

**STRUCTURAL CHANGES INDUCED BY HE-NE  
LASER ON THE CHICK EMBRYO OVARY.**

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**SUMMARY**

The morpho-histochemical alterations that occur in the chicken ovary at 7 days of incubation after irradiation with He-Ne laser of a potency of 5 mw and at a wavelength of 632.8 nm were studied. The embryos were irradiated for 5 minutes through a window opened in the eggshell and aseptically maintained in incubator for 24 hours. The gonads were dissected out and processed for the following techniques: H/E, PAS, Alcian blue, and Toluidine blue. Controls: The ovaries were formed by a germinative or superficial epithelium, with germ and epithelial cells, and by primary sex cords compressed between them, although separated by a reduced stroma. The cords contained germ cells. The surface coat of the germinative epithelium presented a thin layer of PAS positive, alcianophilic at pH 2.5 and orthochromatic material. Basement membranes and intercord extracellular substance were also PAS positive. Problems: Disorganization of the tissue structure was well manifest in irradiated gonads, accompanied by negativization of the histochemical reactions. A lymphocytary infiltration was also found. No structural alterations were observed in germ or epithelial cells. It is concluded that laser radiations would act producing decrease of the muco-

substances associated to the plasma membrane and basement membrane. They would also provoke the appearance of an inflammatory mononuclear infiltration.

Key words: chick embryo - ovaries - He-Ne laser irradiation.

**INTRODUCTION**

Studies on the use of low potency lasers are related for the most part, to their therapeutic actions, having been employed as anti-inflammatory, antialgic and biostimulador agents in reparation tissues<sup>16</sup>.

With respect to the reproductive system, Villaplana-Torres<sup>17</sup> demonstrated alterations in Leydig cells from male albino rats after irradiation of the adenohipophysis with He-Ne. Other workers<sup>11</sup> have observed an increase in spermatogenesis after infrared laser irradiation of the seminiferous epithelium of male rats. A biostimulatory action of lasers on interstitial, connective muscle and nerve cells has also been found<sup>8</sup>.

Rasmussen and coworkers<sup>13</sup> have reported the cytotoxicity and mutagenesis produced by excimer lasers at 193, 254 or 308 nm on the germ cells of the hamster ovary.

Among the few studies made on embryos, Puchades-Orts and coworkers

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kers<sup>12</sup> described diverse somatic malformations in the chick between the stages 10 and 20 of Hamilton and Hamburger after He-Ne irradiation. Mims and McKinnell<sup>9</sup> employed laser radiations on the chick embryo at the stages 6 and 7 of Hamilton and Hamburger and showed absence of germ cells in the gonadal ridge after irradiation of the germinal crescent or the endodermic area that produces germ cells.

In studies made at our laboratory, the sequence of morphologic and biochemical changes that occur during differentiation of female chicken gonads was established<sup>1-5,15</sup>.

Also, we have recently described the appearance of alterations in the chick embryo mesonephros after irradiation with He-Ne laser, which are quite similar to those found in chronic interstitial nephritis<sup>6</sup>.

In the present work, the purpose was to study the morphologic and histochemical changes that take place in the ovary of the chick during its embryogenesis after He-Ne irradiation.

## MATERIAL AND METHODS

Forty chick embryos at 7 days of incubation, stage 31 of Hamilton and Hamburger, were employed. Half of the embryos (problems) were irradiated with He-Ne laser with a potency of 5 mw and at a wavelength of 632.8 nm for 5 minutes through a window opened in the eggshell. Only a window was made in the other non irradiated 20 embryos (controls), being both groups incubated for 24 hours under aseptic conditions.

The right and left gonads of control and problem groups were dissected out to perform the following studies<sup>14</sup>:

1. Routine staining with the Hematoxylin-eosine technique of the material previously fixed in Bouins.

2. Cytochemistry: PAS, PAS/amilase, PAS/sialidase, for glycoproteins demonstration; Alcian blue at pH 2.5 and 1.0, an methylation and saponifica-

tion tests, for the demonstration of sulphated and non-sulphated glycosaminoglycans; Toluidine blue at pH 3.8, to demonstrate basophilic, metachromatic and alcohol-resistant mucosubstances.

## RESULTS

### A — Controls (non irradiated embryos):

At 7 days the ovaries were formed by a superficial or germinative epithelium and by primary sexual cords or cords of the primary proliferation. The germinative epithelium presented epithelial and germ cells. A thin coat of material that was PAS, alcianophilic at pH 2.5 and orthochromatic basophilic with Toluidine blue stood out in this epithelium. Primary sexual cords were seen compressed against each other, although separated by a reduced stroma with scarce interstitial cells. They were approximately straight, adopted a perpendicular disposition with respect to the germinative epithelium and were composed of epithelial and germ cells (Fig. 1A). A PAS positive and alcianophilic coat surrounded the cords, while the inter-cord extracellular substance was PAS positive.

### B — Problems (irradiated embryos):

Disorganization of tissue structures was evident in irradiated gonads, whose primary sexual cords appeared disarranged and separated by wider spaces with some clear areas of edema and others occupied by an inflammatory lymphocytic infiltration (Fig. 1B).

The mononuclear infiltration was accompanied by a few plasmocytes, while macrophages were only occasionally found. The epithelial and germ cells of the cords did not exhibit manifest structural lesions. However, the decrease in the amount of germ cells with respect to controls was remarkable.

In edematous areas, epithelial cells were observed either to be separated by clear spaces among them or isolated.



Besides, when the degree of edema was severe, interstitial cells presented a scant cytoplasm surrounding a picnotic nucleus.

The lack of response to the histochemical reactives tested was well evident, both at the level of the membrane coats and basement membranes as well as in the intercord extracellular substance.

## DISCUSSION

He-Ne laser has been employed in medical practice disregarding possible harmful effects on organs and tissues. In the chick embryo mesonephros, we have described alterations that are similar to those found in the present work. Besides, these results are in accordance with those obtained by other authors in different animal species and organs. Puchades-Orts and coworkers<sup>12</sup> have demonstrated that irradiation of chick embryos at the stages 10 to 20 of Hamilton and Hamburger with He-Ne at 632.8 nm, produces cephalic hypoplasia, rudimentary extremities or their absence, anophthalmia and microphthalmia as well as other somatic anomalies.

The scarce amount of germ cells found by us in affected areas would agree with the results obtained by Mims and McKinnell<sup>9</sup>, who showed that irradiation with rubi laser of the region that produces germ cells at early stages of development, leads to a posterior lack of these cells in the genital ridge.

Severe malformations have been produced in rat embryos<sup>7</sup> and fertile eggs prior to incubation<sup>10</sup> by the rubi laser. Furthermore, Rasmussen and coworkers<sup>13</sup> found a cytotoxic and mutagenic effect of the excimer laser on hamster germ cells.

It is of interest that the reduction of mucosubstances in irradiated embryos is in agreement, at least in part, with the findings of Zhang and coworkers<sup>18</sup>, who reported a reduction of the products of peroxidation of membrane lipids in the mouse.

From these preliminary results, it becomes clear the need of continuing the analysis in depth on the effects of laser radiations by means of other techniques.

## RESUMEN

Se estudiaron las modificaciones morfohistoquímicas que ocurren en el ovario de pollo de 7 días de incubación por irradiación con laser He-Ne de 5 mw de potencia y 632,8 nm de longitud de onda. Los embriones fueron irradiados durante 5 minutos a través de una ventana abierta en la cáscara del huevo y mantenidos asépticamente durante 24 horas en incubadora. Se diseccionaron las gónadas y se procesaron para las técnicas de H/E, PAS, Alcian Blue y Azul de toluidina.

Controles: Los ovarios estaban formados por un epitelio germinativo o superficial, con células germinales y epiteliales, y por cordones sexuales primarios apretados unos contra otros, aunque separados por un estroma muy reducido.

Los cordones contenían células germinales. La cubierta superficial del epitelio germinativo presentaba una delgada capa de material PAS positivo, alcianófilo a pH 2,5 y ortocromático. Las láminas basales y la sustancia extracelular intercordonal también eran PAS positivas.

Problemas: En las gónadas irradiadas era bien manifiesta la desorganización de la estructura tisular, acompañada de una negativización de las reacciones histoquímicas. También se observó un infiltrado linfocitario. En las células germinales y epiteliales no se observaron alteraciones estructurales. Se concluye que las radiaciones laser actuarían produciendo disminución de las mucosustancias asociadas a la membrana plasmática y membranas basales. Provocarían además, la aparición de un infiltrado inflamatorio mononuclear.

Palabras clave: embrión de pollo - ovarios - irradiación con laser de He-Ne



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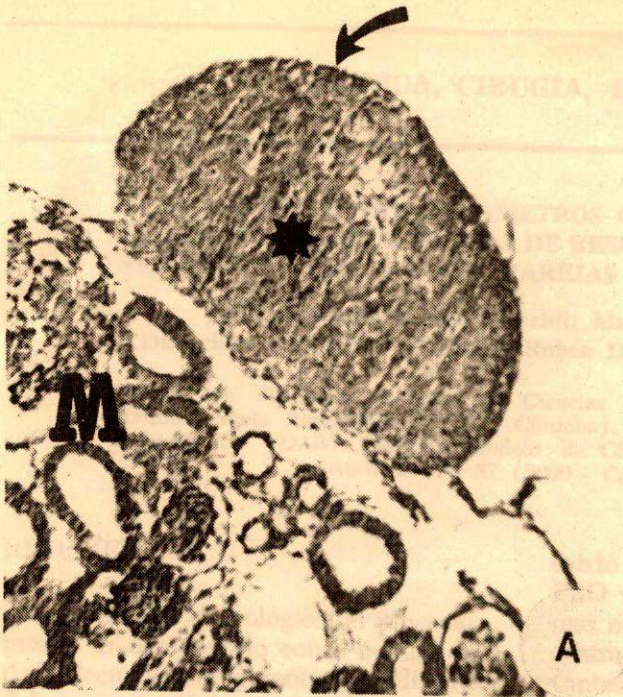


FIGURE 1:

A: Control ovary of chick embryo at 7 days of in ovo development. Germinative epithelium (arrow). Primary sexual cords (as terisk). Mesonephros (M). Hematoxylin-eosine stain. 250 X.

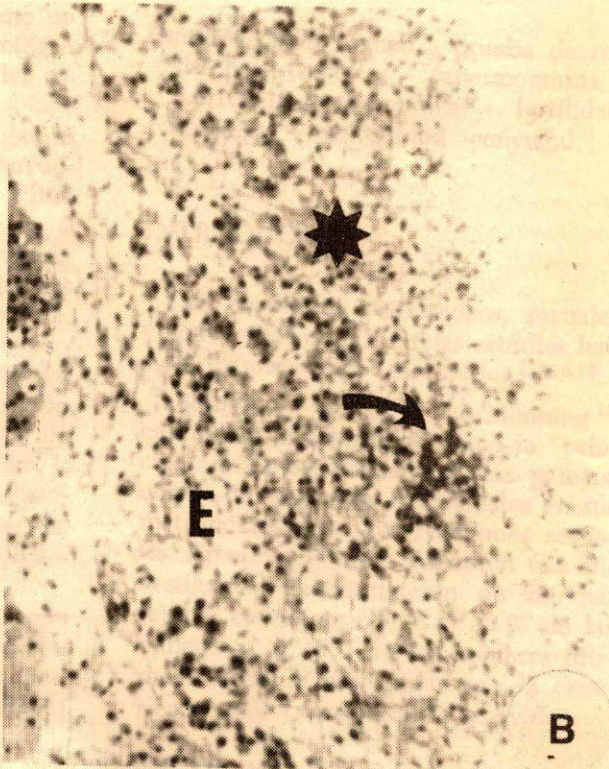


FIGURE 1:

B: Irradiated ovary. Disorganized sexual cords (asterisk). Zones of edema (E). Focuses of inflammatory infiltration (arrow). Hematoxylin-eosine stain. 400 X.