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SERUM LEVELS OF PROLACTIN AND ALPHA-MELANOTROPHIN AND STRUCTURE OF THE OVARY DURING THE REPRODUCTIVE CYCLE OF *Columba livia* (Domestic pigeon).

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SUMMARY

Given the importance of the hormones prolactin and alpha-melanotrophin in the regulation of the mammalian reproductive cycle, it was decided to assess their serum levels during the reproductive cycle of *Columba livia* (domestic pigeon).

Female pigeons were utilized in the stages of egg-laying, incubation and rearing. Simultaneously, the structural changes of the ovary in the aforementioned periods were studied. The levels of prolactin and alpha-melanotrophin showed similar behaviour. The low concentrations found in the period of egg-laying ($x \pm s = 4.3 \pm 0.6$; 3.5 ± 0.26 ng/ml) increased significantly during incubation ($x \pm s = 6.5 \pm 0.7$ ng/ml ; 6.6 ± 1.7 ng/ml) to reach the maximun value in the rearing period ($x \pm s = 10.33 \pm 1.8$ ng/ml ; 13.6 ± 1.9 ng/ml). Accompanying these changes a marked predominance of the medullary zone over the cortex and lack of developing follicles were found in the rearing state.

Key words: prolactin - alpha-melanotrophin - reproductive cycle - ovarian structure - *Columba livia*

INTRODUCTION

Vertebrate reproductive cycles are known to be governed by a group of hormones whose functions have been relatively well defined. However, there are aspects as yet non elucidated, thus remaining under continuous study.

Prolactin (PRL) has been classically considered a lactation or mammotrophic hormone and it is now known to play a role in several other functions related to both male and female reproductive cycles, such as influence on the induction of puberty in female and male rats (Döhler and Von Zür Mücklen, 1977 (3), suckling (Selmanoff and Gregerson, 1980 [11]), the onset of incubation in different classes of birds (Cheng and Burke, 1983 [2]; Lea and Chadwick, 1981[9]; Etches et al., 1979[4]) and the onset of laying in the domestic turkey (Etches et al, 1979[4]). On the other hand, other authors have described a decrease of plasmatic levels of prolactin during nest-building and midincubation and an increase during the first day of hatching in breeding pigeons (Buntin and Forsyth, 1979[1]). However, the control of clutch size in pigeon is independent of prolactin levels (Haywood, 1993[6]).

Furthermore, alpha-melanotrophin (α-MSH), considered as the melanotrophic hormone, has been shown to influence the reproductive cycle of mammals. Its secretion is similar to prolactin during the estrous cycle in the rat (Vivas and Celis, 1977[15]). On the other hand, α-MSH plays a role in the induction and maintenance of pseudopregnancy in rats (Volosin and Celis, 1984[16]).

Most of the studies have been done in mammals, but in fact, little is known about their action in avian species (Etches et al., 1979[4]; Lea and Chadwick, 1981[9]; Halawani and Behnke, 1983[5]; Milano, 1984[10]).

Consequently, we considered of interest to choose an avian model that would allow us a characterization of the levels of these hormones during the different stages of the reproductive cycle, and to correlate them with the morphologic changes of the ovary. The domestic pigeon (*Columba livia*) was selected, because it shows perfectly defined and recognizable reproductive cycle periods: laying, incubation and breeding.

MATERIAL AND METHODS

In this work we used thirty adult female domestic pigeons (*Columba livia*) kept in a dovecoat in the Universidad Nacional de Río Cuarto with food and water available *ad libitum*. In order to standardize the samples the animals were chosen in the following manner: laying (15-20 h after the first egg of the two-egg clutch was laid), incubation (8-9 days after the first egg was laid) and breeding (4-5 days after hatching) were selected. The specimens were decapitated and the blood samples were collected. Then the ovaries were immediately removed and fixed in Bouin to process them by conventional histological techniques.

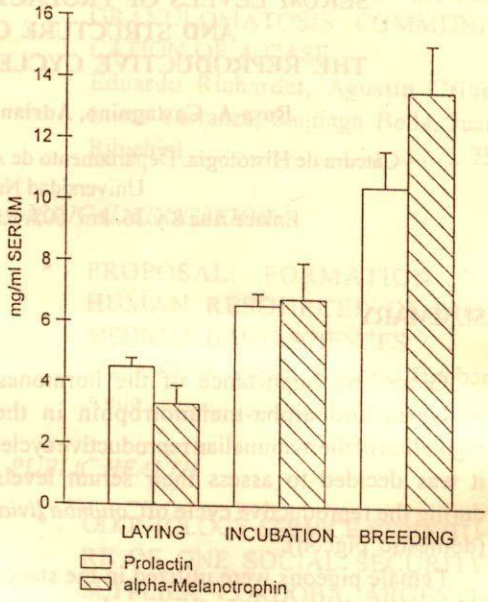
Blood samples were centrifuged to separate serum which was immediately frozen until processing. Prolactin was determined by heterologous radioimmunoassay (Diagnostic Products Corporation) with an intra-assay coefficient of 1.7% and a coefficient of variation of 0.8%. The α-MSH assay followed the procedure of Taleisnik and Orias, 1965 (14). This assay uses toads skin and α-MSH synthetic as standard. The estimated precision of the assay was 0.105. The design used was 2 x 2 (2 doses of reference preparation and 2 doses of the test preparation).

The results were subjected to an analysis of variance ($p < 0.05$) (Sokal and Rolhf, 1979[13]). Thereafter, the Tuckey's test was applied.

RESULTS

The results of serum prolactin and alpha-melanotrophin determinations throughout the different periods of the reproductive cycle are shown in Fig. 1. It can be seen that both hormones increased at the laying period, throughout incubation and reached the highest level at the breeding period (Fig. 1).

Figure 1: Serum levels of Prolactin and alpha-melanotrophin during the reproductive cycle of the domestic pigeon.



Vertical bars represent the mean ± ESM. (n=10). All means were compared with each other, according to the Tuckey's test, p<0.01

The morpho-histological characteristics of the ovaries in the different stages of the reproductive cycle showed remarkable changes with regard to the quantity, size and kind of ovarian follicles. A considerable difference in the proportion of cortical and medullary tissues was also noticed.

In the laying period (Fig. 2 a, b), an important development of the parenchymal or cortical zone could be seen, presenting ovarian follicles in different growth stages: a few previtellogenic follicles and abundant vitellogenic follicles. The previtellogenic follicles presented cylindrical follicular cells which started secreting in the follicular epithelium or granular layer, coinciding therefore with the appearence of lipid vacuoles in the oocyte cytoplasm. In this phase the thecas began to differentiate. In the vitellogenic follicles the important development of the oocyte with its high vitellus content could be observed. Around the oocyte, the follicular cells of the granulosa layer conformed a cylindrical pseudostratified epithelium, well differentiated from the thecas, the last follicular layer. The cortical stroma, of loose connective tissue, was scarce due to the large quantity of follicles. The vascular or medullary zone was reduced to a core of vascularized connective tissue.

During incubation, the cortical zone contained primordial follicles, a few previtellogenic ones, and regressing follicles. A predominance of the cortical stromal tissue could be also noticed. The primordial follicles were the smallest, having a single cubic follicular epithelium and the thecas were not differentiated (Fig. 2 c). Follicles in regression were characterized by an interruption of the oocyte contour through wich vitellus apparently scaped (Fig. 2 d).

During breeding, the parenchymal or cortical zone was less developed and there were mainly primordial follicles (Fig. 2 e). The most evident feature in this stage was the predominance of the vascular or medullary zone, with a very vascularized connective tissue and thick strands of smooth muscular tissue (Fig. 2 f).

DISCUSSION

The changes in PRL seen in this study are comparable to those obtained by other authors in other avian species. For instance, studies performed in species whose broods utilize free food, such as the domestic chicken (Lea and Chadwick, 1981[9]), show that the serum levels of PRL begin to increase slowly from the moment of mating until it reaches its highest peak at hatching, decreasing afterwards. In the pigeon, on the other hand, the increase continues and reaches its maximal value in the breeding period (Buntin and Forsyth, 1979[1]).

Concerning alpha-melanotrophin no data are known about this hormone in birds. In studies carried out in female rats (Vivas and Celis, 1977[15]) it has been shown that the hypophyseal α-MSH level reaches its highest level peak in diestrus. Khorram and Mc Cann (1984 a, b [7, 8]) considered that α-MSH is a neuromodulator of PRL secretion. Volosin and Celis (1984[16]) working with pseudopregnant rats, showed that there were coincident PRL and α-MSH peaks.

Taking into account the values of PRL and α-MSH and the ovarian histology above described, in the different periods of the pigeon reproductive cycle, the following scheme may be presented: in the laying period, when the lowest serum levels of PRL and α-MSH are found, the ovary shows a predominant cortical or parenchymal zone with previtellogenic and vitellogenic follicles, where the large amount of vitellus stands out, while the medullary or vascular zone is scarce. In the period of incubation the ovary shows a regression of the vitellogenic follicles, accompanied by a higher serum concentration of PRL and α-MSH. These hormones reach their highest level in serum during the breeding stage, coinciding with the predominance of primordial follicles in the ovary and an important devolopment of the medullary zone (Shani et al. 1973[12]).

According with these results, it can be concluded that: a) prolactin and alpha-melanotrophin serum levels show a parallel behaviour in the different stages studied: laying, incubation and breeding, similar to that observed in pseudopregnancy in rats. b) The highest concentration of both hormones is attained in the breeding period.

RESUMEN

Dada la importancia de prolactina y alfa-melanotrofina en la regulación del ciclo reproductivo de mamíferos, se decidió investigar sus perfiles de secreción en el ciclo reproductivo de *Columbia livia* (paloma doméstica), y su comparación con las variaciones morfológicas del ovario.

Se utilizaron palomas hembras en diferentes estadíos de su ciclo reproductivo: postura - incubación - cría. Las muestras ováricas se procesaron por las técnicas histológicas convencionales, mientras que los dosajes hormonales se realizaron mediante ensayo biológico para alfa-melanotrofina y radioinmunoensayo para prolactina.

Se obtuvieron diferencias significativas en los distintos períodos tanto en lo que respecta a la estructura ovárica como a los valores hormonales de prolactina y alfa-melanotrofina. Ambas muestran un comportamiento similar en los respectivos estadíos del ciclo reproductor. Las concentraciones más bajas se encontraron en el período de Postura (x ±s = 4,3 ± 0,6; 3,5 ± 0,26 ng/ml), aumentando significativamente en el de Incubación (x ± s = 6,5 ± 0,7 ng/ml; 6,6 ± 1,7 ng/ml) y alcanzando los máximos valores en el de Cría (x ± s = 10,33 ± 1,8 ng/ml; 13,6 ± 1,9 ng/ml). Concomitantemente, se observaron variaciones morfológicas en ovario con marcado desarrollo del estroma medular.

Palabras clave: prolactina - alfa-melanotrofina - ciclo reproductor - estructura ovárica - *Columba livia*.

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Figure 2. Microphotographs of ovarian transversal sections at the different stages of the reproductive cycle

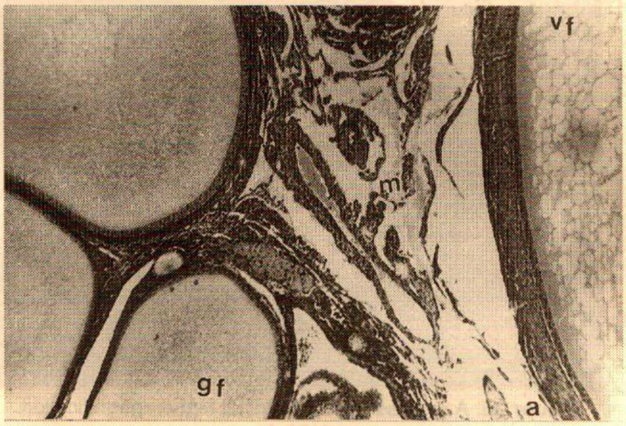

Figure 2a. Ovary during the laying period: cortical development with growing (gf) and vitellogenic (vf) follicles and reduced medullary (m) axis prevail. (160x).

Figure 2b. A vitellogenic follicle (vf) at higher magnification. The oocyte cytoplasm with its rich vitelline content stands out. (160x).

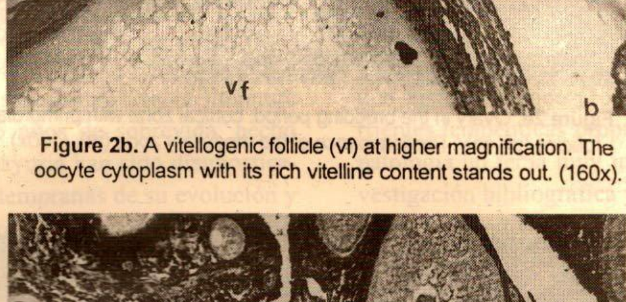

Figure 2c. Ovary in the incubation period: cortex with primordial (pf) and previtellogenic (pvf) follicles. Differentiated medullary tissues (m). (160x).

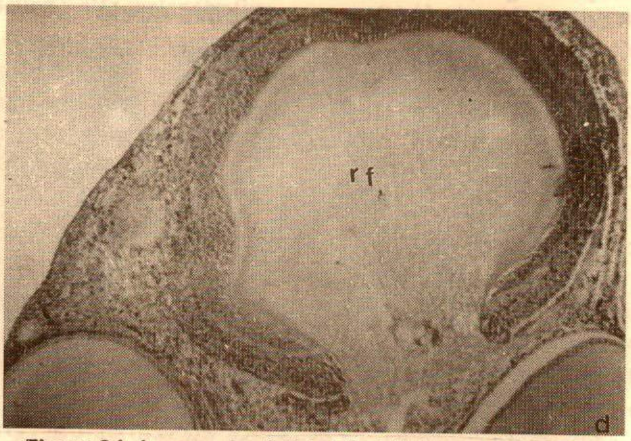

Figure 2d. A regressing follicle (rf) at higher magnification. (400x)

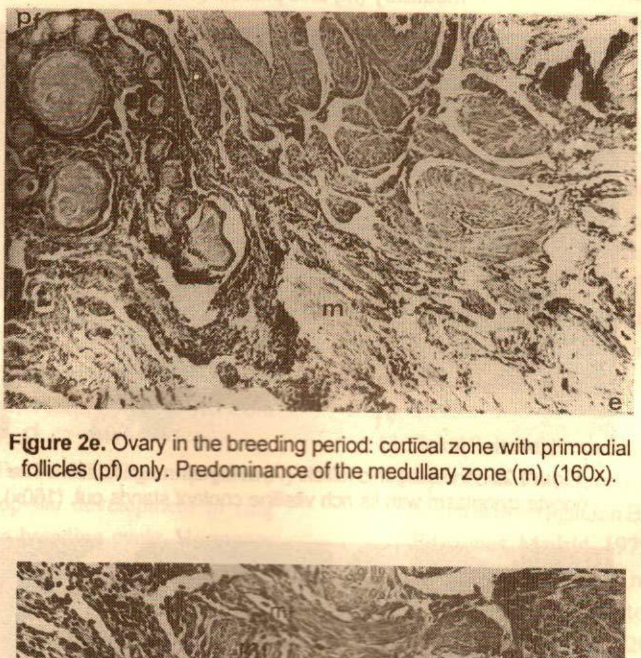

Figure 2e. Ovary in the breeding period: cortical zone with primordial follicles (pf) only. Predominance of the medullary zone (m). (160x).

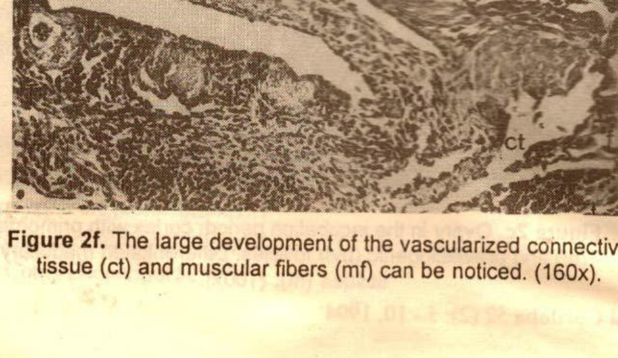

Figure 2f. The large development of the vascularized connective tissue (ct) and muscular fibers (mf) can be noticed. (160x).