IN VITRO EFFECTS OF GONADOTROPHIC AND STEROID HORMONES ON THE INTERSTITIAL CELLS OF THE CHICK EMBRYO OVARIES

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SUMMARY

The embryonic gonads produce and secrete the steroid hormones that induce secondary sex differentiation in different species [1-2]. The production of estrogens, testosterone and progesterone by interstitial cells of the chick embryo ovary has been demonstrated in experiments in ovo and in vitro [3-4-5]. Estrogens would be responsible at the 7th day of in ovo incubation, of the evolution of the functional left ovary [6-7-8]. According to Gasc [9], only the cells of the germinative epithelium of the left ovary possess receptors for estrogens, thus originating the formation of a cortex in this functional gonad and not in the right one.

On the other hand, extragonadal factors, such as gonadotrophins, would participate in gonadal differentiation. Involvement of the hypophysis in the morphological and functional differentiation of the ovary in the chick embryo, has been considered in several studies [10-11-12]. Woods and Week [13] established the functional hypophyseal-gonadal axis at 13 days of in ovo incubation. However, the effects of individual gonadotrophins on the interstitial cells of the chick embryo remain unclear.

Avila et al. [7] and Grassi Milano [14] observed that when the female gonads were cultured without steroid or gonadotrophic hormones at the start of differentiation an hermaphrodite left ovary and a male right one were formed. Kyparissi and Vakolopoulu [15] observed that the inoculation in ovo of FSH at the end of embryonic development led

INTRODUCTION

Several studies have shown the importance of steroid production by the ovarian interstitial cells of the chick embryo in ovo as well as in vitro. The authors wished to analize the modifications produced by LH, hCG, 17-β-estradiol, testosterone propionate and progesterone in vitro on the interstitial cells of the chick embryo female gonads. Explants from right and left ovaries of 7 to 19 developmental days in ovo were cultured and processed to be structurally and ultrastructurally studied. In the control cultures, nest of interstitial cells increased with age in both ovaries, cells presenting abundant smooth endoplasmic reticulum, mitochondria with tubular cristae and lipidic inclusions. Under the action of LH and hCG, the grouped interstitial cells had increased lipidic inclusions and organoids related to the steroid synthesis in the right ovary and in the medulla of the left ovary at all studied ages. On the contrary, in the presence of progesterone, estrogen and testosterone, isolated or grouped interstitial cells were observed, having scanty organoids and lipidic inclusions. We conclude that steroid hormones would reduce the interstitial cell activity while performing that cellular function, LH and hCG would act on such cells stimulating the synthesis of steroids which would be the intrinsic factor responsible for the sexual differentiation of the functioning left ovary and for the atrophy of the right ovary.

Key words: Chick embryo - Ovaries - Interstitial cells - Hormones - In vitro effects.

to an increment in the number and size of follicles in the left ovary. Pittini and Grassi Milano [16] added FSH and LH to cultures of 15 days-old chick embryos testicles and ovaries and found that only male gonads developed similar to normal embryos. In previous works [8] we demonstrated in vitro that LH or hCG induced, at the start of differentiation, the development of cortical-like regions in right gonads. On the contrary, when FSH was added to cultures, both ovaries exhibited regression of their different components. Teng and Teng [17], using biochemical methods, detected an increment in the production of steroids by isolated interstitial cells of the chick embryo ovaries when cultured in the presence of hCG.

In view of these data, we proposed to analize the structural changes induced in vitro by gonadotrophins and steroid hormones on the interstitial cells of the chick embryo gonads during embryogenesis.

MATERIALS AND METHODS

Left and right ovaries from 7, 11, 15 and 19 days-old Cobb's White Rock chick embryos were used. The embryo sex was established from skin karyotypes and cartilage cells in cultures [18].

Ten gonads for each age and side were employed for each experimental group. Organ cultures were prepared following the procedure of Fabro et al. [19]. Each gonad was cut into small pieces that were cultured separately in Eagle's medium supplemented with 10 % foetal calf serum, 100 units/ml of penicilin, 5 μg/ml of streptomycin sulphate and 1 % L-glutamine. The explants were incubated 4 days at 37 ºC in a humidified atmosphere of 95 % air and 5 % CO_2 on substrate. The culture media were changed after 2 days at which time fresh hormones were added again when needed.

The following experiments were carried out: a) controls without hormones, b) with human LH purity: hLHa 2 % by radioimmunoassay (30 μg/ml), c) with hCG from human pregnancy urine. Inmmunological potency: 9000 IU/mg (lst IRP International standard) (15 UI/ml), d) with 17 β-estradiol (1 μg/ml), e) with testosterone propionate (1 μg/ml), f) treated with progesterone (10 μg/ml). United States Biochemical Corporation supplied the hormones. Gonadotrophins were dissolved in 0.9% NaCl and a stock solution was made of each steroid in absolute ethanol. The solvent was tested at the highest concentration used experimentally (1.4 x 10^{-2} mM), and found to have no effect on explants.

At the end of each culture period the explants were fixed in Karnovsky solution [20] for 2 hours, and then postfixed for 1 hour in 1 % osmium tetroxide. The gonads were then dehydrated in acetone and embedded in Araldite. Thick sections, stained with 1 % toluidine blue, were photographed using a Zeiss Photo II microscope. Thin slices were stained with uranyl acetate contrasted with lead citrate and examined with a Siemens E 101 electron microscope at magnifications between 2.000 to 20.000 X.

RESULTS

A. - *Controls*

Interstitial cells presented an aspect similar to that found in ovo in both ovaries. These cells were polimorphous, and in thick sections numerous vacuoles were found that surrounded an irregular nucleus with euchromatine and that had one or two nucleoli.

At the four ages studied, the interstitial cells in the left ovary were grouped in the deep medullary region, whereas in the right gonad, they were either isolated or grouped between ovocytes and epithelial cells of the lacunar walls that form the structure of this organ. Ultrastructurally, interstitial cells contained an abundant smooth endoplasmic reticulum (SER), Golgi apparatus, mitochondria with tubular cristae and a scarce rough endoplasmic reticulum (RER), (Fig. 1 A and B).

B. - *Effects of LH or hCG*

In ovaries extracted from 7 and 11 days-old embryos and cultured for 4

days, the two hormones exerted a similar effect on both gonads. An increment in the amount of nests of interstitial cells was observed. Cells were round and at a difference with controls, they had multiple cytoplasmic vesicles.

In ovaries from 15 and 19 days-old embryos the response to these hormones was lower.

The left ovary presented abundant cellular groups both in the juxtacortical medulla and in the deep region, that were not seen in controls.

The right ovary showed also abundance of nests of interstitial cells between epithelial and germ cells.

The ultrastructural characteristies of these cells were similar in both ovaries. There was an increase of the SER and of mitochondria with tubular cristae. The vesiculo-tubular SER was related to lipidic drops that were more numerous that in controls (Fig. 1 C and D).

An increase of pinocytotic vesicles was also seen. The RER and free ribosomes were more scarce that in controls, the same as dense bodies.

C. - *Effects of steroid hormones*

Addition of estradiol to cultures of both ovaries produced at the four ages studied, a diminution of interstitial cells. We observed often interstitial cells undergoing necrosis, and the SER as well as mitochondria and lipidic drops were less developed than in controls.

After addition of testosterone or progesterone to cultures of left and right ovaries from 7 and 11 days-old embryos, interstitial cells were less developed, the same as their organoids.

These cells contained mitochondria with a matrix of higher electronic density that were near lipidic drops. Lipidic drops presented a more dense content with a peripheral halo that was more electrodense than the inner content. They were circumscribed by membranes and their contours were irregular. However, some cells had lipidic drops with an homogeneous electrodense content, surrounded by the SER (Fig. 2 A).

Multivesicular bodies near the plasma membrane and the "lining bodies" were more conspicuous than in controls and than in cultures treated with estradiol (Fig. 2 B). In cultures from gonads of 15 and 19 days-old embryos, no differences with respect to controls were found.

DISCUSSION

Interstitial cells of both ovaries respond to the action of LH and hCG, as shown by the increment of the organoids involved in the synthesis of steroid hormones, that lead to the accumulation of numerous lipidic drops in their cytoplasm. These results are similar to those obtained by Labarbera and Ryan [21] on pig thecal cells cultured in the presence of LH or hCG. Dahl [22] reported similar results in thecal cells of the domestic fowl when these were injected with pregnant mare serum gonadotrophin or hCG. Dimino [23] detected in the ovary, an increase of mitochondrial steroidal activity when rats were injected with pregnant mare serum. Soto et al. [1] showed similar morphological changes in human thecal cells cultured in the presence of LH. Besides, the presence of numerous pinocytotic vesicles would be related to the increase in metabolic activity by these cells under gonadotrophic actions [24]. The similar response obtained with LH or hCG would be due to the fact that these hormones share a common membrane receptor in target cells, as shown by Bahl [25] in bovine luteal cells.

The steroids used, on the contrary, produced a diminution of the organoids and inclusions of interstitial cells. According to Soto et al. [1], the presence of scarce lipidic vacuoles with a content of high electronic density would be due to the presence of intermediate lipids in steroid synthesis that would not reach their final metabolic pathway "in vitro". Furthermore, the multivesicular bodies in the interstitial cells of ovaries cultured with testosterone or progesterone would participate in the mecha-

nism of release of steroids [26]. Besides, the increment of "lining bodies" would be related to the presence of high concentrations of estrogen in the culture, as suggested by Carlon and Erickson [27] in prefollicular cells of the chick left ovary cultured with testosterone. Dahl [28] demonstrated the atrophy of thecal cells after injection of steroid hormones to the domestic fowl.

Weniger [2] showed an increased steroid secretion by the foetal mammal ovary in vitro under the action of LH or hCC. We think that the stimulatory effects of gonadotrophins on interstitial cells would increase the production of steroid hormones; these would in turn contribute to the development of the cortex in the left ovary

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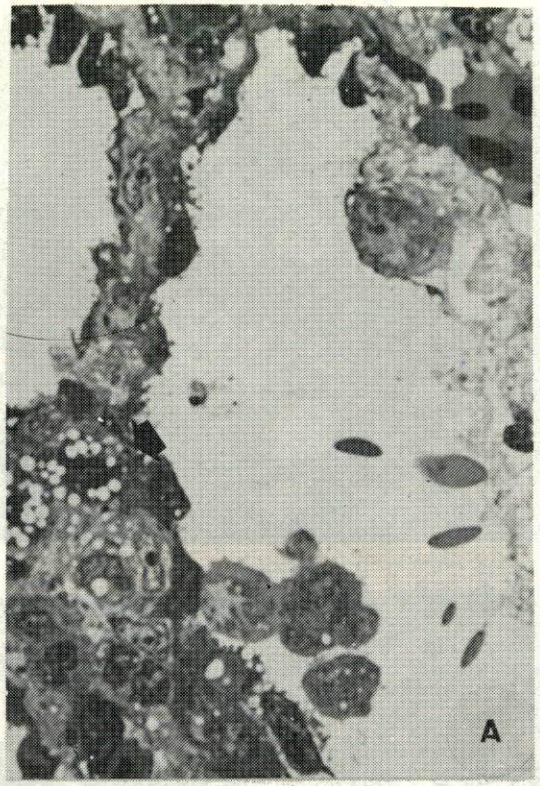
RESUMEN

Diversos estudios demostraron la importância de la producción de esteroides por las células intersticiales del ovario del embrión de pollo tanto in ovo como in vitro. El presente trabajo se efectuó para analizar las modificaciones que LH, hCG, 17-β-estradiol, propionato de testosterona y progesterona producen in vitro sobre las células intersticiales de gónadas femeninas del embrión de pollo. Explantos de ovarios derecho e izquierdo de 7 a 19 días de desarrollo in ovo fueron cultivados y procesados para su estudio estructural y ultraestructural. En los cultivos controles los nidos de células intersticiales aumentaron con la edad, en ambos ovarios, presentando aquellas abundante REL, mitocondrias con crestas tubulares e inclusiones lipídicas. Por acción de LH y hCG, las células intersticiales agrupadas incrementaron sus inclusiones lipídicas y organoides relacionados con la síntesis de esteroides en el ovario derecho y en la médula del ovario izquierdo en todas las edades investigadas. En cambio, en presencia de progesterona, estrógeno o testosterona se observaron células intersticiales aisladas o en grupos, con escasos organoides e inclusiones lipídicas. Se concluye que las hormonas esteroideas deprimirían la actividad de las células intersticiales supliendo aquéllas la función de las mismas, mientras que la LH y hCC actuarían sobre dichas células estimulando la síntesis de esteroides los que serían el factor intrínseco responsable de la diferenciación sexual del ovario izquierdo funcionante y de la atrofia del ovario derecho.



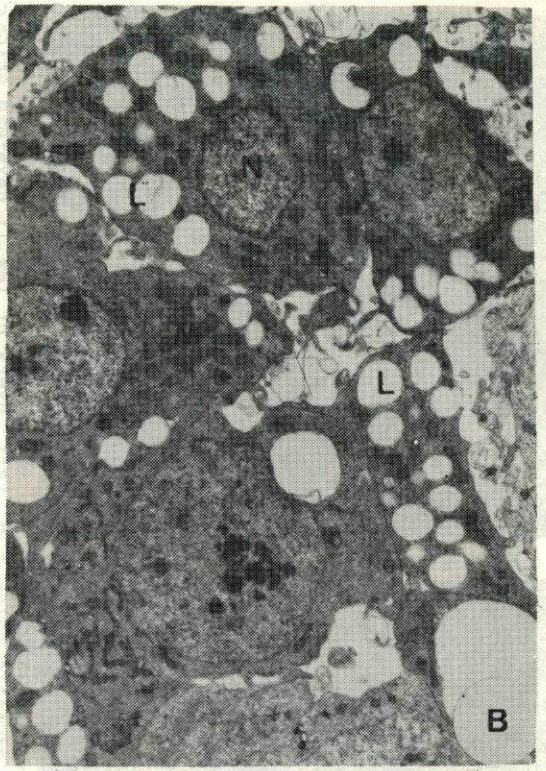
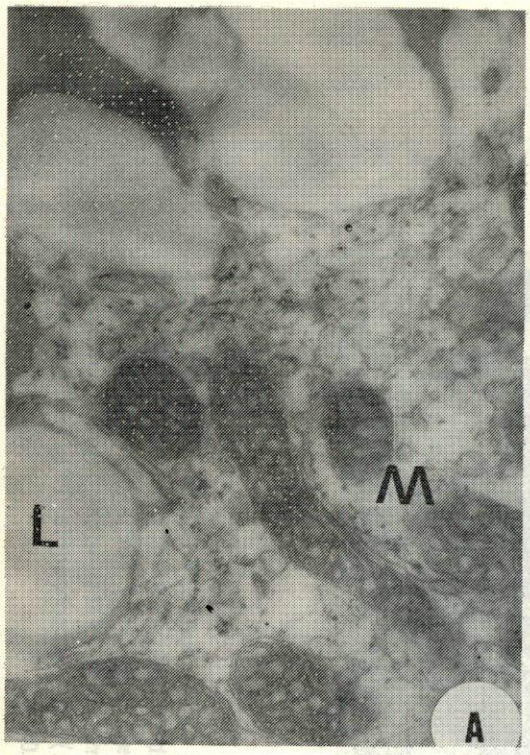


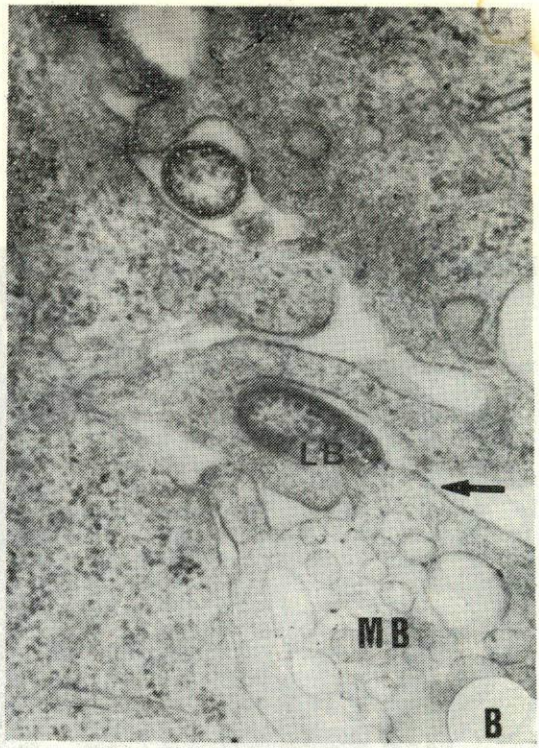
Figura 1:

A: Left ovary of the 7 days-old embryo cultured in a medium without hormones (control) for 4 days. Nest of interstitial cells in the lacunar walls of the deep medullary regions are indicated (arrows). 400X.

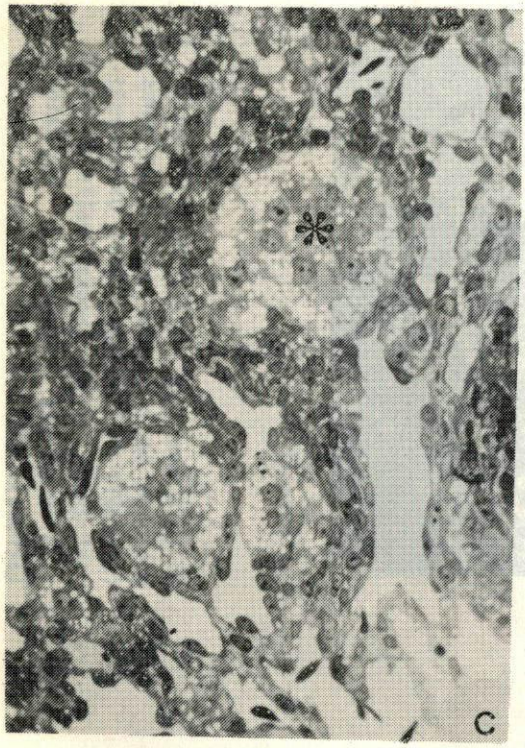
B: Left ovary of the 7 days-old chick embryo cultured in a medium without hormones (control) for 4 days. Group of interstitial cells with lipidic vacuoles (L) and mitochondria (M). Nucleous (N). 5,000X.



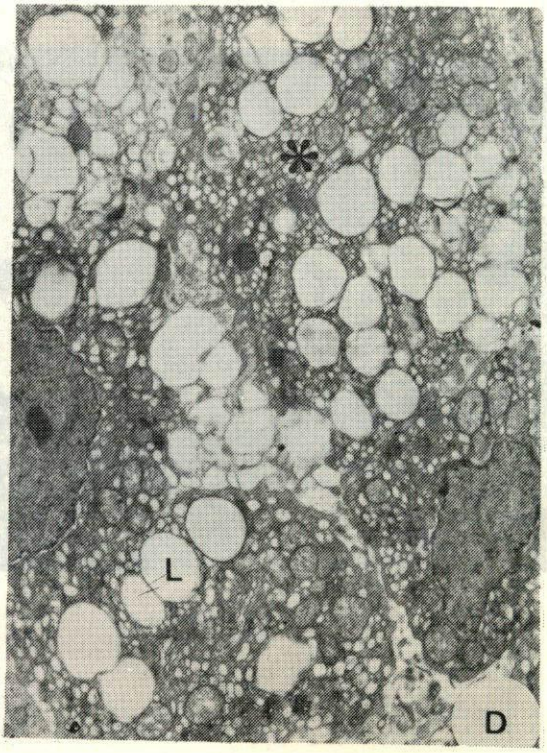
A: Right ovary of the 11 days-old chick embryo cultured in a medium with testosterone for 4 days. Part of the cytoplasm of an interstitial cell. Mitochondria with tubular cristae (M). Lipidic droplets (L). 30,000X.



B: Left ovary of the 11 days-old chick embryo cultured in a medium with testosterone for 4 days. Multivesicular bodies (MB) and "Lining bodies"(LB) in relation with the membrane (arrow) 30,000X.



C: Left ovary of the 7 days-old chick embryo culture in a medium with LH for 4 days. An increment in the number of nests of interstitial cells with numerous lipidic vacuoles is observed (asterisk). 400X.



D: Left ovary of the 7 days-old chick embryo cultured in a medium with LH for 4 days. Note the abundance of lipidic droplets (L) and SER (Asterisk) in the cytoplasm of interstitial cells. 5,500X.

Figure 2