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Histopathological changes of Albino Rat ovary treated with Clomiphene citrate

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Abstract

This study was designed to evaluate the effect of clomiphene citrate (Clomid drug) on Albino Rat ovary. A total of thirty healthy rats at three months of age, weighing 200– 250 g on average and having regular estrous cycles for four days were divided into two groups (each of 15 rats). Control group was administered orally saline, treated group was administered orally Clomid drug at 50 mg/kg for a period 14 day. The animals were sacrificed at ovaries were removed. Histological section of treated group with clomid showed elevating in numbers of developing follicles underlying atretic changes such as denoted oocyte and dissociation of theca externa and theca interna layers from ovary stromal tissue with increased incidence of apoptosis and numbers of inflammatory cells. Ovary cortex consisted of many congestion spots, other sections showed ovarian tissue replaced by dense fibrous connective tissue that is slightly vascularized cytoplasm and pynotic cell nuclei. In addition, to karyorrhexis in ovary stroma tissue and degenerative cells. Depending on the results of this study, it was concluded that treating albino rats with clomid for 14 days at a concentration of 50 mg / kg can increase the number of follicles, which leads to stimulation of ovulation without clear histopathological changes.

Keywords: Clomiphene citrate. Clomid. Ovulation induction. Rat.

Introduction

The ovulation process is essential in terms of women's health and fertility during the childbearing period. Ovulatory dysfunction is one of the most common causes of reproductive failure in women of childbearing age [1]. An ovulatory infertility may be due to Polycystic Ovary Syndrome (PCOS) a disorder common to 6-20% of women of childbearing age [2,3], obesity, hypothalamus, hypothalamic dysfunction, hyperprolactinemia, pituitary adenomas or diseases hypothyroidism in humans [4]. Unexplained infertility contributes to 20%-25% of all female infertility [5]. In the absence of other important infertility factors in couples, successful ovulation often restores normal human fertility [6].

Clomid or clomiphene citrate is regularly prescribed to patients to treat ovulatory dysfunction in women who want to become pregnant, Patients with polycystic ovary syndrome [7], climacteric syndrome, galactorrhea, psychiatric illness, menorrhagia, amenorrhea after taking oral contraceptives, and some cases of secondary amenorrhea of undetermined etiolog [8]. Clomiphene citrate is a selective estrogen receptor modulator, which stimulates ovulation by inhibiting a negative, endogenous, estrogen feedback on the hypothalamic-pituitary axis, which leads to increased FSH secretion, follicular growth, and ovulation [9].

The current study aimed to evaluate the effects of clomiphene citrate (clomid drug) compound on ablino

rats ovary at 50 mg/kg dosing, to determining whether clomid causes histopathological changes in the ovary.

Materials and Methods

The experimental animal

This study was conducted in the animal laboratory of the factually of Education for Girls, University of Kufa. on September 1, 2021 until April 1, 2022, with the approval of the Ethics Committee of the same university, and following the ARRIVE checklist: (Available on: https://arriveguidelines.org/arrive-guidelines. Accessed on: March 12, 2022). A total of thirty healthy rats at three months of age weighing 200– 250 g on average and having regular estrous cycles for four days were used. Animals were kept on a 12-hour light/dark cycle at 22°C and had free access to food and water. Sawdust bed from the rat cages were changed three times a week and cages changed it has been cleaned and sterilized.

Experimental design

The animals used in this study were divided into two groups. Each group consisted of 15 rats. The first group was considered as a control administered orally daily with saline. The second group was administered orally daily with 50 mg clomid / kg of body weight for 14 days.

Histological examination

Treated experiment animals and controls were sacrificed by cervical dislocation, their ovaries were rapidly removed, and fixed in Bowen's fluid. After 24 hours. The sections were prepared according to Samare-Najaf et al., 2020 **[10]**. Histological section was examined by light microscope at a magnification 100x and 40x.

Results

Control group

Histopathological study of Samples from control rats stained with H&E showed the normal structure of the ovary in the form of an outer cortex containing the follicles and the central medulla. The cortex showed a primordial follicle with flattened follicular cells, primary follicles with cuboidal follicular cells with the pellucid zone around the oocyte, antral follicles with cavities between the follicular cells, graafian follicles with antrum, and much of the corpus luteum composed of mature luteinizing cells with clear eosinophilic cytoplasm and a large nucleus with chromatin arranged in cords around the sinusoids. Some atrial follicles with discrete dark picnotic granule cells are found in the follicular antrum (Figure 1 A, B and C).

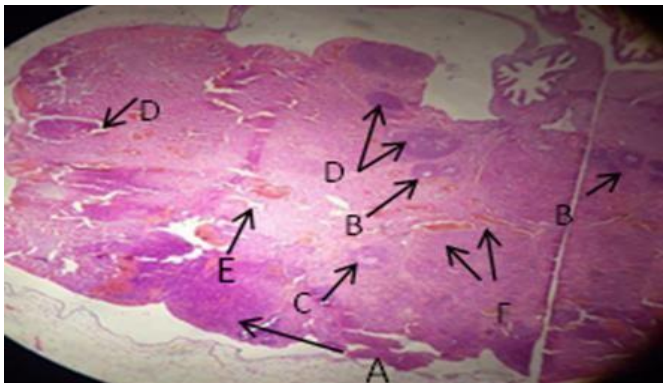

Figure 1 A: Histological section from control group ovary shows: A- ovary cortex , B- secondary follicles, C- antral follicles, D- growing and developing corpus luteum , E- ovary medulla and F- blood vessels. (H ɑ E) 40X. Source: Own authorship.

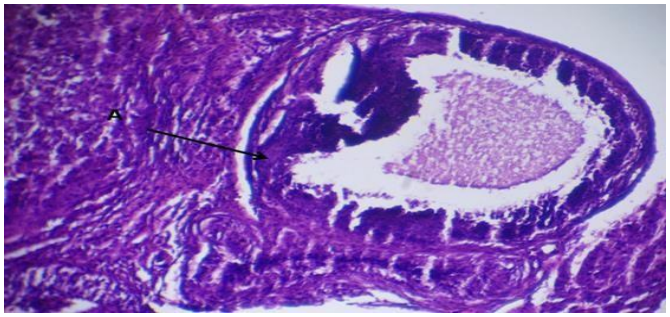

Figure 1 B: Corpus luteum (A). Ovary cortex in control group. .(H&E)100 X. Source: Own authorship.

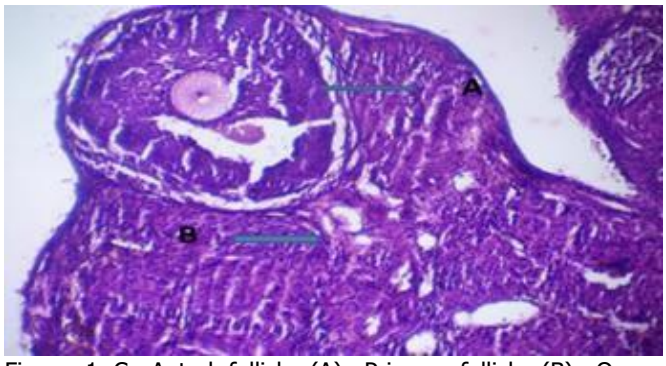

Figure 1 C: Antral follicle (A). Primary follicle (B), Ovary cortex in control group. (H&E)40 X. Source: Own authorship.

Effects clomid on the ovarian tissue

The results of the current study showed that oral dosing of white female rats with clomid at a concentration of 50 mg /mL of body weight for 14 days led to changes in ovarian tissue. Histological sections of the ovarian tissue showed elevating in numbers of developing follicles underlying atresic changes some of these follicle were primordial atretic follicle with denoted oocyte other were congestion primordial atretic follicles with denoted oocyte Figures 2-4, section also showed ovary cortex which consisted numbers of secondary follicle Figures 2-4,7. Ovary cortex consisted numbers of antral follicles underlying

atresic changes such as denoted oocyte and dissociation of theca externa and theca interna layers from ovary stromal tissue Figure 3, also there were numbers of antral follicles underlying atresic changes like denoted oocyte and zona pellucida ,degenerative corona radiata cells and granulosa cells in the region of cavity are also reduced in numbers and scattered with increased incidence of apoptosis Figure 6, other graffian atretic follicles contained antrum surrounded by multiple vacuoles with numbers of inflammatory cells in side it Figure 5. Other section showed antral follicles with different atretic signs such as Lack of clarity between theca externa and theca intern layers with degenerative and pale cells Figure 7, showed ovary cortex consisted of many congestion spots Figure 7, ovarian tissue replaced by dense fibrous connective tissue that is slightly vascularized Figure 4. Ovary stroma cell contained Considerable amounts of - fatty degenerative cell Figure 4, Vacuolation of the cell cytoplasm and pyknotic cell nuclei. In addition to karyorrhexis in ovary stroma tissue and degenerative cells, Figures 5 and 6.

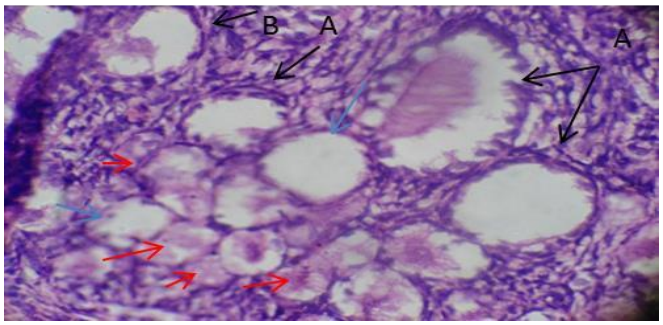

Figure 2. Histological section from a treated ovary showing elevating in numbers of developing follicles underlying atresic changes some of this follicle under stages of primordial atretic follicle with denoted oocyte (blue arrows) other were congestion primoredial atretic follicls with denoted oocyte (red arrows) A-primary atretic follicles B- secondary atretic follicles (H& E 400 X). Source: Own authorship.

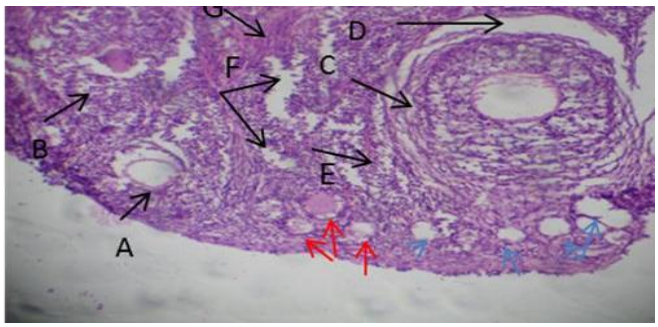

Figure 3. Histological section from a treated ovary showing elevating in numbers of developing follicles some of these follicle under stages of primordial atretic follicle with denoted oocyte AND Vacuolated cells (blue arrows) other were congestion primoredial atretic follicles(red arrows).section also shows A – primary atretic follicles B-secondary atretic follicles C-prenatal atretic follicles with D- dissociation of theca externa and theca interna layers from ovary stromal tissue E- necrosis area.F- Oedema and cavitation G- inflammatory cell infiltrations (H& E 40 X). Source: Own authorship.

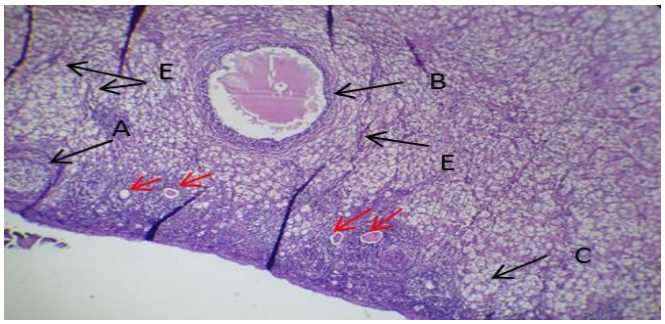

Figure 4. Histological section from a treated ovary shows: cortical region contains multiple stages of atretic follicles A- secondary atretic follicles B-grafian atretic follicles and numbers of congestion primordial atretic follicles (red arrows). ovary stromal cell tissue contains c- fatty degenerative cell E- vascularized dense fibrous connective tissue(H& E 400 X). Source: Own authorship.

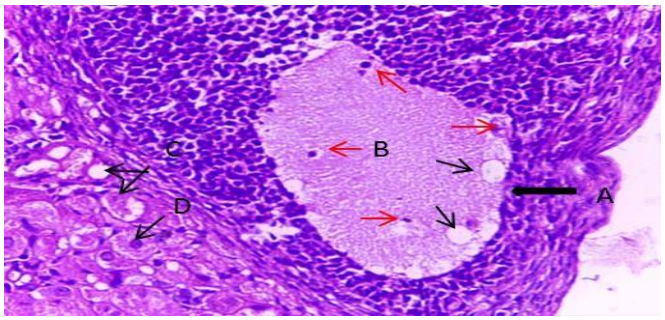

Figure 5. Histological section from a treated ovary shows: A- atretic antral follicles with denuded oocyte B - Follicle antrum surrounded by multiple vacuoles (black arrows) and numbers of inflammatory cell infiltrations also noticed in side it (red arrows), ovary stroma cell contain C-Vacuolation of the cytoplasm of stromal cells and D- pynotic cell nuclei (400X, H&E). Source: Own authorship.

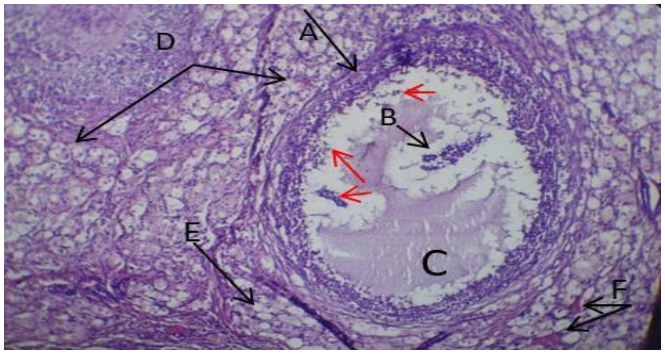

Figure 6. Histological section from a treated ovary shows: A- atretic graafian follicle B- denuded oocyte and decay zona pellucida and corona radiata cells C- follicular antrum with scattered and increased incidence of granulosa cells (red arrow) D- karyorrhexis in ovary stroma tissue and degenerative cells. E- Vacuolated cells. F- congestion in ovary cortex cells (100X, H&E). Source: Own authorship.

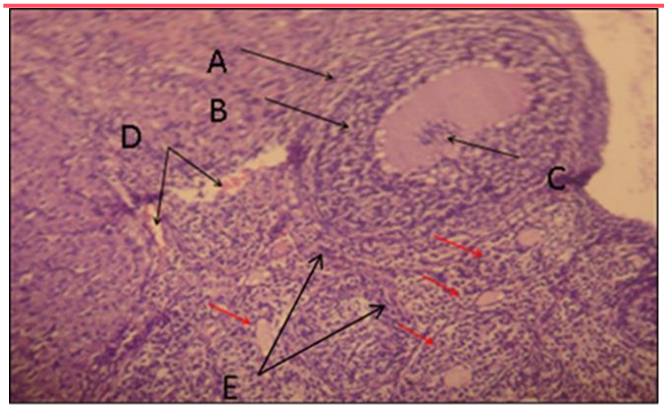

Figure 7. Histological section from a treated ovary shows A-antral follicles undergoing atretic changes such as B-Lack of clarity between theca externa and theca intern a layers C-denuded oocyte and zona D- congestion in ovary cortex with expansion of blood sinusoids. Pellucida E- inflammatory cell infiltrations. Section also shows many secondary follicles undergoing the same atretic changes (red arrows) s (red arrows).100X (H&E). Source: Own authorship.

Discussion

The results of the current study showed that oral administration of albino female rats with clomid at a concentration of 50 mg/mL of body weight for 14 days led to changes in ovarian tissue, through its activity to inducing ovulation. These results are agreed with Lombardi et al., 2020 **[11]** they showed a significant increase in the number of primary and antral follicles, in clomiphene citrate treated rats, Alkushi et al., 2015 **[12]** study showed that Clomiphine induced folliculogenesis in rat ovary and increased the number of growing ovarian follicles, Chaube et al. 2017 **[13]** that demonstrate The clomiphene citrate has good ovulation induction ability in anovulatory women, and in other study by Al-Shammary 2008 **[14]** showed that clomiphene citrate stimulates ovulation in females domestic hens.

These results suggest that clomiphene citrate (clomid) has a direct ovarian effect. That may be related to an estrogenic action of clomiphene citrate to enhance sensitivity to gonadotropin releasing hormones (GnRH) for its direct estrogenic effect. The action of clomifene citrate is determined by its binding to three sites. The primary site is in the hypothalamus, where it inhibits the negative feedback of estrogens, causing enhanced release of gonadal hormones. A second site is located in the pituitary gland, where clomiphene citrate enhances release of FSH by stimulating GnRH, and another site in the ovary. It is similar to what has been described for the pituitary gland, as it senses the granulosa cells of the follicle to the action of gonadotrophins and regulates the activity of aromatase **[15]**.

The result of the current study also showed numbers of antral follicles underlying atresic changes like denoted oocyte and zona pellucida, degenerative corona radiata cells and granulosa cells in the region of cavity are also reduced in numbers and scattered with increased incidence of apoptosis. This result may attribute to induce reactive oxygen species (ROS) mediated granulosa cells as well as oocyte apoptosis within the follicle of the ovary. The follicular growth and development may leading to induce susceptibility of oocytes towards apoptosis and decline oocyte quality after ovulation. Some studies have indicated that use of Clomid may stimulate the accumulation of reactive oxygen species in the ovaries, perhaps by inhibiting the activity of catalase, which leads to apoptosis in oocytes in rats **[16-18]**.

In the current study the results are differed with Lamfon and Al-matrafi 2013 **[19]** who found treating rats with clomid causing abnormal graafian follicles appeared with enlarged antrum and degenerated zone pellucida, and decrease in the number of ovarian follicles this difference may be attributed to the dose given that reached 100 mg/kg body weight. The dose given and the duration of the dose may also be a fundamental reason for the difference in the results of the studies. In the use of high dosage and long-term clomiphene citrate treatment, ovulation induction can lead not only to higher incidences of ovulation failure but also poor pregnancy rates Ozdemir et al. 2005 **[20]**. It is known that ovulation occurs in 70% of cases, while conception occurs only in about 25-30% of cases. The decreased pregnancy rate is due to the antiestrogenic effects of clomiphene citrate on the cervix, which makes it difficult for sperm to penetrate and the lining of the uterus to grow which in turn leads to the non-receptiveness of the embryo **[21]**. Idemudia and Ataman, 2024 **[22]** found results regarding ovarian function and histomorphology.

Although clomid drug is a commonly used treatment for infertile women, it is not without side effects that must be taken into account, especially if it is used for a long time and repeatedly, these sid effects include increased risk of cancer, thin endometriums and Uterus, programmed changes in the reproductive organs.

Conclusion

It was concluded that the treating albino rats with Clomid for 14 days at a concentration of 50 mg / kg can increase the number of follicles, which leads to stimulation of ovulation without distinct histopathological changes.

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Author contributions

All authors contributed to the conception and design of

the study. Material preparation, data collection, and laboratory analyses were performed by all authors. The first draft of the manuscript was prepared by the corresponding author, and all authors critically reviewed, edited, and approved the final version of the manuscript for submission.

Acknowledgment

Not applicable.

Ethical Approval

This study was conducted in the animal laboratory of the factually of Education for Girls, University of Kufa. on September 1, 2021 until April 1, 2022, with the approval of the Ethics Committee of the same university, and following the ARRIVE checklist: (Available on: https://arriveguidelines.org/arrive-guidelines. Accessed on: March 12, 2022).

Informed Consent

Not applicable.

Funding

Not applicable.

Data Sharing Statement

The datasets generated and analysed during the current study are not publicly available because of confidentiality of patient information, but are available from the corresponding author on reasonable request, subject to institutional approval and compliance with applicable data-protection regulations.

Conflict of Interest

The authors declare no competing interests.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

References

1. N Karaca YK, Akpak A, Cakır M, Marasli HAktun. Effects of nicotine exposure on clomiphene citrate induced rats. morphological and immunohistochemical analysis in the ovaries2019: 389.
2. March W, Moore V, Willson K et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod., 2010,25 (2): 544-51.
3. Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. Int. J. Womens Health . 2011; 3:25–35.
4. Tripathy S, Mohapatra S, Muthulakshmi M, Chandrasekhar A. Induction of Ovulation with Clomiphine citrate Versus Clomiphene with Bromocriptine in PCOS Patients with Normal Prolactin: A Comparative Study. Journal of Clinical and Diagnostic Research, 2013; 7, 2541-2543.
5. Tadesse TM. Clomiphene citrate in the management of anovulation: a review of mechanisms, outcomes, and clinical challenges. Gynecol Endocrinol. 2026 Dec 31;42(1):2646774. doi: 10.1080/09513590.2026.2646774..
6. Practice Committee of the American Society for Reproductive Medicine Use of Clomiphene Citrate in Women.Fertility Sterility, 2003; 80, 1302-1308.
7. Abu Hashim H. Clomiphene citrate alternatives for the initial management of polycystic ovary syndrome. An evidence. 2012.
8. Practice Committee of the American Society for Reproductive Medicine. Use of clomiphene citrate in infertile women: a committee opinion. Fertil Steril. 2013; 100(2):341–348.
9. Emily SJungheim MD, Anthony O Odibo MD. Fertility treatment in women with polycystic ovary syndrome: a decision analysis of different oral ovulation induction agents). Fertility and Sterility, 2010; 94(7):2659- 2664.
10. Samare-Najaf M, Zal F, Safari S, Koohpeyma F and Jamali N Stereological and histopathological evaluation of doxorubicin induced toxicity in female rats' ovary and uterus and palliative effects of quercetin and vitamin E. Hum. Exp. Toxicol. 2020; 39 (12), 1710-1724
11. Lombardi LA , Mattos LS, Simões RS, Florencio-Silva R Simões MJ , Franco PC, Carbonell AF, Sasso GRS, Baracat EC, Soares-Jr JM. Histomorphometric and immunohistochemical

changes in interstitial cells and ovarian follicles of rats with polycystic ovaries treated with clomiphene citrate. Gynecological Endocrinology 3.

12. Alkushi AG, Mustafa M, Sinna MM, Azab BA, AbdEl GE. SalamEA , Essam, S M. Eid EM and . ElSawy NA .Effect of Clomiphene Citrate On The Ovary Of Adult Rat . Jokull Journal, 2015, vol 65, No. 12.
13. Chaube SK , Tiwari M , Gupta A, Sharma A, Prasad S, PandeyAN , Yadav PK, Khatun S and Shrivastav TG. Clomiphene Citrate and Oocyte Quality Glob J Reprod Med, 2017, 1(4): GJORM.MS.ID.555570.
14. Al-Shammary HA . Effects of Clomiphene citrate on the histological structure of the Iraqi domestic chicken's ovaries. Journal of Thi-Qar University, 2018, Vol.13 No.3.
15. Practice Committee of the American Society for Reproductive Medicine (PCASRM). Use of clomiphene citrate in infertile women: a committee opinion. Fertil Steril, 2013;100(2):341-
16. Chaube SK, Prasad PV, Thakur SC, Shrivastav TG. Estradiol protects clomiphene citrateinduced apoptosis in ovarian follicular cells and ovulated cumulus-oocyte complexes. Fertil Steril, 2005; 84(Suppl 2):1163-72.
17. Chaube SK, Tiwari M, Gupta A, Sharma A, Prasad S,.Clomiphene Citrate and Oocyte Quality. Glob J Reprod Med, 2017; 1(4).
18. Ege S, Bademkıran MH, Peker N, Özgün Z, Bağlı İ, Erdem S, Erel Ö, Özgökçe Ç, Gül E Increased oxidative stress is associated with thiol/disulphide homeostasis in clomiphene citrate resistant polycystic ovary syndrome. J Obstet Gynaecol, 2021 Apr;41(3):467-470.
19. Lamfon, HA and Al-matrafi SS. Clomid induced hormonal and histological alterations in ovary of albino rats. JES, 2013; 9(12).
20. Ozdemir I, Ustundag N, Guven A, et al. Effect of clomiphene citrateon ovarian, endometrial, and cervical histologies in a rat model. Gynecol Obstet Invest. 2005; 60(4):181–185.
21. Speroff L, Glass R, Kase N. Clinical Gynecologic Endocrinology and Infertility (eds.) Lippincott Williams and Wilkins; 2004. pp. 487-523.
22. Idemudia, OU, Ataman JE. Evaluation of Ovarian Histomorphology and Function Following Clomiphene Citrate and Human Chorionic Gonadotropin Administration in Wistar Rats. Journal of Applied Sciences and Environmental Management, 2024, 28(3), 715-723.