# Effects of Clomiphene citrate on the histological structure of the Iraqi domestic chicken's ovaries

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## Abstract

Clomiphene citrate is commonly used to induce ovulation in women. However, the effects of clomiphene citrate on the domestic chikens ovary's have not been fully elucidated. Therefore, we used histological analyses to examine the ovaries of Iraqi domestic chickens administered clomiphene citrate (25 mg/kg of body weight) daily for 30 and 60 days. Our results demonstrated that the ovaries from the hens that were administered clomiphene citrate for 30 and 60 days were not morphologically or histologically distinct. However, compared to the control group, both these groups showed a significant treatment effect on ovarian diameter and the number of follicles ($P < 0.05$), from the result we can conclusion that the clomophene citrate stimulating the ovalation in domestic chikens .

**Keywords:** Clomiphene citrate, chicken ovary, histological structure.

## Introduction

Clomiphene citrate, commonly used to induce ovulation in women, is an anti-estrogenic agent that stimulates the secretion of pituitary gonadotrophic hormones. It stimulates ovulation by blocking the effect of estrogens at the receptor sites in the hypothalamus and pituitary gland (Kerin etal ,1985). The structure of clomiphene is similar to that of estrogen. Similar to other hormones, estrogen exerts its effects throughout the body by occupying its receptors; the occupied receptor signals the organ. In the brain, estrogen directs the release of follicle-stimulating hormone (FSH) (reference). Clinical studies have demonstrated that while clomiphene citrate induces ovulation in 80% of physically capable

women, it results in a pregnancy rate of only 40% (Speroff *et al.*, 1983). O'Keefe and Marrone (1986) reported that clomiphene citrate inhibits androgen and estrogen production in thecal cells in vitro by decreasing the activity of C17–C20 lyase and aromatase. The effects of clomiphene citrate on the hypothalamic-pituitary axis are widely accepted.

Clomiphene citrate antagonizes estrogen receptor (ESR) signaling in female reproductive tissues including the ovaries in rodents (Chaube *et al*., 2006; London *et al*., 2000), fallopian tubes in chickens (Sutherland *et al*., 1977), and endometrium in humans (Gonen and Casper 1990; Homburg *et al*., 2006). It also acts as a strong agonist in non-reproductive tissues such as bone and the liver (Turner *et al*., 1998). In rats, clomiphene citrate induces apoptosis of ovarian granulosa cells, which can be res
cued by 17ß-estradiol (E2) in vivo (Chaube *et al.,* 2006, Nagao and Yoshimura, 2001). Thus, clomiphene citrate acts as an ESR antagonist that regulates apoptosis in the tissue and cellular targets of estrogen signaling. Apoptotic changes in human chorionic villi and decidual tissues may increase the risk of tubal ectopic pregnancy (Kokawa *et al*., 1998).

Clomiphene citrate may impair fertility by affecting the cervical mucus and causing endometrial dysfunction. The drug is as efficacious at a dose of 50 mg as it is at a dose of 100 mg; moreover, the lower dose helps avoid some undesirable side effects. However, other adverse effects may occur at this dose, including multiple pregnancies, an increase in the rate of multiple births, and ovarian hyperstimulation. Ovarian cancer may also develop owing to 50 mg clomiphene citrate administration; however, this claim is yet to be substantiated (Sovino *et al*., 2002).

The aim of the present study was to assess the effects of chronically administered clomiphene citrate on ovulation in domestic chickens. We used the left ovary and oviduct to represent the reproductive organs of the hen. The ovary consists of an outer cortex enveloping a vascular medulla and containing ovarian follicles of various sizes. Covering the cortex is a layer (germinal epithelium) of cuboidal or flattened cells. Below the epithelium is the tunica albuginea, composed of

dense connective tissue, and below the tunica albuginea is a stroma of loose connective tissue (Bacha and Bacha, 2000).

## Materials and Methods

Thirteen Iraqi domestic hens, *Gallus gallus domesticus*, aged 30 weeks were subjected to a daily 14-h light to 10-h dark cycle. The hens were provided water and commercially available chicken feed. They were treated daily for 30 and 60 days with clomiphene citrate (25 mg/kg of body weight). After killing the hens, the ovaries were removed, fixed in 10% formalin, dehydrated, and embedded in paraffin. Sections (5 mm) were prepared and stained with hematoxylin and eosin (H & E; Luna, 1960). The histological structure of the ovary was examined using light microscopy, and the data were recorded and analyzed. Data are expressed as mean ± standard error of the mean (SEM) and analyzed by two-way analysis of variance followed by Fisher's least significant difference analysis. Differences among means were considered significant at $P < 0.05$ Analyses were performed using Statistical Product and Service Solutions, 2006, software (IBM Corporation, Armonk, NY, USA).

## Results

No significant histopathological differences were observed in the ovaries of hens following administration of clomiphene citrate (25 mg/kg of body weight) daily for 30 or 60 days in comparison with those of the control group (Fig. 1, 2, and 3). However, histometric evaluation showed that clomiphene citrate administration for 30 and 60 days stimulated ovulation and significantly affected the diameter of the follicles, compared to the control group ($P < 0.05$). The diameter of the ovarian follicles in the group of hens treated with clomiphene citrate during all stages of follicular maturation (follicle selection, slow-growing white follicles, and final differentiation) was similar at 30 and 60 days of treatment but significantly greater than that for hens in the control group, as shown in Table 1. While there was no significant difference in the number of follicles (10 follicles in 15 $mm^2$) between 30 and 60 days of treatment, administration of clomiphene citrate significantly increased the

number of ovarian follicles in both these groups compared to the number in the control hens (6 follicles in 15 $mm^2$, $P < 0.05$) (Fig. 4, 5, and 6).

We observed numerous small immature follicles on the surface of the stalks in the cortical tissue (Fig. 7) as well as on the surface of mature large follicles (Fig. 8 and 9). There was no histopathological effect of clomiphene citrate on the layers of the ovaries between the two experimental groups (30 and 60 days of treatment), which is consistent with an increase in the yolk-laden oocytes with rounded nuclei (germinal vesicles). The oocytes were surrounded by several layers. Large follicles were suspended from the surface of the ovary by stalks of cortical tissue comprising follicle of varying sizes (Fig. 7). The surface of many large follicles had one or two small immature follicles (Fig. 8).

**Table 1. Diameter of ovarian follicles following daily chronic administration of clomiphene citrate to domestic hens.**

| Period | **30 days** | **60 days** | |
|---|---|---|---|
| | **Diameter (mm2)** | **Diameter (mm2)** | |
| **Control** | 5.0 ± 0.543 a | 5.10 ± 0.543a | 5.05±0.53 |
| **Clomiphene citrate(25mg/kgbody weight)** | 10.7 ± 0.232 b | 10.1 ± 0.248 b | 10.4±0.23 |
| **Mean** | 7.85±0.44 | 8.05±0.540 | |
| **Interaction LSD** 4.02 | | | |

*****Values represent mean ± SEM. Values with nonidentical superscripted letters (a–b) are considered significantly different $P < 0.05$.

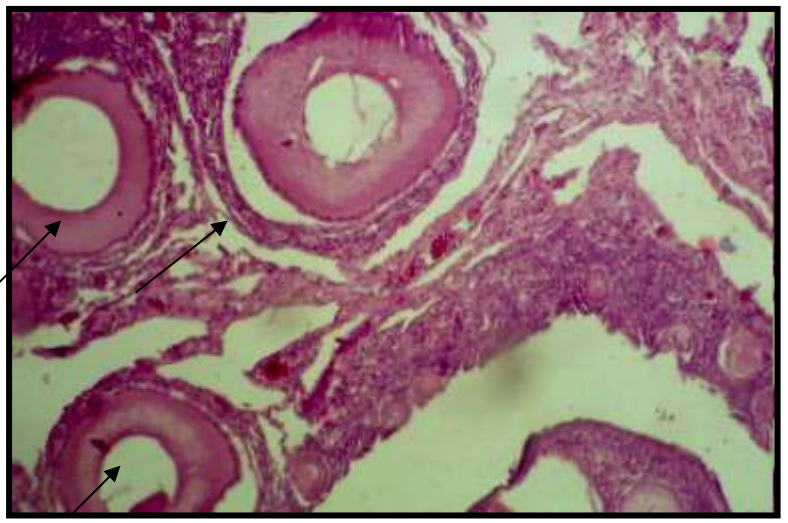

Fig. 1. Tissue from a hen in the control group showing ovarian follicles (arrows). H & E stain, magnification ×400.

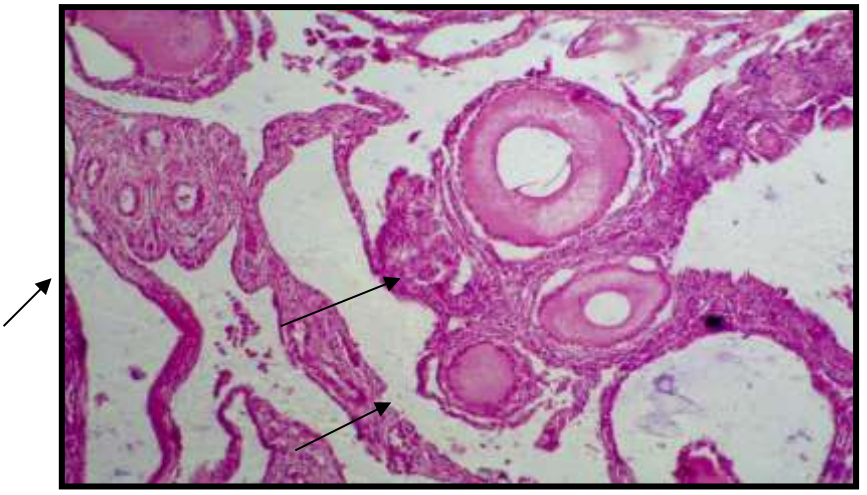

Fig. 2. Tissue from a hen administered clomiphene citrate (25 mg/kg body weight) daily for 30 days showing the various sizes of ovarian follicles (arrows). H & E stain, magnification ×400.

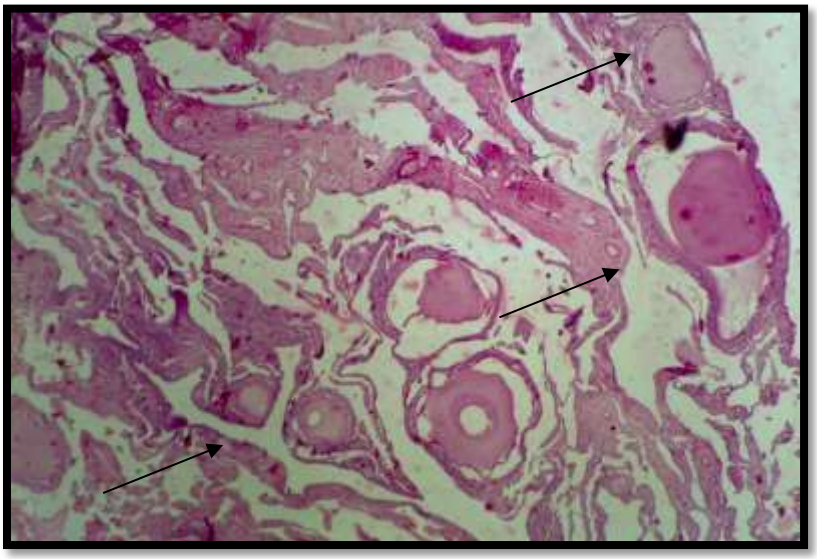

Fig. 3. Tissue from a hen administered clomiphene citrate (25 mg/kg body weight) daily for 60 days showing the various sizes of ovarian follicles (arrows). H & E stain, magnification ×400.

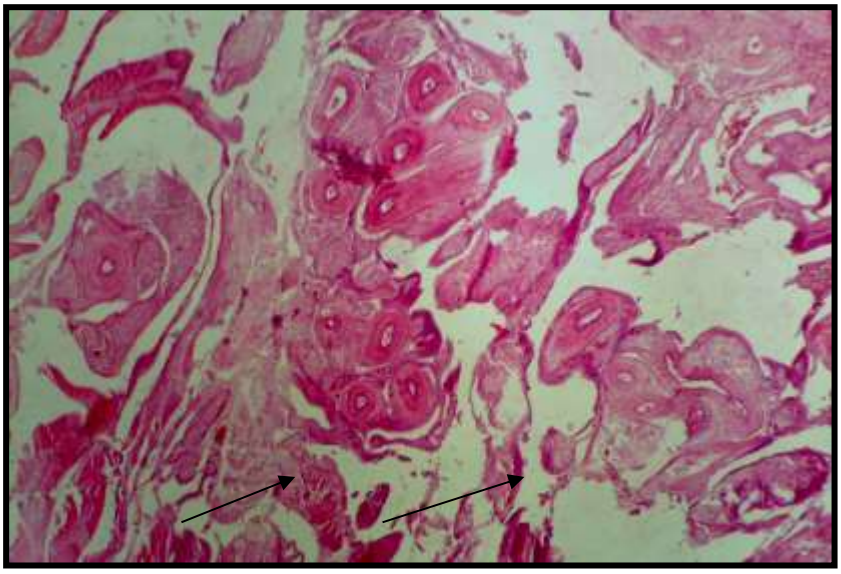

Fig. 4. Tissue from a hen in the control group showing numerous ovarian follicles (arrows). H & E stain, magnification ×170.

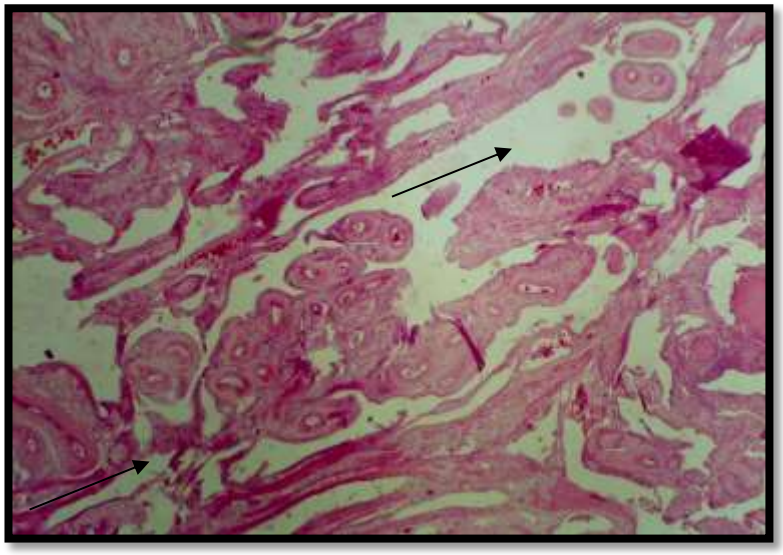

Fig. 5. Tissue from a hen administered clomiphene citrate (25 mg/kg body weight) daily for 30 days showing numerous ovarian follicles (arrows). H & E stain, magnification ×170.

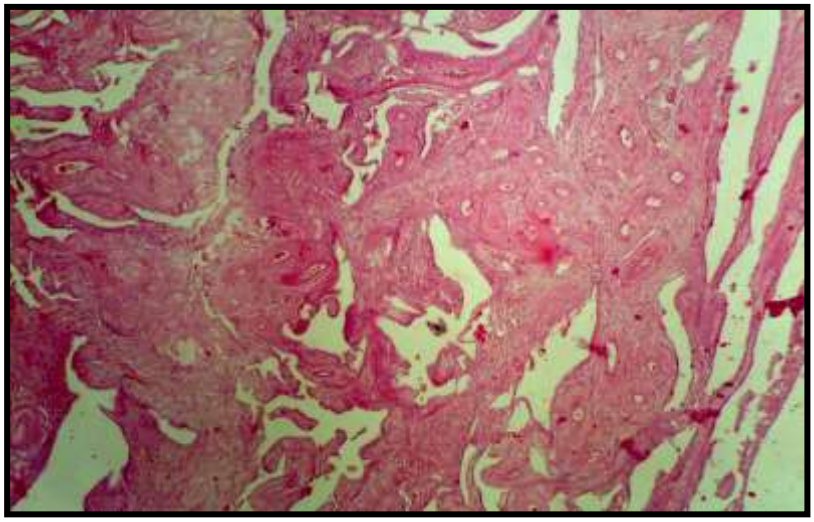

Fig. 6. Tissue from a hen administered daily clomiphene citrate (25 mg/kg body weight) for 60 days showing numerous ovarian follicles (arrows). H & E stain, Magnification ×170.

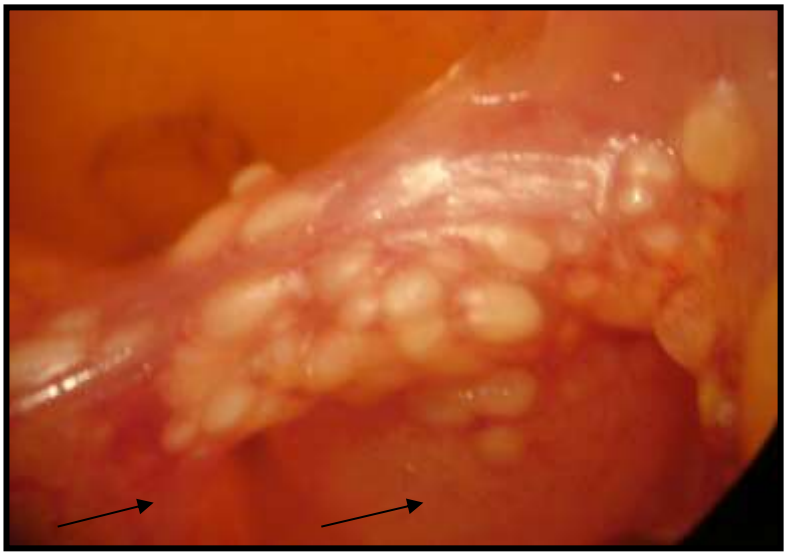

Fig. 7. Image taken during morphological analysis showing the growth of numerous ovarian follicles on the surface of ovarian stalks (arrows). H & E stain,30 x.

Fig. 8. Image taken during morphological analysis showing small follicles on the surface of mature follicles (arrows).

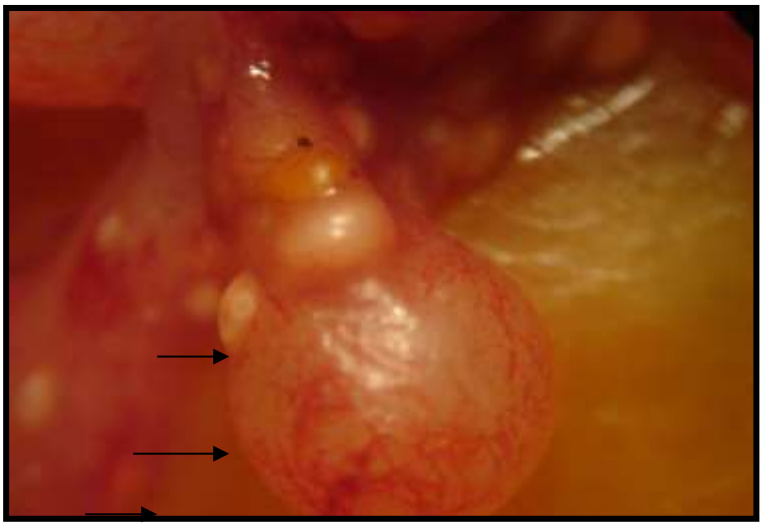

Fig. 9. Image taken during morphological analysis showing numerous immature follicles on the surface of one follicle (arrows), 30 x .

## Discussion

Our results demonstrated that the ovaries from Iraqi domestic chickens administered clomiphene citrate (25 mg/kg of body weight) daily for 30 days and 60 days were not morphologically or histological distinct. However, we found a significant effect of treatment for both of these groups in comparison with hens in the control group on ovarian diameter and on the number of follicles.

Clomiphene citrate exhibits estrogenic and antiestrogenic effects and induces ovulation in a complex fashion. Therefore, clomiphene citrate could have a direct estrogenic effect on gonadotrophs, enhancing sensitivity to gonadotropin-releasing hormone (GnRH). Moreover, clomiphene has a direct estrogenic effect on the ovary, which is similar to that described for the pituitary gland. It sensitizes the granulosa cells in the follicle to the action of gonadotrophins and upregulates aromatase activity. The agonistic or antagonistic effects of clomiphene citrate on the

effectors in the genital tract may also be due to different populations of α and β estrogen receptors in those tissues (Goldstein *et al*., 2000). Adashi (1984) showed that there are three sites of action for clomiphene citrate. The primary site is in the hypothalamus, where clomiphene citrate blocks the negative feedback of estrogen, thereby enhancing the release of gonadotrophins. The secondary sites are in the pituitary, where clomiphene citrate enhances the GnRH-stimulated release of FSH, and in the ovary, where its effects have not been fully elucidated.

Clomiphene citrate inhibits progesterone synthesis in rat follicles (Laufer *et al.*, 1982) and granulosa cells (Welsh *et al.*, 1984), in monkey luteal cells (Westfahl and Resko, 1983), and in hen granulosa cells (Sgarlata *et al*., 1984). O'Keffe and Marrone (1987) conducted an in vitro study to show that clomiphene citrate inhibits luteinizing hormone-stimulated androstenedione and estrogen production in a dose-dependent manner in hen theca cells.

Shao *et al.* (2009) showed that during chronic clomiphene citrate administration in rats, isthmus-specific apoptosis of epithelial cells and activation of ESR2α in cilia act in parallel to block gamete and embryo passage through the fallopian tube, eventually resulting in tubal ectopic pregnancy. Kokawa *et al.* (1989) showed that the apoptotic changes in human chorionic villi and decidual tissues may increase the risk of tubal ectopic pregnancy. However, clomiphene citrate causes ovarian and uterine abnormalities (Nagao and Yoshimura, 2001) and may have adverse effects on the fallopian tube or on apoptotic signaling pathways in this tissue (Kokawa *et al.*, 1998).

In this study we are find that the clomiphene citrate had a good effect on hens ovaries which causes significantly increase of number and diameters of the follicles and increased early egg production .

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## تأثير عقار كلوميفين سترات على التركيب النسيج لمبايض الدجاج المحلي العراقي

**هيفاء عبد علي الشمري**

**قسم التحليلات المرضية \كلية العلوم جامعة ذي قار**

يستخدام عقار كلوميفين سترات عادة للحث على الإباضة عند النساء. ان تاثير عقار كلوميفين سيترات على مبايض الدجاج لم توضح تماما.لذلك اجريت هذه الدراسة النسيجية لفحص تاثير عقار كلوميفين سترات بجرعتين (25 ملغم / كغم من وزن الجسم) يوميا لمدة 30 و 60 يوما على التركيب النسيجي للمبايض. أظهرت النتائج عدم وجود اي تاثيرات مرضية نسيجية لمبايض الطيور المعالجة بالجرعتين من عقار كلوميفين سترات لمدة 30 و 60 يوما ، كما وأظهرت النتائج ان هذه الجرع لها تأثيرمعنوي عند مستوى احتمال ($P < 0.05$) على قطر وعدد بصيلات المبيضية .من هذه النتائج نستنتج بأن عقار كلوميفين سترات يحفز التبويض في مبايض الدجاج المحلي.