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Immunohistochemical localization of progesterone receptor isoforms and estrogen receptor alpha in the chicken oviduct magnum during development

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ABSTRACT

In this work, the immunohistochemical expression of progesterone receptor (PR) isoforms and estrogen receptor alpha (ER-α), as well as the histomorphometric changes of the magnum region of the left oviduct from 8-day-old chicken embryos to one-month-old chickens were evaluated. Results indicate evident histological changes in the oviduct magnum during development mainly in the magnum's mucosa. Immunohistochemical analysis showed that the oviduct magnum from 8-day-old chicken embryos to one-day-old chickens did not present any PR isoform, but the oviduct magnum of one-week and one-month-old chickens expressed PR in the nuclei of all cell types. In epithelial cells, PR-B was the only isoform expressed; in muscle and serosa cells, PR-A isoform was the only isoform expressed; and stromal cells expressed both isoforms. The results also demonstrate positive ER-α immunostaining in the nuclei of different cells from embryonic life to later developmental stages of the oviduct magnum. Data indicate that the variations of ER-α or PR expression or dominance of either PR expression is differentially regulated depending on the cell type, the development of the oviduct, and in an age-specific manner. These variations in sex steroids hormone receptors are related with histological changes of the oviduct magnum through development.

1. Introduction

In the fowl, as in most avian species, generally, only the left ovary and oviduct develop in females. The left Müllerian duct continues to grow and develops into the functional oviduct of the mature female chicken, whereas the right duct undergoes a relatively slow involution after day 12 of incubation (Teng, 1987) disappearing completely by the time of hatching (Romanoff, 1960; Jacob and Baskst, 2007).

It is known that growth, differentiation, and function of the normal oviduct are under the control of steroid sex hormones, mainly estrogen and progesterone (Kohler et al., 1969; Oka and Schimke, 1969; Palmiter and Wrenn, 1971; Kiell et al., 1982). Most of these processes are mediated by specific intracellular receptors: progesterone, and estrogen receptors (PR and ER, respectively), which belong to a superfamily of ligand-induced transactivators that regulate a number of physiological and morphological processes by interacting with DNA-specific sequences, called hormone responsive elements (Savouret et al., 1991; Tsai and O'Malley, 1994).

Chicken PRs are expressed as two isoforms: PR-A (79 kDa) and PR-B (110 kDa). PR-A is an N-terminally truncated form of PR-B (Conneely et al., 1987a,b; Gronemeyer et al., 1987). It has been shown that the PR isoforms regulate different genes and exert distinct functions (Tora et al., 1988). Extensive studies exist regarding the effect of estrogens or progesterone treatment on concentrations of PR-A and PR-B in chicken immature oviduct (Syvala et al., 1996, 1997; Joensuu, 1990). Most studies on PR distribution in the chicken oviduct are restricted to sexual maturation (Ylikomi and Tuohimaa, 1988; Isola, 1990) and newly-hatched chickens (González-Morán and Camacho-Arroyo, 1998, 2003), as well as to the chicken embryo during the early development of the urogenital tract (Gasc, 1991).

Two forms of ER-α protein have been reported in chicken tissues: ER-α form I (66 kDa), and ER-α form II (61 kDa). The two chicken ER-α forms differ in their ability to modulate transcription

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activity of estrogen target genes in a promoter and cell type-specific manner (Griffin et al., 1999).

Most studies on ER-α distribution in the chicken oviduct have focused on the chicken embryo during early development of the left Müllerian duct, which is reported to contain estrogen receptors from day 8 of incubation onwards (Teng and Teng, 1975a,b, 1976, 1978; Gasc and Stumph, 1981) and during sexual maturation (Joensuu and Tuohimaa, 1989). However, there is no information of PR isoforms and ER-α distribution in different cell types in the chicken oviduct along development during normal growth in chickens. Therefore, the objective of this research was to examine, through immunohistochemistry, the presence of PR isoforms and ER-α in different cell subpopulations and correlate the localization of these receptors with the sequence of histomorphometric changes of the magnum region of the left oviduct in female chicken from the early development of the Müllerian duct (8-day-old embryos) until the immature oviduct developmental stage (one-month-old chickens).

2. Materials and methods

2.1. Animals and morphometric analyses

Fertile white leghorn eggs (Babcock-B300) were incubated at 38 °C in a forced-draft incubator. Some newly hatched chickens were housed in brooders at 30 °C under a 14:10 light–dark cycle (lights on 06:00–20:00) with food and water *ad libitum* until the age of one month. The experiments were performed under the guidelines of the Mexican Law of Animal Protection.

Embryos (8- and 13-day-old) and one-day, one-week and one-month-old chickens, 10 per age group, were killed by decapitation, the left oviduct was excised immediately and cleaned of adhesive tissue, recording the wet weight and length. Pieces of cephalic region of the oviduct magnum were fixed in 4% paraformaldehyde for 4 h, dehydrated, and embedded in paraplast. Transverse sections of 5 μm in thickness were stained with hematoxylin-eosin for histological and morphometric evaluations under light microscopy.

For the morphometric study three sections by animal ($n = 10$ animals per age group) were analyzed. Three oviduct magnum areas were analyzed in all age groups: luminal, epithelial, and stromal area, total area corresponded to the addition of these 3 areas. The number of cells in the magnum's epithelial area was determined in three representative fields scattered in the center and extremities of each section per animal.

All areas were digitalized by using the Image-Pro plus 6 image analysis program. Epithelium thickness was measured every 75 μm at high magnifications using an ocular micrometer, across the whole epithelial area in each section.

2.2. Immunohistochemistry

The immunehistochemical detection of PR isoforms and ER-α was carried out by using the Universal Dako Lsab+peroxidase kit (Dako Corporation, CA, USA). The monoclonal antibodies used were PgR Ab-8 (clone hPRa2+hPRa3) or PgR Ab-6 (clone hPRa6) (NeoMarkers, CA, USA), the former recognized both PR isoforms whereas the latter only recognizes the PR-B isoform. Also used monoclonal antibody against the ligand-binding domain of the alpha-ER (ER Ab-10) (clone TE111.5D11) (NeoMarkers, CA, USA), which recognized both ERα forms. Antibodies PgR Ab-6 and ER Ab-10 were company validated on chicken and other animal. The reactivity of PgR Ab-8 with chicken has been tested previously in our laboratory. We perform a comparative immunohistochemical analysis with several independent antibodies: Let 81 (Groyer-Picard et al., 1990), alpha PR-22 (Syvala et al., 1996) (these antibodies cross reactivity with PR in chicken), and PgR Ab-8. Immunohistochemical analysis showed that PgR Ab-8 cross-reacted with PR in chicken.

Oviduct magnum sections of 5-μm thickness were mounted on slides coated with poly-L-lysine (Sigma, St. Louis, MO, USA). Three sections by animal ($n = 10$ animals per age group) were analyzed. They were deparaffinized, rehydrated through graded concentrations of alcohol to distilled water. The slides were thereafter incubated in a microwave oven with 10 mM citric acid, pH 6.0, for two cycles at 750 W for 10 min each. Ten minutes were left between cycles. Then, slides were washed twice with 0.05 M sodium phosphate buffer (PBS) at pH 7.4. The slides were incubated in 3% hydrogen peroxide for 10 min; 0.5% Triton X-100 in PBS for 20 min; 1% normal swine serum in PBS for 20 min, and monoclonal antibody PgR Ab-8 or Ab-6 or alpha-ER (ER Ab-10) (4 μg/ml) in PBS containing 0.3% Triton X-100 and 0.1% gelatin during 72 h for PR, and 24 h for ER at 4 °C in a humid chamber. Sections were incubated with biotinylated secondary antibody for 30 min at room temperature, and then with streptavidin-peroxidase conjugated for 30 min. Sections were washed twice with PBS between incubations. Peroxidase activity was revealed with 3,3′-diaminobenzidine chromogen solution in the presence of hydrogen peroxide. After washing, sections were dehydrated and mounted with Canada Balsam without counterstaining. Immunohistochemical negative controls consisted of adjacent sections incubated with preimmune serum instead of the primary antibody.

One of the most important factors involved in the biological responses to progesterone mediated by its receptors is the existence of PR isoforms which regulate the expression of different genes and therefore different functions in cells (Mulac-Jericevic et al., 2000). The differences in the transcriptional activity of PR isoforms are related to their structure. In the N-terminal region PR-B possesses a unique Activation Function that is absent in PR-A. The Inhibitory Function located next to Activation Function 1 is only active in PR-A, and PR-B exhibits more phosphorylation sites than PR-A (Knotts et al., 2001). Another element participating in the contrasting transcriptional activity of both PR isoforms is their differential bound cofactors, since PR-A presents a higher affinity to corepressors such SMRT, whereas PR-B exhibits a higher affinity to coactivators such as SRC-1 (Giangrande et al., 2000).

Total number of PR isoforms and ER-α immunostained cells was determined in three representative fields scattered in the center and extremities of each section, in epithelial, stromal, muscle and serosa cells of the cephalic region of the oviduct magnum per each section (3 sections by animal) in 10 oviducts per age group, using a 100× objetive. Immunopositive cells were considered those with brown staining of the nucleus. All areas were digitalized by using the Image-Pro plus 6 image analysis program.

Due to the fact that there are no antibodies that recognize PR-A, immunohistochemical analysis of PR-A isoform was performed by subtractive inference between the number of immunoreactive cells incubated with PgR Ab-8, which recognizes both isoforms (PRAB), minus the number of immunoreactive ones incubated with PgR Ab-6 that only recognizes PR-B.

2.3. Statistical analyses

Results were analyzed by one-way ANOVA. Differences among groups were determined by Tukey's multiple comparison test. Differences among means were considered significant at $P < 0.05$.

3. Results

3.1. Oviduct dimensions

Data indicate that the left oviduct of chickens showed a marked increase in wet weight and length with age, increasing from