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# Effect of luteinizing hormone on the right regressing ovary of newly hatched chicks treated during embryonic development

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#### Abstract

We studied the histologic and stereological changes induced in the right ovary of newly hatched chicks treated with LH during their embryonic development. Results indicate that LH administration causes a diminution in size and total volume (P < 0.01) of the right ovary, as well as a decrease in the total volume of lacunar channels, blood vessels, and interstitium. Other changes obtained after LH treatment were a reduction (P < 0.001) in the number of germ cells, as well as an increase in the total volume of interstitial cell cords (P < 0.01). This expansion is due to the increase of cellular volume of interstitial cells (P < 0.001) and not to their number, which decrease in the LH-treated right ovary. All these modifications were similar to those occurring in the regressing right ovary during development. The findings suggest that the right ovary of the newly hatched chick is able to respond to LH treatment during embryonic development, inducing marked histologic changes that accelerate its regression. © 2012 Elsevier Inc. All rights reserved.

Keywords: Chick; Right ovary; Luteinizing hormone; Development

#### 1. introduction

In avian species, synthesis of sex steroids by embryonic gonads is regulated by two pituitary gonadotropins: LH and FSH, composed of common  $\alpha$  [1], but distinct  $\beta$  subunits [2]. The cDNA sequence for the avian LH $\beta$  [3,4] has been determined. The gene encoding LH $\beta$  is expressed in chickens beginning on day 4 of embryonic development and LH $\beta$  immunopositive cells start to appear in the anterior pituitary gland on day 8 of egg incubation [5,6]. Woods et al [7] reported

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that LH is present in the plasma of male and female embryos as early as day 10.5, and LH-positive cells exist inside the left ovary during development.

Studies on the effect of LH on both left and right ovaries have focused mainly on the synthesis of sex steroids. Some investigations have found that both growing and regressing ovaries of the chick embryo, under organ culture conditions, respond to mammalian LH (eLH and oLH) with an increase in cyclic AMP concentration and in 17- $\beta$  estradiol and testosterone production [8,9]. Histologic and quantitative studies in the chick embryo have been restricted to the effect of LH on the left functional ovary, but not on the right one, which regresses. Only Avila et al [10] reported the effect of LH on both left and right ovaries of chick embryos in organ culture, mainly focusing on the ultrastructure of interstitial cells.

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A previous report from our laboratory described morphologic changes and variations in the different cell subpopulations [11,12] of the left ovary of newly hatched chicks by treatment with LH during embryonic development, but the effect of LH on the right ovary, under the same conditions, has not been assessed yet.

Therefore, the purpose of the present study was to evaluate the histologic and stereological changes induced in the diverse structures and variations in the number of cell subpopulations of the right ovary of newly hatched chicks treated in vivo with LH during their embryonic development.

#### 2. Materials and methods

#### 2.1. Animals and experimental procedures

Fertile eggs from White Leghorn chicks (Babcock B-300) were obtained from a hatchery of the state of Puebla, Mexico, and incubated at 38°C and 58.3% relative humidity in a forced drafts incubator. Embryos

were divided into three groups. Group I was treated with 0.9% NaCl (control), group II received 1  $\mu g$  of equine LH per embryo, and group III received 10  $\mu g$  of equine LH (NIH-LH-SI) per embryo, dissolved in 0.9% NaCl, applied to the chorioallantoic membrane through a small opening in the egg shell, on days 13, 15, and 17 of incubation, in a volume of 100  $\mu$ L. We used eLH because some studies have shown that eLH preparations exhibit gonadotropic activity in birds [9,11–13], as well as for its commercial availability. Chicks were killed by decapitation 24 h after hatching. The sex of the newly hatched chicks was determined through in situ microscopic examination of the gonads. The right ovaries of 23 chicks per group were dissected, cleaned from adhesive tissue, and measured (length and width).

### 2.2. Histologic and stereological evaluations

Immediately afterward, eight right ovaries per group were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraplast. Cross sections (5- $\mu$ m) were

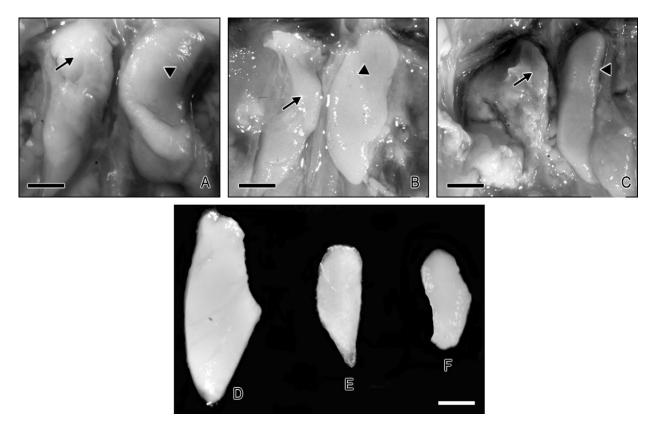


Fig. 1. Stereomicroscopic view of left and right ovaries of newly hatched chicks, control and treated with LH. Note their position in the body cavity and disparity in size between both ovaries. Control (A), 1  $\mu$ g LH-treated (B), and 10  $\mu$ g LH-treated (C) chicks. Note the differences in size among the right ovary of control (D), 1  $\mu$ g LH-treated (E), and 10  $\mu$ g LH-treated (F) chicks. Magnification is represented by a 1.13-mm scale bar (A–C) and 0.7-mm scale bar (D–F). Right ovary ( $\rightarrow$ ), left ovary ( $\triangleright$ ).

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