The Effect of Clomid (Clomiphene Citrate) on Chickens

II. Histo-Physiological Study

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ABSTRACT

A group of 16 Single Comb White Leghorn-type pullets were force-fed 2 mg of Clomid[2]/bird/day. Overstimulation of the ovarian and oviductal activities was observed. The germinal epithelia exhibited high cuboidal cells and the cortices contained numerous developing follicles.

In the oviduct, at the funnel magnum junction, the cilia became crowed and the secretory cells were hyperplastic with cytoplasms full of secretions. Sections taken from the magnum showed that the epithelium mucus cells were ciliated and loaded with secretions. The arrangement of these cells was irregular giving the entire epithelia a pseudostratified-like pattern. The glandular cells were hypertrophic and hyperchromatic.

The shell gland exhibited epithelium of highly pseudostratified columnar cells. Some of these cells were hyperplastic; the others were hypertrophic. The cell's cytoplasms were full of secretions.

INTRODUCTION

In a previous experiment, which was initiated and conducted in this department, it was found that Clomid significantly improved feed intake and egg production of laying pullets (Shanawany *et al.*, 1978).

The objective of the experiment reported here was to investigate the histo-physiological effects of Clomid in laying pullets.

MATERIALS AND METHODS

Thirty-two (32) Single Comb White Leghorn-type pullets, 24 weeks of age, were housed in individual laying cages, one bird per cage, employing standard feeding and management practices.

After being in production for one month, the pullets were then divided equally into two groups. Each pullet in the first group was force-fed 2 mg daily of clomiphene

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citrate (Clomid) suspended in 1 ml sterile saline. Similarly, pullets in the second group, acting as control were force-fed 1 ml sterile normal saline daily.

After one week of force feeding, five pullets of each experimental group were killed. Thyroids, thymi, adrenals, kidneys, ovaries, oviducts, spleens and livers were removed, trimmed from adherent tissues, dehydrated in a graded series of alcohol solutions and embedded in paraffin wax. Sections 5 micron thick were prepared and stained with Hematoxlin-Eiosin for microscopic study.

RESULTS AND DISCUSSION

The ovary

Figures 1 and 2 reveal that the ovarian epithelia of clomiphene fed pullets possessed high cuboidal cells and that the arrangement of the cells was irregular, whereas, the epithelia of the control pullets showed simple cuboidal cells having boxes or cube-like shapes regularly arranged.

Cortices of clomiphene citrate fed pullets contained developing follicles, whereas, the control pullets exhibited the same histology, but to a lesser extent (Figs. 3 and 4).

This finding coincides with a previous anatomical investigation reported by Shanawany *et al.* (1978). They reported that clomiphene citrate increased the number of follicles and that the ovary of clomiphene fed pullets were heavier than those of the control.

The funnel

In both clomiphene fed and control pullets, the funnel epithelia were simple columnar, ciliated and non-secretory. At the funnel-magnum junctions, the cilia

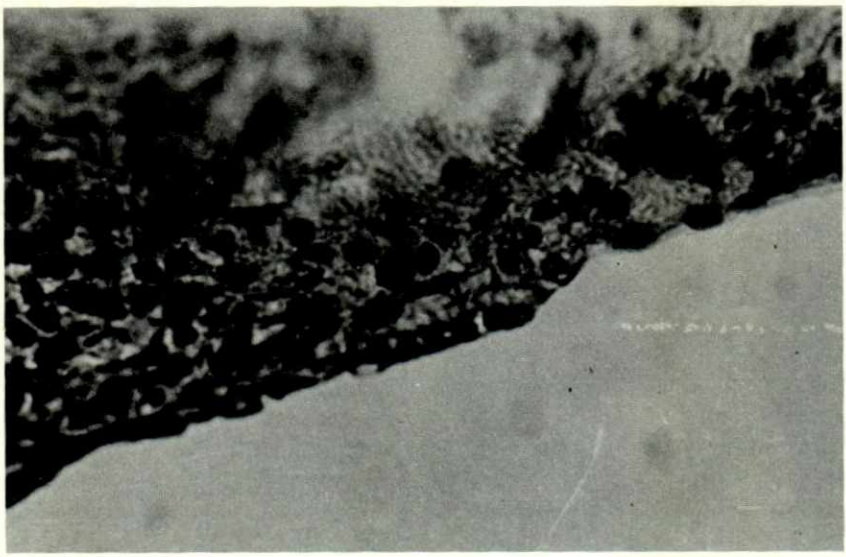

Fig. 1. Germinal epithelium of Clomid-fed pullet. The epithelium consists of high cuboidal cells (× 70).

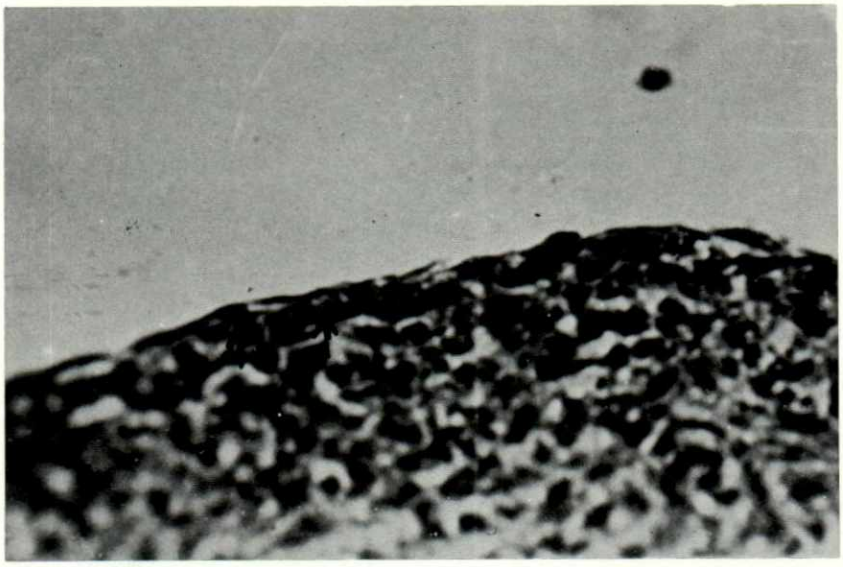

Fig. 2. Germinal epithelium of control pullet. The epithelium consists of low cuboidal cells. (× 70).

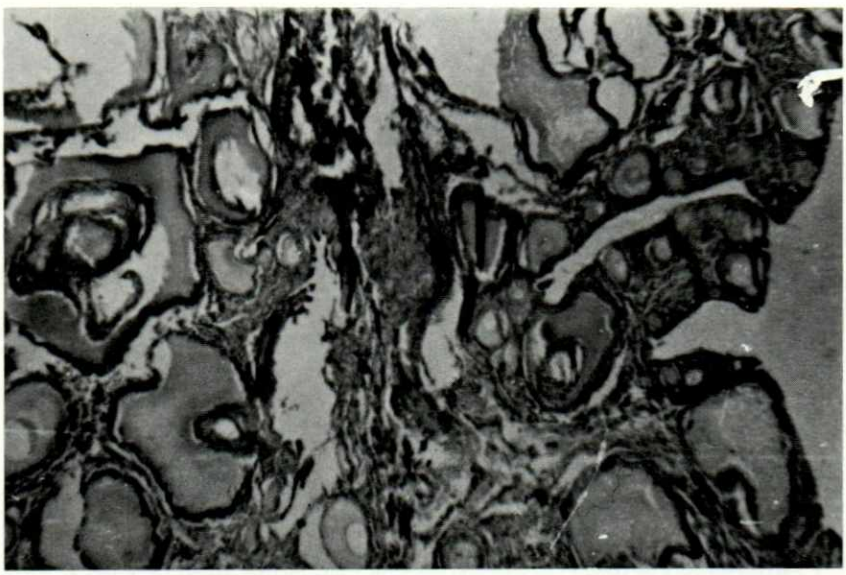

Fig. 3. Ovary of Clomid-fed pullet. Numerous developing follicles are in evidence (× 200).

became crowded and the secretory cells were in evidence. Aitkins (1971) reported that in the funnel of the laying hen, virtually all cells are ciliated and non-secretory and that the secretory cells appear toward its caudal end.

The funnel-magnum junctions of climiphene fed pullets exhibited pronounced hyperplastic secretory cells with their cytoplasm full of secretion (Figs. 5 and 6). This histological change was attributed to the indirect influence of the drug.

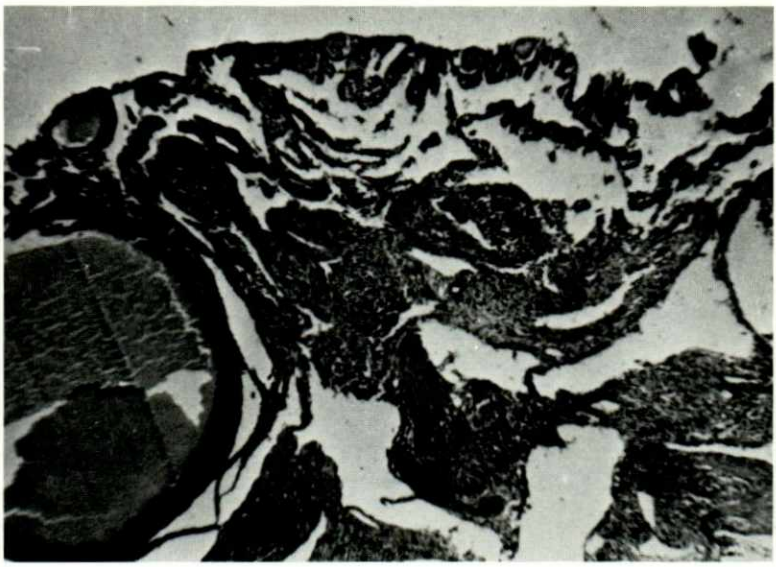

Fig. 4. Ovary of the control pullet (× 200).

Over stimulation of the ovary by the drug seems to increase the activity of the funnel. Each time the ovulated ovum passes it is grasped and engulfed by the funnel. Thus, in successive ovulations, the activity of the funnel increases.

The magnum

Sections from this part of the oviduct showed that clomiphene fed pullets possessed epithelia which consisted of cuboidal cells that were highly ciliated and loaded with mucus secretion (Fig. 7). The arrangement of these cells was irregular giving the entire epithelia a pseudostratified like pattern (Fig. 8).

The glandular cells were hypertrophic and hyperchromatic. The cytoplasms, which were full of secretion droplets, were compressing and depressing the nuclei thus making them appear crescent like (Figs. 10 and 11). The increased volume of secretion compressed the connective tissue making it indistinguishable.

Successive ovulations, due to the drug, stimulate the magnum in an attempt to add albumen each time the ovulated ovum reaches this region during the course of egg formation. Thus, the cells become hyperplastic and hyperchromatic.

Gilbert (5) reported that three possibilities might be responsible for the release of albumen into the lumen of the magnum:

(a) The developing egg, either by direct mechanical stimulation or by some chemical substances diffusing from the yolk;

(b) by some humoral agents which synchronize albumen release as the egg passes down the oviduct; or

(c) that a neural co-ordinating mechanism could be involved.

The isthmus

Sections taken from the isthmi of clomiphene force-fed and control pullets did not reveal any histological changes that could be attributed to the influence of the drug.

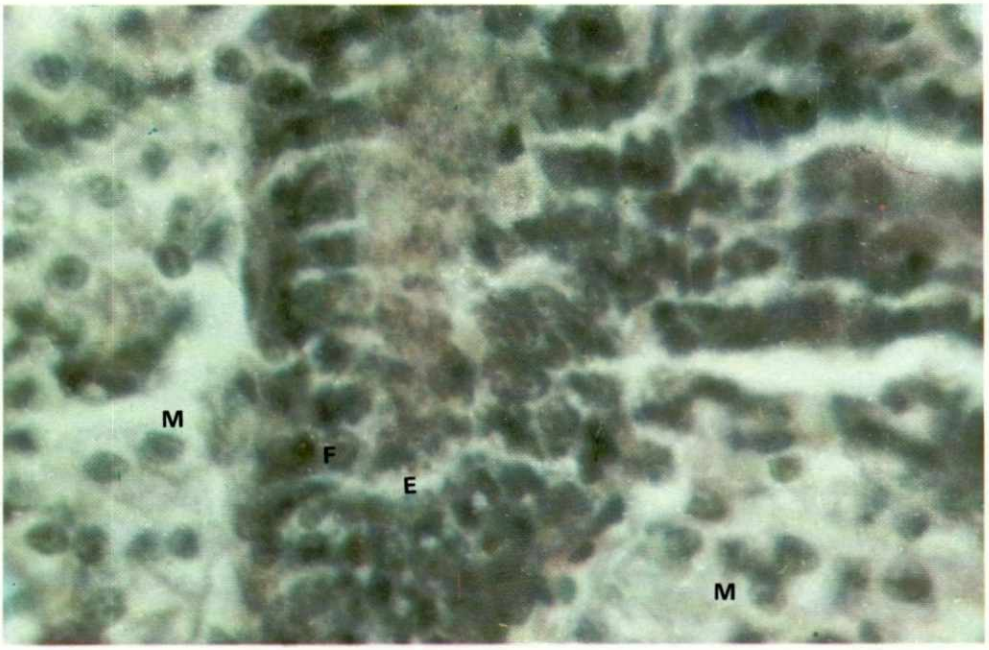

Fig. 5. Funnel-magnum junction (F-M) of Clomid-fed pullet. The funnel secretory cells are hyperplastic. H and E ($\times 1200$).

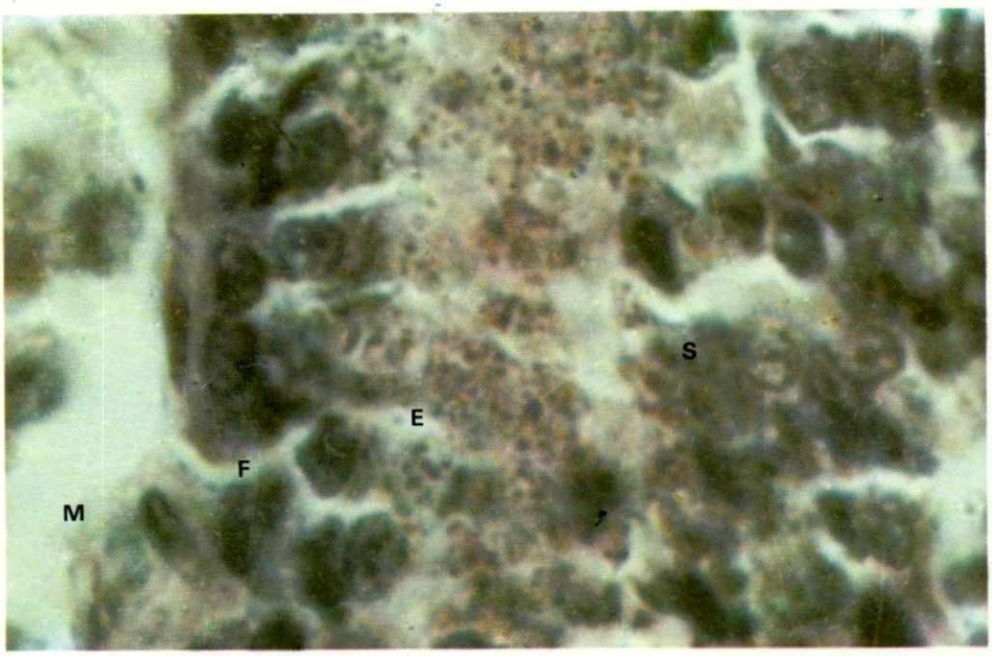

Fig. 6. Funnel-magnum junction (F-M) of Clomid-fed pullet. The secretory cells (S) exhibited hyperactivity with increased volume of secretion. H and E. ($\times 2000$).

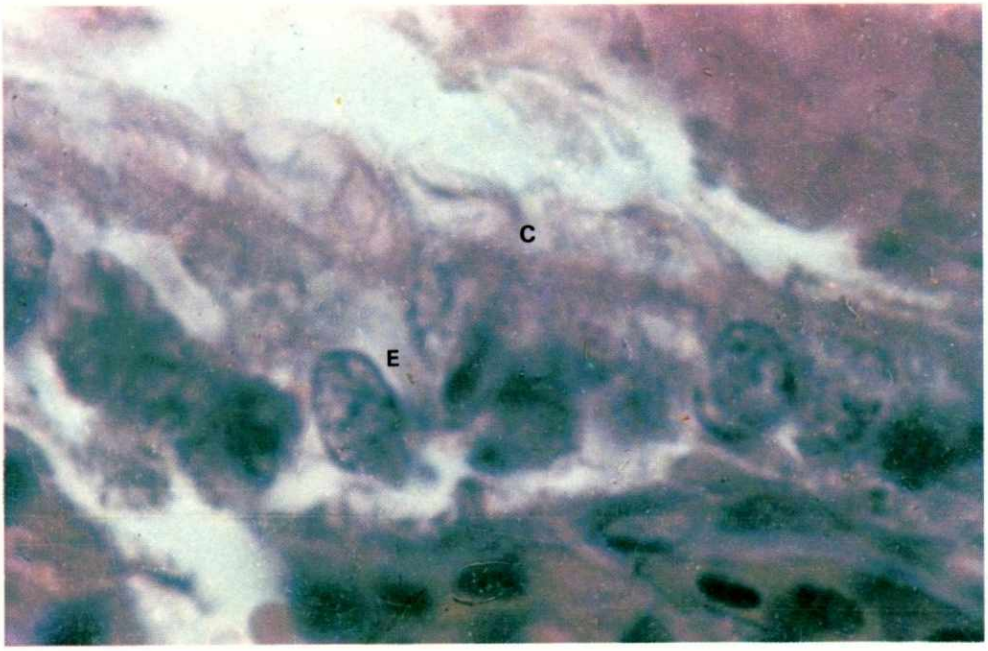

Fig. 7. Magnum of Clomid treated pullet. The cells are highly ciliated (c) and the secretory cells are loaded with secretion (e). H and E. (× 2000).

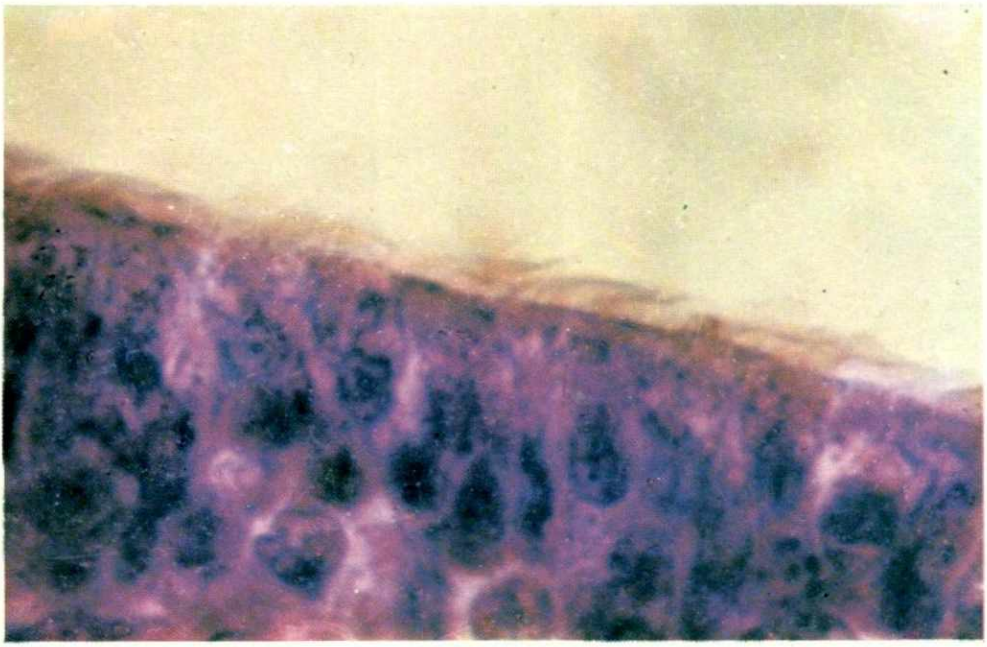

Fig. 8. Magnum of Clomid treated pullet. The lining epithelium is irregularly arranged. H and E. (× 2000).

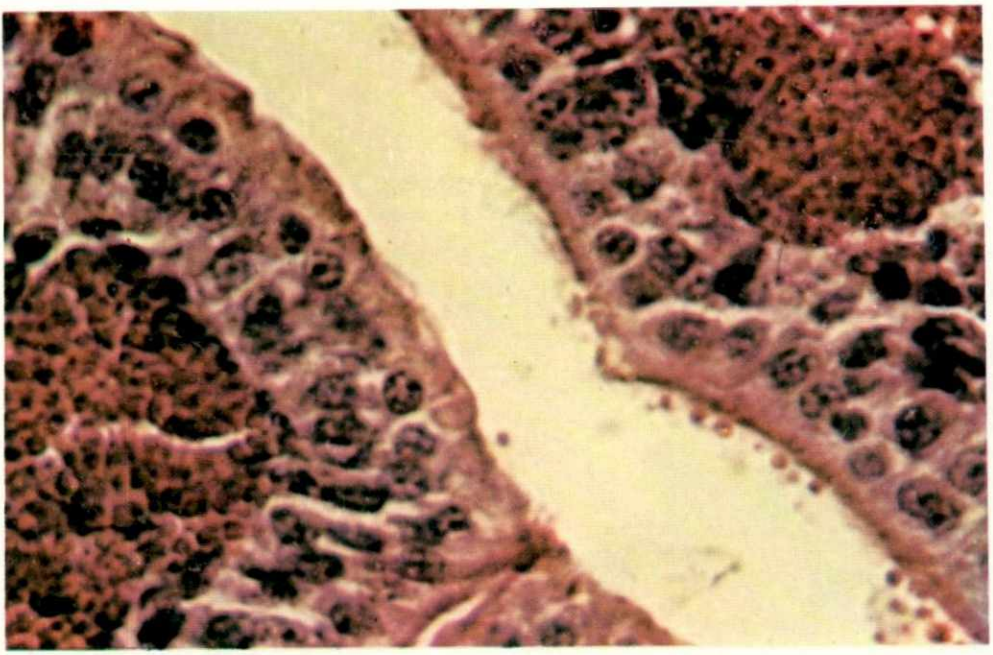

Fig. 9. The magnum of a control pullet. The lining epithelium consists of ciliated and secretory cells. The reduction in height of the cells is characteristic of low activity. H and E. (× 1200).

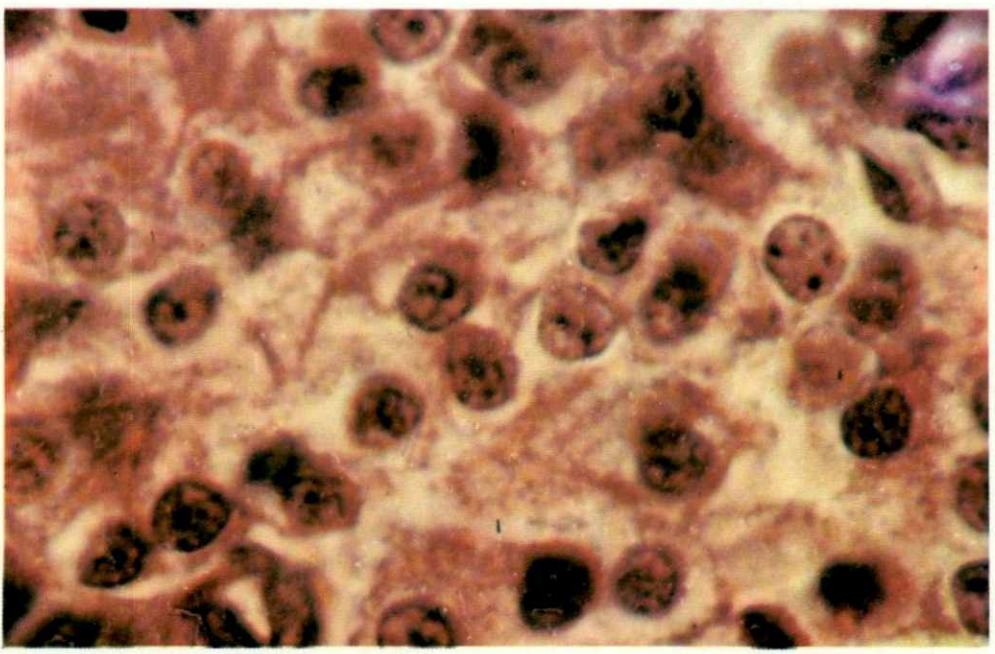

Fig. 10. Magnum of treated pullet. The cells are hypertrophic and hyperchromatic. H and E. (× 2000).

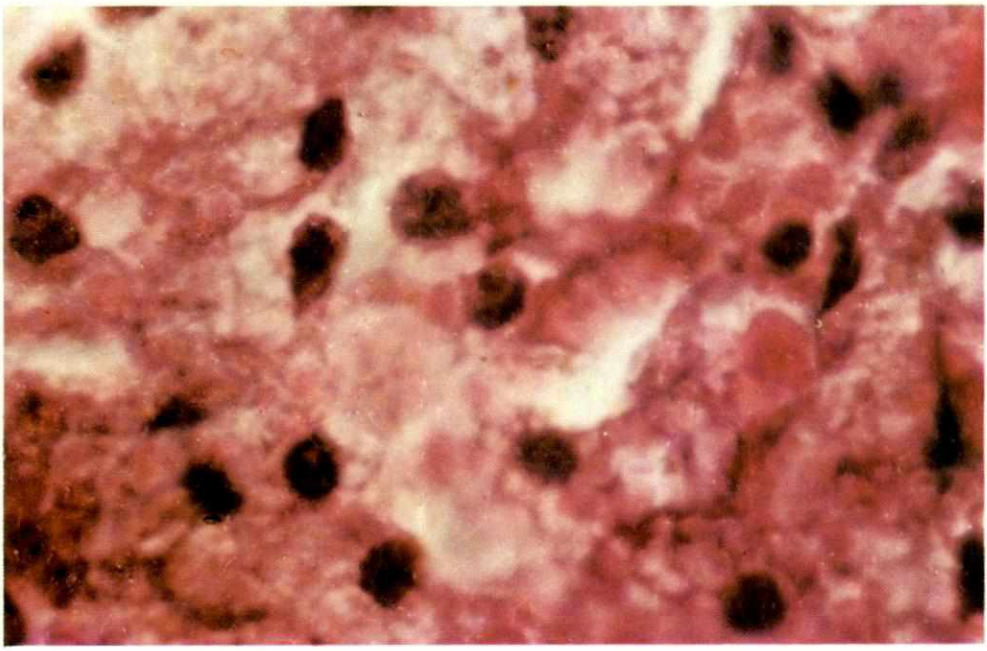

Fig. 11. Magnum of treated pullet. The glandular cells are full of secretion making some of the nuclei and the connective tissue undistinguishable. H and E. (× 2000).

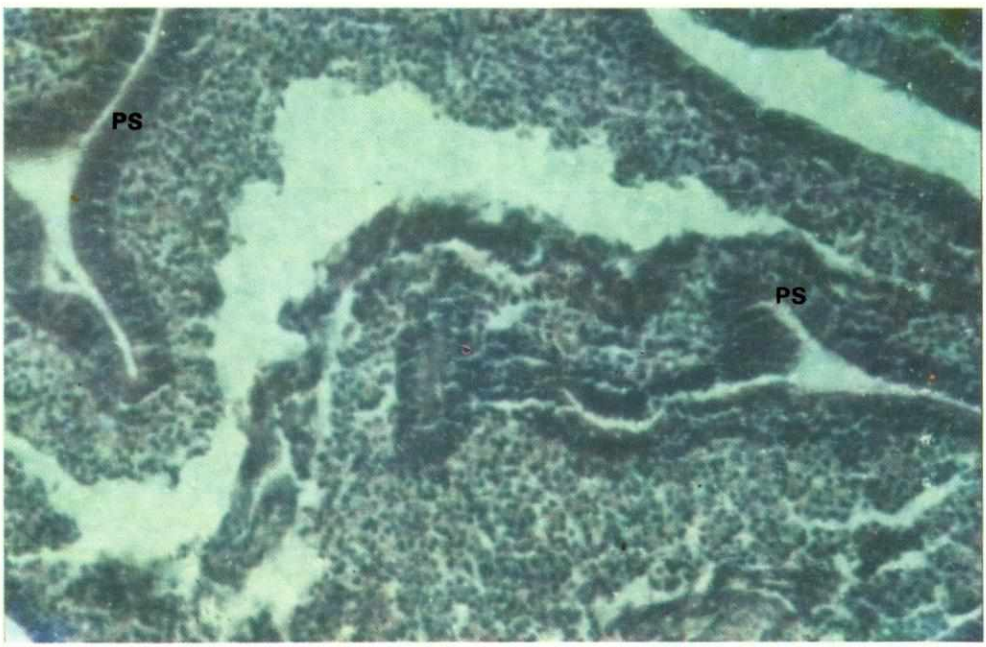

Fig. 12. Shell gland of a Clomid-fed pullet. The epithelium exhibites pseudostratified ciliated columnar cells (PS). The cells are dense and crowded. The glandular cells are hyperplastic. H and E. (× 200).

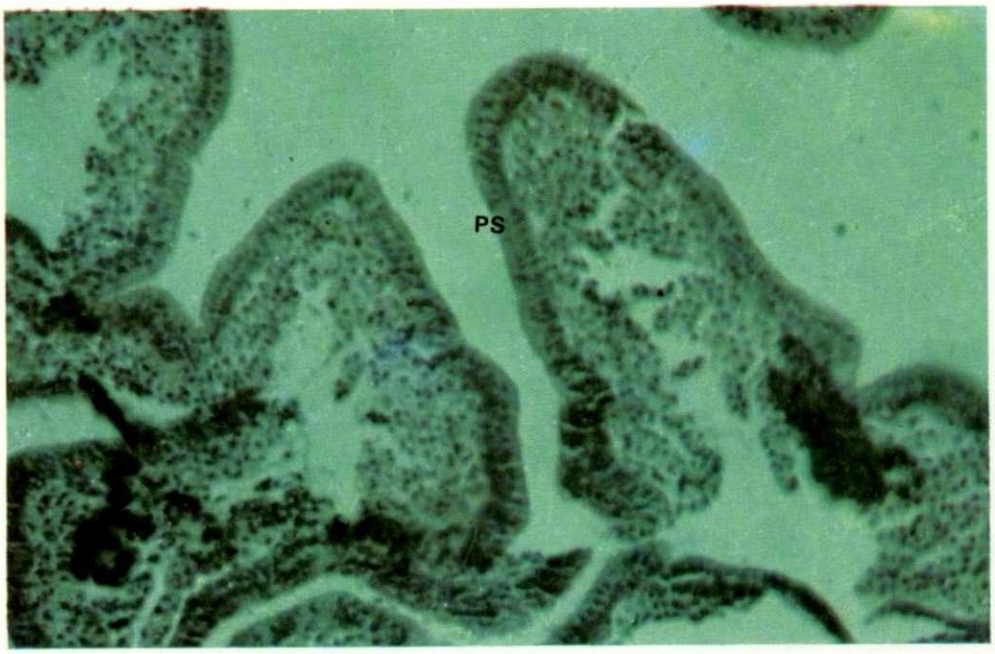

Fig. 13. Shell gland of a control pullet. The epithelium exhibits pseudostratified ciliated columnar cells. The cells are uniform. The glandular cells are normal when compared with Figure 12. H and E. (× 200).

Isthmi of clomiphene force fed and control pullets possessed ciliated columnar epithelia. The glandular cells were full of secretion.

The shell gland

In clomiphene force-fed pullets the epithelia covering the folded shell region exhibited psuedostratified columnar cells. The nuclei were lying at different levels, as seen on Hematoxlin-Eiosin section. The cells were abundant and highly ciliated, their lumens were full of secretion as shown in Figures 14 and 15. Some of these cells were hyperplastic and some were hypertrophic.

The presence of successive eggs in the shell gland, due to overstimulation of the ovary by clomiphene citrate, activates the ciliated and the secretory cells to absorbe and deposit more calcium.

Hobman and Schraer (1969), as reported by Simkiss and Taylor (10), found that the shell glands ciliated epithelia of the laying hen actively move calcium ions from the blood stream. The calcium ions are temporarily stored in the mitochondria when calcification is not occurring, but are moved out and transported outside of the cells via the endoplasmic reticule during shell formation.

Gay and Schraer (1967), as reported by Simkiss and Taylor (10), found that the mitochondria of the shell gland accumulates calcium to a much greater extent than do those of the liver, but their ability to accumulate calcium is greater when the shell is not being calcified.

The two sources of calcium for shell formation are the medullary bones and food. Under the influence of clomiphene citrate, calcium mobilization from the medullary bones increases. At the same time, calcium absorption from the intestine increases,

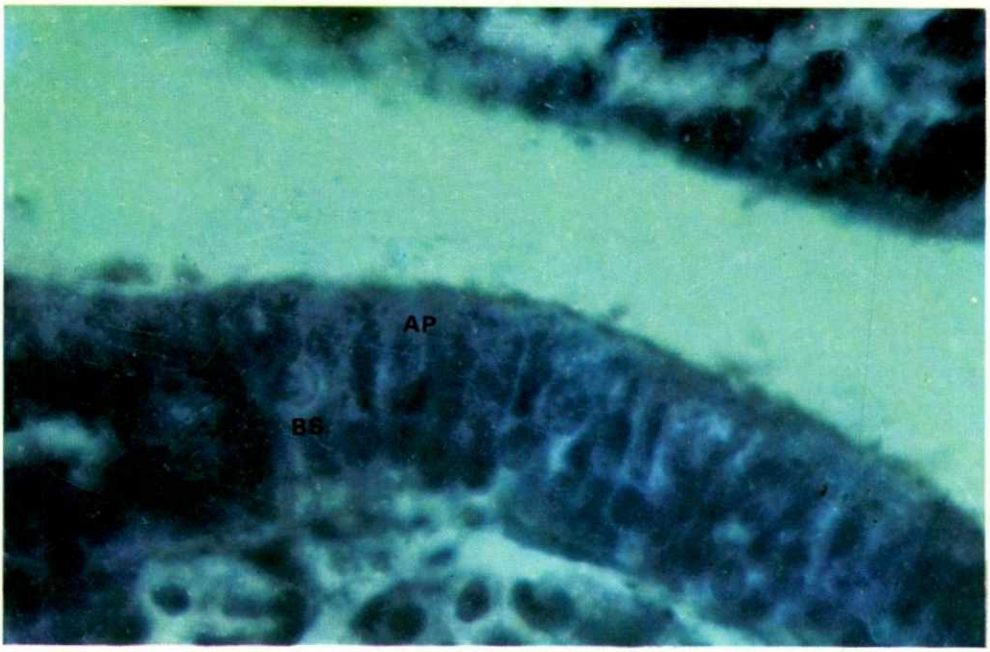

Fig. 14. Shell gland of Clomid-fed pullet. The pseudostratified ciliated columnar epithelium is in evidence. The cell near the gland lumen are ciliated apical (AP), the cell near the basement membrane are non-ciliated basal. H and E. (× 2000).

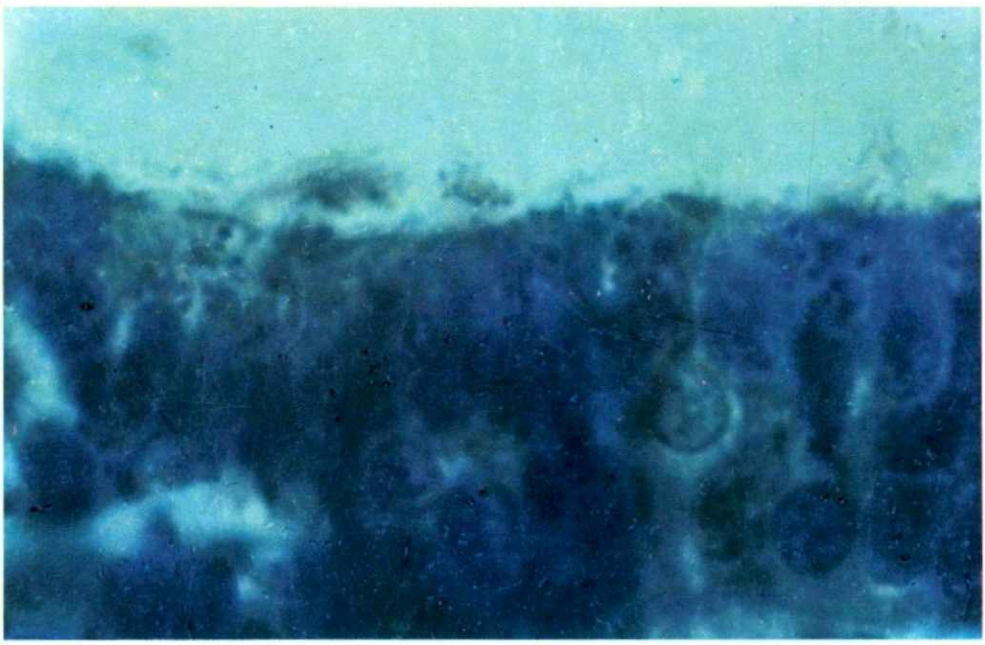

Fig. 15. Shell gland of Clomid-fed pullet. Both apical and basal cells are active, abundant and their cytoplasms are full of excretion. H and E. (× 2000).

thus, the demand on calcium for shell formation is met by these two sources.

McGinnis and Wallace (6) reported that clomiphene citrate increases blood calcium in chickens.

Shanawany *et al.* (9) found that clomiphene citrate slightly increased shell thickness in the treated pullets as compared with those of the control. Feed intake was significantly increased in birds force fed clomiphene citrate. The treated birds consumed 42 grams of feed per day more than the control.

Bloom *et al.* (1958) as reported by Sturkie (11), showed that in high producing hens the medullary bones undergo a sequence of bone formation and destruction during the laying cycle. In the early stages of shell formation, when calcium is being absorbed from the gut in large amounts, both osteoblasts and osteoclasts are abundant. In the advanced stages of shell calcification, when supplies of dietary calcium may not be so plentiful, the bone trabeculae are surrounded by huge numbers of osteoclasts and the osteoplasts are rare. When the shell calcification was not in progress, the cell population was very variable.

Comar and Driggers (1949), as reported by Sturkie (11), found that 60 to 75% of the calcium in the egg shell comes from the food.

Mobilization and inhibition of calcium from the medullary bones are uner hormonal control. Parathormone from the parathyroid triggers calcium mobilization, whereas, calcitonin from the ultimobranchials inhibit further calcium resorption.

Al-Khazraji (3) found that removal of ultimobranchial glands resulted in a reduction in the efficiency of calcium utilization of laying pullets.

Al-Khazraji *et al.* (2) found that at 19 weeks of age, the ultimobranchial cells of pullets exhibited some degree of hyperplasia and hypertrophy. These changes were pronounced and accompanied with hyperchromatic nuclei at 36 weeks of age, i.e. at the peak of egg production.

Other tissues

No histological changes due to clomiphene citrate were observed on sections taken from thyroids, thymi, adrenals, kidneys, spleens or livers.

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تأثير عقار الكلوميد على الدجاج
٣ — دراسة هستو فسيولوجية

ع . ك . الخزرجي ، م . م . شنواني ، ع . حامد

المستخلص

لقد أعطى عقار الإخصاب (كلوميد) لدجاج بيوض بعمر ٢٤ أسبوع بمعدل ٢ ملجم / لكل طير / لكل يوم . وقد اتضح من النتائج الهستولوجية بأن هذا العقار سبب تحفيز خلايا كلّ من المبيض وقناة البيض ، وزاد من فعاليتها الوظيفية .

والصور المنشورة طى البحث توضح نوعية هذا التحفيز وتأثيراته الفسيولوجية والهستولوجية .