Immunohistochemical distribution of prolactin containing cells in the pituitary of the chickens

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ABSTRACT: The objective of the current study was to determine the immunohistochemical distribution and density of prolactin containing cells in the pituitary of the female and male chickens during postnatal developing period after hatching for five months. The modified labelled avidin-biotin method with monoclonal mouse antihuman prolactin as a primary antibody was used to detect prolactin positive cells. At the end of each month, prolactin containing cells were identified in the pars distalis of the pituitary glands of chickens. The number of the prolactin positive cells were found to be increased depending on the monthly development period, while the reaction density was found to be decreased in all groups. Additionally, males had less prolactin positive cells than that of females.

Keywords: pituitary; pars distalis; prolactin containing cells; chicken

The hypothalamopituitary axis plays a central role in the endocrine system. Most of the hormones from the anterior pituitary are regulated by negative-feedback inhibition mechanism: The pituitary hormones in the circulation interact with their target tissues, which are stimulated to secrete various hormones that inhibit the release of the pituitary hormones (Brook and Marshall, 2001).

Prolactin (PRL) stimulates lactation in the postpartum period of mammalians. In birds, it stimulates crop-milk production, which forms the basis of an *in vivo* bioassay for PRL, and also nest-building activity (Dellmann, 1998). In reptiles, amphibian and teleosts, PRL acts as an osmoregulator (Dellmann, 1998; Brook and Marshall, 2001). Prolactin secretion increases during sleep, exercise, orgasm, stimulation of nipple, brood and pregnancy. It is effective in the formation of spermatozoon and it stimulates the growth of testes as well. Increased prolactin levels during pregnancy period of mammalians and incubation period of the birds pressure FSH and LH secretions (Yilmaz, 1999). Lactotropes (prolactin cells) give positive immunohistochemical reaction for prolactin. Their staining affinity increases when the cells are hypertrophied, as in pregnancy and lactation (Dellmann, 1998). Prolactin serum level increases as a consequence of the experimental photostimulations on Peking ducks, pigeons and quails (Farner et al., 1973). Proudman et al. (1999) has demonstrated that prolactin containing cells are active and rare in birds.

The purpose of the current study was to determine the immunohistochemical distribution and density of prolactin containing cells in the pituitary of the female and male chicks during postnatal developing period after hatching for five months.

MATERIAL AND METHODS

Animals

Total of fifty (n = 25 males and n = 25 females) one-day-old chicks (Isobrown) were used in the present study. At the end of each month for

Supported by funds of University of Uludag (Project No. 2000-12).

5 months (between February and June), ten chickens (n = 5 males, n = 5 females) were chosen randomly to sacrifice by cervical dislocation for further immunohistochemical analysis.

Immunohistochemical analysis

The pituitary gland was removed and immediately fixed in buffered neutral formalin. Then, they were dehydrated through a graded alcohol series, cleared in xylene and embedded in paraffin. Prolactin containing cells were identified in the pars distalis by the modified the labelled avidin-biotin (LAB) technique (Hsu et al., 1981; Erdost, 2004) using monoclonal mouse antihuman prolactin (Novocastra NCL-PRO) as a primary antibody and using Histostain-Plus Bulk Kits (Zymed 2nd Generation LAB-SA Detection System). The sections were deparaffined in xylene (2 times, 5 min each) and dehydrated in graded ethanol series (3 times each, 96% to 70%), tap water and then phosphate-buffered saline (PBS, pH 7.4) (3 times, 2 min each). For antigen retrieval, they were put into buffered citrate (pH = 6) for primary antibody of prolactin in a microwave oven (Siemens model HF11921) at 700 watt for 5 min for 3 times. Then, they were treated with 3% hydrogen peroxide for 8–10 min to block endogenous peroxidase activity. After washing in PBS for three times, 10% goat non-immun serum (Reagent A, Histostain-Plus Bulk Kits, Zymed 2nd Generation LAB-SA Detection System) was applied for 10 min to prevent non specific binding of the antibodies. After diluting with antibody diluent (1/50, Novocastra NCL-PRO), monoclonal mouse antihuman prolactin was applied for 1 h in a humid chamber at 37°C. Then the slides were washed in PBS for three times, biotinylated secondary antibody (Reagent B, Histostain-Plus Bulk Kits, Zymed 2nd Generation LAB-SA Detection System) were applied for 10 min. After washing with PBS for three times, they were incubated with the streptavidin-biotin-peroxidase conjugate as outlined by the manufacturer (Reagent C, Histostain-Plus Bulk Kits, Zymed 2nd Generation LAB-SA Detection System) for 10 min at room temperature. After washing with PBS (3 times, 2 min each), the peroxidase staining was performed with 3,3-diaminobenzidine/hydrogen peroxide, until desired color density was developed at room temperature. The slides were counterstained with Hematoxylin. Then slides were rinsed in tap water (10 min) and mounted.

For each group, negative and positive tissue control slides were processed at the same time. The negative control was carried out by incubating sections with antibody diluent instead of the primary antibody. The positive control was also conducted with tissue section from sheep pituitary known to contain the hormones of studied in the present study. Images were captured using the light microscope and video camera (Ikagemidae) coupled with a computer.

Statistical analysis

Prolactin positive cells were classified depending on the density of the staining as weakly stained (+), moderate (++) and strongly stained (+++). They were counted in 10 fields per each 1 μm^2 and calculated with Image Tool analysis. Probability values were calculated by using The Mann Whitney U analysis method (Sumbuloglu and Sumbuloglu, 1994) in minitab computer program.

RESULTS

Prolactin immunoreactive cells

Prolactin positive cells as single or double – triple groups were detected in both zones of the pars distalis. Cells being in polymorph shape, some of them had an ovoid shape while some others had the cytoplasmic processes (Figure 1). While euchromatic big nucleus was found to have an eccentric localisation in cytoplasm, it was also found to have a central localisation. It was determined that the structure and localisation of those cells were not subject to changes irrespective of their sex or age for 5 months development period.

Table 1. The number of prolactin positive cells (in 1 μm^2 area) for five months development period

Groups/Months	♀ ($\bar{x} \pm s_{\bar{x}}$)	♂ ($\bar{x} \pm s_{\bar{x}}$)
I	13.60 ± 1.1	12.70 ± 0.54
II	14.80 ± 0.33	13.70 ± 0.47
III	15.40 ± 0.58	13.70 ± 0.40
IV	15.20 ± 0.80	14.20 ± 0.66
V	16.40 ± 0.64	14.60 ± 0.45

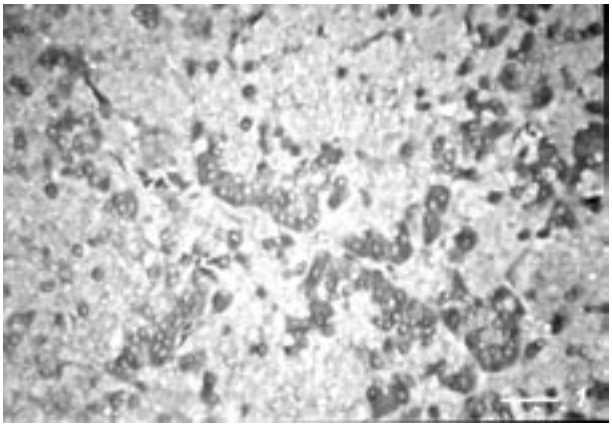

Figure 1. Prolactin positive cells in one-month-old male; bar 70 μm

The cell numbers and reaction density of prolactin immunoreactive cells (as unit per area) in the pituitary glands of male and female chicks are given in Tables 1 and 2. Females contain more prolactin positive cells than that of males for each month (Table 1). As the cell numbers increase with monthly development, males were observed to contain less prolactin positive cells than that of females. According to monthly evaluations carried out, structural and cellular distribution of PRL containing cells in male and female groups were found to be nearly identical for overall experimental period.

There was not any difference between females and males at the same ages in terms of the reaction density of PRL cells (Table 2). In the first month, PRL positive cells for both groups were stained almost strong reaction density (+++) (Figure 1). In the following months, it was seen that the number of cells with reaction density of the moderate stained (++) increased (Table 2, Figures 2, 3). The positive tissue control (sheep hypophyses) and the negative control (chicken hypophyses) were processed at the same time (Figures 4, 5).

Table 2. Reaction density of prolactin positive cells

Groups/Months	♀	♂
I	+++	+++
II	++	++
III	++	++
IV	++	++
V	++	++

Weakly stained (+), the moderate stained (++), the strongly stained (+++)

DISCUSSION

Mikami et al. (1988) stated that prolactin positive cells were localised in the central and in the dorso–caudal zones of pars distalis as small groups when primary antibodies prepared against sheep prolactin was used. They also indicated that the number of prolactin immunoreactive cells increased during pregnancy and lactation period, while they showed a sparse distribution in the central zone of pars distalis in bats during hibernation. While prolactin positive cells showed a scattered distribution in pars distalis of mice pituitary, (Nakane, 1970), they were found in the cephalic zone of pars distalis of tortoises pituitary (Mikami et al., 1985). The number of prolactin positive cells of mature female mouse was greater than those of males (Sasaki and

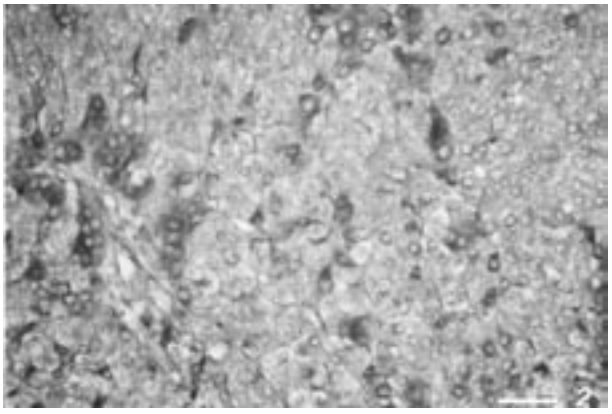

Figure 2. Prolactin positive cells in five-month-old male; bar 70 μm

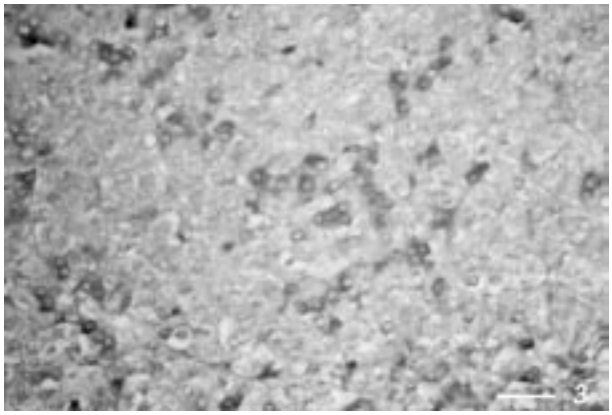

Figure 3. Prolactin positive cells in five-month-old female; bar 70 μm

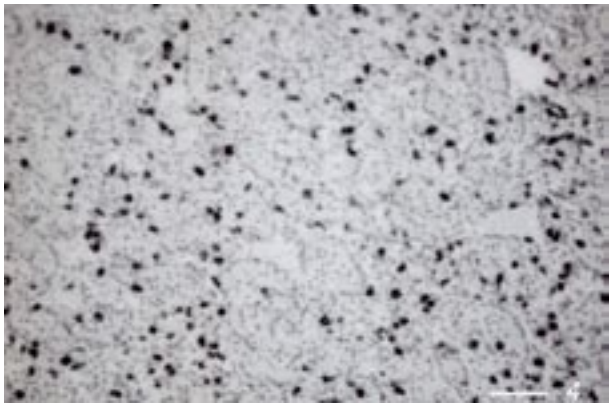

Figure 4. Positive tissue control (sheep pituitary); bar 140 μm

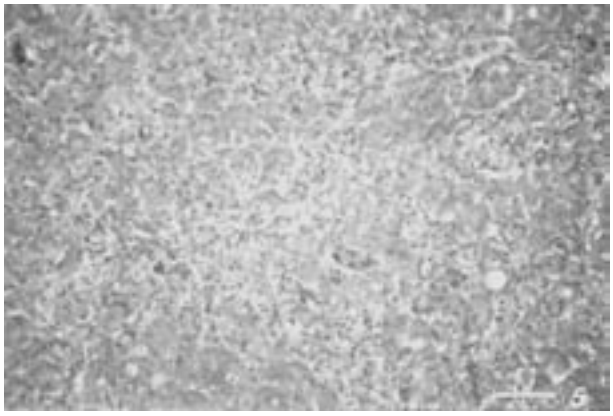

Figure 5. Negative control (chicken pituitary); bar 140 μm

Iwama, 1988). Lactotrops were found only in cephalic zone of hyphophysis in turkeys during incubation and egg laying period (Ramesh et al., 1996). Some studies showed that localisation of prolactin positive cells in the cephalic zone of pars distalis in the chicken (Mikami et al. 1975; Berghman et al., 1992; Ramesh et al., 1996; Proudman et al., 1999), while some other studies showed that they were localised in both zones (Barabanov, 1985; Kansaku et al., 1995). Additionally, prolactin containing cells were shown to be dense in cephalic zone of adenohypophysis, whereas they are rare in caudal zone of chicken embryos (Barabanov, 1985). Prolactin mRNA was found to be located in both cephalic and caudal zones of pars distalis of hypophysis in domestic cocks (Kansaku et al., 1995). In our study, prolactin positive cells were located in both zones of pars distalis as well. These findings indicate that prolactin containing cells in domestic birds have similar immunohistochemical localization with the mammalian prolactin cells (Barabanov, 1985).

In the present study, the reaction density of prolactin positive cells was seen to be decreased in all male and female groups while the number of those cells were found to be increased depending on the monthly development. When the number of prolactin positive cell were compared between male and female groups for 5 months development period, males were found to contain less prolactin positive cells than those of females. We are in the opinion that the increase in cell number is shaped depending on the effect of the genital development and increasing sunlight whereas the decrease in the reaction density is realised by the negative feed back effect, active FSH and LH hormones.

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Received: 05–03–24
Accepted: 05–05–12

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