

Cytoarchitectural differences in reproductive organs of some polycystic ovary-like induced animal models

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common gynaecological, endocrine disorder that occurs during reproductive age and is a significant cause of anovulatory infertility. Letrozole is an aromatase inhibitor which negates the action of the aromatase enzyme, which results in the buildup of male hormones (testosterone) in the females, causing hyperandrogenism, which is a hallmark of Polycystic Ovarian Syndrome. Mifepristone (RU486) is a progestin antagonist that acts to arrest the actions of the progesterone hormone, resulting in follicular atresia and anovulation. DHEA is an androgen which was also administered in a bid to cause hyperandrogenism in the rats. This study aimed to evaluate the effects of these hormones on the cytoarchitecture of the ovaries and uterus to assess their various PCOS-like histological features. Animals were grouped mainly into three: Letrozole, Mifepristone and DHEA groups, which were further divided into two subgroups each, administered low and high doses of letrozole orally, Mifepristone and Dehydroepiandrosterone (DHEA) subcutaneously. Each of the subgroups also had a comparison control group. Following the completion of administration, the Wistar rats were euthanized, and their ovaries and uterus were collected for histological analysis. Increased proliferation of ovarian follicles was noted in the treated groups compared to control, as well as thickening of the endometrial layer.

1. Introduction

Polycystic ovarian syndrome (PCOS) is a complex endocrine and metabolic disease with a global prevalence of 4 %-20 % (Jabeen Ayesha et al., 2022). It is a leading cause of infertility characterised by polycystic ovarian morphology, hormonal imbalance, hyperandrogenemia and anovulation (Jabeen Ayesha et al., 2022). Other prominent features include weight gain, mood swings, hirsutism, and acne. PCOS is classified as a chronic systemic disease characterized by obesity and insulin resistance and is often associated with long-term complications such as Type 2 diabetes mellitus, endometrial carcinoma and cardiovascular disease (Pachiappan et al., 2017). About three-quarters of individuals with PCOS have elevated androgen levels and this is said to be

genetically acquired and also a result of environment on hypothalamic-pituitary-ovarian axis (Yang et al., 2018).

The hypothalamic-pituitary-ovarian axis is responsible for the synthesis of gonadotropins. GnRH is released in a pulsatile manner into the pituitary portal blood vessels for delivery to the anterior pituitary for the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH). The pulsatile pattern of GnRH secretion is essential for proper coordination of gonadal function (Limonta et al., 2018). The LH stimulates theca cells outside of the follicle produce androgens that are transported into the developing antral follicles with subsequent conversion into estrogens via aromatase activity on FSH stimulation. FSH is the primary signalling molecule responsible for increasing the expression of the CYP450arom enzyme in granulosa cells (Barbieri, 2014).

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¹ Conceptualisation of The work

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P450 aromatase is an enzyme expressed in the placenta, ovary, adrenal gland and testis. It converts androgens (androstenedione and T) transported from the theca cells into estrogens (estrone and E2) (Limonta et al., 2018).

While the aetiology of the syndrome is yet to be wholly understood, previous studies have shown PCOS to be linked to hereditary, premature fetal development, lifestyle and environmental factors such as stress (Lee et al., 2018). It has also been identified that a decrease in the activity of cytochrome P450 enzyme could contribute to the development of PCOS. Deficiency in aromatase activity result in disturbance in steroidogenesis that triggers ovarian failure, such as in PCOS. Aromatase catalyses the biosynthesis of estrogens from androgens; decreased activity of this enzyme could cause hormonal imbalance, intraovarian androgen excess that could result in polycystic ovaries and circulating hyperandrogenism (Abdallah et al., 2023). In the ovary, androgens derived from theca cells are converted to estradiol under the influence of aromatase produced by granulosa cells (Lee et al., 2018; Abdallah et al., 2023). Abnormal follicular maturation and multiple cystic follicles arranged at the periphery of the ovary have been reported in the presence of elevated ovarian androgen levels (Barbieri, 2014).

Letrozole is an aromatase inhibitor that negates the action of the aromatase enzyme, which would cause a buildup of male hormones (testosterone) in females, resulting in hyperandrogenism (Lee et al., 2018). Disturbances in the FSH hormone feedback mechanism in the hypothalamus are a consequence of hyperandrogenic conditions.

Mifepristone (RU486) is a progestin antagonist which, as its name implies, acts to arrest the actions of the progesterone hormone. Progesterone is crucial in gonadotropin regulation, follicular development, and ovulation. If the actions of progesterone are arrested, this would cause follicular atresia and anovulation (Tamura et al., 2009). DHEA is an androgen which was also administered in a bid to cause hyperandrogenism in the rats, as it has been used in previous research in inducing PCOS in female rats.

The study aimed to investigate the effects of letrozole, mifepristone, and DHEA on the histomorphology of the ovary and uterus, comparing their impact to elucidate PCOS-like features.

2. Methodology

Forty-five adult female Wistar rats, 180–200 g, were purchased from a standard animal breeding farm in Ogbomoso, Oyo State, Nigeria. All rats were kept in the animal facility of the College of Health Sciences, University of Ilorin, where they were allowed to acclimatize for seven days prior to the commencement of the experiment. Animals had free access to standard pelleted rat feed and clean water that was regularly removed and replaced on a 24-hour basis during and after the acclimatization period. The pelleted rat feed was purchased from a standard production company in Ilorin, Kwara State, Nigeria.

The management of the rats complied with the ARRIVE guidelines and the requirements of the ethical board of the University of Ilorin, Ilorin, Nigeria.

Letrozole containing 2.5 mg fine-coated letrozole and Mifepack containing 200 mg mifepristone tablets produced by Ranbaxy (UK) Limited, as well as DHEA containing 50 mg dehydroepiandrosterone tablets obtained from Sigma-Aldrich, USA, were purchased from the general drug centre in Ilorin, Kwara State, Nigeria.

3. Ethical statement

This research work complied with the ARRIVE guidelines and it was approved with the code: UERC/ASN/2020/1994 by the University of Ilorin Ethical Review committee to be in compliance with the Institutional Animal Care and Use Committee (IACUC).

4. Administration

Forty-five adult female animals weighing 180–200 g were grouped into three broad groups for each of the three models. Each group was subdivided into two groups: a high dose and a low dose. Each group also had its control rats, which were not treated during the experiment. The dosage used is as follows;

Letrozole, a low dose of 0.2 mg/kg, was administered orally for 21 consecutive days (Ghagane et al., 2022).

Letrozole, a high dose of 0.4 mg/kg, was administered orally for 21 consecutive days (Abdallah et al., 2023).

Mifepristone, a low 2 mg/100 g dose, was administered subcutaneously for 9 consecutive days (Coelho junior, 2012).

Mifepristone, a high dose of 4 mg/100 g, was administered subcutaneously for 9 consecutive days (Meera et al., 2020).

A low dose of DHEA at 4 mg/kg was administered subcutaneously for 21 consecutive days (Pandey et al., 2010).

A high dose of 6.5 mg/kg of DHEA was administered subcutaneously for 21 consecutive days (Weber and Weber, 2015; Enya et al., 2022).

5. Tissue processing

After the administration was completed, the animals were sacrificed. The rats were weighed and anesthetized with 20 mg/kg ketamine before sacrifice. The abdomen was incised and dissected to excise the uterus and ovaries, which were then fixed in preparation for the histological analysis.

The tissues harvested were fixed in 10 % formalin and passed through ascending grades of alcohol, starting with 50 %, 70 %, and then 90 %, with an interval of 2 hours each. The tissues were then passed through two series of absolute (100 %) alcohol to get rid of all formalin. In preparation, the tissue for embedding was cleared in xylene to prevent a reaction between the alcohol and the paraffin wax at 56 degrees Celsius until all tissues were embedded at a similar orientation. Ultra-thin slides (4 µm) of the tissues were obtained using a microtome and then mounted on glass for viewing under the microscope.

The tissue sections were mounted on glass slides, stained with Hematoxylin and Eosin (H&E) stains, and cover-slipped. Sections were viewed and captured using an Olympus binocular research microscope connected to an Amscope camera and examined for changes in ovarian morphology.

6. Results

(Fig. 1a and 1b) Tissues from control animals showed relatively healthy ovarian cytoarchitecture, exhibiting follicles at different levels of development, including secondary follicles, graffian follicles, and corpora lutea. The treated groups generally had more preantral and fewer matured follicles (secondary and graffian) than the control groups.

The Letrozole-treated group had an increased number of preantral follicles compared to the control group, and this effect was more pronounced in the low-dose group. Compared to the low-dose group, the high-dose group showed cystic follicles with some evidence of atresia and large cysts without granulosa cells.

Ovarian follicles in the mifepristone-treated animals show decreased secondary and graffian follicles and increased preantral follicles compared to the control group. Enlarged cysts without oocytes and hyperplasia of the theca layer are also noted in the low-dose treated group. The high-dose treated group showed smaller cystic follicles and

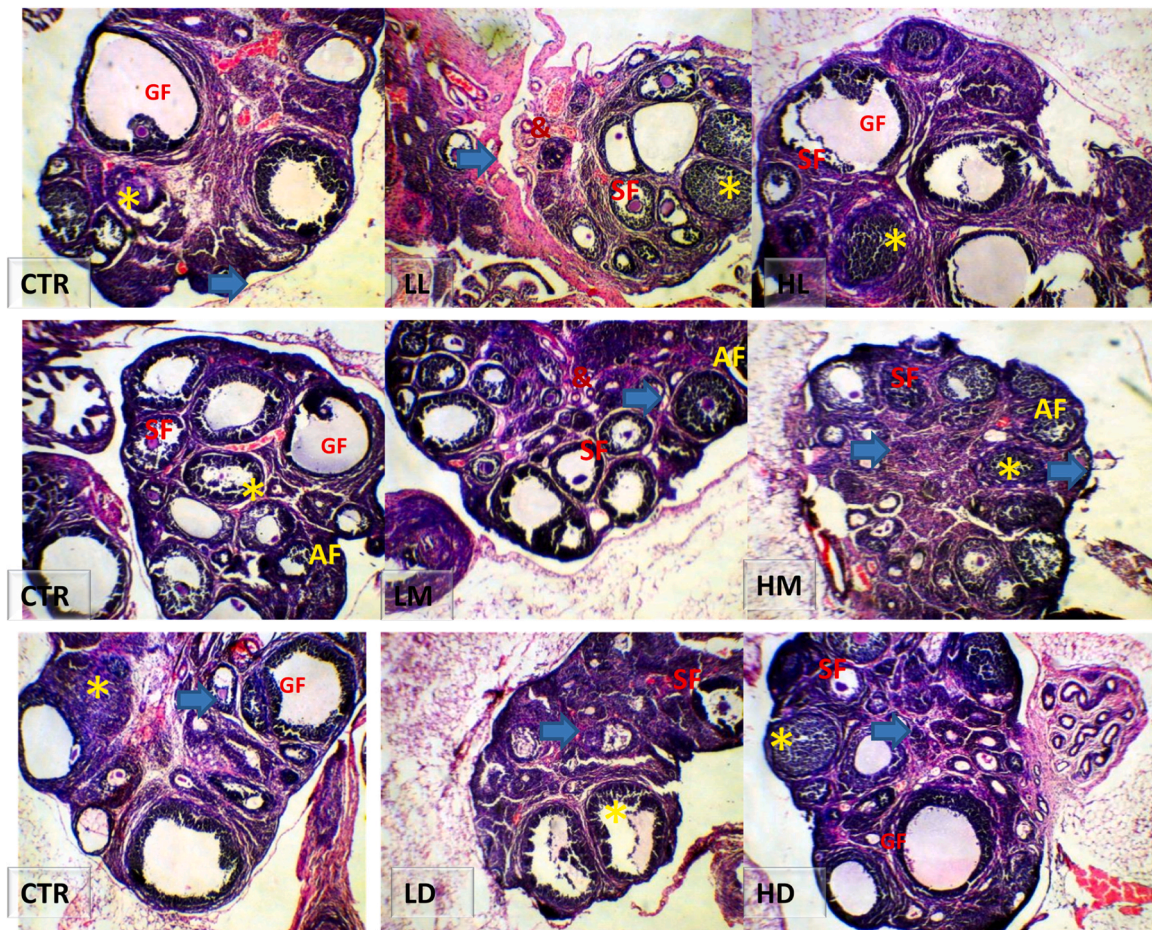


Fig. 1a. Photomicrograph showing the rate of folliculogenesis in female animal rats administered Letrozole, Mifepristone and DHEA * - corpus luteum, arrow – primary follicle, AT – atretic follicle, SF-Secondary follicle, GF – Graafian follicle, &-Primordial follicle. Image showing the ovarian structure of the control group (CTR), the low-dose letrozole group (LL), the high-dose letrozole group (HL), the control group (CTR), the low-dose mifepristone group (LM), and the high-dose mifepristone group (HM), the control group (CTR), and the low-dose DHEA group (LD) and high-dose DHEA group (HD).

more atretic oocytes than the low-dose group.

The treated group shows an increased number of ovarian follicles, and a highly reduced number of secondary or graafian follicles is noted. They also show large cysts without oocytes and a decreased number of corpus luteum compared to the control. (Fig. 2a)

The uterus of the control group displayed normal, typical cytoarchitecture, while the Letrozole-treated group showed endometrial thickening accompanied by uterine narrowing compared to the control. Groups treated with mifepristone have a thickened endometrial lining (more than the letrozole-treated group) with congested stroma. In contrast, the DHEA-treated groups show an endometrial layer similar to the control but with congested stroma.(Fig. 2b)

7. Discussion

Polycystic ovarian syndrome (PCOS) is an endocrine disorder characterised by hyperandrogenism and chronic anovulation. It is a multi-glandular syndrome that involves the hypothalamic, pituitary and ovarian axis, and it results in reproductive disturbances with metabolic complications such as obesity and hyperinsulinemia (Teede et al., 2006). Gonadotropins are released in a pulsatile manner, LH stimulates theca cells to produce androgens that are converted into estrogens via aromatase activity on FSH stimulation. Thus, deficiency in aromatase activity disrupt ovarian steroidogenesis which triggers development of PCOS.

Several studies have documented the efficacy of letrozole, mifepristone, and DHEA in PCOS establishment. To contribute new insights

into the dose-specific effects of these treatments on ovarian morphology, we explored the effects of varying doses of letrozole, Mifepristone, and DHEA on ovarian structure, revealing distinct histological changes that are dose-dependent.

The reduced number of follicles and increased atretic follicle and corpus luteum observed in this study are signs of ovarian damage and dysfunction. These align with previous studies by Juengel and co-workers, who suggest differential effects on ovarian morphology and follicular dynamics (Juengel et al., 2021). However, oxidative stress could contribute to ovarian damage and hormonal imbalance by increasing accumulation of free radical in the ovary, though this study did not assess biochemical parameters. Seyedeh-Roza et al., (Seyedeh-Roza Tafarishi Nejad et al., 2021 Jul 1) reported increased oxidative parameters in ovarian damage resulting from ovarian torsion.

Letrozole-treated groups resulted in an increased number of ovarian follicles, particularly in the low-dose group (Torres et al., 2019). Kafali et al. (2004) reported multiple ovarian cysts, growing follicles and oocytes, corroborating our study. Furthermore, we observed oocytes that lack granulosa cell layer, few theca cell layers and corpus lutea, contrary to the report of (Ahmed et al., 2023). The increase of follicles aligns with letrozole's mechanism of action, which involves the inhibition of estrogen synthesis resulting from deficient aromatase enzyme activity due to lack of FSH stimulation on granulosa cell layer, thereby disrupting the feedback mechanism on the hypothalamic-pituitary-gonadal axis. This disruption leads to an increase in gonadotropin-releasing hormone (GnRH) and, subsequently, follicle-stimulating hormone (FSH), promoting folliculogenesis (Tang et al., 2020). The high dose of letrozole

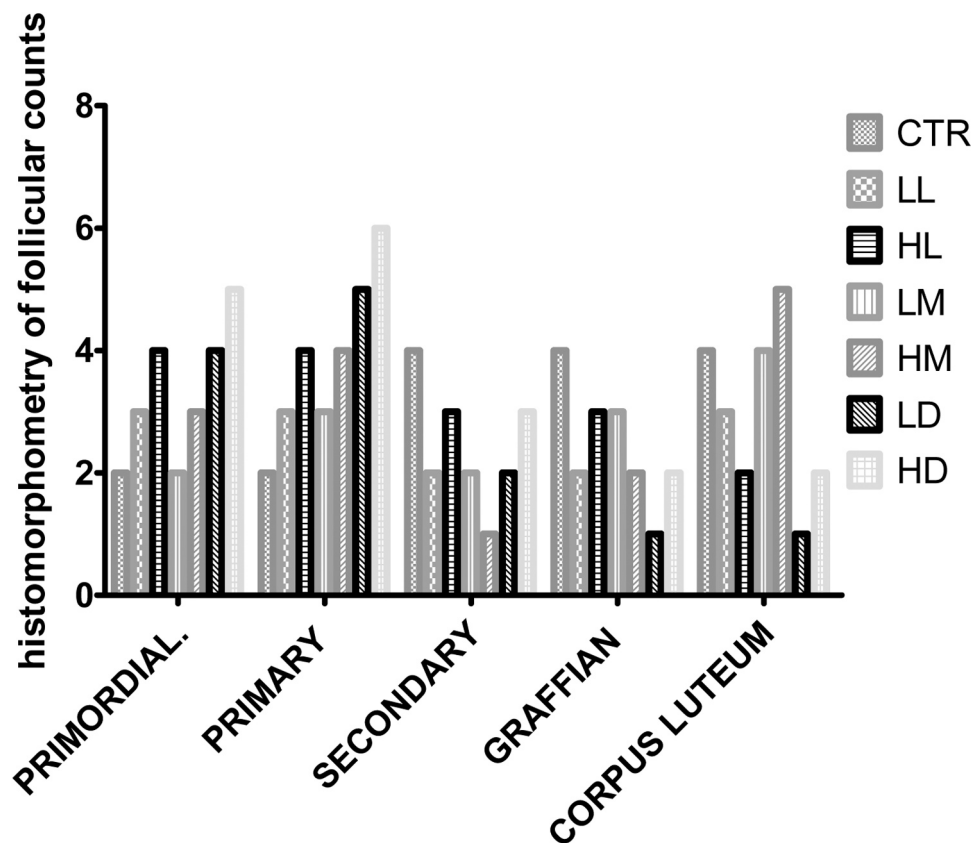


Fig. 1b. Showing histomorphometry of follicular counts.

resulted in cystic follicles and atresia, indicating a potential over-suppression of estrogen, leading to an imbalance in follicle maturation and atrophy.

Histological micrographs of ovarian cells in mifepristone-induced PCOS rats resulted in decreased secondary and Graafian follicles, as observed in both low (LM) and high-dose (HM) groups. This reduction aligns with the works of (Rezvanfar et al., 2012), who also noted follicular reduction following mifepristone administration. Additionally, the presence of enlarged cysts without oocytes in the low-dose group and small cystic follicles in the high-dose group reflects the findings by (Adams and Singh, 2021), suggesting a disruption in follicular maturation. Progesterone plays a vital role in facilitating follicular development and maturation. On the other hand, Mifepristone is known for its anti-progestogenic properties. Thus, the mechanisms by which Mifepristone induced these morphological changes observed in this study could be related to its ability to disrupt progesterone's facilitative action on follicular development and maturation (Tamura et al., 2009).

DHEA-treated groups (LD and HD) demonstrated an increased number of ovarian follicles but a notable absence of secondary or Graafian follicles. This result supports the hypothesis that (Nautiyal et al. (2022) proposed that DHEA supplementation can lead to an altered follicular environment. Moreover, the observed large cysts without oocytes and a decreased number of corpus luteum could indicate impaired ovulation, as suggested by Pivonello et al. (2020). It is well-known that hyperandrogenemia prevents the average follicular growth and manifests as a polycystic ovary. The underlying mechanism of DHEA could be due to the overproduction of androgens that impede the normal follicular maturation process.

The histological analysis of the uterus in letrozole-treated animals shows myometrial narrowing with constricted uterine glands accompanied by endometrial thickening similar to the Mifepristone-treated groups, which also exhibits endometrial hyperplasia with congested stroma as reported by (Enitome et al. (2020)). This phenomenon can be

attributed to letrozole's reduction in estrogen levels. Estrogen is a critical regulator of endometrial growth. With decreased estrogen synthesis, a compensatory mechanism may lead to endometrial hyperplasia, as the endometrium responds differently to the altered hormonal environment (Murugesan et al., 2020a; Rajan et al., 2017). Furthermore, uterine narrowing may result from altered myometrial responses to this hormonal imbalance (Roberts and Gammill, 2005).

Mifepristone-treated groups exhibited thickened endometrial lining with congested stroma. Mifepristone, a progesterone receptor antagonist, disrupts progesterone's standard modulation of the endometrium (Meera et al., 2020). Progesterone typically opposes estrogen's proliferative effects on the endometrium. In its absence or reduced action, as induced by Mifepristone, the endometrium may exhibit unregulated growth, leading to thickening (Murugesan et al., 2020b). The congested stroma observed could be indicative of increased vascularisation or oedema, potentially a response to disrupted endometrial homeostasis.

The DHEA-treated groups showed an endometrial layer similar to the control but with congested stroma. DHEA, a precursor to sex hormones, may not directly induce endometrial growth but could influence the local intrauterine environment. The congested stroma suggests a possible alteration in vascular dynamics or localised inflammatory response, which might be secondary to the hormone's conversion to estrogen or testosterone within the uterine tissue (FitzPatrick, 2022).

This summary highlights how letrozole, Mifepristone, and DHEA affect ovarian function and uterine physiology. Letrozole and DHEA increase ovarian follicle numbers, but their effects on follicle maturity and cyst formation differ. In contrast, Mifepristone reduces follicle numbers and promotes follicular atresia (Osuka et al., 2019). These effects are vital for understanding how these compounds might be used therapeutically in conditions such as polycystic ovary syndrome (PCOS), infertility, and other ovarian disorders. Similarly, the impact of these drugs on the endometrium is varied, with letrozole and Mifepristone causing endometrial thickening, while DHEA shows a more nuanced

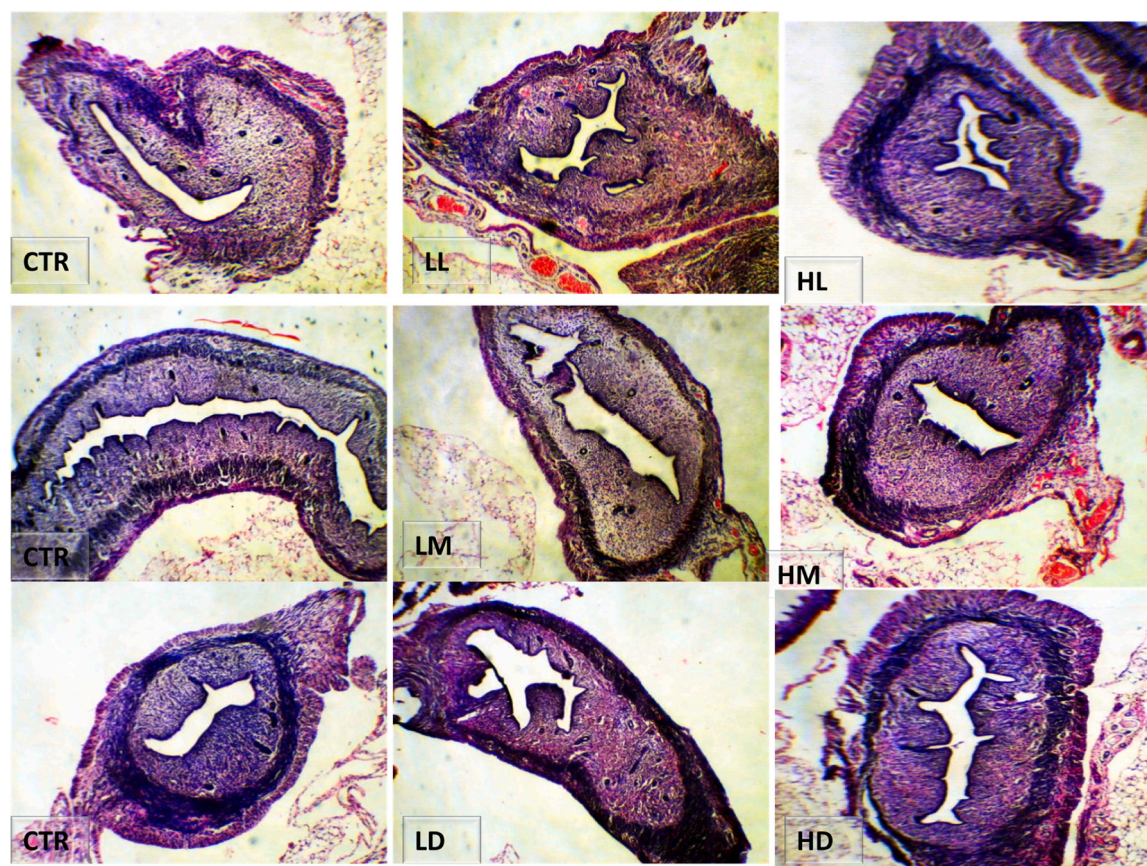


Fig. 2a. Image shows the uterine endometrial thickness of the control group (CTR) low, dose letrozole group (LL) and high dose letrozole group (HL), control group (CTR); low dose mifepristone group (LM) and high dose mifepristone group (HM), control group (CTR), low dose DHEA group (LD) and high dose DHEA group (HD).

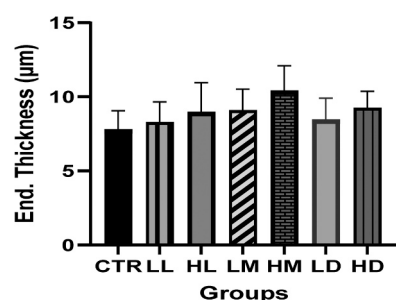


Fig. 2b. Showing histomorphometry of endometrial thickness. All treated groups had thickened endometrium compared with the control group, while it is thickest in animals administered high-dose Mifepristone. There is no statistically significant difference in the endometrial thickness of control animals compared to experimental animals.

effect (Arentz et al., 2014). This diversity in drug action underscores the complexity of hormonal regulation within the uterus and is particularly relevant for treating conditions like endometrial hyperplasia, uterine fibroids, and infertility treatments. Understanding these differential effects is crucial for optimising hormonal therapies in gynaecological conditions (Bulun et al., 2005).

8. Conclusion

The experimental models we employed could replicate one of the critical features of PCOS, which was the proliferation of ovarian follicles on the histological assessment. Although the efficacies varied from one to the other, the histology results alone suggest that the mifepristone

model was the most effective of the three models. However, we recommend further analysis to check the other features, such as hormonal and biochemical assays and assessment of the hypothalamic-pituitary-ovarian axis, for further comparison of these models.

Authors statement

This study examined the effect of Letrozole, Mifepristone and DHEA on ovarian and uterine cytoarchitecture of adult female Wistar rats in order to assess their various polycystic ovary-like histological features. Polycystic ovary syndrome (PCOS) is a common gynecological and endocrine disorder that occurs during reproductive age and it is a major cause of anovulatory infertility. Letrozole is an aromatase inhibitor, Mifepristone a progestin antagonist and DHEA is an androgen, all of which were administered to the rats in a bid to cause hyperandrogenism which is the hallmark of PCOS. The ovarian cytoarchitecture shows proliferation of ovarian follicles, various levels of follicular maturation in a substance-dependent and dose-dependent manner while also showing features of follicular atresia. Thickened endometrium with congested stroma and some uterine narrowing was observed in the uterine cytoarchitecture of the treated animals. The contributions of this study to scientific literature is expression of Letrozole, Mifepristone and DHEA effect on female reproductive functions especially cytoarchitecture of the ovaries and uterus.

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CRediT authorship contribution statement

Moyosore Salihu Ajao: Supervision. **Abdulmalik Omogbolahan Hussein:** Project administration, Methodology. **Ismail Adetayo Lawal:** Visualization. **Eniola Risikat Kadir:** Writing – review & editing, Methodology, Data Curation, Conceptualization. **Azeezat Dagbo Yakub:** Writing – original draft, Visualization. **Rukayat Jaji-sulaimon:** Methodology. **Lekan Sheriff Ojulari:** Methodology.

Data Availability

No data was used for the research described in the article.

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