REVISTA DE ZOOLOGIA (5) 13-22,1993.

HISTOMORPHOLOGICAL CHANGES IN THE CHICKEN OVIDUCT AFTER 17β-ESTRADIOL TREATMENT DURING EMBRYONIC DEVELOPMENT.

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RESUMEN

La administración de bajas dosis de 17β-estradiol (200 ng/huevo) a embriones de pollo, los días 15 y 17 de incubación, induce un aumento en la densidad celular de la mucosa del magnum y la formación de un epitelio pseudoestratificado con invaginaciones de las células epiteliales hacia el estroma iniciando la formación de glándulas tubulares, así como un incremento en el área de la pared y la luz del magnum en los pollos recién nacidos. Estos resultados demuestran que la administración de estradiol induce cambios en el oviducto del embrión de pollo provocando citodiferenciación y modificaciones en la mucosa del magnum durante el desarrollo, por lo que el oviducto puede ser considerado como un sistema útil para evaluar los efectos de las hormonas esteroides.

Palabras clave: Oviducto. Magnum. Embrión de pollo. Estrógenos.

ABSTRACT

A low dose of 17β-estradiol (200 ng/egg), administered at 15 and 17 days of embryonic development, induced an increase in the cellular density of the magnum's mucosa and the formation a pseudostratified epithelium with invaginations of epithelial cells into the stroma, which initiates the formation of the tubular glands. It also caused an increment in the wall and lumen areas of the magnum in the newly hatched chicken. These results demonstrate that administration of exogenous estradiol induces changes in the chick embryo oviduct, producing cytodifferentiation and modifications of the magnum's mucosa during development. Therefore this tissue can be considered a useful system to evaluate the effects of steroid hormones.

Key words: Oviduct. Magnum.Chick embryo. Estrogens.

INTRODUCTION

Mullerian duct development in the chick embryo is complex and poorly understood, progress has been slower in the chick because of the asymetry of the female genital tract.

Both the male and female 5-6 day chick embryos have undifferentiated pairs of Mullerian ducts. In the male embryo, the right and left Mullerian ducts start involution on the 8th day of incubation and disappear by the 13th day. In the female embryo, the right Mullerian duct starts to regress on the 9th day of incubation and appears as a tiny cloacal stump at the time of hatching. The left Mullerian duct, however, continues to develop, eventually becoming the functional oviduct of the mature female chick. (Hutson, et al., 1983).

Genetic programming and gonadal secretions are considered to be the major factors responsible for this asymmetrical development of the embryonic organ. The involvement of steroid hormones in Mullerian duct development has long been an interesting topic for study, yet the important questions about the interaction of the sexual steroids and this embryonic target organ are still unanswered. (Teng and Teng, 1975c; Hutson et al., 1985).

The chick oviduct is an excellent target model for gonadal hormone studies. In recent years, the effect of 17β-estradiol and progesterone on cell proliferation, cytodifferentiation, and synthesis of specific proteins has been extensively studied (Kohler et al., 1969; Palmiter, 1972; Niemela and Elo, 1983; Pageaux et al., 1986; Perche and Sandoz, 1988).

Characterization of the 17β-estradiol and progesterone receptors as well as their genetic expression has been achieved in the chick oviduct (Teng and Teng, 1975a; Rodriguez et al., 1989; Tuohimaa et al., 1989; Isola, 1990).

Binding of 17β-estradiol to cytoplasmic and nuclear fractions of the Mullerian duct has been detected in the chick embryo from 8 days of incubation onward (Teng and Teng, 1975b, 1978).

Autoradiographic studies have shown that 17β-estradiol binding to the nucleus of the stromal cells of the oviduct occurs on day 15th of development (Gasc and Stumpf, 1981).

Explanted oviducts of 13 and 16-day-old chick embryos respond to treatment with 17β-estradiol in the culture medium by developing epithelial crypts containing goblet cells, and an edematous stroma; whereas hydrocortisone shows a cooperative effect on tubular gland formation (Kiell et al., 1982).

This work was aimed at studying the effects of a low dose of 17β-estradiol administered *in ovo* on the left oviduct of newly hatched chicken.

MATERIALS AND METHODS

Fertile white Leghorn eggs (Babcock B-300) were incubated at 37.8°C in a forced draught incubator. The embryos received 200 ng of 17β-estradiol (Sigma Chemical Co, St Louis) dissolved in 2% ethanol on the chorioallantoic membrane on days 15 and 17 of incubation. This dose was selected because of its maximal effect, according to a previous dose-response study (Sotelo,

1985). Control embryos received only the vehicle. Chickens were decapitated within 24 hrs afther hatching.

A total of 87 left oviducts were dissected, recording the wet weight. For morphological studies, the magnum region of 15 left oviducts was dissected and fixed in Bouin's fluid for 1 h., included in paraffin and serial transverse sections of 4 μm thickness were made. The histological sections were stained with hematoxylin-eosin. The total area of the sections, the areas of the oviduct's lumen and of the wall were determined using a computer (HP, Mod 9885M) system aided by a digitalizer (HP, Mod 9825) and a specially designed program to calculate areas based on algorithms of infinitesimal small triangles (Burden and Fayres, 1985). Areas are reported in square micrometers.

The thickness of the epithelium was measured at high magnification, using a micrometric lens. One measurement was taken every 75 μm, sampling the whole epithelial area in each section.

The density of nuclei per area (13,576 μm^2) was determined for both the epithelium and the stroma of the mucosa; in each section different regions of the epithelium and the stroma were digitalized to obtain their areas. In each area, the nuclei were counted and divided by the area, in this way we were able to calculate the number of nuclei in each 100 μm^2 of epithelium and stroma of the magnum's mucosa. These measurements were performed in three regions selected at random from each section. Sections were obtained at three levels of the magnum: Level I corresponded to the cephalic region; level II, to the medial region; and level III, to the caudal region close to the isthmus.

Statistical evaluations were performed by means of the variance analysis (ANOVA) and Student's "t" test. (Schefler, 1969).

RESULTS

The morphometrical and histological changes observed in the oviduct showed a difference in the areas of the magnum's wall and lumen and in the thickness of the epithelium. This made us suggest three levels along the magnum in an infundibulum-uterine direction. Morphometrical measurements in the control group showed that the areas of the lumen and the wall of the oviduct decreased from level I to level III, whereas the thickness of the epithelium increased in the same direction (Table 1).

These three levels were also identified in the magnum of the treated oviducts. The morphometric changes were observed mainly in the lumen and wall of the magnum, recording a significant increase in their areas in the treated oviducts. The epithelium also increased in height in the 17β-estradiol treated embryos, but the difference is not significant when compared with the controls (Table 1).

The most evident morphological changes in the treated group were found in the magnum's mucosa. The treatment induced a pseudostratified epithelium and

invaginations of the epithelial cells into the supepithelial stroma. (Figs. 3,4)

This was more evident in level I of the magnum, where the epithelial surface of the non-stimulated oviducts consists of a columnar epithelium, wich rests upon an undifferentiated stroma. Whereas treated oviducts showed an uneven pseudostratified epithelium with small invaginations of the epithelium into the stroma from which tubular glands are to be formed. (Figs. 1,2,3,4).

Besides, an increase in the density of nuclei per unit-area was observed both in the epithelium and the stroma of the magnum's mucosa for level I and II. For level III this increase was only significant in the epithelium. (Table 2).

The wet weight of the oviduct increases slightly with the hormonal treatment but he increase is not statistically significant (Fig. 5).

TREATMENT	Level	n	Magnum's wall area	Magnum's lumen area	Total area	Epithelium thickness (μm)
Control	I	6	4.9 ± 0.3	0.9 ±0.2	5.8 ± 0.3	11.6 ±0.8
17β-estradiol	I	6	6.3 ± 0.3**	1.8 ± 0.3*	8.1± 0.4**	12.5 ± 0.3
Control	II	6	4.2 ± 0.2	0.7 ± 0.1	4.8 ± 0.2	14.9 ± 1.1
17β-estradiol	II	6	4.6 ± 0.2	1.1 ± 0.1*	5.7 ± 0.2	17.4 ± 1.1
Control	III	4	3.6 ± 0.2	0.3 ± 0.04	3.9 ± 0.2	26.6 ± 0.2
17β-estradiol	III	8	4.9 ±0.4*	0.7 ± 0.07***	5.6 ± 0.4*	28.2 ± 1.9

Values are expressed as mean ± SEM.
Levels of significance: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 1. Morphometric measurements of the oviduct's magnum from newly hatched chikens, wich received 17β-estradiol (200 ng/egg) at 15 and 17 days of incubation. (Area X 10^4 μm^2).

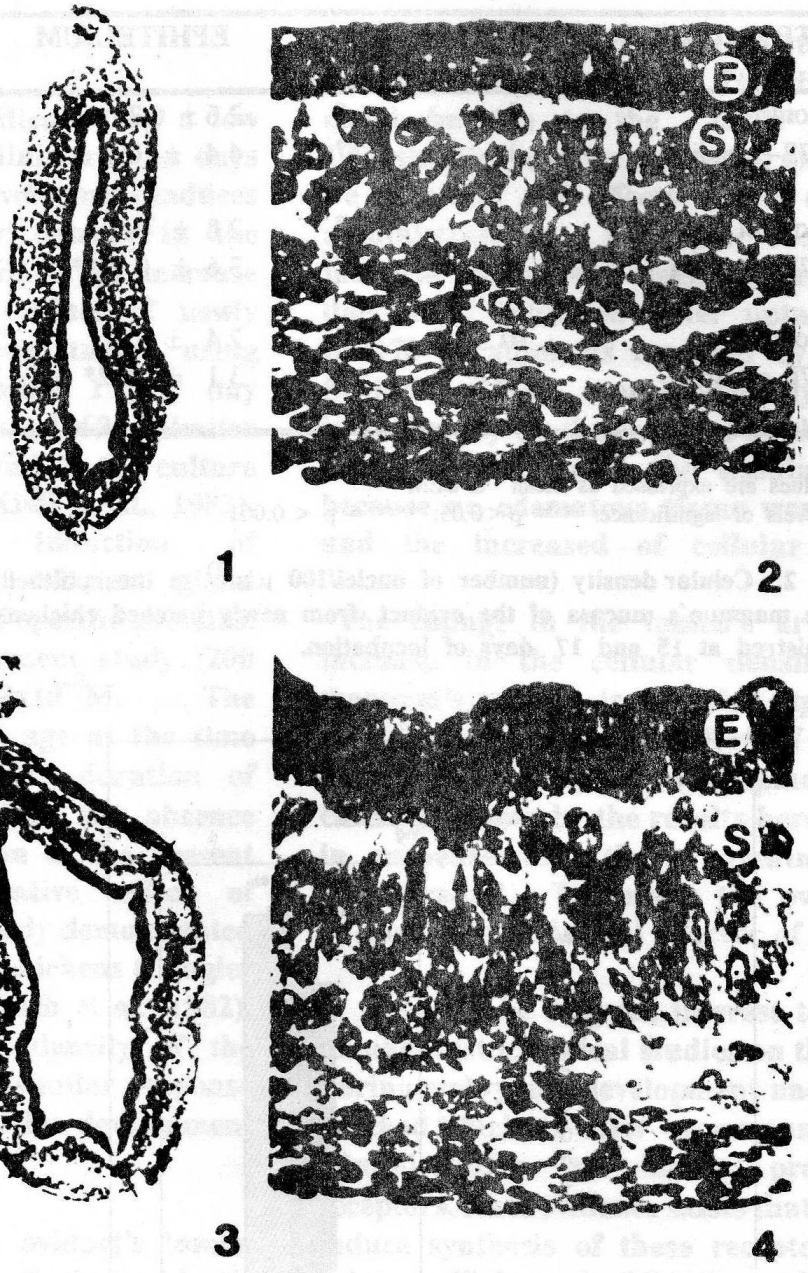

Fig. 1 Transverse section at Level I of the oviduct's magnum from a newly hatched control chicken. X169.

Fig. 2 High-magnification of the magnum's mucosa from newly hatched control chicken. Transverse section at Level I. Ephithelium (E) and stroma (S). X864.

Fig. 3 Transverse section at Level I of the oviduct's magnum from a newly hatched chicken, after treatment with 17B-estradiol. Note the increase in the areas of the magnum's wall and lumen, and epithelial invaginations (arrows). X169.

Fig. 4 A detail of the magnum's mucosa of a newly hatched chicken treated with 17β-stradiol. Transverse section at Level I. Note the morphology of the region, the epithelium (E) and stroma (S) appear to consist of tightly compacted cells. X864.

TREATMENT	LEVEL	n	EPHITELIUM	STROMA
Control	I	6	2.6 ± 0.2	2.1 ± 0.1
17β-estradiol	I	7	4.4 ± 0.3	3.4 ± 0.2***
Control	II	7	2.8 ± 0.1	2.2 ± 0.1
17β-estradiol	II	7	3.6 ± 0.1***	3.5 ± 0.2***
Control	III	4	2.4 ± 0.1	2.0 ± 0.2
17β-estradiol	III	5	3.1 ± 0.2*	3.6 ± 0.2

Values are expressed as mean ± SEM
Levels of significance: * = $p < 0.05$; *** = $p < 0.001$

Table 2. Celular density (number of nuclei/100 μm^2) in the ephithelium and the stroma of the magnum's mucosa of the oviduct from newly hatched chickens. 17β-estradiol was administred at 15 and 17 days of incubation.

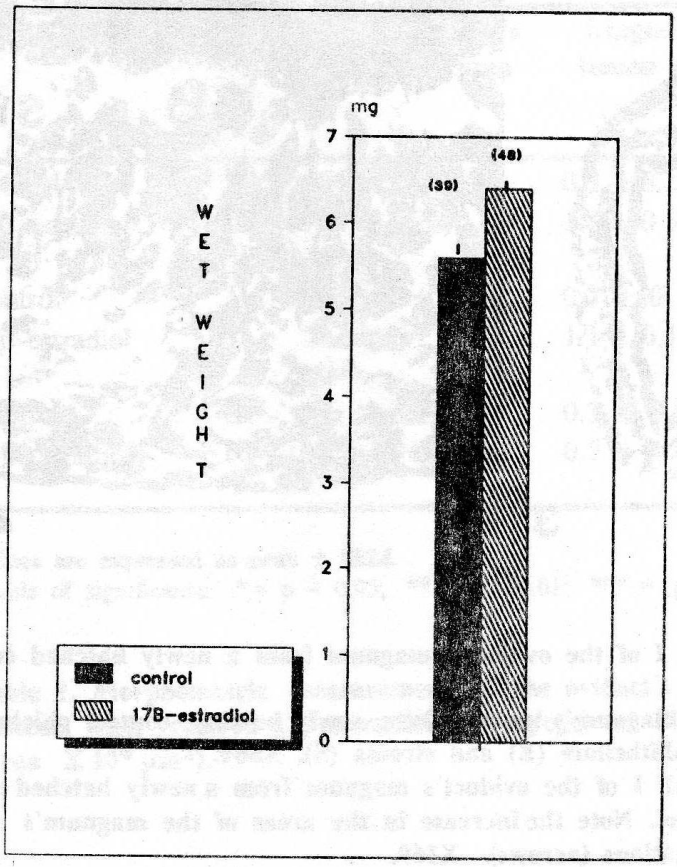

Fig. 5. Wet weigth of the oviduct in a newly hatched chicken. The number of oviducts per group is indicate in parentheses. Values are expressed as mean ± SEM.

DISCUSSION

The results obtained indicate that a low dose of 17β-estradiol administered on days 15 and 17 of embryonic development induces an increased cellular density in the magnum's mucosa, as well as an increase of the lumen of the oviduct of newly hatched chicken. Previous studies, using high doses of 17β-estradiol 1 mg/ day onward (Oka and Schimke, 1969; Palmiter and Wrenn, 1971) or 1μg/ml in the culture medium of the oviduct (Kiell et al., 1982), have reported the induction of cytodifferentiation with tubular gland formation and synthesis of specific proteins. The dose used in the present study (200 ng/egg) corresponds to $2x10^{-8}$M. The dose of 17β-estradiol, the age at the time of administration, and the duration of the treatment could explain the absence of tubular gland formation in the present study. The proliferative effect of 17β-estradiol has been clearly demonstrated in the oviduct of immature chickens (Laugier et al., 1983; Bouixvieux-Ulrich et al., 1982). The increase in cellular density of the oviduct's mucosa shows a similar response to that observed during the development of the chick embryo.

The enlargement of the oviduct's lumen could be a consequence of the oviduct's growth, but it could also be explained by water, sodium, and chloride retention in the lumen as described for the mammalian oviduct (Astwood, 1938; Talbot et al., 1940) or for the laying and nonlaying hens treated with estrogens (Cecil et al., 1970). Kohler et al., in 1969, confirmed that the tissular water uptake causes an edematous stroma, however our findings reveal a stroma with increased cellular density. This discrepancy could be due to the lower dose of 17β-estradiol used in this work and because we recorded the effects a few days after administration. We also observed an increase in the wet weight that could be due to an increased water uptake caused by 17β-estradiol, as reported by Oka and Schimke (1969), morphologically revealed in this study by the increase in the lumen's area. As well as to the tissular growth, because no edematous tissue was observed and the increased of cellular density.

The change in the lumen's area or the increase in the cellular density of the magnum's mucosa is a useful experimental model to evaluate the effect of estrogens during the embryonic development of the chick, as shown by the results here obtained in response to the treatment with 17 -estradiol. Therefore, the oviduct can be used as a biological effector of estrogens.

It would be of great interest to perform immunohistochemical studies on the oviduct during embryonic development and in newly hatched chickens after exogenous estradiol administration to locate progesterone receptors, since evidence exists that estrogens induce synthesis of these receptors in the oviduct (Toft and O'Malley, 1972; Hora et al., 1986; Laugier et al., 1991) and that the formation of tubular glands does not being before mucosal stromal cells express progesterone receptors (Joensuu, 1990).

ACKNOWLEDGEMENT

The authors thank Biol. Flora García Formenti for revising the manuscript.

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Fecha de recepción: 1 de Marzo de 1993.

Fecha de aceptación: 23 de Agosto 1993.