developments of gonads. The reptilian (a lizard, *Trachydosaurus rugosus*, a tortoise *Chelodina longicollis* and a crocodile, *Crocodylus johnstoni*) thyroid found active at high temperatures (Hulbert AJ and Williams CA, 1988). In *Uromastix hardwickii* the follicular cell-height, nature of colloid and weight of the gland showed a seasonal variation being active in summer when the environmental temperature was high, while in winter when the environmental temperature was low, the thyroid was almost inactive. This was associated with the active and inactive phases of reproduction (Sinha AK and Choubey BJ, 1981).

Due to accumulation of colloid content the follicles measured maximum in their diameter in recrudescent period of *M. trijuga.* Follicular epithelium was often obscure and measured minimum of all the periods. Brittle/ragged colloid of recrudescent period and thinning of epithelium indicate hypo activity of the follicle (Figure 3 & 3a). The thyroid gland of this period appeared opaque due to brittle colloid. The gland on an average weighed moderate. It appears that the weight of the gland is positively correlated with weight of the animal (Table. 1). Thyroid hormones are important for regulating a variety of developmental processes in vertebrates, including growth (Huang WT et al. 2009).

Melanochelys trijuga did not undergo hibernation during winter unlike *Lissemys punctata* (Chandavar VR and Naik PR, 2004). Instead *M. trijuga* during the winter months of recrudescent period bask under sun to rise body temperature. They being continuous feeders and active throughout the winter, plasma glucose rose to higher level (113.52±2.71 mg/dl). In *L. punctata* plasma glucose measured least of 91.39 ±2.76 mg/dl during winter which corresponded with recrudescent period (Chandavar VR and Naik PR, 2004). Thyroid hormone from accumulated colloid of recrudescent period maintained the basal metabolic rate and thus promoted break down of fat and liver glycogen to provide energy in this period of winter months. During winter, night temperature was 11^{o}C, and the turtles were maintained in outdoor enclosures. The follicles of this period showed least epithelial height. Turtle *Lissemys p. pucntata* exposed to low ambient temperature (10^{o}C for 15 days) showed a significant decrease in relative thyroid weight and epithelial height in both the peripheral and central follicles of the gland, with colloid materials in the follicular lumen (Sengupta A et al. 2003).

The follicle diameters vary in different reptiles from 50µ to 300µ and may reach much larger sizes in large turtles, and the thyroid weigh between 0.044g to 0.362 g (Lynn WG. 1970). In the present investigation in *M. tijuga*, the gland weighed between 0.150 g to 0.300 g and follicles measured between 120 to 350 µ while in *L. punctata* (Chandavar VR and Naik PR, 2010) the thyroid gland weighed between 0.120 to 0.246 g, and follicle diameter measured from 200 µ to 500 µ. The epithelial lining of the follicle varied in height from flat to columnar depending upon the functional state of the follicles and the amount of the colloid present in its lumen.

Thyroid activity was mainly dependent on environmental temperature. Thyroid activity and sexual system are interdependent in many reptilian species. Period of breeding corresponds with a greatest body activity in reptiles (Lynn WG. 1970). It is suggested that photoperiod exerts its action on thyroid activity presumably via gonads and/or

pineal-gonadal axis in *L. p. punctata* (Sarkar S et al. 2007).

In the present investigation the thyroid appeared to increase its activity from regenerative to reproductive period when gonads are active and days are longer. During recrudescent period stored colloid was in use for metabolic activities and to increase the body temperature both in *M. trijuga* and *L. punctata* (Chandavar VR and Naik PR, 2010). *M. trijuga* and *L. punctata* exhibit similar pattern of thyroid activity and reproductive cycle through the former is non-hibernating turtle and later is winter hibernating turtle.

The studies on cyclic changes in the thyroid in connection with the reproductive cycle indicate that thyroid activity may play a part in sexual behaviour as well as in gonad maturation depending on day length and atmospheric temperature.

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REFERENCES

1. Anonymous. Guidelines for care and use of animals in scientific research. New Delhi. Indian National Science Academy. 2000:1-31.
2. Chandavar VR and Naik PR. Variation in plasma glucose and pancreatic β cells in the turtle, Lissemys punctata (Order: Chelonia; Family: Trionychidae). Acta Zool (Stockholm). 2004; 85:113-118.
3. Chandavar VR and Naik PR. Seasonal variation in thyroid activity of turtle Lissemys punctata with respect to annual cycle of reproduction. J Appl Natu Sci. 2010; 2(2): 194-199.
4. Chow SY, Woodbury DM and Yen-Chow YC. Relation between pH regulation and iodide transport in turtle thyroid glands. J Endocrinol. 1990; 127(1):85-101.
5. Das I. Turtles and Tortoises of India. World Wide Fund for Nature-India. Bombay. Delhi. Oxford University Press. 1995: 1-176.
6. Hotchkiss RD. Histochemistry theoretical and Applied. Edinburgh (Churchhill Living stone): c 1968. Chapter, The Periodic Acid Schiff (PAS) Technique; p. 659-660.
7. Huang WT, Chien JT, Weng CF, Jeng YY, Lu LC and Yu JY. Ovine thyroid stimulating hormone (TSH) heterologously stimulates production of thyroid hormones from Chinese soft-shell turtle (Pelodiscus sinensis) and bullfrog (Rana catesbeiana and Rana rugulosa) thyroids in vitro. Comp Biochem Phys A. 2009; 153(2):167-173.
8. Hugenberger JL and Licht P. Characterization of thyroid hormone 5′-monodeiodinase activity in the turtle (Trachemys scripta). Gen Comp Endocr. 1999;113(3):343-59.
9. Hulbert AJ and Williams CA. Thyroid function in a lizard, a tortoise and a crocodile, compared with mammals. Comp. Biochem. Phys A. 1988; 90(1):41-48.
10. Kohel KA, MacKenzie DS, Rostal DC, Grumbles JS and Lance VA. Seasonality in plasma thyroxine in the desert tortoise, Gopherus agassizii. Gen Comp Endocr. 2001;121(2):214-22.
11. Licht P, Pavgi S and Denver RJ. The role of hormone binding in the cold suppression of hormone stimulation of the pituitary, thyroid, and testis of the turtle. Gen Comp Endocr. 1990; 80(3):381-92.
12. Licht P, Denver RJ and Pavgi S. Temperature dependence of in vitro pituitary, testis, and thyroid secretion in a turtle, Pseudemys scripta. Gen Comp Endocr. 1989;76(2):274-285.
13. Lynn WG. Biology of the reptilia. London (New York): Academic Press; c1970. The thyroid; p. 201-234.
14. Machado VL, Wassermann GF and Marques M. In vitro effect of insulin on the uptake of glucose and alpha-aminoisobutyric acid in the thyroid gland of the turtle (Chrysemys dorbigni). Gen Comp Endocr. 1991; 82(1): 8-13.

15. Nakao N, Ono H and Yoshimura T. Thyroid hormones and seasonal reproductive neuroendocrine interactions. Reproduction. 2008; 136(1):1-8.
16. Pavgi S and Licht P. Measurement of plasma thyroxine binding protein in relation to thyroidal condition in the turtle, Trachemys scripta, by radioimmunoassay. Gen Comp Endocr. 1992; 85(1):147-155.
17. Sarkar S, Sengupta A, Chaudhuri-Sengupta S and Maiti BR. Thyroid responses to altered photoperiod in the soft-shelled turtle Lissemys punctata punctata bonnoterre. Acta Biol Hung. 2007;58(1):11-19.
18. Sengupta A, Ray PP, Chaudhuri-Sengupta S and Maiti BR. Thyroidal modulation following hypo- and hyper-thermia in the soft-shelled turtle Lissemys punctata punctata Bonnoterre. Eur J Morphol. 2003;41(5):149-54.
19. Sinha AK and Choubey BJ. Seasonal changes in the thyroid gland of Indian spiny tailed, sand lizard, Uromastix hardwickii (Gray) male. Zellforsch Mikrosk Anat. 1981; 95(1):72-80.
20. Trinder P. Determination of blood glucose using oxidase peroxidase system with a non carcinogenic chromogen. J Clin Patho. 1969;22:158-161.