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Original Article

Preliminary Assessment of Clomiphene Citrate and Human Chorionic Gonadotropin Activity on Uterine Histology in Female Wistar Rats

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ABSTRACT

Anovulation, a common cause of infertility in Polycystic Ovary Syndrome (PCOS) women, prompts the use of anti-estrogenic drugs like clomiphene citrate (CC). However, there are conflicting reports on the effect of CC, and accordingly, this study investigated the effects of clomiphene citrate and human chorionic gonadotropin (hCG) on uterine histomorphology. 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 body weight (BW) of hCG on day one, followed by 0.7 mg/kg BW of CC twice daily for five days, and sacrificed on day 7; Group C received 0.7 mg/kg BW of CC twice daily for five days and sacrificed on day 19; Group D received 0.7 mg/kg BW of CC twice daily for five days and sacrificed on day 23. Thereafter the histology of the uterus across experimental groups was examined for possible alterations. Findings revealed normal uterine architecture in all groups, with consistent features such as a patent lumen, three-layer composition (endometrium, myometrium, perimetrium), and intact endometrial glands. These findings suggest that the treatments did not alter the basic histological structure of the uterus when compared to the control group. Taken together, these findings contribute to the understanding of the effects of these drugs and further research is necessary to explore their long-term effects.

Keywords: Clomiphene citrate, human chorionic gonadotropin, Uterus, Histology

INTRODUCTION

Infertility is a complex medical condition that affects 10-15% of couples worldwide. It is influenced by the intricate regulation of reproductive function, which involves the synchronized functions of neural and endocrine systems (Genazzani, 2005; Mansori *et al*., 2016; Ruder *et al*., 2008; Saraswathi *et al*., 2012). Anovulation brought on by hormonal imbalances is a common cause of infertility in women with PCOS, a common endocrine illness that affects 5–10% of women of reproductive age (Kamel, 2013; Amudha and Rani, 2016). According to epidemiological data, 10–15% of couples have trouble getting pregnant. The World Health Organisation (WHO) reports that 37% of women have female infertility, 8% of men experience it, 3% of both male and female infertility, and 5% of infertile couples are unable to conceive (Unuane *et al*., 2011).

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Anti-estrogenic drugs are the main medical strategy for inducing ovulation; clomiphene citrate (CC) is the first-line regimen and is well-known for its low side-effect rate and cost-effectiveness (Van Santbrink *et al*., 2005; Saruhan *et al*., 2014; Barbieri, 2019). However, the use of clomiphene citrate has been linked to negative side effects, such as thrombosis and pancreatitis, as well as ovarian enlargement and vasomotor flashes (Yasar and Ertugrul, 2009; Lamfon and Al-matrafi 2013). Meijer *et al*. (2006) found a link between specific birth abnormalities and clomiphene citrate. Anovulatory women undergo ovulation when exposed to the anti-estrogen MRL-41, which was identified in 1961 (Greenblatt *et al*., 1961). According to Robert *et al*. (2013), clomiphene citrate (CC) is prescribed medically for cases of oligoovulation and anovulation. In cases without a known aetiology, it has shown efficacy with a clinical pregnancy rate of 5.6% per cycle (Guzik *et al*., 1998). Oral administration of CC, which contains zuclomiphene and enclomiphene, is used; however, response varies, and patients who are obese, insulin-resistant, or hyperandrogenic are more likely to be unable to induce ovulation (Wu and Winkel, 1989; Imani *et al*., 1998; Homburg, 2002; Rostami-Hodjegan *et al*., 2004). Although the primary effect of CC is to indirectly stimulate GnRH secretion, it can also raise

LH concentrations, which may jeopardise the likelihood of pregnancy (Homburg *et al*., 1988; Shoham *et al*., 1990; Homburg, 2002).

Human chorionic gonadotropin (hCG), is widely used to stimulate ovulation because it acts on the LHCG receptor and aids in maintaining the corpus luteum during the early stages of pregnancy (Hoermann *et al*., 1990; Kovacs *et al*., 2004; Cole, 2009). Kovacs *et al*. (2004) reported that increased hCG levels in non-pregnant individuals could indicate paraneoplastic diseases or malignancy. Theories suggest that the hCG's negative charge repels the mother's immune cells, protecting the developing baby. Additionally, it might play a role in the proliferation and differentiation of cells, which could lead to apoptosis (Askling *et al*., 1999; Kayisli *et al*., 2003). Adjuvants such as dexamethasone and hCG are used in CC treatment with the goal of increasing its effectiveness (Daly *et al*., 1984; Agarwal and Buyalos, 1995). However, there are conflicting reports and insufficient data on the effects of CC and hCG on the histology of the uterus in experimental animals; accordingly, this study investigated such activity using female 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

The female rats weighing between 150 – 170 g were randomly assigned to four groups. Group A (control) received only feed and water; Group B 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 sacrificed on day 7; Group C received 0.7 mg/kg BW of CC twice daily for five days and sacrificed on day 19; Group D received 0.7 mg/kg BW of CC twice daily for five days and sacrificed on day 23..

Animal Sacrifice and Histological Assessment

At the end of administration, the animals were sacrificed under chloroform anaesthesia. The uterus of the experimental rats was immediately excised, weighed, fixed in Bouin's solution and processed through the Hematoxylin and Eosin staining methods as previously described (Drury and Wallington, 1980).

RESULTS

Plate 1 A-D show normal uterus displaying a patent lumen, inner endometrium (En) which is the thickest layer, middle myometrium (My) and outer perimetrium (Pe). The endometrium is lined by simple columnar epithelium (Ep) beneath which there is a thick lamina propria (LP) containing several endometrial glands (EG). The myometrium is made up of inner circular (IC) and outer longitudinal (OL) smooth muscles.

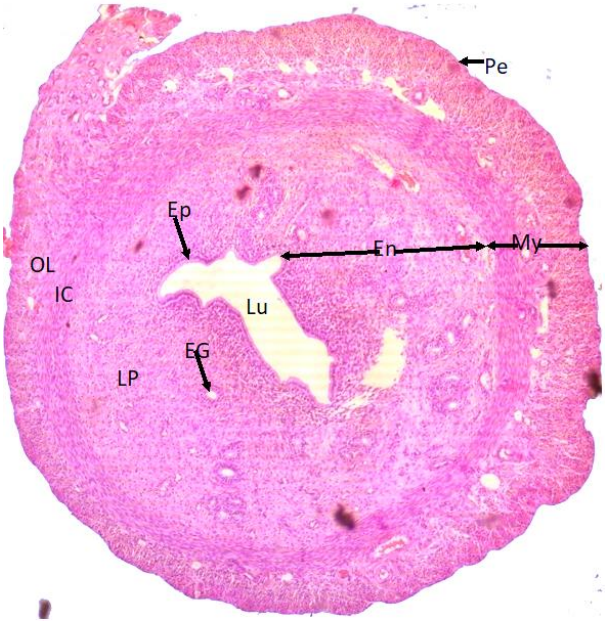

Plate 1: Photomicrograph of the control group showing normal uterus showing a patent lumen, inner endometrium En which is the thickest layer, middle myometrium My and outer perimetrium Pe. The endometrium is lined by simple columnar epithelium Ep beneath which there is a thick lamina propria LP containing several endometrial glands EG. The myometrium is made up of inner circular IC and outer longitudinal OL smooth muscles. H&E; 100X

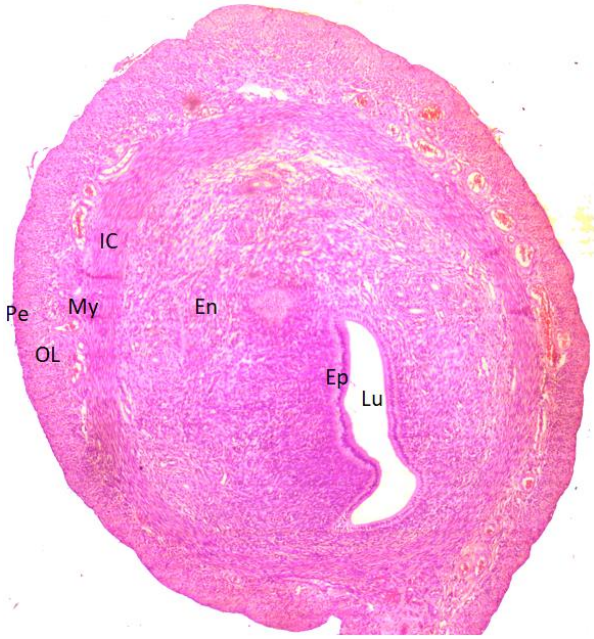

Plate 2: Photomicrograph of Group B showing normal uterus showing a patent lumen, inner endometrium En which is the thickest layer, middle myometrium My and outer perimetrium Pe. The endometrium is lined by simple columnar epithelium Ep beneath which there is a thick lamina propria LP containing several endometrial glands EG. The myometrium is made up of inner circle IC and outer longitudinal OL smooth muscles. H&E; 100X

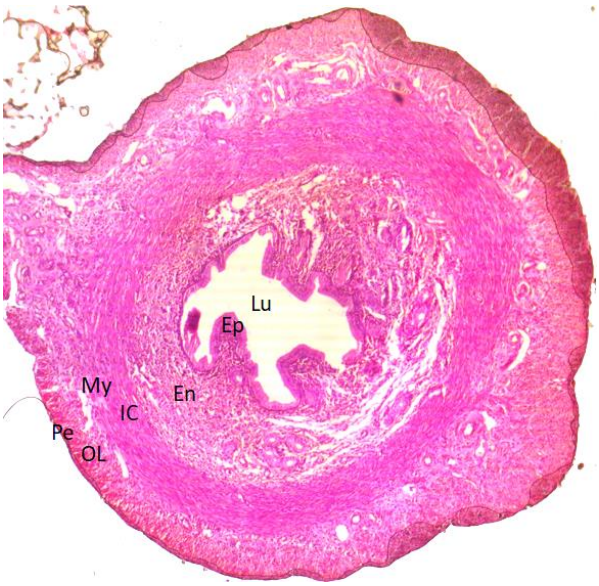

Plate 2: Photomicrograph of Group C showing normal uterus showing a patent lumen, inner endometrium En which is the thickest layer, middle myometrium My and outer perimetrium Pe. The endometrium is lined by simple columnar epithelium Ep beneath which there is a thick lamina propria LP containing several endometrial glands EG. The myometrium is made up of inner circle ICM and outer longitudinal OLM smooth muscles. H&E; 100X

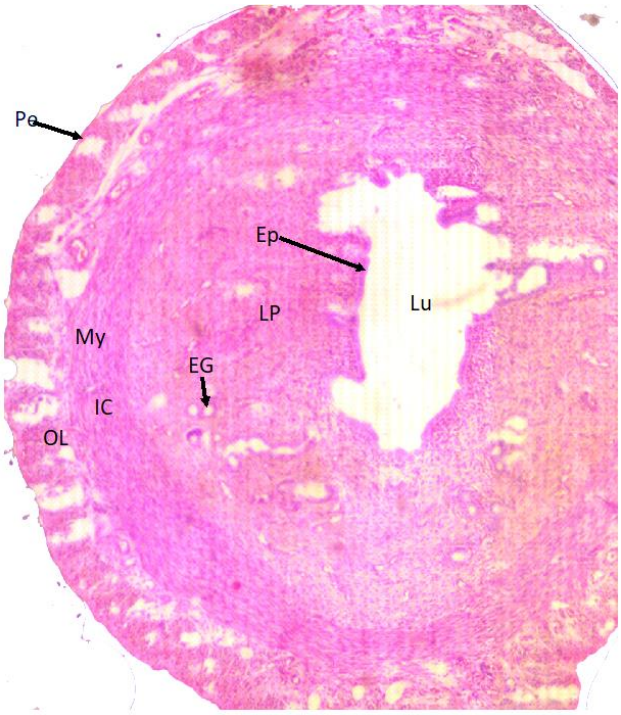

Plate 4: Photomicrograph of Group D showing normal uterus showing a patent lumen, inner endometrium En which is the thickest layer, middle myometrium My and outer perimetrium Pe. The endometrium is lined by simple columnar epithelium Ep beneath which there is a thick lamina propria LP containing several endometrial glands EG. The myometrium is made up of inner circle IC and outer longitudinal OL smooth muscles. H&E; 100X

DISCUSSION

In this study, the histology of the uterus was assessed in female Wistar rats following treatments with human chorionic gonadotropin (hCG) and clomiphene citrate. The histology of the uterus showed normal architecture similar to the control group which suggests that the administration of hCG and clomiphene citrate did not cause any noticeable alterations in the uterine structure within the experimental period.

This contradicts the study by Ónen *et al*, (1997) on the histopathological changes in the uterus of rats treated with clomiphene citrate demonstrating that 100ug/kg doses of hCG-induced polygonal nodular cells in the uterus epithelium and the presence of erythrocytes in the stroma. This contrast may be as a result of the experimental setup differences, as Ónen and colleagues administered neonatally. Also, El-Morshedy *et al*. (2020) reported an increase in the uterine wall thickness with an increase in the height and width of the endometrial folds, dilated lumen with patches of the hypertrophied surface epithelium and pseudo-stratification following administration of 1 mg/kg/day body weight of clomiphene citrate during

the diestrus stage of their menstrual cycle. Similarly, a study by Branham *et al*. (1988) suggested that neonatal clomiphene citrate exposure caused prolonged luminal epithelium hypertrophy and inhibited uterine gland genesis. Worthy of note is that administration was postnatal (Branham *et al*., 1988).

In agreement with our findings, no significant differences were observed in the histology of the uterus following CC treatment in human female subjects (Thatcher *et al.*, 1988). Similarly, in a different study and in agreement with our study, no significant effect on the uterus was demonstrable following CC treatment in baboons (Eley *et al.*, 1991). Kuscu and colleagues reported that CC did not adversely affect endometrial leukaemia inhibitory factor levels in females (Kuşcu *et al.*, 2002).

In conclusion, the administration of hCG and CC, in the given doses, did not cause any noticeable alterations in the uterine structure of the female rats. These findings provide valuable insights into the effects of these substances on the uterus and could have implications for their use in fertility treatments. However, further research is needed to fully understand the implications of these findings and to investigate the potential effects of different doses, treatment schedules, and longer-term effects. It would also be interesting to investigate the effects of these treatments on other aspects of reproductive health, such as hormone levels, ovulation, and fertility.

References

1. Agarwal SK, Buyalos RP. (1995). Corpus Luteum Function and Pregnancy Rates with Clomiphene Citrate Therapy: Comparison of Human Chorionic Gonadotrophin-Induced Versus Spontaneous Ovulation. Human Reproduction; 10: 328-331.
2. Amudha M, Rani S. (2016). Fertility inducing effect of Cadaba fruticosa (L.) Druce in female albino rats. International Journal of Biology, Pharmacy and Allied Sciences; 5(4): 850-863.
3. Askling J, Erlandsson G, Kaijser M, Akre O, Ekbom A. (1999). Sickness in pregnancy and sex of child. The Lancet. 354(9195):2053.
4. Barbieri RL. (2019). Female Infertility. Yen and Jaffe's Reproductive Endocrinology.:556-581.e7.
5. Branham WS, Zehr DR, Chen JJ, Sheehan DM. (1988). Uterine abnormalities in rats exposed neonatally to diethylstilbestrol, ethynylestradiol, or clomiphene citrate. Toxicology. 51(2-3):201–212.
6. Bukhari SAA, Ali S, Zubair M, Ahmad I, Rehman UU. (2016). Effect of clomiphene citrate and human chorionic gonadotropin (hCG) on ovulation induction in prepubertal Sahiwal heifers. Asian Pacific Journal of Reproduction. 5(3):232–235.
7. Chaube SK, Shrivastav TG, Prasad S, Tiwari M, Tripathi A, Pandey AN, Premkumar KV. (2014). Clomiphene Citrate Induces ROS-Mediated Apoptosis in Mammalian Oocytes. Open Journal of Apoptosis; 3: 52-58.
8. Cole LA, Ladner DG, Byrn FW. (2009). The normal variabilities of the menstrual cycle. Fertility and Sterility. 91(2):522–527.
9. Daly, DC, Walters CA, Soto-Albors, Tohan N, Riddick DH. (1984). A randomized study of dexamethasone in ovulation induction with clomiphene citrate. Fertility and Sterility; 41(6): 844-848.
10. Derksen L, Tournaye H, Stoop D, Van Vaerenbergh I, Bourgain C, Polyzos NP, Haentjens P, Blockeel C. (2014). Impact of clomiphene citrate during ovarian stimulation on the luteal phase after GnRH agonist trigger. Reproductive Biomedicine Online; 28(3): 359-368.
11. Drury, R and Wallington, E. (1980). Carleton's histological technique 5th ed. New York: Churchill Livingstone.
12. Eijkemans MJC, Habbema JDF, Fauser BCJM. (2003). Characteristics of the best prognostic evidence: an example on prediction of outcome after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Seminars in Reproductive Medicine; 21(1):39-47.
13. El Morshedy KE, Elmasry TA, Buabeid MA. (2020). Histological Changes in the Endometrium of Female Albino Rat Uterusunder the Effect of Clomiphene Citrate (CC). Journal of American Science, 16(2).
14. Eley, RM, Gould, KG, Eley, DS, Suleman, MA and Tarara, RP. (1991). Effect of clomiphene citrate upon periovulatory endometrial development in the baboon. Journal of medical primatology, 20**,** 49-57.
15. Genazzani AD. (2005). Neuroendocrine aspects of amenorrhea related to stress. Pediatric Endocrinology Review; 2(18): 661-668.
16. Greenblatt RB, Bafrield WE, Jungck EC, Ray AW. (1961). Induction of ovulation with MRL/41. Preliminary report. Journal of American Medical Association; 178: 101-104.
17. Guzik DS, Sullivan MW, Adamson GD, Cedars MI, Falk RJ, Peterson EP, Steinkampf MP. (1998). Efficacy for treatment for unexplained infertility. Fertility and Sterility; 70: 207-213.
18. Hoermann R, Spoettl G, Moncayo R, Mann K. (1990). Evidence for the presence of human chorionic gonadotropin (hcG) and free beta-subunit of hcG in the human pituitary. Journal of Clinical Endocrinology and Metabolism, 71 (1): 179–86
19. Homburg R. (2002). Should patients with polycystic ovarian syndrome be treated with metformin?: A note of cautious optimism. Human Reproduction. 17(4):853–856.

20. Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS. (1988). Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. The British Medical Journal; 297(6655):1024-1026.
21. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. (1998). Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Journal of Clinical Endocrinology and Metabolisim; 83(7):2361-2365.
22. Kamel H. (2013). Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. European Journal of Obstetrics & Gynecology and Reproductive Biology;168(1): 60-63.
23. Kayisli UA, Selam B, Guzeloglu-Kayisli O, Demir R, Arici A. (2003). Human Chorionic Gonadotropin Contributes to Maternal Immunotolerance and Endometrial Apoptosis by Regulating Fas-Fas Ligand System. The Journal of Immunology. 171(5):2305–2313.
24. Kousta E, White DM, Frank S. (1997). Modern use of clomiphene citrate in induction of ovulation. Human Reproduction Update;3(4):359-365.
25. Kovacs P, Matyas S, Bernard A, Kaali SG. (2004). Comparison of Clinical Outcome and Costs with CC + Gonadotropins and GnRHa + Gonadotropins During IVF/ICSI Cycles. Journal of Assisted Reproduction and Genetics. 21(6):197–202.
26. Kuşcu, N, Koyuncu, F, Var, A, Lacin, S, Uyanik, B and Ceylan, E. (2002). Clomiphene citrate does not adversely affect endometrial leukemia inhibitory factor levels. Gynecological endocrinology, 16**,** 151-154.
27. Lamfon HA, Al-matrafi SS. (2013). Clomid-induced hormonal and histological alterations in ovary of albino rats. Journal of American Science; 9(12):39-43.
28. 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 Prod. (inpress):e28802.
29. Meijer WM, de Jong-Van den Berg LT, van den Berg MD, Verheij JB, de Walle HE. (2006). Clomiphene and hypospadias on a detailed level: signal or chance? Birth Defects Res A Clin Mol Teratol. 76(4):249-52.
30. National Research Council. (2010). *Guide for the Care and Use of Laboratory Animals, 8th edition. National Academies Press*.
31. Ónen A, Devecí E, Erdinç M. (1997). Histopathological changes in the uterus of rats treated neonatally with clomiphene citrate. PubMed. 24(4):200–2.
32. Robert R, Samantha P, Marc F, Roger L, Dale McClureR, Jeffrey G, Michael T, Margareta P, Eric W, Glenn S. (2013). Use of clomiphene citrate in infertile women: a committee opinion. Fertility and Sterility. 100(2):341–348.
33. Rostami-Hodjegan A, Lennard M.S, Tucker GT, Ledger WL. (2004) Monitoring plasma concentrations to individualize treatment with clomiphene citrate. Fertil Steril 81,1187–1193.
34. Ruder EH, Terry JH, Jeffrey B, Goldman MB. (2008): Oxidative stress and antioxidants: exposure and impact on female fertility. Human Reproduction., 14: 345-357.
35. Saraswathi CD, Gupta SK, Sreemantula S. (2012). Protective effect of Symplocos racemosa bark on cold restraint stress induced reproductive changes in female rats. Journal of Natural Products; 5: 251- 258.
36. 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 Journal of Veterinary Sciences. 30(3):114–122.
37. Shoham Z, Borenstein R., Lunenfeld B, Pariente C. (1990) Hormonal profiles following clomiphene citrate therapy in conception and nonconception cycles. Clinical Endocrinology (Oxford) 33,271–278.
38. Thatcher, SS, Donachie, KM, Glasier, A, Hillier, SG and Baird, DT. (1988). The effects of clomiphene citrate on the histology of human endometrium in regularly cycling women undergoing in vitro fertilization. Fertility and Sterility, 49**,** 296-301.
39. Unuane D, Tournaye H, Velkeniers B, Poppe K. (2011). Endocrine disorders & female infertility. Best Practical Research Clinical Endocrinological Metabolism; 25(6):861-873.
40. Van Saintbrink, EJ, Eijkemans MJ, Laven JS, Fauser BC. (2005). Patient-tailored conventional ovulation induction algorithms in anovulatory infertility. Trends Endocrinological Metabolism; 16(8): 381-389.
41. Wu CH, Winkel CA. (1989). The effect of therapy initiation day on clomiphene citrate therapy. Fertility and Sterility; 52:564-568.
42. Yaşar HY, Ertuğrul O. (2009). Clomiphene citrate-induced severe hypertriglyceridemia. Fertility and Sterility; 92(1): 396.edition 7-8.