INTERSTITIAL CELLS OF THE OVARIES OF THE CHICK EMBRYO: ULTRASTRUCTURAL ASPECTS OF THEIR INNERVATION

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SUMMARY

In a previous work we demonstrated that germ and epithelial cells of the atrophic right ovary and of the medulla in the left functioning ovary undergo regression while interstitial cells are preserved. The objective of the present study was to analyze the relationship between nerve fibers and nerve endings and interstitial cells from both ovaries during embryogenesis in the chick. To this purpose, left and right ovaries from embryos of 7 to 19 days of development were processed for ultrastructural study and ultracytochemical determination of acid phosphatase. At 7 days, isolated interstitial cells were found in the central region of both ovaries, containing SER, Golgi apparatus, mitochondria with tubular cristae and abundant lipidic vacuoles. Nerve fibers and nerve endings were scarce. From the 11th day, interstitial cells were found grouped in the right ovary and in the medulla of the left one, and were related to nerve bundles surrounded by Schwann cells. The nerve ending, in close contact with the membrane of interstitial cells, contained mitochondria, microtubules and small and big vesicles, some with an electrodense content. This relationship between interstitial cells and nerve endings and fibers was more frequent from the 15th day. The activity of acid phosphatase localized in germ and epithelial cells presented a more intense reaction in the right ovary and in the medulla of the left ovary at 15 days. Enzyme activity was not detected in interstitial cells nor in nerve endings and fibers at the different ages studied. These results suggest that nerve fibers and nerve endings would be necessary for interstitial cells differentiation in both ovaries of the chick embryo.

Key words: Chick embryo - Ovaries - Interstitial cell - Innervation.

INTRODUCTION

In previous works (3,20) we demonstrated that in the atrophic right ovary and in the medulla of the functional left ovary, germ and epithelial cells undergo regression while interstitial cells are preserved. These cells are identified from the 7th day of in ovo development. Ultrastructurally, they are steroid-producing cells, with abundant SER, mitochondria with tubular cristae, lipidic inclusions and 3-β-hydroxysteroid dehydrogenase activity (7, 8, 9, 12, 16, 20). We have also shown that there is acid phosphatase activity in germ and epithelial cells in the right ovary and in the medulla of the left ovary, but not in interstitial cells. This finding allowed us to propose the involvement of this enzyme in the involution of the right ovary and the left medulla (21).

The participation of interstitial cells in the differentiation of estrogen-dependent organs and tissues during embroynic life is currently accepted, although its relationship with the nervous system remains unclear.

Dahl (6) in a study of the innervation of the steroid-secreting theca cells in the domestic fowl, postulated a nervous control of this endocrine secretion.

Amanuma and Yamada (1) studied ultrastructurally ovaries of chick embryos from 12 to 20 days of in ovo development and demonstrated that at the 17th day, nerve endings were related to groups of interstitial cells. These authors also found nervous fibers and endings in male gonads feminized in

ovo by diethylbestrol (DES) while they were absent in control male gonads (2).

In view of these data, we analyzed the ultrastructural relationship between nervous fibers and endings and interstitial cells of the chick ovary during embryogeny in ovo and in vitro in the presence or absence of steroid or gonadotrophic hormones.

MATERIAL AND METHODS

Coob's White Rock female chick embryos at 7, 11, 15 and 19 days of incubation were employed. Developmental stages were determined according to Hamilton an Hamburger (13). The sex of embryos was established through skin karyotypes and cartilage cells in culture (19).

Ten left and right gonads were carefully dissected. Fragments were fixed at room temperature in Karnovsky's fixative (17). Four hours later the fixative was decanted and replaced with 0.1 M cacodylate buffer at pH 7.4 containing 0.003 M calcium chloride; two or more changes of the buffer were made over a 24 hours period. The tissues were then post-fixed in osmium tetroxide in 0.1 M cacodylate buffer for one hour and after a rinse in buffer were dehydrated in acetone; afterwards they were embedded in Araldite resin and polymerized at 60 ºC for 48 hours.

Sections were cut with a Porter Blum MT1 ultramicrotome. Thick sections stained with 1 % Toluidine blue were examined and photographed using a Zeiss Photo II microscope. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 101 electron microscope, at magnifications between 2.000 to 20.000 X.

Chick embryo gonads were also used for the ultracytochemical determination of acid phosphatase in ovo, according to the method of Jones and Fox as previously described (21). Besides, ovaries were cultured in the presence of the following hormones: LH (30 μg/ml), hCG (15 UI/ml); testosterone propionate (1 μg/ml) or 17-β-estradiol 1 μg/ml) for 4 days, with the purpose of studying their effects on the innervation of interstitial cells. The methodology followed was as described in a previous work (5). Ulterior processing of gonads for electron microscopy was as above.

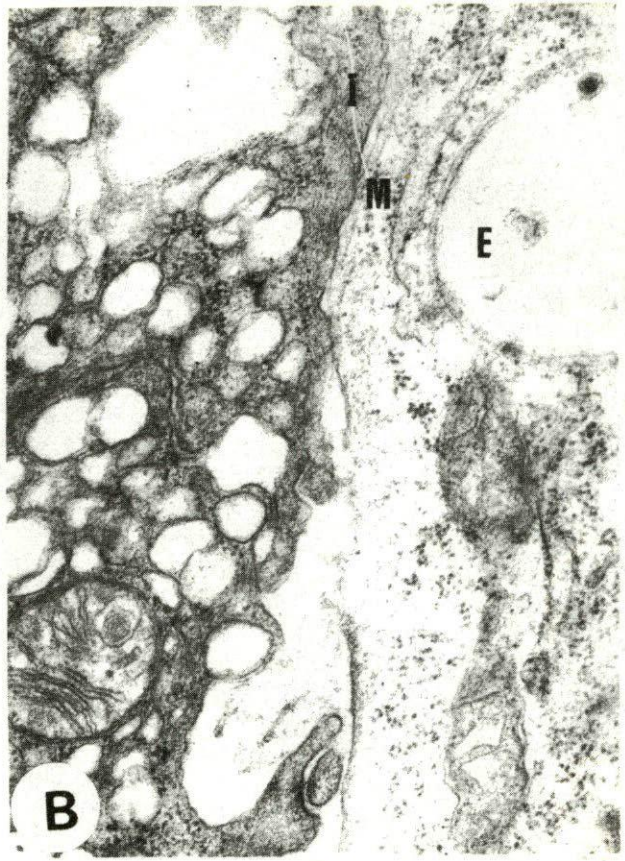
RESULTS

At 7 days of incubation (stage 31) isolated interstitial cells were found in the central region ot both ovaries. These cells were polymorphous, with cytoplasmic processes and they joined other interstitial cells through junctional complexes. The nucleus was oval, with finely granular chromatin and one or more nucleoli. The cytoplasm presented SER, Golgi apparatus, mitochondria with tubular cristae and lipidic droplets. These droplets had an electrolucid content and were surrounded by vesicles of the SER. Nerve fibers were scarce in both the right and left ovaries and were usually constituted by several axons surrounded by Schwann cells (Fig. 1A). Also, in both ovaries, nerve endings were rarely found. When present, they had inner mitochondria, microtubules and vesicles of different sizes, and some vesicles had an electrodense core. Some nerve endings were associated with processes of mesenchymal cells, interposed between interstitial cells and nerve endings (Fig. 1B). Mesenchymal cells were characterized by a scanty cytoplasm with GER, mitochondria with laminar cristae and numerous free ribosomes.

At 11 days (stage 37), groups of interstitial cells were noticeable in the right ovary and in the medulla of the left gonad. These groups of interstitial cells showed an increase of the vesiculotubular SER that surrounded lipidic vacuoles with an electrolucid content. According to their abundance, these cytoplasmic components conditioned the position of the nucleus. The lipidic vacuoles frequently fused with each other and with the plasma membrane. The mitochondria, with tubular cristae, were associated to lipidic vacuoles. The Golgi apparatus was juxtanuclear while both the GER and free ribosomes were scarce. Mesenchymal cells were found surrounding groups of interstitial cells.

There was usually only one nerve fascicle in the periphery of the group of interstitial cells (Fig. 2A). The axons

Fig. 1: 7 days chick embryo
A: Left ovary. Schwann cell (S) surroun ding a nerve fascicle (N). 8,000X.



B: Right ovary. Nerve ending (E). Interstitial cell (I). Note the presence of the cytoplasm of a mesenchymal cell (M). 20,000X.

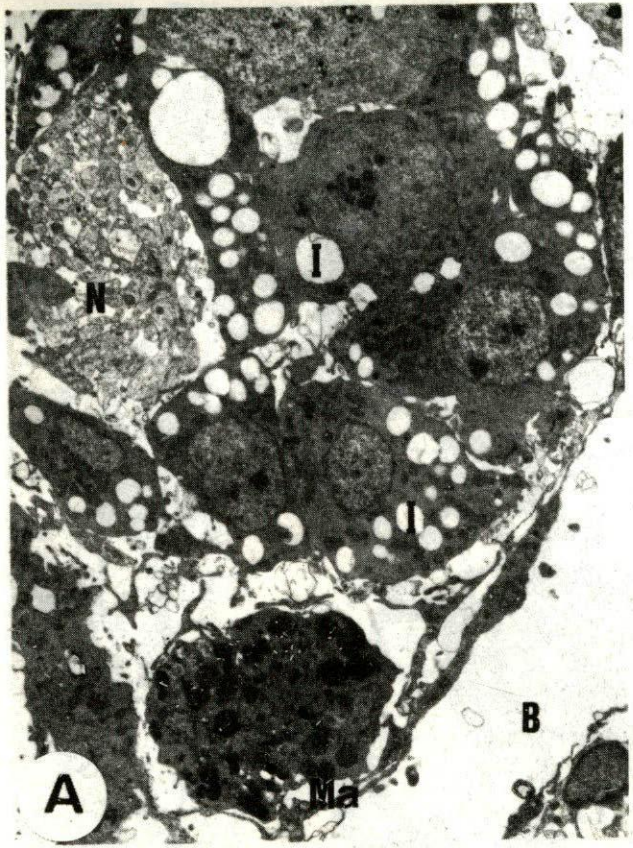
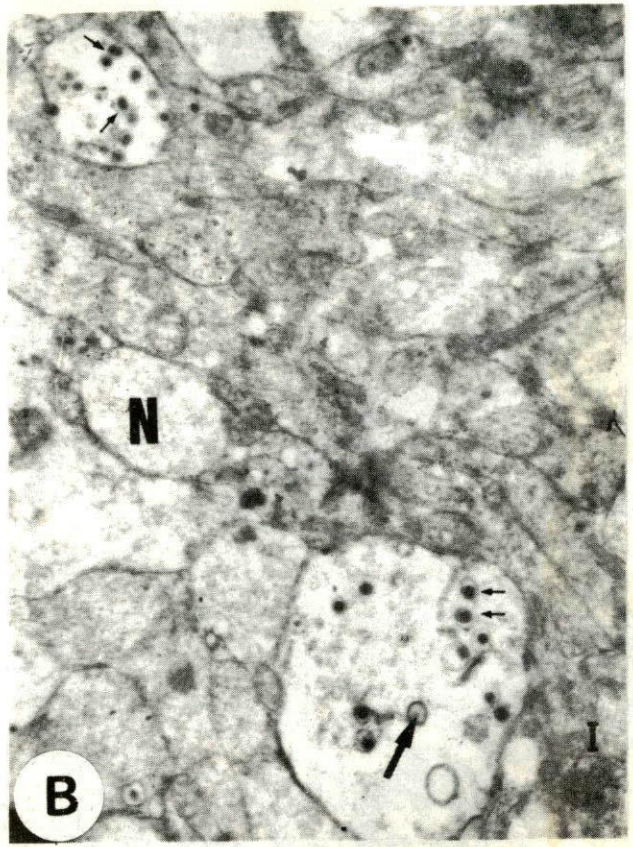


Fig. 2: 11 days chick embryo
A: Left ovary. Medulla. Group of interstitial cell (I). Nerve fascicle (N). Blood vessel (B). Macrophage (Ma). 3,000X.



B: Right ovary. Nerve fibers (N). Two nerve ending containing both electrodense (small arrows) and electrolucid vesicles (large arrow). Partial viewing of interstitial cell (I). 8,500X.

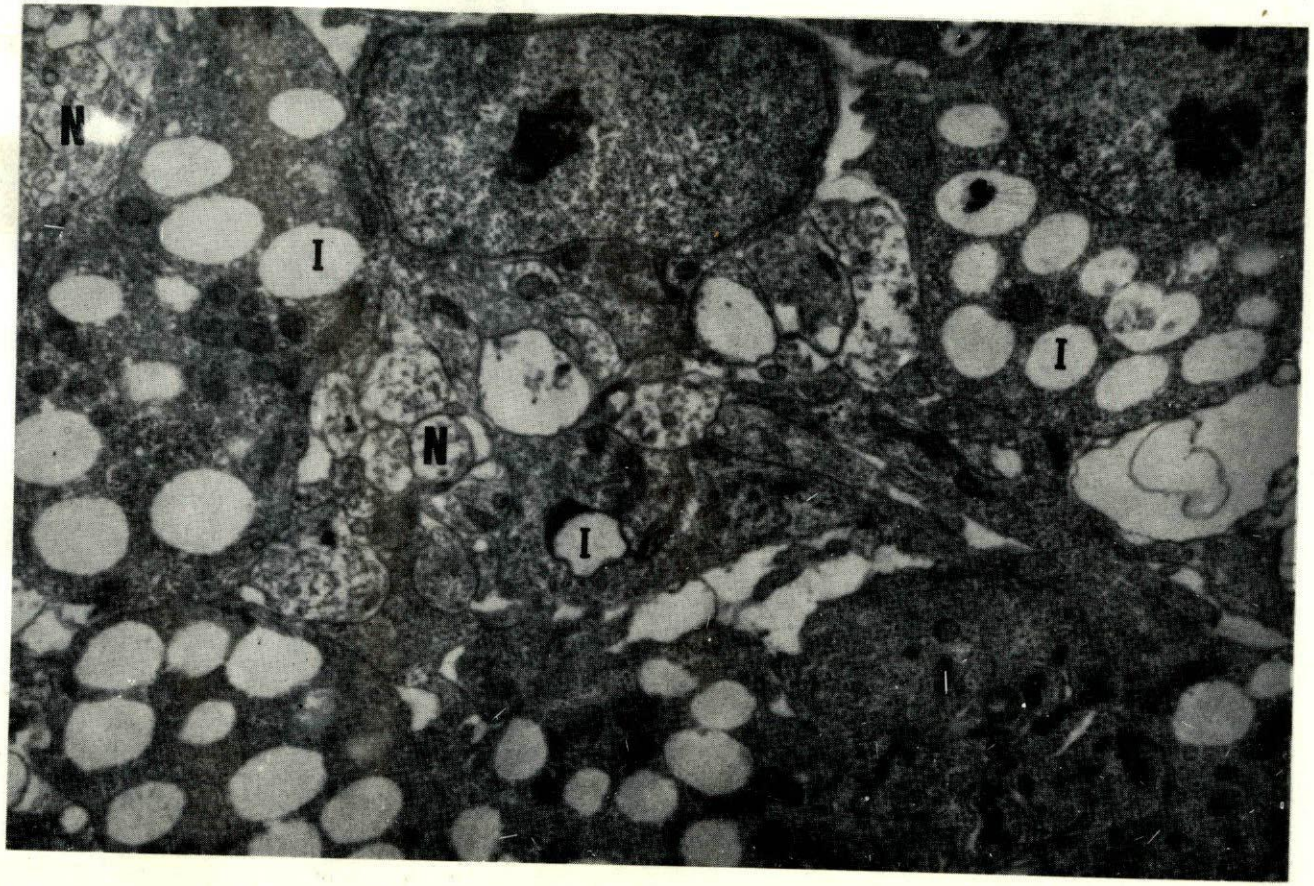


Fig. 3: Left ovary from 15 days chick embryo. Nerve fibers (N) among interstitial cells (I). 4,000X.

inside the fascicles presented numerous microfilaments. A Schwann cell surrounded the nerve fibers. At this age, a greater number of nerve endings was directly related to the interstitial cells. An evident increase of vesicles with an electrodense content was found inside the nerve endings, while only a few presented an electrolucid material (Fig. 2B). No increase in the thickness of the membrane of the nerve endings or of the interstitial cells was observed. Condensation of the cytoplasm near the contact membrane of interstitial cells was not found. Lipidic vacuoles were found near these contacting regions.

From 15 (stage 41) to 19 days (stage 45), there was an increase in the number of groups of interstitial cells. In the right ovary, smaller groups of interstitial cells between typical lacunae were observed. Isolated interstitial cells were also found as part of the walls of lacunae. In the left ovary groups of interstitial cells were found between the medullary lacunae, and also in the juxtacortical medulla.

Nerve fascicles were more frequently seen in this ovary and some were found in the periphery of the groups of interstitial cells. Besides, the presence of nerve fibers among interstitial cells was conspicuous (Fig. 3). Nerve endings appeared grouped and in close contact with interstitial cells. Thickennings of the plasma membranes of the contacting nerve endings and interstitial cells were sometimes found in the gonads at 19 days of development, as well as a condensation of the cytoplasm underlying the contacting membranes.

No acid phosphatase activity was found in interstitial cells or in nerve fibers and endings Furthermore, when ovaries of chick embryos of 7 to 19 days of in ovo development were cultured in vitro in the presence of 17-β-estradiol or testosterone propionate for 4 days, the nervous structure did not undergo modifications, while there was a decrease of organoids involved in steroid-production within the interstitial cells. In ovaries cultured in the presence of LH or hCG, the nervous structure was not modified, while there was an increase in organoids and lipid droplets in the interstitial cells.

DISCUSSION

Several workers have confirmed the importance of steroid production by interstitial cells in the embryonic gonads of different species (10, 12, 15, 16, 18).

Jordanov and Angelova (15, 16) postulated a role of these cells in the differentiation of ovaries from the chick embryo. Amanuma and Yamada (1) who studied interstitial cells in both ovaries from 15 to 20 days of development described at the 17th day of development, their contact with nerve fibers and endings, and attributed them a mesenchymal origin.

In the present work, we have detected from the 7th day of development, nerve endings and fibers that are further developed with increasing embryonic age in both ovaries. The presence of vesicles, some of them containing an electrodense material as well as mitochondria, microtubules and microfilaments, is typical of nerve endings. However, contrary to the finding of Yamada and Amanuma (23), we observed only sporadically the typical synaptic-like structures in embryonic gonads at 19 days of developmen. Thickenning of the joining membranes and condensations of the cytoplasm underlying the plasma membrane of interstitial cells were rarely seen in this research.

Furthermore we found nerve endings containing 2 types of vesicles, with and without an inner electrodense material, at a difference with the observations of Dahl (6), who described 2 types of nerve endings, according to the presence or absence of vesicles with an electrodense content in adult hens. That author considered that nerve endings containing vesicles with electrodense material had adrenergic activity, whereas those possessing empty vesicles had cholinergic activity. We think that both nerve structures would participate in the differentiation of interstitial cells in early embryonic stages. At 19 days of development, the normal adult structures described by Dahl in hens would appear, to be completed after birth, concomitantly with a central control for steroid production (6).

Amanuma and Yamada (2) postulated that the relationship between nerve endings and fibers with interstitial cells constitutes a specific characteristic structural organization in the chick embryo ovaries.

The absence of acid phosphatase activity in nerve fibers and endings found in the present study, indicates that these structures do not undergo regressive changes, differing from the germ and epithelial cells of the right ovary and of the medulla of the left ovary. In ovaries from chick embryos at 7 to 19 days of in ovo development, cultured in the presence of gonadotrophins, the increase of interstitial cells as well as of the typical organoids observed in this work, was not accompanied by variations in the nervous structures. Similar results were reported by Dahl (7) in domestic fowl injected with gonadotrophins, although he only found an hypertrophy of thecal cells in the left ovary. Besides, the results in ovaries cultured with steroid hormones agree with those of Dahl, who demonstrated the atrophy of thecal cells after injections of steroid hormones.

These findings lead us to conclude that in the left and right ovaries of the chick embryo, the presence of nervous fibers and endings seems to be necessary for the differentiation of interstitial cells. These cells could then act on hormone-dependent tissues through their secretion of steroids.

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RESUMEN

En trabajos previos demostramos que en el ovario derecho atrófico y en la médula del ovario izquierdo funcionante del pollo experimentan regresión las células germinales y epiteliales y se preservan las células intersticiales (Avila y col. 1987). El objetivo del presente trabajo fue analizar la relación de las fibras y terminaciones nerviosas con las células intersticiales en ambos ovarios durante la embriogénesis en el pollo. Para ello se utilizaron ovarios izquierdos y derechos de embriones de 7 a 19 días de desarrollo, los que fueron procesados para su estudio ultraestructural y determinación ultracitoquímica de fosfatasa ácida. A los 7 días se encontraron células intersticiales aisladas en la región central de ambos ovarios, las cuales contenían REL, Golgi, mitocondrias con crestas tubulares y abundantes vacuolas lipídicas. Las fibras y terminaciones nerviosas eran escasas. A partir de los 11 días las células intersticiales se encontraron agrupadas en el ovario derecho y en la médula del ovario izquierdo y se relacionaban con fascículos nerviosos rodeados de células de Schwann. Los terminaciones nerviosas, en íntimo contacto con la membrana de las células intersticiales, contenían mitocondrias, microtúbulos y vesículas grandes y pequeñas, algunas con contenido electrodenso. Esta relación entre las células intersticiales y las fibras y terminaciones nerviosas fue más frecuente desde los 15 días. La actividad de fosfatasa ácida localizada en las células germinales y epiteliales, presentó una reacción más intensa a los 15 días en el ovario derecho y en la médula del ovario izquierdo. No se detectó actividad enzimática en las células intersticiales ni en las fibras y terminaciones nerviosas en las distintas edades estudiadas. Estos hallazgos sugieren que las fibras y terminaciones nerviosas serían necesarias para la diferenciación de las células intersticiales en ambos ovarios del embrión de pollo.

Palabras clave: Embrión de pollo - Ovarios - Célula intersticial - Inervación.