Clomid induced hormonal and histological alterations in ovary of albino rats

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Abstract: Clomid is a drug used in treatment of the polycystic ovary syndrome. On the other hand, its use was accompanied with many adverse effects. The present study aims to assess the hormonal and histological changes in ovary of rats given clomid. Treating rats with clomid at doses 10, 50 and 100mg for a week induced degenerative effects in the ovary. The ovarian stroma contained large number of vacuoles, atretic follicles of different sizes and congested blood vessels. The abnormal Graafian follicles appeared with enlarged antrum and degenerated zona pellucid. Morphometrical results indicated significant decrease in the number of ovarian follicles and increase in atretic ones. The results of hormonal analysis revealed significant decrease in the serum level of progesterone and estradiol. These effects may be attributed to the anti-estrogenic effect of clomid.
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Key words. Clomid, ovary, histology, estradiol, progesterone.

1. Introduction

The polycystic ovary syndrome affects 7 to 8% of women and may be the most common cause of female infertility. Women with this syndrome have hyperandrogenism, morphologic changes in the ovary (polycystic), inappropriate gonadotropin secretion (elevated levels of circulating luteinizing hormone), hyperinsulinemia (Homburg et al.1988). Clomiphene citrate (clomid) is among the drugs that used in the treatment of the polycystic ovary syndrome (Burghen et al.1980). It is a selective estrogen receptor modulator that increases production of gonadotropins by inhibiting negative feedback on the hypothalamus and is used mainly in female infertility, in turn mainly as ovarian stimulation to reverse oligoovulation or anovulation, as well as being used for ovarian hyperstimulation, such as part of an *in vitro* fertilization procedure (Ioannidou-Kadis et al. 2006).

Many adverse effects of clomid were recorded such as ovarian enlargement, vasomotor flashes, nausea, vomiting, breast discomfort, headache, abnormal vaginal bleeding, visual symptoms, weight gain and shortness of breath. It has also been reported that clomid induces acute pancreatitis (Siedentopf et al., 1997; Keskin et al., 2007), myocardial infarction (Duran and Raja, 2007), hypertriglyceridemia (Yasar and Ertugrul, 2009), deep vein thrombosis (Benshushan et al., 1995) and pulmonary embolism (Chamberlain and Cumming, 1986). Meijer et al., (2006) reported association between clomid and the birth defects (neural tube defects and hypospadia). Reefhuis et al. (2011) have identified associations between the use of clomid and anencephaly, Dandy Walker malformation, septal heart defects, muscular VSD, coarctation of the aorta, esophageal atresia, cloacal exstrophy, craniosynostosis and omphalocele. Nagao and Yoshimura, (2001) showed that clomid caused ovarian and uterine abnormalities. It also induced apoptosis and degeneration in fallopian tube (Shao et al., 2009). The present work aims to study the effects of clomid on albino rat ovaries.

2. Materials and methods

Adult female albino rats with an average weight of 160±5 g were obtained from the animal house unit of the King Fahd Centre for Medical Research in Jeddah, Saudi Arabia. Animals were placed in plastic cages at room temperature (22 ± 2°C), relative humidity 40-65 %, and alternating light and dark conditions for 12-hour periods. The rats were maintained on commercial food consisting of standard rat chow and had free access to drinking water. All animals received care in accordance with the methods approved under the institutional guidelines for the care and use of laboratory animals at the King Fahd Centre for Medical Research, Jeddah. Rat food pellets were purchased from the Saudi Grain Oils and Floor Mills organisation, Jeddah, Saudi Arabia. The sawdust bedding of the rat cages was changed three times a week and the cages were cleaned and sterilized.

Clomiphene citrate (Clomid)

Clomid is a non-steroidal, ovulatory stimulant designated chemically as 2-[p-(2-chloro-1,2-diphenylvinyl) phenoxy] triethylamine citrate (1:1). A stock solution of clomid was prepared by dissolving clomid in distilled water; three concentrations of 10, 50 and 100 mg/ml (v/v) were prepared. Each concentration of clomid was given to the rats orally by intragastric intubation.

Experimental design

Animals were divided into four groups; each group consisted of 15 rats.

Group 1: The rats of this group were considered as controls. The animals in this group were dissected after two weeks, and the weight of each animal was measured before dissection.

Group 2: The rats of this group were fed daily with 10 mg of clomid/kg body weight for two weeks.

Group 3: The rats of this group were fed daily with 15 mg of clomid/kg body weight for two weeks.

Group 4: The rats of this group were fed daily with 100 mg of clomid/kg body weight for two weeks.

Histological study

Animals from the different groups were dissected, and their ovaries were carefully separated from the adjoining connective tissue and washed in normal saline. Ovaries were fixed in alcoholic Bouin's solution for 48 h and then processed for wax embedding and microtomy. The ovaries were then serially sectioned to a thickness of 6 μm using a rotary microtome. These histological sections were further processed for haematoxylin and eosin staining.

Morphometric data analysis

Haematoxylin and eosin stained sections of ovaries from control and experimental animals were examined histologically and used for morphometric analysis. All serial sections of the ovaries were counted for the various stages of follicle development as described by Bolon *et al*.(1997). Follicles were classified as small (mean diameter < 20 μm), medium (mean diameter 20–70 μm) or large (mean diameter >70 μm) follicles.

Hormonal assays

For hormones determination, blood samples were collected from the inferior vena cava and then centrifuged. Sera were obtained by centrifugation of the blood sample and stored at -20°C. Estradiol and progesterone hormone were determined using radioimmunoassay kits supplied by diagnostic Co, Los Angeles according to Maruyama et al.(1987).

Statistical Analysis

Data were expressed as mean values ± SD and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® 4 Inc., USA).

3. Results

Histological and morphometical results

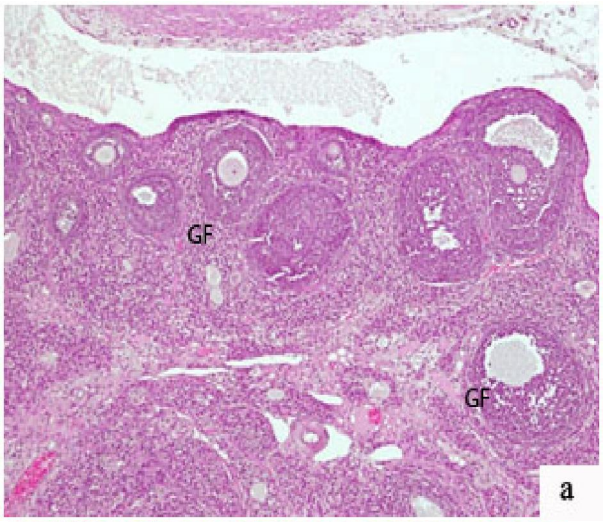
Histological section of ovary of control rat revealed that the developing follicles were well placed and embedded in ovarian stroma together with Graffian follicles, corpus lutea and atretic follicles (Figs.1a&b). Treating rats with 10mg clomid for one week caused degeneration of few follicles (Figs.2a). After treatment with 50 mg, the ovary showed large number of degenerated follicles together with congestion of blood vessels (Fig.2b). Sections in ovaries of rats treated with 100 mg clomid revealed many deleterious histological changes. The germinal epithelium showed abnormal structure including the appearance of many degrees of invaginations along its surface. The cuboidal cells of the germinal epithelium became flattened with deeply stained nuclei and lost their arrangement. The ovarian stroma contained large number of vacuoles, atretic follicles of different sizes and congested blood vessels. Some abnormal Graafian follicles appeared with enlarged antrum and degenerated zona pellucid (Figs.3a,b). The corpora lutea occupied a large area in the section Data in Table (1) showed that treating rats with clomid caused a significant decrease in the number of follicles. The number of small, medium and large follicles was significantly ($P<0.05$) decreased in animals given clomid at doses of 50 and 100 mg.. Conversely, the number of atretic follicles showed a significant increase.

Hormonal results

Data in figure 4a showed that there is a significant decrease in estradiol level in animals given clomid at doses 50 and 100 mg. Similarly, there is a significant decrease in progesterone level in animals given clomid at doses 10 and 50 mg. Highly significant decrease was observed in animals given 100mg (Fig.4b).

4. Discussion

Clomid, a selective estrogen-receptor modulator, presumably works to induce ovulation by inhibiting negative, endogenous, estrogen-feedback on the hypothalamic-pituitary axis resulting in increased FSH secretion, follicular growth, and ovulation (Holtkamp et al.1960). Results of the present work showed that clomid induced many histological alterations in the ovary of rats. The number of follicles decreased with increase of atretic ones. Moreover, estradiol and progesterone decreased in sera of clomid-treated rats. Similarly, Chaube et al.(2005) reported that when immature female rats were injected with clomid the number of ovulated cumulus-oocyte complexes and estradiol level in ovary and serum were reduced, whereas membrane blebbing in oocytes, bax protein expression, and DNA fragmentation in ovarian follicular cells and ovulated oocytes were induced after clomid treatment. Duran and Raja, (2007) reported that clomid treatment caused many adverse effects including ovarian enlargement.



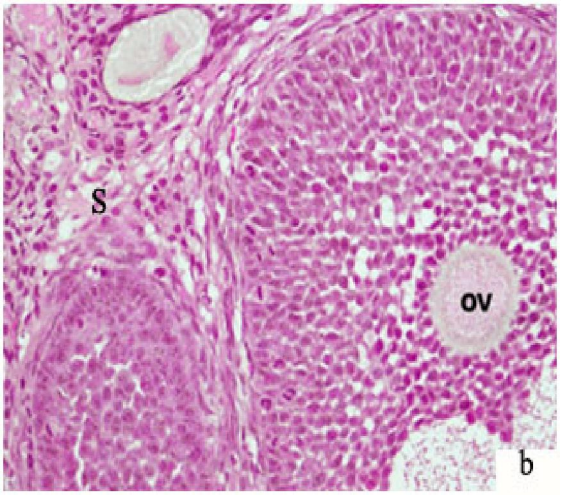


Fig.1. a. Section in ovary of a control rat showing graffian follicles (GF) X100
b. Enlarged graffian follicle, ov: ovum, S: stroma, X200

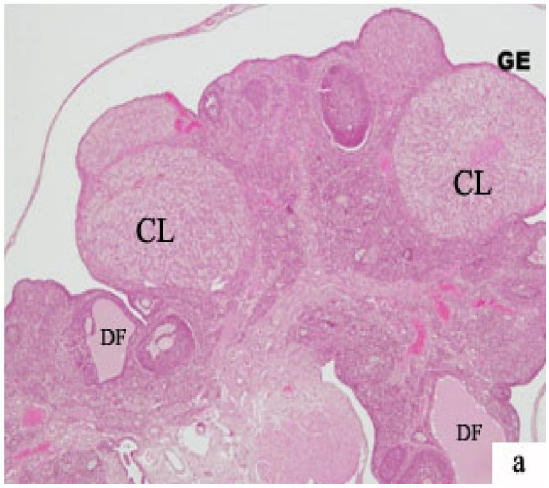
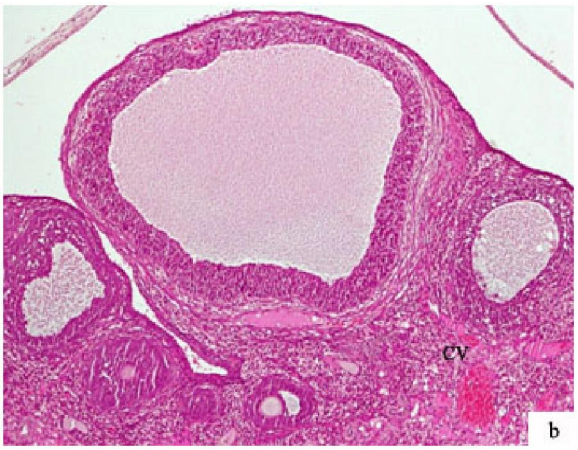
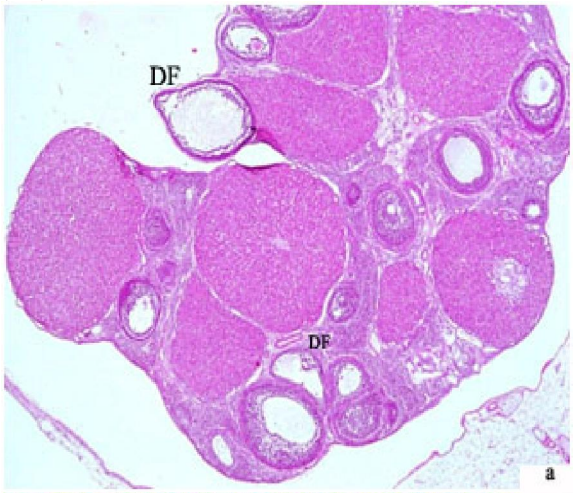


Fig.2.a. Section in ovary of a rat treated with 10mg clomid showing corpus luteum (CL) and degenerated follicles (DF),X100



b. Section in ovary of a rat treated with 50mg clomid showing degenerated follicles and congested blood vessels (CV), X 200



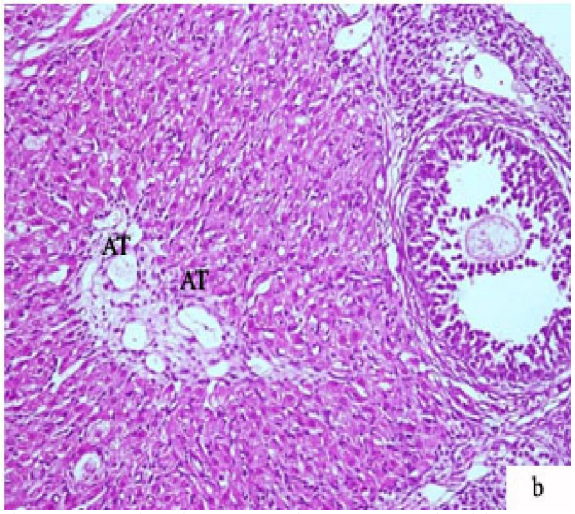


Fig.3. a. Section in ovary of a rat treated with 100mg clomid showing degenerated follicles (DF),X100
b. Enlarged portion of ovary of a rat treated with 100mg clomid showing atretic follicles (AT), X200

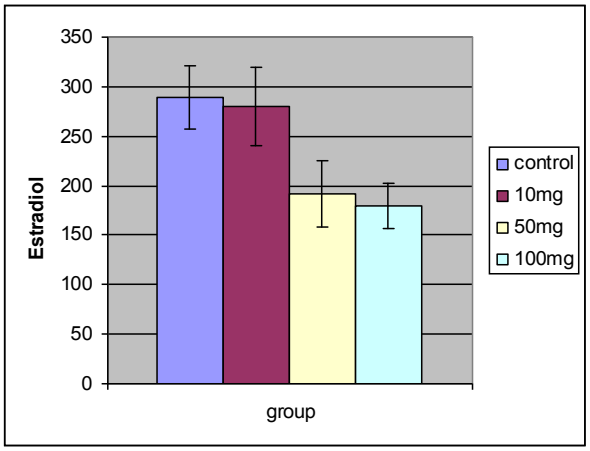


Fig.4a. Change in estradiol (pmol/L) in different animal groups

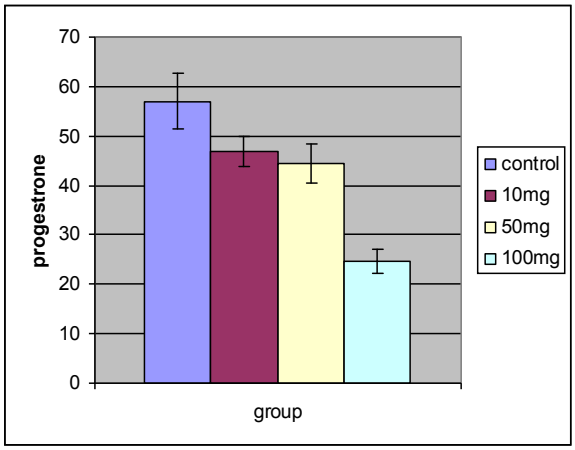


Fig.4b. Change in progesterone (nmol/L) in different animal groups.

Table 1. Effect of clomid on the number of different ovarian follicles

Treatment	No. of rats	Number of follicles			
		small	medium	large	Atretic
Control	5	188 ± 7	43± 3.5	7± 1	16±2
10 mg clomid	5	186 ± 6	42± 4	5 ± 1	18±1.5
50 mg clomid	5	160 ±4.5*	31± 2*	3 ±1*	22 ± 3*
100 mg clomid	5	143± 5.5*	23±2*	3±1*	28 ± 2.5*

(*). Significant at P<0.05

Kauppila et al. (1981) studied the effect of estrogen and clomid on 25 women with climacteric symptoms. Histologic examination revealed endometrial atrophy in 41% of the samples after the first estrogen treatment, whereas after the first and third clomid periods this was increased to 77% and 73%, respectively. The first clomid treatment significantly decreased the concentrations of cytosol estrogen and progestin receptors in endometrium, as compared with the levels recorded at the end of the preceding estrogen therapy. Kettel et al. (1993) reported that clomid -induced ovulation in women with polycystic ovarian syndrome is accompanied by increased secretion of LH and FSH with enhanced estrogen secretion. The increased LH pulse amplitude after clomid, together with decreased pituitary sensitivity to GnRH, suggests a hypothalamic effect. Chaube et al. (2006) found that Coadministration of clomid (10 mg/kg body weight) to immature female rats significantly reduced levels of cAMP, PGE2 in the ovary, ovary and uterus weights, and ovulation rate, whereas FSH and LH levels were not significantly altered. Supplementation of estradiol protected against these inhibitory effects of clomid and augmented levels of FSH and LH in serum.

The effects of clomid on ovulation and ovum maturation were studied using the isolated perfused rabbit ovary. clomid added to the perfusate with human chorionic gonadotropin did not affect ovulatory efficiency, ovulation time, oocyte maturation, or degeneration of ovulated ova and follicular oocytes. During perfusion without human chorionic gonadotropin, the percentage of follicular oocytes with germinal vesicle breakdown was significantly increased in response to clomid, a greater percentage of follicular oocytes was degenerated. Estradiol added to the perfusate reversed the effect of clomid on degeneration of follicular oocytes. Of follicular oocytes from ovaries perfused with clomid, 79.3% were degenerated; in contrast, 25% were degenerated in ovaries treated with clomid plus estradiol. These data suggest that clomid has a direct ovarian effect and that ovum degeneration associated with clomid may be related to an anti-estrogenic action (Yoshimura et al.1985).

Clomid is capable to interact with estrogen-receptor-containing tissues, including the hypothalamus, pituitary, ovary, endometrium, vagina, and cervix. It may compete with estrogen for estrogen-receptor-binding sites and may delay replenishment of intracellular estrogen receptors (Hughes et al.1996). Clomid in the used doses showed anti-estrogenic effect.

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