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Alterations in the ultimobranchial and parathyroid gland of the garden lizard, *Calotes versicolor* after prolactin administration

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In the present study, the effects of prolactin administration have been investigated on the plasma calcium, plasma phosphate, ultimobranchial gland, and parathyroid gland of the lizard *Calotes versicolor.* The lizards were procured and divided into two numerically equal groups A and B. The animals from group A were given daily intraperitoneal injections of 0.1 ml of vehicle (0.8% NaCl solution) per 50 g body weight. Lizards from group B were daily injected intraperitoneally with ovine prolactin (dissolved in 0.8% NaCl solution) in a dosage of 5 I.U. per 50 g body weight. Ten specimens from each group were anaesthetized with chloroform 4 hrs after the last injection on 1st, 3rd, 5th, 10th and 15th day of the treatment and blood samples were collected. After collection of blood, ultimobranchial and parathyroid glands were fixed. Prolactin treatment to *Calotes versicolor* provoked hypercalcemia and hyperphosphatemia. The ultimobranchial gland of prolactin treated lizards exhibit hyperactivity which is evident by a decreased staining response and increased nuclear volume of ultimobranchial cells. Few degenerating ultimobranchial cells have also been observed. The parathyroid gland of prolactin injected lizards became inactivated.

Key words: prolactin, ultimobranchial gland, parathyroid gland, plasma calcium, plasma phosphate, lizard.

INTRODUCTION

Calcium, particularly its ionic form, is of vital importance in many biological processes (Srivastav *et al.*, 2000, 2008; Booher, 2008; de Matos, 2008). Thus, the levels of calcium are precisely regulated by the interplay of several endocrine systems. In terrestrial vertebrates, control of calcium metabolism is maintained by two major hormones –parathyroid hormone (PTH) and calcitonin (CT). Parathyroid hormone is secreted by parathyroid glands. Secretion of PTH is enhanced when the ionic calcium concentration falls below the physiological concentration. CT is secreted by ultimobranchial gland (UBG) in non-mammals and from calcitonin cells (C cells) in mammals. Increased blood calcium concentration stimulates the secretion of CT. These two hormones (PTH and CT) act on the gut, bone and kidney to exert their actions.

Clark (1983) suggested that in aquatic vertebrates pituitary gland was important in the blood calcium regulation and could be of some significance in terrestrial forms as well. Among many pituitary hormones, prolactin has been shown to provoke hypercalcemia in fishes (Pang, 1981; Wendelaar Bonga & Flik, 1982, 1984; Jackson *et al.*, 2005), amphibians (Baksi *et al.*, 1978; Srivastav & Rani, 1991), reptiles (Swarup *et al.*, 1985; Srivastav & Rani, 1990; Srivastav *et al.*, 1994), birds (Baksi *et al.*, 1978) and mammals (Robinson *et al.*, 1975). Recently, Charoenphandhu & Krishnamra (2007) have reported that prolactin acts as a regulator of calcium homeostasis by controlling the in-

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testinal calcium absorption. To the best of our knowledge, there exists a single report regarding the effects of prolactin on the ultimobranchial and parathyroid gland of reptiles – snakes (Srivastav *et al.*, 1994). In the present study, an attempt has been made to investigate the effects of prolactin on the ultimobranchial and parathyroid gland of the garden lizard, *Calotes versicolor* as there exist no information from lizards regarding the activity of these glands in response to prolactin administration.

MATERIALS AND METHODS

Adult specimens of *C. versicolor* (both sexes, body weight 27-34 g) were procured and maintained under laboratory conditions for a week. Initial blood samples (from ten specimens) were taken before the start of the experiment (zero hour). Then the remaining animals were divided into two numerically equal groups –A (vehicle-injected control) and B (prolactin-injected experimental) and were treated as follows for 15 days:

Group A: Lizards were given daily intraperitoneal injections of 0.1 ml of vehicle (0.8% NaCl solution) per 50 g body weight.

Group B: Lizards from this group were daily injected intraperitoneally with ovine prolactin (dissolved in 0.8% NaCl solution) in a dosage of 5 I.U. per 50 g body weight.

During the experiment the animals were not fed. Ten specimens from each group were anaesthetized with chloroform 4 hrs after the last injection on 1st, 3rd, 5th, 10th and 15th day of the treatment. Blood samples were taken by cardiac puncture at each experimental interval in heparinized tubes. Plasma was separated by centrifugation and analyzed for calcium (Sigma kit) and inorganic phosphate (Sigma kit) levels.

After collection of blood, the ultimobranchial gland (the region anterior to the heart) and parathyroid gland (near the carotid bifurcation) from the lizards were taken out. All these tissues were fixed in aqueous Bouin's solution. The materials thus fixed were routinely dehydrated in graded series of alcohols, cleared in xylene and embedded in paraffin. Serial sections were cut at 6 mm and stained with hematoxylin/eosin (HE).

Nuclear (ultimobranchial and parathyroidal cells) indices (maximal length and maximal width) were determined with the aid of an ocular micrometer. Fifty nuclei were measured per animal. The nuclear volume was calculated as:

$$\text{Volume} = 4/3\ \pi\ ab^2$$

where 'a' is the major semiaxis and 'b' is the minor semiaxis.

All data were presented as the mean ± s.e. of ten specimens and Student's t test was used to determine statistical significance. In all studies the experimental group was compared to its specific time control group. ANOVA test followed by Student-Newman-Keul's (SNK) test for multiple group comparisons was also performed.

RESULTS

The mean plasma calcium levels of *C. versicolor* at zero hour, was 10.37 ± 0.14 mg per 100 ml. The plasma calcium level of *C. versicolor* remains unaffected up to day 3 following prolactin treatment. After day 5, hypercalcemia has been recorded which progressively increases till day 10 (Fig. 1). After day 15, the levels decrease slightly although it is still hypercalcemic (Fig. 1). The plasma calcium levels differed significantly ($F = 18.22$; $p < 0.0001$) among the time intervals.

The initial value (zero hour) for plasma phosphate was 4.48 ± 0.05 mg per 100 ml. The plasma phosphate level of *C. versicolor* remains unchanged up to day 3 following prolactin treatment. Prolactin treatment evokes hyperphosphatemia on day 5 which progressively increases up to day 10 (Fig. 2). The levels become almost normophosphatemic after 15 days prolactin treatment (Fig. 2). The plasma phosphate levels differed significantly ($F = 15.28$; $p < 0.0001$) among the time intervals.

The ultimobranchial gland of control *C. versicolor* has been described in detail by Srivastava *et al.* (2008). Histologically the ultimobranchial gland of vehicle-injected lizards contains follicles (one large follicle or few small follicles) and cell cords or cell clumps. The cells, which form compact cell clumps, are all alike (Fig. 3). Their nuclei are ovoid having dense chromatin material. The follicles are lined by epithelium possessing simple cuboidal cells or pseudostratified columnar cells. The lumen of the follicle may contain a variable amount of colloid-like material with desquamated cells and cell debris.

No histological alteration is noticed in the ultimobranchial gland of prolactin-treated lizards up to day 3. The gland depicts signs of hyperactivity on day 5

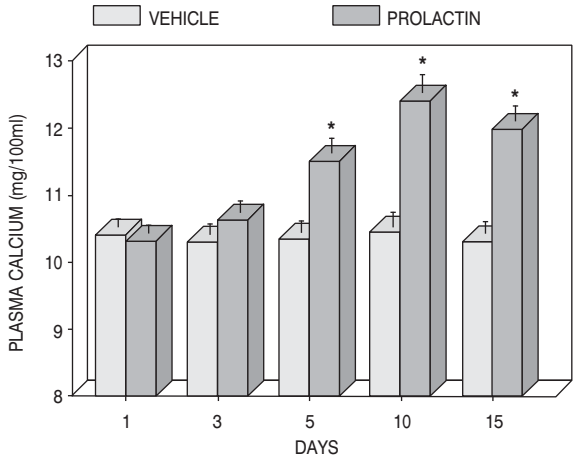

FIG. 1. Plasma calcium levels of vehicle and prolactin treated lizards. Values are mean ± s.e. of ten specimens. Asterisks indicate significant differences ($p < 0.05$) as compared with vehicle-injected lizards.

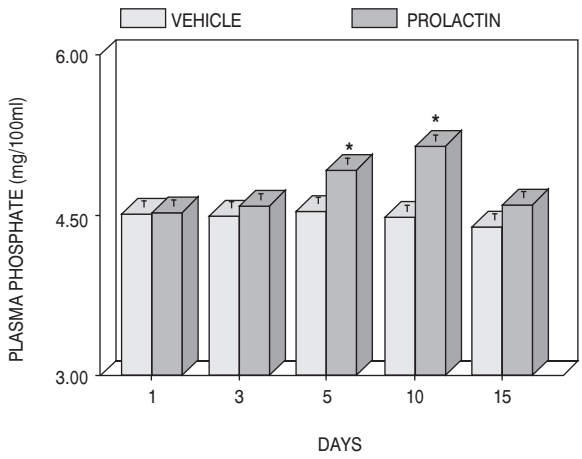

FIG. 2. Plasma phosphate levels of vehicle and prolactin treated *Calotes versicolor*. Values are mean ± s.e. of ten specimens. Asterisks indicate significant differences ($p < 0.05$) as compared with vehicle-injected lizards.

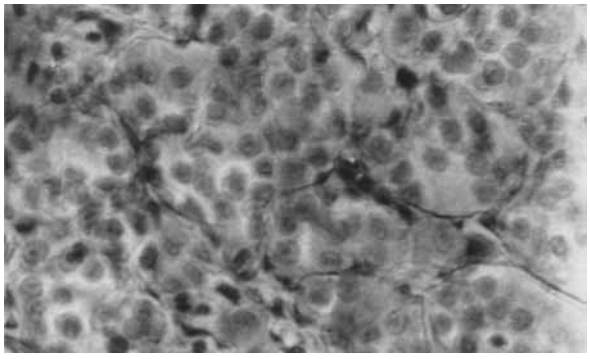

FIG. 3. Ultimobranchial gland of vehicle-injected *Calotes versicolor.* HE ×200.

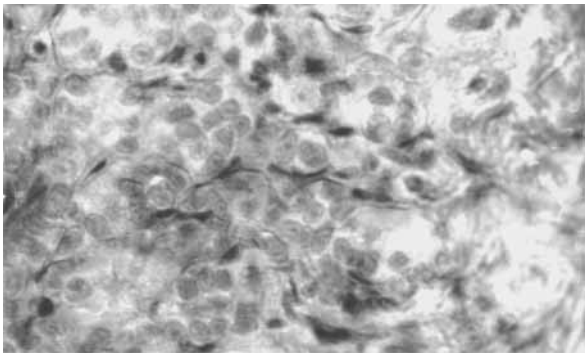

FIG. 4. Ultimobranchial gland of 5 days prolactin treated lizard showing decreased staining response of the cytoplasm. HE ×200.

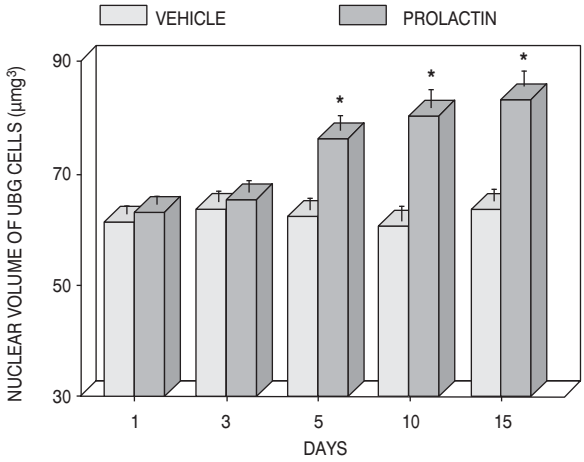

FIG. 5. Nuclear volume of ultimobranchial cells of vehicle and prolactin treated lizards. Asterisks indicate significant differences ($p < 0.05$) as compared with vehicle-injected specimens.

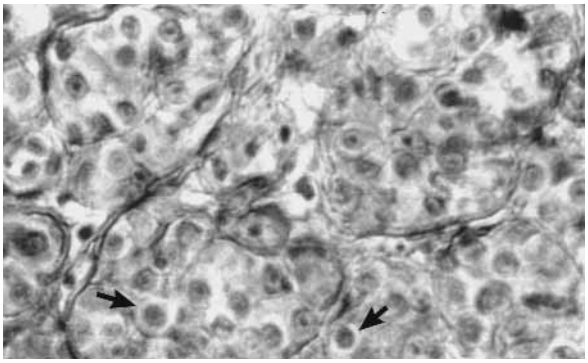

FIG. 6. Ultimobranchial gland of 15 days prolactin treated *Calotes versicolor* showing exhausted cells (arrows). HE ×200.

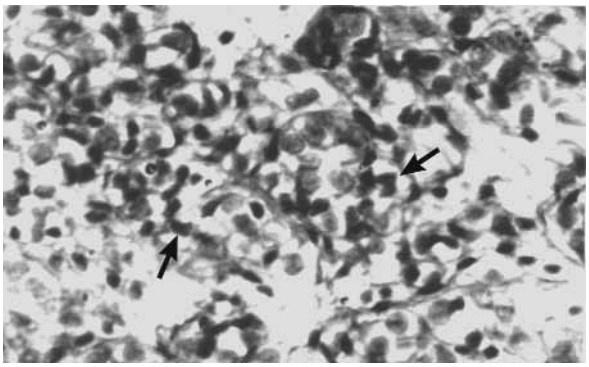

FIG. 7. Degenerating cells (arrows) in the ultimobranchial gland of 15 days prolactin treated *Calotes versicolor*. HE ×200.

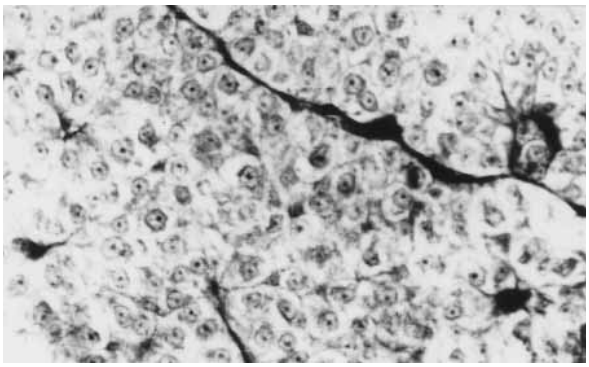

FIG. 8. Parathyroid gland of vehicle-injected *Calotes versicolor*. HE ×200.

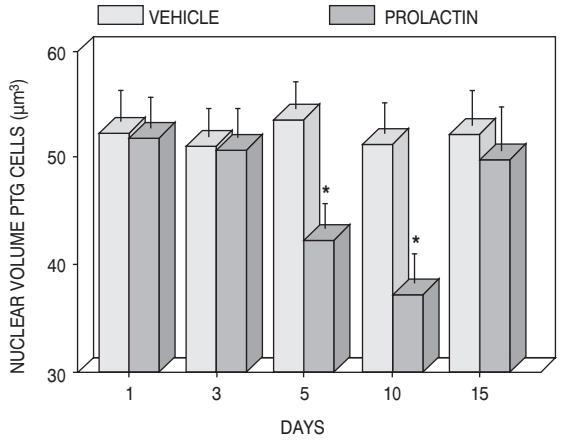

FIG. 9. Nuclear volume of parathyroidal cells of vehicle and prolactin treated lizards. Asterisks indicate significant differences ($p < 0.05$) as compared with vehicle-injected specimens.

which is evident by a decreased staining response of the cytoplasm (Fig. 4) and an increased nuclear volume of ultimobranchial cells (Fig. 5). The nuclear volume records a progressive increase from day 10 onwards (Fig. 5). A few completely exhausted cells (Fig. 6) and degenerating cells (Fig. 7) have also been observed on day 15. Nuclear volume of ultimobranchial cells differed significantly ($F = 7.09$; $p < 0.0001$) among the time intervals.

In vehicle-injected *C. versicolor* one pair of parathyroid glands occur which are located near the bifurcation of the carotid arch in the cervical region. These glands are enclosed by connective tissue. The cells of the parathyroid glands are arranged in compact cords separated by connective tissue strands containing blood vessels. The gland contains a single cell

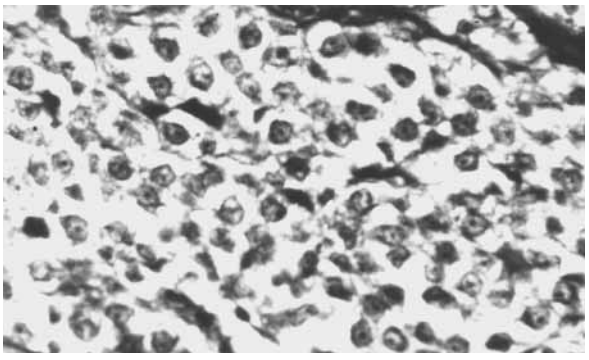

FIG. 10. Parathyroid gland of 10 days prolactin treated *Calotes versicolor* showing decreased staining response of the nuclei. HE ×200.

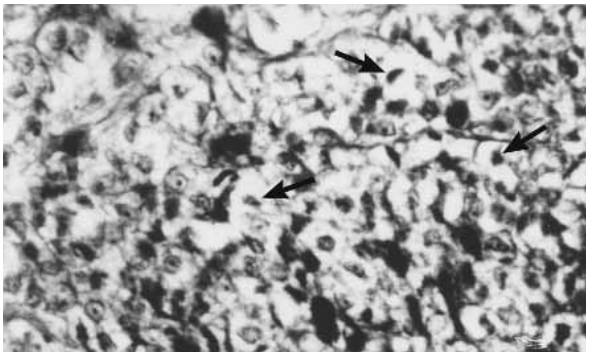

FIG. 11. Degenerating cells (arrows) in the parathyroid gland of 15 days prolactin treated *Calotes versicolor*. HE ×200.

type, which are oval, rounded or irregular in shape. These cells possess indistinct cell boundary (Fig. 8). The parenchymal cells contain scanty cytoplasm and a large centrally located ovoid nucleus with one or more nucleoli (Fig. 8).

Up to day 3 following prolactin treatment there is no histological change in the parathyroid gland of *C. versicolor.* After day 5, the nuclear volume records a decrease which progresses till day 10 (Fig. 9). Also, on day 10 the staining response of nuclei of parathyroidal cells decreases (Fig. 10). After day 15, the nuclear volume is almost similar to the nuclear volume of vehicle-injected *C. versicolor* (Fig. 9). Also, few degenerating cells are noticed on day 15 (Fig. 11). Nuclear volume of parathyroidal cells differed significantly among the time intervals ($F = 2.69$; $p < 0.0082$).

DISCUSSION

Prolactin is effective in inducing hypercalcemia in *C. versicolor*. This is in conformity with the reports of Swarup *et al.* (1985 – *Varanus flavescens*) and Srivastav & Rani (1990 – *Natrix piscator*) who have also noticed increased serum calcium levels after prolactin administration. Similar effects of prolactin have also been reported for rats (Robinson *et al.*, 1975), Japanese quail (Baksi *et al.*, 1978), bullfrogs (Baksi *et al.*, 1978), anurans (Srivastav & Rani, 1991) and teleosts (Pang, 1981; Wendelaar Bonga & Flik, 1982, 1984; Srivastav & Swarup, 1985; Jackson *et al.*, 2005). The hypercalcemia observed in *C. versicolor* could not be attributed to the enhanced calcium absorption in intestine as the lizards were not fed in the present study during experimental period. Thus the hypercalcemic effect of prolactin may be ascribed to the enhanced reabsorption of calcium in the kidney and/or enhanced resorption of bone.

In *C. versicolor* prolactin evoked hyperphosphatemia. This is in agreement with the report of Srivastav & Rani (1990) who were the first to report the hyperphosphatemic effect of prolactin in a snake, *Natrix piscator*. This study is probably the first to report such a response of prolactin from lizards. The elevation of phosphate level could be attributed to the increased bone resorption and/or mobilization of phosphate from soft tissues.

In the present study, *C. versicolor* when subjected to prolactin treatment exhibits hyperactivity of UBG which is evident by the increased nuclear volume and decreased staining response of the cytoplasm of ultimobranchial cells. The hyperactivity of ultimobranchial cells could be attributed to increased release of hypocalcemic factor (CT) to combat the elevated plasma calcium concentration. This explains the slight decrease which has been observed in the plasma calcium level on day 15 following prolactin treatment. The occurrence of degenerating cells may be due to exhaustion of the cells after continuous hyperactivity. There exists a single report regarding the effect of prolactin on ultimobranchial gland of reptile (snake — Srivastav *et al.*, 1994). Moreover, few reports are also available from fish (Srivastav & Swarup, 1985) and amphibia (Boschwitz, 1969; Srivastav & Rani, 1991).

Sherwood (1968) reported that the important factor regulating the secretary activity of parathyroid gland is the blood calcium concentration. As such, hypercalcemia suppresses the release of PTH and renders the parathyroid gland inactive. This supports the observations recorded in the present study regarding the inactivity of parathyroid gland in *C. versicolor* which is evident by the decreased nuclear volume and degeneration among the parathyroidal cells after prolonged hypercalcemic challenge by prolactin. Degeneration of parathyroid gland has also been reported from other vertebrates after experimental hypercalcemia induced by vitamin D or prolactin administration (Swarup & Srivastav, 1979; Koyama *et al.*, 1984; Srivastav & Rani, 1988, 1992; Srivastav *et al.*, 2000, 2008).

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REFERENCES

Baksi SN, Kenny AD, Galli-Gallardo SM, Pang PKT, 1978. Vitamin D metabolism in bullfrogs and Japanese quail: Effects of estradiol and prolactin. *General and comparative endocrinology*, 35: 258-262.

Booher CM, 2008. Effects of calcium availability on reproductive output of big brown bats. *Journal of zoology*, 274: 38-43.

Boschwitz D, 1969. Influence of prolactin on the ultimobranchial bodies of *Bufo viridis*. *Israel journal of zoology*, 18: 277-289.

Charoenphandhu N, Krishnamra N, 2007. Prolactin is an important regulator of intestinal calcium transport. *Canadian journal of physiology and pharmacology*, 85: 569-581.

Clark NB, 1983. Evolution of calcium regulation in lower

vertebrates. *American zoologist*, 23: 719-727.

Jackson LF, McCormick SD, Madsen SS, Swanson P, Sullivan CV, 2005. Osmoregulatory effects of hypophysectomy and homologous prolactin replacement in hybrid striped bass. *Comparative biochemistry and physiology B: biochemistry and molecular biology*, 140: 211-218.

Koyama T, Makita T, Enomoto M, 1984. Parathyroid morphology in rats after administration of active vitamin D3. *Acta pathologica japonica*, 34: 313-324.

de Matos R, 2008. Calcium metabolism in birds. *Veterinary clinics of North America: exotic animal practice*, 11: 59-82.

Pang PKT, 1981. Pituitary and prolactin influences on calcium regulation in the mud puppy, *Necturus maculosus. General and comparative endocrinology*, 44: 524-529.

Robinson CJ, Mahajan KK, Horrobin DF, 1975. Some effects of prolactin on calcium metabolism. *Journal of endocrinology*, 65: 27.

Sherwood LM, 1968. Relative importance of parathyroid hormone and thyrocalcitonin in calcium homeostasis. *The New England journal of medicine*, 278: 663-670.

Srivastav Ajai K, Rani L, 1988. Mammalian calcitonin cells: retrospect and prospect. *Biological structures and morphogenesis*, 1: 117-123.

Srivastav Ajai K, Rani L, 1990. Prolactin-induced hypercalcemia and hyperphosphatemia in the freshwater snake, *Natrix piscator. Canadian journal of zoology*, 68: 2702-2704.

Srivastav Ajai K, Rani L, 1991. Response of ultimobranchial body, parathyroid gland, serum calcium and serum phosphate in the frog, *Rana tigrina* after prolactin administration. *European archives of biology*, 102: 159-163.

Srivastav Ajai K, Rani L, 1992. Ultimobranchial body and parathyroid glands of the freshwater snake, *Natrix piscator* in response to vitamin D_3 administration. *Journal of experimental zoology*, 262: 255-262.

Srivastav Ajai K, Rani L, Sasayama Y, 1994. Influence of prolactin administration on the ultimobranchial body and parathyroid glands of the freshwater snake, *Natrix piscator*. *Okajimas folia anatomica japonica*, 71: 59-66.

Srivastav Ajai K, Das VK, Srivastav SK, Suzuki N, 2000. Amphibian calcium regulation: Physiological aspects. *Zoologica poloniae*, 45: 9-36.

Srivastav Ajai K, Yadav S, Srivastav SK, 2008. The ultimobranchial gland in poikilotherms: morphological and functional aspects. In: Haldar C, Singaravel M, Pandi-Perumal SR, Cardinali DP, eds. *Experimental endocrinology and reproductive biology*. Science Publishers, Enfield, NH, USA: 269-296.

Srivastav SP, Swarup K, 1985. Structure and behaviour of the corpuscles of Stannius, the ultimobranchial gland and the prolactin cells in response to prolactin-induced hypercalcemia in male catfish, *Clarias batrachus* (L.). *Monitore zoologico italiano*, 19: 111-120.

Srivastava B, Mishra D, Srivastav SK, Suzuki N, Srivastav Ajai K, 2008. $1,25(OH)_2D_3$ induced alterations in plasma calcium, inorganic phosphate, ultimobranchial gland and parathyroid gland of the garden lizard, *Calotes versicolor. Zoologica poloniae*, 53: 5-18.

Swarup K, Srivastav Ajai K, 1979. Studies of calcitonin cells and parathyroid gland of house shrew, *Suncus murinus* in response to experimental hypercalcemia. *Zeitschrift für mikroskopisch-anatomische forschung*, 93: 343-352.

Swarup K, Srivastav SP, Srivastav Ajai K, Srivastav SK, 1985. Calcemic responses of *Varanus flavescens* to prolactin administration. *Journal de physiologie*, 80: 42-43.

Wendelaar Bonga SE, Flik G, 1982. Prolactin and calcium metabolism in a teleost fish *Sarotherodon mossambicus.* In: Oguro C, Pang PKT, eds. *Comparative endocrinology of calcium regulation*. Japan Scientific Societies Press, Tokyo: 19-26.

Wendelaar Bonga SE, Flik G, 1984. Hypercalcemic control in the lower vertebrates. *Calcified tissue international*, 36: 12-13.