PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (3) 715-723 March 2024

Evaluation of Ovarian Histomorphology and Function Following Clomiphene Citrate and Human Chorionic Gonadotropin Administration in Wistar Rats

***IDEMUDIA, OU; ATAMAN, JE**

Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

**Corresponding Author Email: eghosa.idemudia@uniben.edu*
**Tel: +234 802 3635 289*

Co-Authors Email: ehiagwina.ataman@uniben.edu
**Tel: +234 812 4919 989*

ABSTRACT: Polycystic ovarian syndrome (PCOS), affecting 5-10% of women, is a leading cause of infertility affecting 10-15% of couples globally. Human chorionic gonadotropin (hCG) and clomiphene citrate (CC) are often utilized for the treatment of PCOS. Accordingly, this study explored the effects of CC and hCG on ovarian histomorphology and fertility parameters. Twenty adult female rats were divided into four groups as follows: Group A (control) received only feed and water; Group B received 0.7 mg/kg BW of CC twice daily for five days and was mated before sacrifice on day 19 (before litter); Group C received 0.7 mg/kg BW of CC twice daily for five days, followed by mating and allowed to litter before sacrifice; Group D received 0.7 mg/kg body weight (BW) of hCG on day one, followed by 0.7 mg/kg BW of CC twice daily for five days, and were mated before sacrifice on day 7 (before litter). Results showed that Group B rats had higher, more than other groups, Follicle-stimulating hormone (FSH), Estradiol, Testosterone and Prolactin levels when compared to the control. Similarly, rats in Group B had higher levels of Progesterone while Group D had higher levels of Luteinizing hormone (LH) when compared to the control group. Histological findings demonstrated diverse impacts on ovarian structures, ranging from congested blood vessels to haemorrhages and follicular cysts. Consequently, this study underscores the complexities of drug interactions in reproductive health and provides preliminary insights into the possible adverse effects of CC and hCG on the ovary and fertility parameters.

DOI: https://dx.doi.org/10.4314/jasem.v28i3.12

Cite this Article as: IDEMUDIA, OU; ATAMAN, JE (2024). Evaluation of Ovarian Histomorphology and Function Following Clomiphene Citrate and Human Chorionic Gonadotropin Administration in Wistar Rats. *J. Appl. Sci. Environ. Manage.* 28 (3) 715-723

Dates: Received: 18 January 2024; Revised: 24 February 2024; Accepted: 12 March 2024 Published: 29 March 2024

Keywords: Polycystic ovarian syndrome; Clomiphene Citrate; Human Chorionic Gonadotropin; Ovary; Infertility.

The intricate regulation of reproductive function involves the synchronized functions of neural and endocrine systems, impacting fertility, which is a complex medical concern affecting 10-15% of couples globally (Saruhan *et al*., 2014; Mansori *et al*., 2016). Polycystic ovarian syndrome (PCOS), a prevalent endocrine disorder affecting 5-10% of reproductive-age women, often leads to infertility due to anovulation caused by hormonal imbalances (Kamel, 2013; Amudha and Rani, 2016). Epidemiological data reveals that 10-15% of couples experience difficulties in conceiving, with the World Health Organization (WHO) reporting female infertility at 37%, male infertility at 8%, both male and female infertility at 3%, and 5% facing unexplained infertility (La Marca and Mastellari 2020).

**Corresponding Author Email: eghosa.idemudia@uniben.edu*
**Tel: +234 802 3635 289*

Female age significantly impacts both spontaneous and treatment-related conception, with fertility declining from the age of 30, particularly in the late 30s and early 40s (Somigliana *et al*., 2016). Smoking is associated with reduced fertility, increasing the time to conceive and lowering conception chances in each cycle for female smokers (Moridi *et al*., 2019; Zakaria 2022). Various drugs, including non-steroidal anti-inflammatory drugs and recreational substances like marijuana and cocaine, affect fertility negatively (Krajka-Kuźniak *et al*. 2022; Armour *et al*. 2020). Weight extremes (BMI over 25 or less than 19) and physical inactivity impact the time to conceive, with obesity linked to decreased pregnancy rates (Gambineri *et al*. 2019; Marinelli *et al*. 2022). Psychological stress may also negatively affect female reproductive performance, though defining and measuring stress poses challenges (Marinelli *et al*. 2022). Inconsistent evidence surrounds the association between caffeine and female infertility, but high caffeine levels may have a negative impact (Krajka-Kuźniak *et al*. 2022; Marinelli *et al*. 2022). Alcohol's effect on female fertility is inconsistent, but excessive consumption is harmful to the fetus (Krajka-Kuźniak *et al*. 2022; Marinelli *et al*. 2022). Environmental pollutants, heavy metals like lead, and pesticides may adversely affect female fertility (Marinelli *et al*. 2022; Bala *et al*. 2020). The primary medical approach for inducing ovulation involves anti-estrogenic medications, with clomiphene citrate (CC) as the first-line regimen, known for its cost-effectiveness and minimal side effects (Barbrieri *et al*., 2019; Maitra and Hegde 2020). Clomiphene citrate (CC) is medically used for anovulation and oligoovulation cases (Robert *et al*., 2013). It has demonstrated efficacy, with a clinical pregnancy rate of 5.6% per cycle in cases without a known etiology (Behnoud *et al*. 2019). CC, containing enclomiphene and zuclomiphene, is administered orally, but the response varies, and inability to induce ovulation is more likely in obese, insulin-resistant, and hyperandrogenic patients (Fontenot *et al*., 2016; Gupta and Khanna 2018; Brown and Farquhar 2016). CC's main action involves indirectly stimulating GnRH secretion but can lead to increased LH concentrations, potentially compromising pregnancy rates (Maggi *et al*., 2015). Human Chorionic Gonadotropin (hCG) is commonly used for ovulation induction, acting on the LHCG receptor and contributing to the maintenance of the corpus luteum during early pregnancy (Smitz and Platteau 2020). hCG stimulates the growth of fewer larger follicles and reduces the risk of ovarian hyperstimulation syndrome in PCOS patients. hCG is encouraged to be given early in the development of follicles to stimulate follicular growth. The ovary, a paired ovoid structure, plays a crucial role in reproduction, changing surface appearance due to regular ovulation and corpus luteal degeneration (Tsui *et al*., 2023; Sinnatamby, 2011). Accordingly, the objective of this study is to investigate the ovarian histomorphology and function following clomiphene citrate and human chorionic gonadotropin administration in Wistar Rats.

MATERIALS AND METHODS

Twenty adult female Wistar rats were obtained from the University of Benin's Animal House of the Anatomy Department in Benin City, Edo State, Nigeria. The animals were given normal care, including free access to water and Vital Grower's feed, which was made in Benin City. The National Institute of Health and the National Academy of Sciences Guides for the Use of Laboratory Animals (NRC, 2010) served as the basis for the ethical principles for the care and use of animals.

Experimental Design: Experimental Design: Rats were divided into four groups as follows: Group A (control) received only feed and water; Group B received 0.7 mg/kg BW of CC twice daily for five days and was mated before sacrifice on day 19 (before litter); Group C received 0.7 mg/kg BW of CC twice daily for five days, followed by mating and allowed to litter before sacrifice; Group D received 0.7 mg/kg body weight (BW) of hCG on day one, followed by 0.7 mg/kg BW of CC twice daily for five days, and were mated before sacrifice on day 7 (before litter).

Procedures for Hormonal Profiling: Blood samples were obtained through the inferior vena cava for hormonal profile assessment as follows:

Estrogen, Progesterone, Prolactin and Testosterone Assay: Enzyme-linked immunosorbent assay (ELISA) method was used for hormone measurement (Kemppainen, 2023). Briefly, a desired number of coated wells was secured in the holder. Fifty microliters of standards, specimens, and controls were dispensed into the appropriate wells. Additionally, 100 microliters of Enzyme Conjugate Reagent was dispensed into each well, and thorough mixing for 30 seconds was carried out, recognizing the crucial nature of complete mixing in this setup. The incubation period was set for 60 minutes at room temperature. Following the incubation, the wells were washed with wash buffer. Subsequently, 100 microliters of Substrate Solution was added to each well, and a 15-minute incubation at room temperature ensued.

Further, 50 microliters of Stop Solution were added to each well. The absorbance at 450nm was then read using a microplate reader (Engvall and Perlmann, 1972).

Follicle-stimulating hormone and Luteinizing Hormone Assay: Micro-particle Enzyme Immunoassay MEIA method was used for hormone measurement (Gay *et al*., 1970). Briefly, the probe/electrode assembly was delivered into the sample and anti-hormone coated micro-particles to the incubation well of the reaction cell. Subsequently, the hormone was bound to the anti-hormone-coated micro-particles, forming an antibody-antigen complex. An aliquot of the reaction mixture, containing the antibody-antigen complex bound to the micro-particles, was then transferred to the glass fibre matrix. The micro-particles were irreversibly bound to the glass fiber matrix. Following this, the matrix underwent a washing step with a wash buffer to remove unbound materials. Next, the anti-hormone: alkaline phosphatase conjugate was dispensed onto the matrix and bound with the antibody-antigen complex. Another washing step was performed to eliminate unbound materials. To complete the process, the substrate, 4-methylumbelliferyl phosphate, was added to the matrix, and the fluorescent product was measured by the microparticle enzyme immunoassay optical assembly (Gay *et al*., 1970).

Animal Sacrifice and Histological Assessment: Rats were sacrificed under chloroform anaesthesia. The ovaries were accessed through a midline abdominal incision and immediately excised, weighed, fixed in Bouin's solution and processed through the Hematoxylin and Eosin staining methods as previously described by Drury and Wallington (1980).

RESULTS AND DISCUSSION

Hormonal profile findings: Hormonal assay findings revealed significant differences in various hormone levels among different treatment groups (Figure 1 – 6). Group B, treated with clomiphene citrate and exposed to mating, showed significantly higher levels of follicle-stimulating hormone (FSH) compared to group D, which received a dose of hCG on the first day followed by clomiphene citrate (Figure 1). Luteinizing hormone (LH) levels were significantly elevated in groups C and D compared to the control group, while group B showed no significant difference (Figure 2). Group C exhibited significantly lower progesterone levels than the control group, whereas groups B and D showed no significant difference (Figure 3). Estradiol levels were significantly higher in groups B, C, and D compared to the control group (Figure 4).

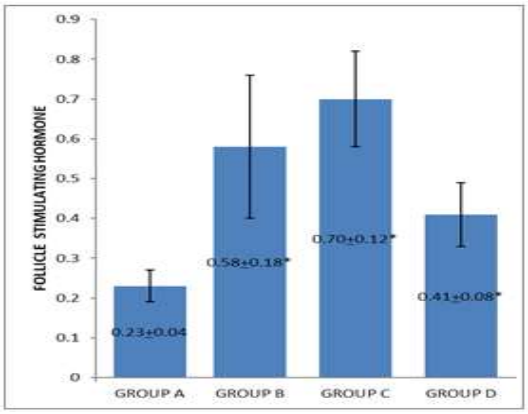

Fig 1: Bar chart showing comparison of follicle stimulating hormone level between different groups. Values are expressed in mean ± Standard error of mean (SEM) and * represents significance taken at p<0.05.

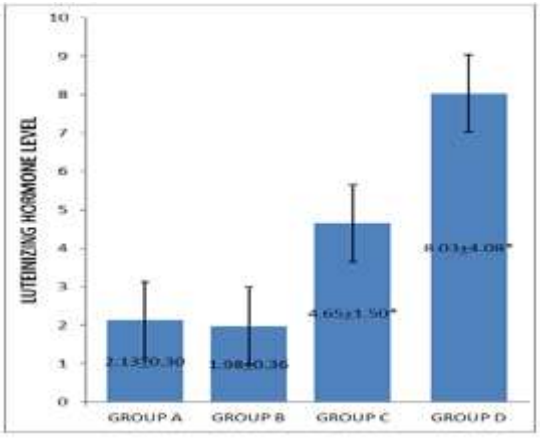

Fig 2: Bar chart showing the comparison of luteinizing hormone level between different groups. Values are expressed in mean ± Standard error of mean (SEM) and * represents significance taken at p<0.05.

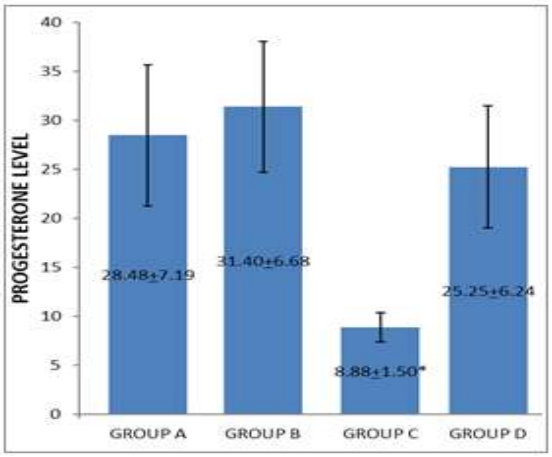

Fig 3: Bar chart showing the comparison of progesterone level between different groups. Values are expressed in mean ± Standard error of mean (SEM) and * represents significance taken at p<0.05.

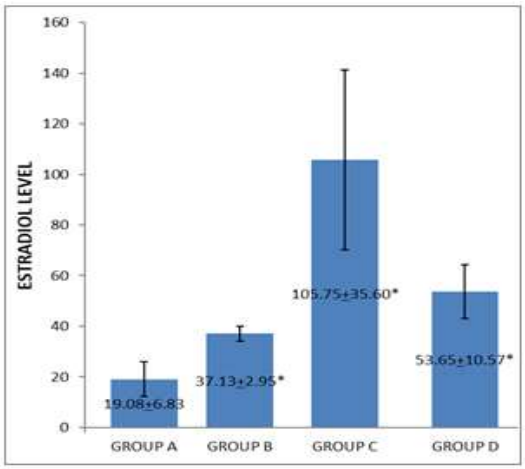

Fig 4: Bar chart showing comparison of estradiol level between different groups. Values are expressed in mean ± Standard error of mean (SEM) and * represents significance taken at p<0.05.

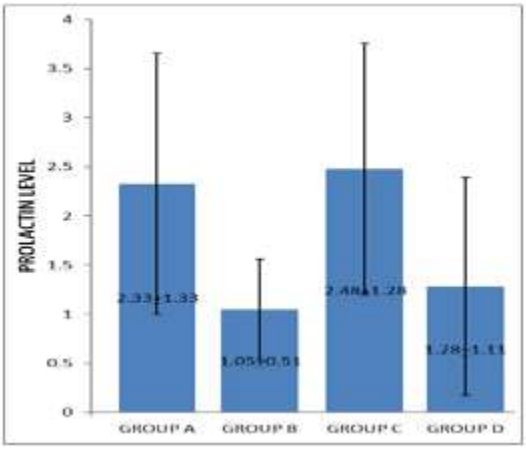

Fig 5: Bar chart showing the comparison of prolactin levels between different groups. Values are expressed in mean ± Standard error of mean (SEM) and * represents significance taken at p<0.05.

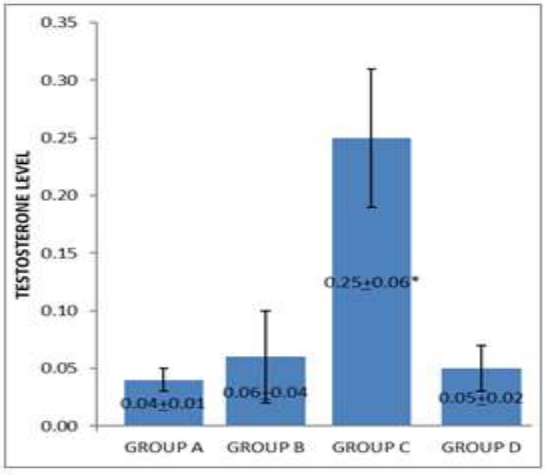

Fig 6: Bar chart showing comparison of testosterone level between different groups. Values are expressed in mean ± Standard error of mean (SEM) and * represents significance taken at p<0.05.

Prolactin levels showed no significant differences between the treatment groups and the control group (Figure 5). Testosterone levels were significantly higher in groups B, C, and D compared to the control group (Figure 6).

Histological Findings: The histological examination of the control group (A) revealed a healthy ovary with various structures including germinal epithelium, maturing follicles, Graafian follicles, ovarian stroma, zona pellucida, liquor folliculi, cumulus ovaricus, and blood vessels (Plate 1). Group B exhibited signs of ovarian distress, such as follicular atresia, cortical degeneration, and hyperaemic stroma (Plate 2).

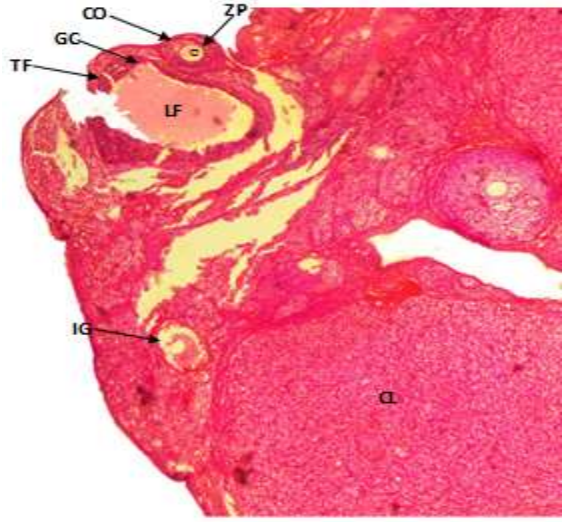

Plate 1: Control slide showing normal ovary with corpus luteum CL and graafian follicle containing oocytes O, zona pellucida ZP, granulosa cells GC, theca folliculi TF, cumulus oophorus CO and interstitial gland present in the medulla H and E X100

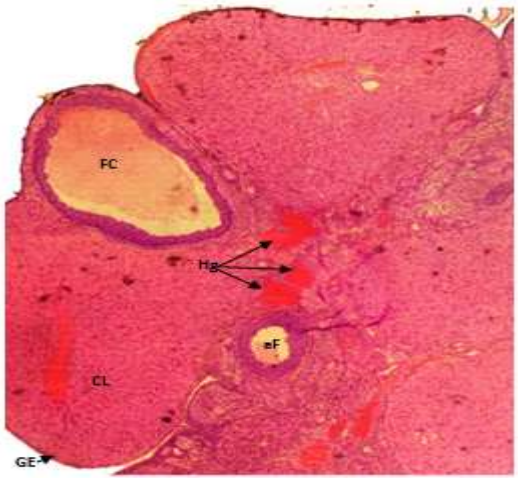

Plate 2: Group B slide ovary treated with clomiphene citrate showing atretic follicle aF, follicular cyst FC, petechial haemorrhage Hg in the medulla, corpus luteum CL, germinal epithelium GE. H and E X100

In contrast, Group C displayed increased folliculogenesis, featuring primary, secondary, graafian, and atretic follicles, alongside vascular congestion. Additionally, Group C showed matured Graafian follicles, cortical degeneration, and petechial haemorrhage (Plate 3). Group D, treated with hCG and clomiphene citrate, manifested degenerative changes in the ovarian stroma, along with primary follicles, haemorrhage, vascular congestion, and developing/atretic follicles (Plate 4). Overall, the histological findings provided insights into the structural changes within the ovaries under different treatments, suggesting varied impacts on folliculogenesis, stromal integrity, and vascular dynamics.

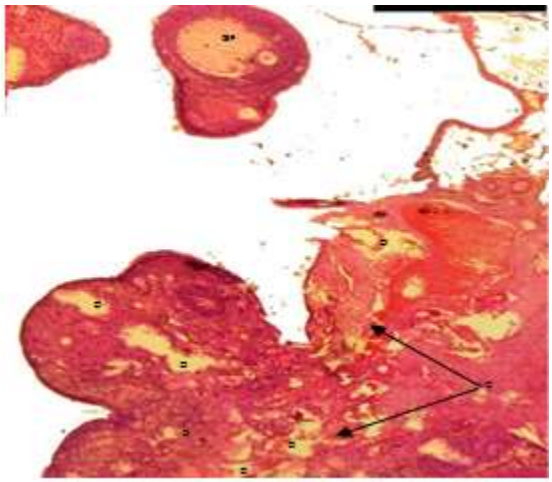

Plate 3: Group C slide ovary treated with clomiphene citrate showing matured graafian follicle GF, marked degenerative changes in the luteal tissues as well as the medulla D, congested blood vessels and petechial haemorrhage in the medulla. H andE X100

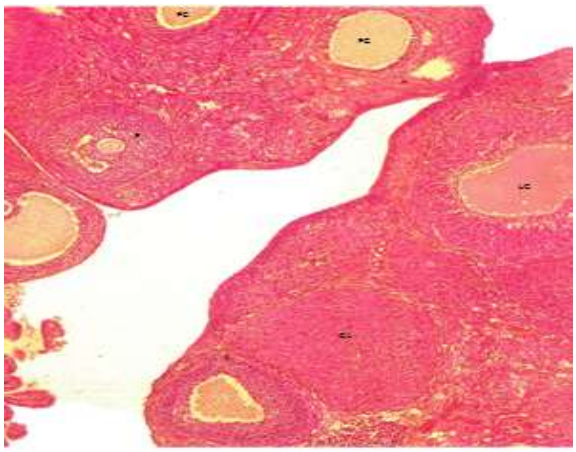

Plate 4: Group D slide ovary treated with clomiphene citrate and hCG showing secondary follicle as well corpora lutea CL, follicular cyst FC and luteal cysts LC in the cortex. H and E X 100

This study investigated the effects of CC and hCG on ovarian histomorphology and fertility parameters in female Wistar rats. Findings from this study revealed that the follicle-stimulating hormone level was significantly higher in groups B and C, which were treated with clomiphene citrate, compared to group D and the control group. The higher FSH levels in groups B and C suggest that clomiphene citrate could be effective in stimulating the production of this hormone. FSH is crucial for the growth and maturation of ovarian follicles in females and spermatogenesis in males, but high FSH levels have been known to be associated with primary gonadal (ovarian and testicular) failure (Raphael, 2023). High FSH has been reported to adversely affect reproductive health, primarily causing infertility in both males and females and may also contribute to symptoms like menstrual irregularities and lowered libido (Raphael, 2023). Therefore, clomiphene citrate could potentially cause infertility. This finding agrees with previous studies (He *et al*. 2023; Tian, 2009).

Similarly, Luteinizing Hormone was significantly higher in groups C and D compared to the control group. High levels of the luteinizing hormone have been previously reported to be associated with reproductive conditions such as Polycystic Ovary Syndrome (PCOS), premature ovarian failure (Society for Endocrinology 2021) and other conditions that can affect the ovaries and testes including exhaustion of the ovaries and testes to produce estrogen and testosterone (Cleveland Clinic 2022a). The progesterone level was significantly lower in group C compared to the control group, but there was no significant difference in groups B and D. This suggests that clomiphene citrate might have a variable effect on progesterone levels, which are essential for maintaining pregnancy (Melish *et al*. 2022). The lower progesterone level in group C suggests that clomiphene citrate might harm progesterone production, which is essential for maintaining pregnancy (Melish *et al*. 2022). This could potentially lead to complications in pregnancy and may need to be considered when using clomiphene citrate in fertility treatments (Melish *et al*. 2022). This aligns with previous studies by Mohammed *et al*. (2021) on the effect of Clomiphene citrate versus letrozole on pregnancy rate in women with polycystic ovary syndrome.

Estradiol level was significantly higher in groups B, C, and D compared to the control group. This indicates that clomiphene citrate can stimulate the ovaries to secrete more estrogen, which is crucial for the

menstrual cycle and pregnancy (Liao *et al*. 2022). Moreover, high levels of estradiol have been suggested to cause irregular periods and may worsen conditions that affect reproductive health, such conditions include PCOS (Cleveland Clinic 2022b) This could potentially make clomiphene citrate detrimental to reproductive health (Liao *et al*. 2022). A previous study by Hussein *et al* (2017), reported an increase in estradiol level on the day HGC was administered, thus suggesting that HGC could be responsible for the increased level of estradiol. Testosterone levels were significantly higher in groups B, C, and D compared to the control group. This suggests that clomiphene citrate can stimulate testosterone production, which is important for male characteristics and functions (Afaq *et al*. 2016). This could potentially make it a useful treatment for conditions related to low testosterone levels (Liao *et al*. 2022). However, a study suggested high testosterone levels could lead to Polycystic Ovary Syndrome, suggesting it to be a hormonal disorder that can impact women's capability to reproduce. It is also reported that PCOS is associated with symptoms such as irregular or prolonged periods, unwanted body hair growth and enlarged ovaries that may not function properly. High testosterone levels could lead to women developing metabolic syndrome which may cause weight gain, high cholesterol levels, high blood pressure and a future risk of developing diabetes and heart diseases such as stroke if they are not controlled (Srirangalingam 2022). Consequently, our findings suggest that while Clomiphene citrate can stimulate testosterone production, it could alternately lead to reproductive health problems.

For the histological findings, Group B showed signs of follicular atresia, a process where a follicle fails to develop, preventing it from ovulating and releasing an egg. Follicular Atresia is a process where most follicles in the ovary undergo degeneration and death (Palomba *et al*., 2018). This can lead to a decrease in the number of viable eggs available for fertilization, potentially affecting fertility (Palomba *et al*., 2018). This group also showed signs of Cortical degeneration of the ovary. This refers to the degeneration of the ovarian cortex, which contains the ovarian follicles (Palomba *et al*., 2018). Degeneration could lead to a decrease in the number of healthy follicles, potentially affecting fertility (Palomba *et al*., 2018). Hyperaemic Stroma, which is an increase in the blood flow to different tissues in the ovaries could lead to a decrease in the number of healthy follicles, potentially affecting fertility (Palomba *et al*., 2018). Thus suggesting that the conditions observed in this group suggest ovarian distress and could lead to female infertility. Group C showed evidence of increased folliculogenesis, the maturation of the ovarian follicle (Baerwald *et al*., 2011). Increased Folliculogenesis refers to the increased development and growth of ovarian follicles. While this is a normal part of the ovarian cycle, an excessive increase could potentially lead to conditions such as Polycystic Ovary Syndrome (PCOS), characterized by the presence of numerous small antral follicles (Wilson *et al*. 2021). This could lead to hormonal imbalances, irregular menstrual cycles, and potential fertility issues (Wilson *et al*. 2021). There were also signs of marked cortical degeneration, petechial haemorrhage in the medulla, and vascular congestion, the swelling of bodily tissues caused by increased vascular blood flow and a localized increase in blood pressure (Clifford 2011). Graafian follicles are mature ovarian follicles from which the ovum is released during ovulation. While the presence of matured Graafian follicles is a normal part of the menstrual cycle, an excessive number could potentially indicate ovarian hyperstimulation syndrome, a condition often associated with fertility treatments (Amelia *et al*. 2016). Cortical Degeneration refers to the degeneration of the ovarian cortex, which contains the ovarian follicles (Wilson *et al*. 2021). Degeneration could lead to a decrease in the number of healthy follicles, potentially affecting fertility (Wilson *et al*. 2021). Group D showed degenerative changes in the ovarian stroma and a few primary follicles. Haemorrhage with developing and atretic follicles is observed, along with evidence of vascular congestion and petechial haemorrhage in the medulla. Clomiphene citrate is commonly prescribed for ovulation induction and can lead to successful pregnancy (Usadi and Fritz 2009). However, these results suggest some adverse effects on the ovary. Degenerative changes in the stroma can disrupt this support, potentially affecting the growth and development of follicles and leading to decreased fertility (Esencan *et al*. 2022). Primary follicles are an early stage in the development of ovarian follicles. If there are issues with the development of primary follicles, it could lead to a decrease in the number of mature follicles and eggs, potentially affecting fertility (Esencan *et al*. 2022; Gurevich, 2019). Haemorrhage, or excessive bleeding, can cause shock and other complications. In the context of the ovaries, it could indicate inflammation or injury, which could potentially disrupt normal ovarian function and affect fertility. Vascular Congestion could indicate an abnormal increase in blood supply to the ovaries, potentially leading to conditions such as Pelvic Venous Congestion Syndrome (PVCS). PVCS is

characterized by chronic pelvic pain and can affect fertility (Kashef *et al*. 2023). Atretic follicles are follicles that have stopped developing and are undergoing degeneration. An increase in the number of atretic follicles could indicate a problem with folliculogenesis, the process of follicle development, which could potentially lead to decreased fertility (Esencan *et al*. 2022; Priya *et al*. 2021; Afreen *et al*. 2023). These findings are in agreement with the study of Ara and Asmatullah (2011) as there are notable observations of petechial hemorrhagic spots found in the ovaries.

Conclusion: This study underscores the complexities of drug interactions in reproductive health and provides preliminary insights into the possible adverse effects of CC and hCG on the ovary and fertility parameters.

REFERENCES

Afaq, S; Tahir, M; Lone, K; Latif, W (2016). Effect of Clomiphene Citrate on Rectus Femoris Histology and Spermatogenesis in rats. *Pak. J. Med. Health Sci.* 10(3): 1060–1064

.

Afreen, V; Hashmi, K; Nasir, R; Saleem, A; Khan, MI; Akhtar, MF (2023). Adverse health effects and mechanisms of microplastics on female reproductive system: a descriptive review. *Environ. Sci. Pollut. Res.* 30(31): 76283–76296. DOI: 10.1007/s11356-023-27930-1

Amelia, CLM; Kyle, DD; McGinnis, LA; Lee, HS; Aldana, N; Robinson, DN; Evans, JP (2016). Cortical mechanics and myosin-II abnormalities associated with post-ovulatory aging: implications for functional defects in aged eggs. *Mol. Hum. Reprod.* 22(6): 397–409. DOI: 10.1093/molehr/gaw019

Amudha, M; Rani, S; (2016). Fertility inducing effect of *Cadaba fruticosa* (L.) Druce in female albino rats. *Int. J. Biol. Pharm. Allied Sci.* 5(4): 850-863

.

Armour, M; Sinclair, J; Noller, G; Girling, J; Larcombe, M; Al-Dabbas, M. A; Johnson, N (2020). Illicit Cannabis Usage as a Management Strategy in New Zealand Women with Endometriosis: An Online Survey. *J. Womens Health.* 30(10): 1485–1492. DOI: 10.1089/jwh.2020.8668

Baerwald, AR; Adams, GP; Pierson, RA (2011). Ovarian antral folliculogenesis during the human menstrual cycle: a review. *Hum. Reprod. Update*. 18(1): 73–91. DOI: 10.1093/humupd/dmr039

Bala, R; Singh, V; Rajender, S; Singh, K (2020). Environment, Lifestyle, and Female Infertility. *Reprod. Sci.* 28(3): 617–638. DOI: 10.1007/s43032-020-00279-3

Behnoud, N; Farzaneh, F; Ershadi, S (2019). The Effect of Clomiphene Citrate Versus Letrozole on Pregnancy Rate in Women with Polycystic Ovary Syndrome: A Randomized Clinical Trial. *Crescent J. Med. Biol. Sci.* 6(3): 335–340

.

Boyar, RM; Perlow, MJ; Kapen, S; Lefkowitz, GK; Weitzman, ED; Hellman, LM (1973). The Effect of Clomiphene Citrate on the 24-Hour LH Secretory Pattern in Normal Men. *J. Clin. Endocrinol. Metab.* 36(3): 561–567. DOI: 10.1210/jcem-36-3-561

Brown, J; Farquhar, C (2016). Clomiphene and other antioestrogens for ovulation induction in polycystic ovarian syndrome. *Cochrane Database Syst. Rev.* 12(12). DOI: 10.1002/14651858.CD002249.pub5

Casarini, L; Riccetti, L; De-Pascali, F; Nicoli, A; Tagliavini, S; Trenti, T; Simoni, M (2016). Follicle-stimulating hormone potentiates the steroidogenic activity of chorionic gonadotropin and the anti-apoptotic activity of luteinizing hormone in human granulosa-lutein cells in vitro. *Mol. Cell. Endocrinol.* 422: 103–114. DOI: https://doi.org/10.1016/j.mce.2015.12.008

Cleveland Clinic. (2022a). High Estrogen: Causes, Symptoms, Dominance & Treatment. Retrieved from Cleveland Clinic website: https://my.clevelandclinic.org/health/diseases/22363-high-estrogen. Accessed 11/12/2023

Cleveland Clinic. (2022b). Luteinizing Hormone: Levels, Function & Testing. Retrieved from Cleveland Clinic website: https://my.clevelandclinic.org/health/body/22255-luteinizing-hormone. Accessed 11/12/2023

Clifford, PS (2011). Local control of blood flow. *Adv. Physiol. Educ.* 35(1): 5–15. DOI: https://doi.org/10.1152/advan.00074.2010

Dando, I; Carmona-Carmona, CA; Zampieri, N (2021). Human Chorionic Gonadotropin-Mediated Induction of Breast Cancer Cell Proliferation and Differentiation. *Cells*, 10(2): 264. DOI: https://doi.org/10.3390/cells10020264

Drury, R and Wallington, E. (1980). Carleton's histological technique 5th ed. New York: Churchill Livingstone.

Engvall, E; Perlmann, P (1972). Enzyme-Linked Immunosorbent Assay, Elisa. *J. Immun*. 109(1): 129–135

.

Esencan, E; Beroukhim, G; Seifer, DB (2022). Age-related changes in Folliculogenesis and potential modifiers to improve fertility outcomes - A narrative review. *Reprod. Biol. Endocrinol*. 20(156). DOI: https://doi.org/10.1186/s12958-022-01033-x

Fontenot, GK; Wiehle, RD; Podolski, JS (2016). Differential effects of isomers of clomiphene citrate on reproductive tissues in male mice. *Br. J. Urol.* 117(2): 344–350. DOI: 10.1111/bju.13244

Gambineri, A; Laudisio, D; Marocco, C; Radellini, S; Colao, A; Savastano, S (2019). Female infertility: which role for obesity? *Int. J. Obes. Suppl.* 9(1): 65-72. DOI: 10.1038/s41367-019-0009-1

Gay, VL; Midgley, AR; Niswender, GD (1970). Patterns of gonadotrophin secretion associated with ovulation. *Fed. Proc*. 29(6): 1880–1887

.

Gupta, MC; Khanna, J (2018). Clomiphene citrate: the changing landscape. *Int. J. Basic Clin. Pharmacol*. 7(8), 1437. DOI: https://doi.org/10.18203/2319-2003.ijbcp20183011

Gurevich, R (2019). How Many Ovarian and Antral Follicles You Should Have for Fertility? https://www.verywellfamily.com/follicle-female-reproductive-system-1960072. Accessed 13/12/2023

He, Y; Wei, X; Liu, Q; Liu Hui-ping; Yang, S (2023). Exploring the mechanism of clomiphene citrate to improve ovulation disorder in PCOS rats based on follicular fluid metabolomics. *Naunyn-Schmiedeb. Arch. Pharmacol*. DOI: 10.1007/s00210-023-02750-9

Hussein, Z; Al-Obaidi, MT; AL-Saadi, WI; Selman, MO (2017). Comparison of the Effect of Clomiphene citrate and Letrozole on the Endometrial Parameters of PCOS Women. *Int. J. Pharm. Sci.* 9(11): 2291–2295

.

Kamel, HH (2013). Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. *Eur. J. Obstet. Gynecol. Reprod. Biol*. 168(1): 60-63

.

Kashef, E; Evans, E; Patel, N; Agrawal, D; Hemingway, AP (2023). Pelvic venous congestion syndrome: female venous congestive syndromes and endovascular treatment options. *CVIR Endovasc*. 6(1). DOI: https://doi.org/10.1186/s42155-023-00365-y

Kemppainen, RJ (2023). Measurement of Hormones in Animals - Endocrine System - MSD Veterinary Manual. *MSD Veterinary Manual*

.

Krajka-Kuźniak, V; Papierska, K; Narożna, M; Jelińska, A; Majchrzak-Celińska, A (2022). Cannabidiol and Its Combinations with Nonsteroidal Anti-Inflammatory Drugs Induce Apoptosis and Inhibit Activation of NF-κB Signaling in Vulvar Squamous Cell Carcinoma. *Molecules*, *27*(24), 8779–8779. DOI: https://doi.org/10.3390/molecules27248779

La Marca, A; Mastellari, E (2020). Infertility. *Endocrinology*, 211–233. DOI: 10.1007/978-3-030-14782-2_11

Liao, Y; Chang, YK; Wang, SM; Chang, HC (2022). Ceiling effect of clomiphene citrate on the testosterone to estradiol ratio in eugonadal infertile men. *PLoS One*, 17(1): DOI: https://doi.org/10.1371/journal.pone.0262924

Maggi, R; Cariboni, AM; Marelli, MM; Moretti, RM; Andrè, V; Marzagalli, M; Limonta, P (2015). GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. *Hum. Reprod. Update.* 22(3): 358–381. DOI: https://doi.org/10.1093/humupd/dmv059

Maitra, C; Hegde, R (2020). Comparison of the role of letrozole & clomiphene citrate as a first line ovulation induction drug in infertile women with polycystic ovary syndrome. *Indian J. Obstet. Gynecol. Res*. 7(1): 12–15. DOI: https://doi.org/10.18231/j.ijogr.2020.003

Marinelli, S; Napoletano, G; Straccamore, M; Basile, G (2022). Female obesity and infertility: outcomes and regulatory guidance. *Acta Biomed. Ateneo Parm.* 93(4):e2022278. DOI: 10.23750/abm.v93i4.13466

Mansori, E; Ghasemiboroon, M; Samani, MA; Alamiri, F; Larky, DA; Farkhad, NK; Kooti, W; Hardani, A; Zargar, AA (2016). The Effect of Hydro-Alcoholic Extract of *Apium graveolens* L. Leaf on Delivery Rate in Female Rats, andWeight and Gender Ratio of Infants. *Jundishapur. J. Nat. Pharm.* 12(1):e28802. DOI: https://doi.org/10.5812/jjnpp.28802

.

Melish, ED; Chukwuebuk, N; Yabrade, T; Ehizokhale, E; Richard, O; Temitope, O; Peter, A (2022). Prenatal Restraint Stress-Induced Maternal Exposure and Clomifene Citrate Administration Modulates Reproductive Programming in Female Wistar Rats. *Pak. J. Biol. Sci*. 25(12): 1066–1076. DOI: 10.3923/pjbs.2022.1066.1076

.

Mahmoud, YAA; Abdel, RMS; Nourhan, AAE (2021). Clomiphene Citrate versus Letrozole for Induction of Ovulation in Infertile women having Polycystic Ovarian Syndrome (Randomized Controlled Trial). *J. Women Health Iss.* 4(7): 01–09. DOI: https://doi.org/10.31579/2642-9756/085

Moridi, A; Roozbeh, N; Yaghoobi, H; Soltani, S., Dashti, S; Shahrahmani, N; Banaei, M (2019). Etiology and Risk Factors Associated with Infertility. *Int. J. Women's Health Reprod. Sci.* 7(3): 346–353. DOI: 10.15296/ijwhr.2019.57

National Research Council. (2010). *Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press*

.

Palomba, S; Daolio, J; Romeo, S; Battaglia, FA; Marci, R; La Sala, GB (2018). Lifestyle and fertility: the influence of stress and quality of life on female fertility. *Reprod. Biol. Endocrinol*. 16(1). DOI: 10.1186/s12958-018-0434-y

Priya, K; Setty, M; Babu, UV; Pai, KSR (2021). Implications of environmental toxicants on ovarian follicles: how it can adversely affect the female fertility? *Environ. Sci. Pollut. Res.* 28(48): 67925–67939

.

Raphael, A (2023). Understanding High FSH Levels: Causes, Implications, and Management. *Medriva.* https://medriva.com/health/understanding-high-fsh-levels-causes-implications-and-management/ Assessed 25/11/2023

Robert, R; Samantha, P; Marc, F; Roger, L; Dale, McClure. R; Jeffrey, G; Randall, O (2013). Use of clomiphene citrate in infertile women: a committee opinion. *Fertil. Steril.* 100(2): 341–348

.

Saruhan BG, Sağsöz H, Akbalık ME (2014). Distribution and density of mast cells in the bovine reproductive tract during the follicular and luteal phases. *Eurasian J. Vet. Sci*. 30(3): 114–122. DOI: 10.15312/EurasianJVetSci.201436509

Sinnatamby, CS (2013). Last's anatomy: regional and applied, 12th edn. *Ann. R. Coll. Surg. Engl.*, 95(3): 230–230

.

Smitz, J; Platteau, P (2020). Influence of human chorionic gonadotrophin during ovarian stimulation: an overview. *Reprod. Biol. Endocrinol.* 18(80). DOI: https://doi.org/10.1186/s12958-020-00639-3

Society for Endocrinology. (2021). Luteinising hormone | You and Your Hormones from the Society for Endocrinology. https://www.yourhormones.info/hormones/luteinising-hormone/ Accessed 20/11/2023

Somigliana, E; Paffoni, A; Busnelli, A; Filippi, F; Pagliardini, L; Vigano, P; Vercellini, P (2016). Age-related infertility and unexplained infertility: an intricate clinical dilemma. *Hum. Reprod.*, 31(7): 1390–1396. DOI: https://doi.org/10.1093/humrep/dew066

Srirangalingam, U (2022). Polycystic ovarian syndrome: consequences of increased testosterone (C. Pano, Ed.). https://www.topdoctors.co.uk/medical-articles/polycystic-ovarian-syndrome-when-there-is-too-much-testosterone. Assessed 25/11/2023

Tian, Z (2009). Clomiphene Citrate | The Embryo Project Encyclopedia. https://embryo.asu.edu/pages/clomiphene-citrate. Assessed 23/11/2023

Tsui, EL; Harris, CJ; Rowell, EE; Laronda, MM (2023). Human ovarian gross morphology and sub-anatomy across puberty: insights from tissue donated during fertility preservation. *F. S. Rep.* 4(2): 196–205. DOI: 10.1016/j.xfre.2023.02.008

Usadi, RS; Fritz, MA (2009). Induction of Ovulation with Clomiphene Citrate. *The Global Library of Women's Medicine*.

Wilson, D; Le Heron, C; Anderson, T (2021). Corticobasal syndrome: a practical guide. *Pract. Neurol.* 21(4): 276–285. DOI: https://doi.org/10.1136/practneurol-2020-002835

Zakaria, M. (2022). Smoking and Infertility: Female Health-Care and Reproductive Risks Associated with Smoking. *Medicon Med. Sci.* 2, 03-10. DOI: 10.55162/MCMS.02.038