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Histomorphometric Studies of Oral Contraceptives on the Ovaries of Adult Female Wistar Rats *(Rattus Norvegicus)*

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Contraception is the intentional prevention of fertilization from taking place through various means. Histomorphometry is the study of microscopic organization and structure of a tissue especially by computer-assisted analysis of images formed by a microscope. The aim of the study was to check the effects of oral contraceptives on histomorphometrical parameters in adult female Wistar rats. Twenty-four (24) adult female Wistar rats of 3months old weighing 150 g - 200 g were used for the study. They were divided into four (4) groups of 6 rats each. Group A received 0.6 mg/kg b.w. of norgestrel, group B received 0.6 mg/kg body weight (b.w) of ethinylestradiol / levonorgestrel, group C received 0.6 mg/kg b.w of ethinylestradiol / levonorgestrel and 0.6 mg/kg b.w of ferrous fumarate orally for 21 days oestrus cycle (Estrus phase) in 5-day cycle (4-day treatment with 1-day break) while group D served as the control group received 0.3 ml of distilled water for 21days. Histomorphometrical analysis of the ovary was carried out. The findings revealed that non-combination contraceptives (non COC) left the ovary intact, while combined (COC) oral contraceptives and the concurrent administration of ferrous fumarate adversely distorted the morphology of the ovary. Luteal cell counts showed significant decrease ($P<0.01$, $P<0.05$) in lutea cell count/ovary in groups B and C when compared to control while significant increase in corpora luteal cell count/corpus luteum was observed in group A when compared with control. The study shows that combined oral contraceptives (COC) with ferrous fumarate is more effective than single contraceptives (Non COC) in preventing pregnancy occurrences.

Key words: Oral contraceptives, luteal cell count, ovaries, corpora luteal cell count/corpus luteum.

INTRODUCTION

The development of oral contraceptives worldwide has changed couples view on family planning drastically (Pincus et al., 1958). Combined oral contraceptive (COCs) pills contain both progestin and oestrogen. The COCs are acceptable and readily available in developing countries to prevent child mortality, morbidity and to reduce population growth arising from unwanted pregnancies (John et al., 2008).

Combined oral contraceptives suppress ovulation by diminishing the frequency of gonadotropin-releasing

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hormone pulses and eliminating the luteinizing hormone surge at mid-cycle (Nelson and Cwiak, 2011). They change the consistency of cervical mucus leading to less sperm penetration thereby making the endometrial lining less receptive to implantation and altering tubal transport of both sperm and oocytes. If used correctly, prevention rate of 99% could be achieved while incorrect pill usage leads to contraceptive failure (Trussell *et al.,* 1980).

Active exogenous progestin and oestrogen are the active compounds of combined oral contraceptives preparations. Increased physiologic levels of progestin and oestrogen have shown to cause a negative feedback effect on the hypothalamo-pituitary (HPA) axis (Wan *et al.,* 1981).[] Follicle-stimulating hormone (FSH), luteinizing hormone (LH) and endogenous gonadotropin-releasing hormone (GnRH) reduction suppresses the development of ovarian follicular thereby preventing ovulation and conception. Progestin, a component of OCs inhibit ovulation and LH surge (Van Heusden *et al*., 2000) while growth inhibition of pre-antral and medium-sized antral follicles in primates was by the action of oestrogens through inhibition of FSH secretion (Koering *et al.,* 1994). Van Heusden and Fauser (2002) stated that ovarian follicle development was not completely inhibited during standard use of combined OCs and the degree of follicular activities depend on the dose and type of steroids used, administration regimen, user compliance, and the pharmacogenomics of the individual taking the hormones (Fitzgerald *et al.,* 1999). Follicular cysts, which include enlarged follicles, functional ovarian cysts and luteinized unruptured follicles (LUFs) have been documented during oral contraceptive use (Broome *et al*., 1995).

Non-pathologic follicular cysts, unspecified ovarian cysts and corpus luteum cysts measuring above 20 mm in diameter detected by ultrasonographic or surgical examination are called functional ovarian cyst (Holt *et al*., 1992) while follicles that fails to ovulate and luteinize and have reached pre-ovulatory diameters are called luteinized unruptured follicles (LUFs).

The aim of this study was to analyze the histomorphometrical changes in the ovary of adult female Wistar rats following administration of oral contraceptives.

MATERIALS AND METHODS

A total of twenty four (24) adult female Wistar rats of 3 months old weighing between 150g and 200g were procured and housed in the Animal House of the Faculty of Health Science, University of Ilorin, Nigeria. They were raised at normal room temperature and fed with pelletized grower feed and water *ad libitum.* The animals were randomly divided into four groups of six (6) rats in each group and they were subjected to two weeks of acclimatization before treatment. The body weights (g) of the rats were taken using the High Precision Electronic Analytical Weighing Balance in the Department of Anatomy, University of Ilorin. All rats were assessed, screened and confirmed to ascertain their health status during the acclimatization period. The experimental protocol was approved by the University ethical review committee of the University of Ilorin, Ilorin, Nigeria. The research was approved to be in compliance with the Institutional Animal Care and Use Committee (IACUC).

Toxicology Studies

A toxicology studies was carried out using the Organization for Economic Cooperation and Development (OECD, 2000) Guidance Document on Humane End points which seeks to reduce the overall animal sufferings in this type of toxicity test. The test used was the limit dose test of the up and down procedure. A total of 6 rats were weighed and individually identified. The 6 rats were given a test dose of 1.5 mg Levonorgestrel, 1.5 mg Ethinylestradiol while 1.5 mg Ferrous Fumarate. The results were evaluated as follows (S = Survival, X = death), 30 minutes after dosing, each rat was observed individually and periodically during the first 24 hours. Special attention was paid during the first 5 hours, and daily thereafter for a total period of 7 days. All observations were systematically recorded with individual records maintained for each animal.

Animal Grouping and Drug Administration

The animals were grouped into four (4) groups. Group A received 0.6 mg/kg b.w. of norgestrel (ROGOTINOR) for 21 days estrous cycle of Wistar rats.Group B received 0.6 mg/kg b.w. of ethinylestradiol / levonorgestrel (DUOFEM) in 5-day cycles (4-day treatment with a 1-day break) for 21 days oestrous cycle of Wistar rats.Group C received 0.6 mg/kg b.w. of ethinylestradiol/levonorgestrel in 5-day cycles (4-day treatment with a 1-day treatment break) plus 0.6 mg/kg b.w. of ferrous fumarate repeatedly for 21 days oestrous cycle of Wistar rats.Group D served as the control group received 0.3 ml of distilled water. The drugs were administered using an orogastric cannula. Each Combination 3 cycle contains 28 pills; each tablet 0.15 mg Levonorgestrel and 0.03 mg Ethinylestradiol while each brown tablet contains 75 mg Ferrous fumarate. The doses of the COCs is the minimum required that will reliably suppress FSH sufficiently to prevent the growth of an ovulatory follicle (Jain *et al.,* 2000).

Animal Sacrifice

After 21days of treatment, the animals were sacrificed on the 22nd day. An abdominopelvic incision was carried out to expose the thorax, abdomen, and the pelvic region. The organ of study (ovary) was excised for the histological studies. Macroscopic examination was done but no changes occurred.

Tissue Processing for Histological Studies

The ovaries were excised and fixed in Bouin's fluid for corpora lutea count. The excised ovaries were placed inside a tissue cassette and then subjected to the manual routine tissue processing procedure as explained below. Ovarian tissues were subjected to the processes of fixation, dehydration, clearing, infiltration, embedding in Paraffin wax, sectioning at 5µm thick and staining was done using Haematoxylin and Eosin while mounting in diphythalate xylene (DPX) and examination on glass slides as described (Bancroft and Stevens, 1996).

Light Microscopy

For light microscopic studies, the ovarian sections on glass slides were captured using Olympus binocular research microscope

Table 1. Showing weight changes in the treatment and control groups.

Group	Initial.(g)	Final (g)	Diff. (g)
A	162 ± 3.4	162.9±2.40	.9±0.021
B	170±2.9	177.89±3.2	.89±1.23
C	165±3.8	171.94±3.5	.94±0.03
D	175±0.4	179.44±1.4 4	.44±1.76

(Olympus, New Jersey, USA) which was connected to a 5.0MP Amscope Camera (Amscope Inc, USA) at ×100 magnification.

Histomorphometric Analysis

Morphometric evaluation involved 3D reconstruction and volume measurement of the ovary while corpora lutea and luteal cell stereological analysis was performed using different sampling procedures and different microscopic magnifications. For luteal cell stereological analysis, the sampling procedure applied by Meyer and Bruce (1979) was slightly modified. Every 8th section was used and inspected with a Zeiss Projection Microscope at a magnification of x1060. In each section, profiles were drawn of luteal cells (and their nuclei) located in two test fields (one near the periphery and the other near the center of the corpus luteum) from 3 corpora lutea. Profiles of the luteal cells and their nuclei were then traced using a digitizer connected to a microcomputer. The surface area of the cross-section of the luteal cells and their nuclei, the length of long and short axes of cells, the volume fraction and the number of cells per test area was measured; the area of test field was also recorded using a 5.0MP Amscope Camera (Amscope Inc, USA). The number of luteal cells was estimated using the primary data as described by Meyer and Bruce (1979).

Statistical Analysis

Data from both groups were statistically analysed using Tukey's Adhoc test of Analysis of Variance (ANOVA) with the aid of SPSS software (V20, USA) followed by subsequent analysis by GraphPad Prism v5.01 (GraphPad Software Inc. USA) with statistical significance set at P<0.05* and p<0.01**.The outcomes were represented in bar charts with error bars to show the mean and standard error of mean respectively.

RESULTS

Body weight changes

A significant decrease (P<0.05) in body weight changes was observed in groups A and C when compared to control group D (Table 1). Values are expressed as mean ± SEM showing the level of significance in *P< 0.05 compared to the control group.

A significant increase in corpora luteal cell count/corpus luteum was observed in group A when compared with the control group (Figure 2) while significant reduction (P<0.05) in groups B and C when compared to group A (Figure 3).

DISCUSSION

A significant decrease in body weight changes observed showed a reduction in food consumption. This corroborated Holmes and Mandl (1962) stated that growth rates in rats may be reduced as a result of COCs administration. An inhibition of pituitary somatotropin after administration of COCs in large doses has been implicated (Pincus, 1966).

The presence of numerous corpus luteum coupled with follicular cyst observed in this group showed that norgestrel is not an effective means of preventing pregnancy. Oestrogen is responsible for the suppression of follicle stimulating hormone, hence follicular development occurs (Swerdloff and Odell 1969) and this corroborates Obasi et al., (2016), who observed the presence of numerous corpora lutea after administration of oral contraceptive in rabbits after artificial insemination. Boiti et al. (1996) stated that the increased number of corpora lutea after contraceptive administration in rabbits was due to high plasma progesterone. This was in agreement with Spona et al., (1996) who reported that the formation of follicular cyst, functional ovarian cysts, luteinized unruptured follicles (LUFs) and enlarged follicles were as a result of the administration of oral contraceptives.

However, the severe damages to groups B could be as a result of COCs dosage leading to reduced follicular cells and a reduction in oestrogen secretion. This was in agreement with Fauser and Van Heusden (1997), who reported that the extent of pituitary-ovarian suppression appear to be related to the dosage of Ethinylestradiol (EE) during combined OC rather than the progesterone dosage and types. This showed that contraceptives in combined form are very effective in preventing pregnancy. However, results observed in group C showed that the effects of combining fumarate with COCs were devastating to the ovaries. This therefore highlighted its efficiency in preventing pregnancy or conception totally.

The decrease in corpora luteal cell count/ovary in groups B and C showed the deleterious effects of the combined oral contraceptive on the ovaries . These corroborated results from the histological studies carried out (Figure 1) while the results in group A compared to the control group showed that the single administration of oestrogen alone cannot prevent pregnancy (Swerdloff and Odell, 1969).

The increase in corpora luteal cell count/CL showed that ovulation occurred in group A corroborating reports from the histological findings.Luteotropin might be implicated because it helps in the sustenance of luteal function through follicular production of estradiol

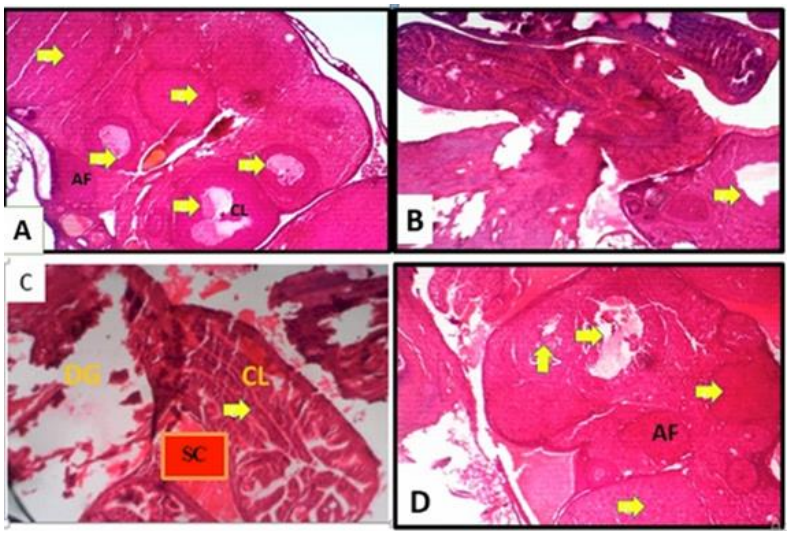

Figure 1. Photomicrographs of the ovaries of female Wistar rats treated with Norgestrel (A), COC (B), COC plus ferrous fumarate (C), Control (D). CL-Corpus luteum, O-Oocyte, AF-Atretic follicle, GF- Graafian follicle, DG-Cellular degeneration, Sc- stroma cells p.

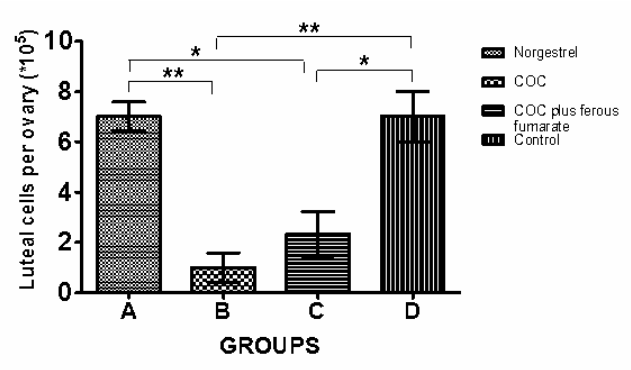

Figure 2. The corpora luteal cell count per ovary across experimental and control groups. *= (P<0.05), **= (P<0.01)-statistical significance compared to the control group.

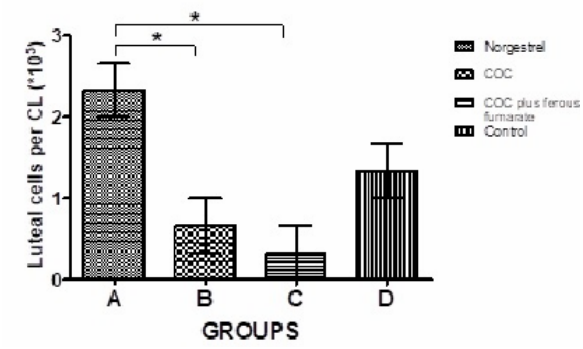

Figure 3. The corpora luteal cell count per corpus luteum across experimental and control groups. *(P<0.05) - statistical significance compared to the control group.

(Dharmarajan et al., 1999) while significant reductions in group B and C corroborated results from the histological studies indicating reduced production of estradiol which helps sustain luteal function leading to a degenerated and damaged ovary.

CONCLUSION

Results showed that Norgestrel alone had little impact on the histoarchitecture of the ovary while the use of combined oral contraceptives and/or fumarate, causes severe damage to the histoarchitecture of the ovary, thereby preventing pregnancy in its totality

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