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Establishing PCOS in Wistar rats: A reliable model for understanding polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a complex endocrine disorder affecting reproductive-age women, characterised by metabolic and reproductive abnormalities. This study aimed to develop and evaluate a comprehensive rat model of PCOS that accurately replicates both the metabolic and reproductive facets of the syndrome. Female Wistar rats were treated with dehydroepiandrosterone (DHEA), high-fat diet (HFD), and a combination of both for 20 and 30 days. The study assessed body weight, estrous cyclicity, serum biochemistry, hormone levels, and ovarian histology. The DHEA+HFD combination model effectively mimicked PCOS characteristics and exhibited significant increases in body weight, disrupted estrous cycles, blood glucose, lipid levels, testosterone, estrogen, and LH levels, with decreased FSH levels. Liver and kidney function markers were also altered, indicating systemic effects. Further, histological examination of ovaries revealed cyst-like follicles and reduced corpus luteum formation, resembling PCOS ovarian morphology. Moreover, DHEA alone induces reproductive changes without significant metabolic alterations, and HFD alone showed slow progression of metabolic features, but the combination group rapidly induced both metabolic and reproductive abnormalities within 20 to 30 days. The synergistic effect highlights the potential role of diet in exacerbating PCOS symptoms. Current study presents a rat model that comprehensively replicates PCOS features in a shorter timeframe. This combination model is valuable for investigating PCOS pathophysiology and potential therapeutic interventions. Furthermore, these findings underscore the importance of considering nutritional factors in PCOS management and open new avenues for research into the intricate relationship between PCOS-related metabolic and reproductive abnormalities.

Keywords: PCOS, Estrous cycle, Hyperandrogenism, Metabolic alterations, Obesity, Dietary factors

Polycystic ovarian syndrome (PCOS) is among the most prevalent endocrinopathies and a major contributor to infertility among women of reproductive years[1]. PCOS is often marked by hyperandrogenism, manifesting as hirsutism and acne. It also entails menstrual irregularities stemming from ovulatory dysfunction. Recently, it was reported that women with PCOS have hypertrophic adipocytes, increased abdominal fat, and a greater incidence of fatty liver disease in comparison to age-related and weight-matched healthy women. Thus, PCOS is now considered to be a significant metabolic illness in addition to reproductive morbidity[2,3].

Following Stein & Leventhal's mid-1930s description of PCOS, efforts were made to determine the etiologic mechanisms behind PCOS development[4]. The precise aetiology of PCOS remains elusive; however, it is postulated to arise from a multifaceted interplay of hormonal, environmental and familial factors[5]. The most recent NIH report claims that the numerous criteria employed are confusing and impede the advancement of understanding the illness, highlighting that the diagnostic criteria for PCOS are still up for debate[6]. Using the inclusionary criteria established in Rotterdam, the committee recommended stratifying the data into four characteristics: (1) androgen excess combined with ovulatory dysfunction; (2) androgen excess coupled with polycystic ovarian morphology; (3) ovulatory malfunction coupled with polycystic ovarian morphology; and (4) androgen surplus coupled with ovulatory malfunction coupled with polycystic ovarian morphology[7].

Being a multifactorial syndromic condition with an unclear aetiology and is influenced by genetic, hormonal, and environmental factors. Genetic susceptibility plays a key role in PCOS, as evidenced by multiple associated genetic variations and familial clustering of symptoms[8]. Another pivotal factor is insulin resistance (IR), with many women exhibiting impaired sensitivity that leads to elevated

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insulin levels, stimulating increased androgen production in the ovaries. This hyperandrogenism leads to anovulation and irregular menstrual cycles[9]. Furthermore, environmental factors such as obesity and lifestyle choices exacerbate IR and hormonal imbalances.

Essentially, women's health is severely impacted, leading to a range of reproductive and metabolic complications. Women with PCOS commonly experience irregular menstrual cycles and anovulation, which are primary contributors to infertility, making it one of the leading causes of reproductive challenges and further increasing the risk of insulin resistance, obesity, type 2 diabetes and cardiovascular diseases[10]. Additionally, psychological repercussions are also very prevalent, with many women reporting instances of anxiety and depression as a result of hormone imbalances[11]. Overall, the interaction of various environmental, hormonal, and genetic factors results in a variety of PCOS symptoms that call for all-encompassing management approaches to address its effects on mental and physical health.

Due to the limited human experimentation in both logistical and ethical ways, animal models are suitable for studying the pathophysiology of PCOS. Hyperandrogenism is one of the primary traits of PCOS and has been extensively studied using various rodent models. These models are primarily developed by administering androgenic hormones, such as dihydrotestosterone (DHT) or dehydroepiandrosterone (DHEA), estrogenic hormones, or aromatase inhibitors to simulate the hyperandrogenic state observed in PCOS[12]. However, none of the animal models fully replicate the disease's metabolic and reproductive phenotypes; in other words, while some animal models can accurately show the reproductive phenotype, they fail to duplicate the metabolic phenotype[13,14]. The potential of a single model to depict the pathophysiology in all dimensions is diminished by the disease's vast range of symptoms and complex aetiology. Thus, it is crucial to have an appropriate animal model that mimics many, if not all, of the reproductive and metabolic characteristics for a better understanding of the disease's pathophysiology.

In this context, the central focus of this study is to undertake a meticulous analysis of the PCOS-induced rat model encompassing metabolic abnormalities, hormonal disruptions and ovarian function linked to reproductive impairments. It evaluates the benefits and potential drawbacks of extrapolating these models to human PCOS. Thus, the present study builds on these existing models by employing a novel design that includes distinct experimental groups. This approach aims to better simulate the interplay between hyperandrogenism and metabolic dysregulation over varying durations of exposure. Including short-term (20-day) and long-term (30-day) DHEA treatments, as well as the combined DHEA+HFD model, allows for a more comprehensive investigation of the reproductive and metabolic phenotypes of PCOS. Unlike traditional models that focus on isolated aspects of the disease, this study's design provides a more integrative framework to explore the pathophysiological mechanisms underlying PCOS, simultaneously addressing its reproductive and metabolic dimensions.

Materials and Methods

Chemicals, solvents and drugs

DHEA was procured from APExBIO (B1375-10000), with purity >98% and Metformin from Hi-Media Laboratories Pvt. Ltd. (RM10257). The study used high-quality chemicals, pharmaceuticals, and reagents procured from SRL and Hi-Media.

Animals

Healthy, adult female Wistar rats (1-1.5 months/70-100g) were procured from Central Animal House, Panjab University, Chandigarh and housed in polypropylene cages in a room with controlled lighting, a 12 h light and dark cycle, an ambient temperature of 23-25°C and accessibility to food and water. All animal procedures were approved by the Institute Animal Ethics Committee (IAEC) of Panjab University, Chandigarh complied with the guidelines provided by the Committee for Control and Supervision of Experiments on Animals (CCSEA) and followed by IAEC-approved animal study proposal (Ref. No. PU/45/99/CPCSEA/IAEC/2023/770 & 848).

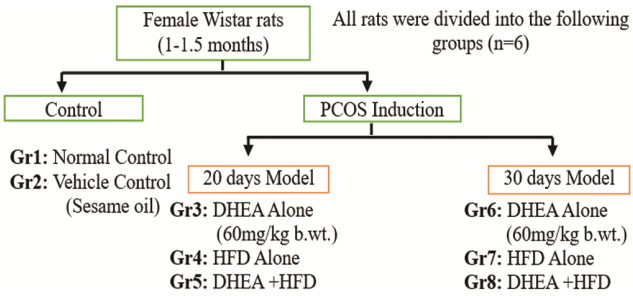
High Fat Diet (HFD)

The high-fat diet was commercially procured from M/s Ashirwad Industries, Punjab Hindustan Lever, India. The high-fat diet contains 414.0kcal/100g, with 44% as carbohydrates, 16% as protein, and 40% as fat. The diet comprises 65% regular rat chow pellets, 20% dried milk powder (Nestle), 6% soyabean oil (Fortune), and 9% pure ghee (Aashirvaad pure ghee). PCOS model rats were fed HFD with 40% of the calories derived from fat[15]. The prepared HFD was given for 20 and 30 days, respectively, to induce PCOS.

Induction of PCOS

After acclimatisation, all rats were randomly divided into the following groups (n=6 in each group): control, DHEA, 40% HFD, and DHEA+HFD groups. The DHEA group received daily oral administration of DHEA (60 mg/kg body weight dissolved in 0.2 mL of sesame oil)[16] and were fed a normal diet. The DHEA+HFD groups were orally gavaged with the DHEA following the same dosage along with the 40% HFD[17]. The control and HFD alone groups received a normal diet and 40% HFD respectively. Further, the reproductive like estrous cycle disruption, ovarian morphology, and hormonal imbalance (testosterone, LH, FSH, estradiol) and metabolic features like body weight, glucose level and lipid profiles were evaluated in 20 and 30-day PCOS-induced groups.

Experimental design



Body weight measurement

During the experimental period, the rats were weighed every second day from the day of PCOS induction until the end.

Estrous cycle determination

Vaginal smear tests were done daily from the 10th day of PCOS induction to ascertain the stage of cyclicity. Vaginal walls were cleaned with saline (0.9% NaCl) to collect cells, which were then smeared on the glass slides. The slides were assessed under a microscope to determine the prevailed cell type in the vaginal smears during proestrus (round, nucleated epithelial cells), estrus (cornified squamous epithelial cells), metestrus (cornified squamous epithelial cells and leukocyte predominance), and diestrus (nucleated epithelial cells and leukocyte predominance)[18].

Biochemical and hormonal analysis

The blood without anticoagulant was collected by myocardial puncture. Blood samples were set aside at room temperature for half an hour. After that, the serum was separated by centrifugation for 15 min at 4°C at 1000 rpm. The serum was analysed for glucose levels, lipid profile, liver function test (LFT), and renal function test (RFT), which were measured by an autoanalyser (Erba Chem 7 plus) using the Erba diagnostic kits, West Bengal (India). The serum hormonal levels (estrogen, androgen, FSH, and LH) were evaluated using Vacmen Acess2 ELISA kits, USA.

Histological examination

For histological evaluation[18] the rats were ethically euthanized by barbiturate overdose (intraperitoneal injection, 60 mg/kg pentobarbital sodium)[19] and the ovaries were expunged and preserved in 4% formaldehyde overnight. Subsequently, the ovaries were immersed in 70% ethanol and fixed in paraffin. Following fixation, the ovarian tissues were cut into serial longitudinal sections of 6 µm thickness and stained for microscopic assessment using haematoxylin and eosin (H&E). The obtained sections were then examined under the light microscope (Progress C7, Camera) to determine ovarian morphology, focusing on the alterations in follicular development and the presence of cystic follicles.

Statistical analysis

The statistical analysis was done using Graph-Pad PRISM 8 and expressed as mean±SD. The differences between the multiple groups were analysed using a two-way analysis of variance (ANOVA) followed by Tukey's multi-comparison *post hoc* test to compare the effects among various groups. P value ≤0.05 was considered to be statistically significant.

Results

DHEA+HFD treatment increases body weight and blood glucose levels

In the 20-day model groups, the increase in body weight was non-significant in DHEA and HFD alone groups. Still, a significant gain was noted in the combination (DHEA+HFD) group paralleled to the control (P≤ 0.01). Further, a notable increase was seen between the combination and DHEA alone (P≤0.01) group (Fig. 1A; P=0.0041). A progressive increase in body weight was observed in groups during the 30-day model period. Still, a significant rise in the DHEA+HFD group related to the control (P≤ 0.01; P=0.0021). Further, there was a substantial increase in the combination group compared to the HFD and DHEA alone groups (Fig. 1B; P=0.0043). Based on the inclusion of HFD,

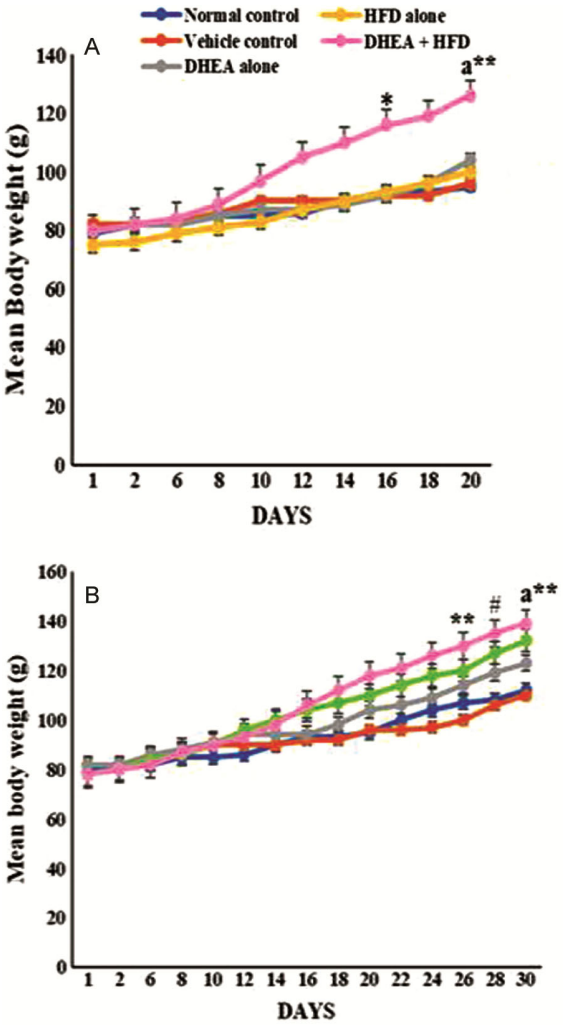


Fig. 1 — Mean body weight during the (A) 20 days and (B) 30 days model induction period. DHEA+HFD significantly increased body weight.

results showed that rats treated with DHEA experienced aberrant fat accumulation.

Reproductive cycles of rats

Vaginal smears were examined under a microscope from the 10th day of PCOS induction to evaluate reproductive cycles. Findings established that the control group had regular cycles, whereas, in the HFD alone group, a protracted but complete cycle was observed. However, nearly all the DHEA and DHEA+HFD groups presented a continual diestrus smear, and both groups experienced irregular (prolonged/arrested diestrus stage) estrous cycles (Fig. 2A & 2B).

Effect of DHEA and HFD on sugar level and lipid profile

There was a steady elevation in the blood sugar levels in each group compared to the control, however, a significant increase in DHEA, HFD and DHEA+HFD 30-day model groups ($P \leq 0.01$) was noted. Whereas, in the 20-day model groups, the levels were increased but not as much as in the 30-day model groups. Further, results showed that DHEA treatment in all groups leads to a surge in cholesterol and lipid levels in comparison to the control. The DHEA+HFD 20 and 30-day model groups and DHEA alone 30-day group exhibited markedly elevated serum levels of total cholesterol ($P \leq 0.01$; $P=0.0012$) and lipids than the DHEA and HFD alone in 20-day model groups, suggesting that HFD caused the elevated serum cholesterol levels in combination groups. Further, the total lipid levels were considerably higher in the 30-day model groups for DHEA and DHEA + HFD ($P \leq 0.01$; $P=0.0038$). However, there was an increase in the 20-day DHEA and DHEA+HFD model groups, but the increase was insignificant in comparison to the control group (Table 1).

Effect of the DHEA and HFD on the liver and kidney function tests

Liver and renal function tests were done to investigate the effects in DHEA and HFD-induced rats. For renal function analysis, creatinine, uric acid and blood urea levels were determined, which are reliable markers of kidney function. Creatinine and uric acid levels in 30-day induced DHEA and DHEA+HFD groups were substantially increased in comparison to the control, and a marked increase was seen over the day 20 and 30 DHEA alone groups ($P \leq 0.05$; $P=0.0023$). Further, there was a notable increase in creatinine levels on days 20 and 30 in DHEA and DHEA+HFD groups and an extremely significant rise in uric acid levels between days 20 and 30 ($P \leq 0.001$; $P=0.0003$). Further, the urea levels were significantly increased in the DHEA+HFD group in both the 20 ($P \leq 0.05$) and 30-day ($P \leq 0.01$) model groups in comparison to control and a highly significant increase between the 20 and 30-day combination groups ($P=0.0041$). This suggested that DHEA administration may be indicative of renal impairment, altered renal excretion capacity and potential metabolic dysfunction in the combination group.

Serum alkaline phosphate, SGPT and SGOT levels were determined for liver function analysis. Alkaline phosphate concentrations indicated a noteworthy shift in the DHEA+HFD between the day 20 and 30 model group ($P \leq 0.01$) and when compared to the control

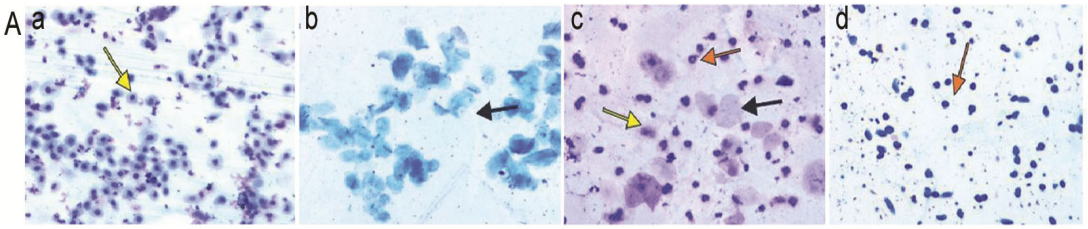


Fig. 2 — (A) Representative images showing estrous cycle stages as detected by vaginal smear analysis (a) Proestrus stage with a predominance of nucleated cells (Yellow arrow) (b) Estrus stage with a cluster of cornified cells (Black arrow) (c) Metestrus stage with a population of all three cell types-nucleated cells, cornified cells and leukocytes (d) The diestrus stage shows numerous prominent leukocytes (Orange arrow).

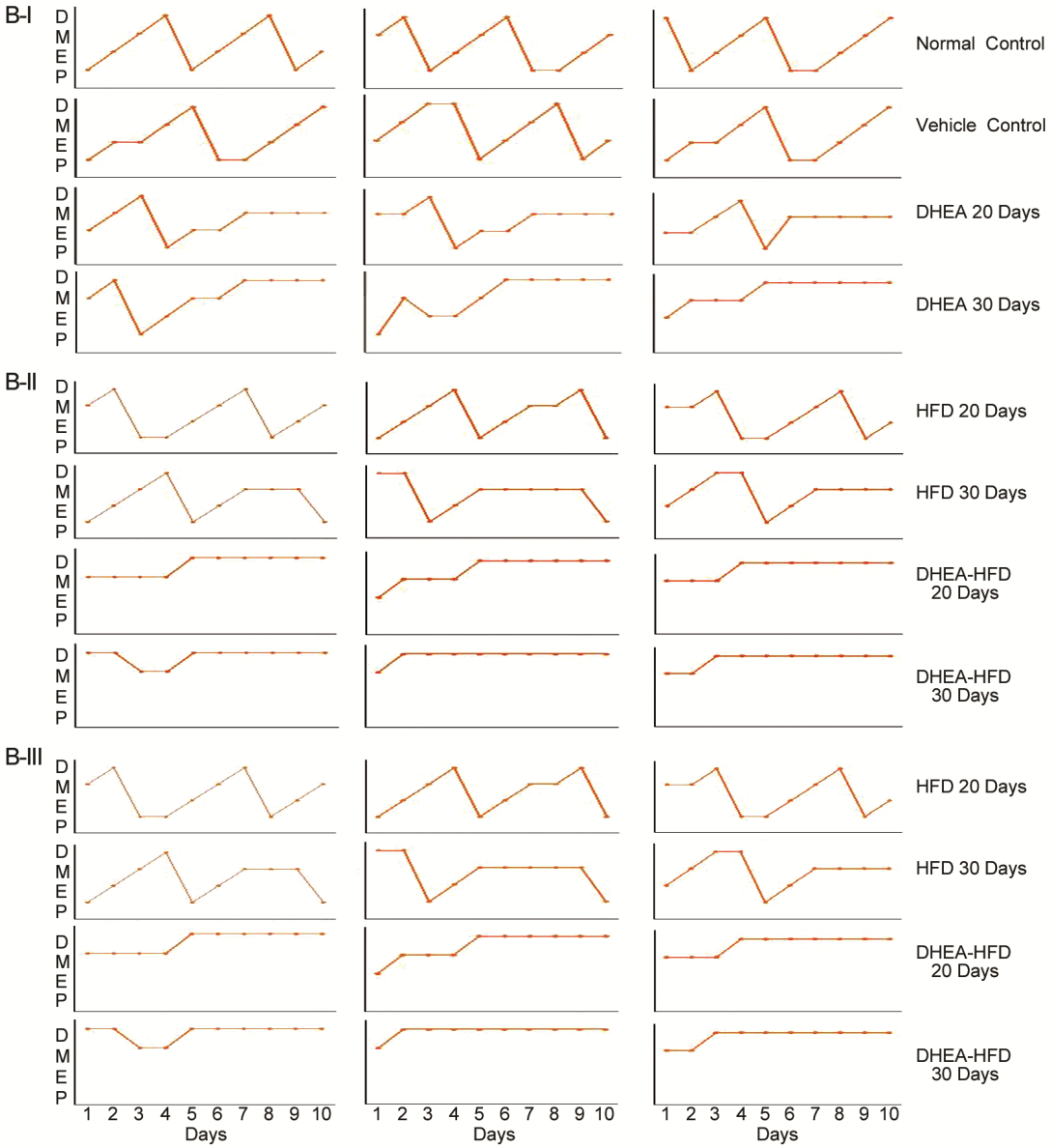


Fig. 2 — (B) Graphical representation of the dynamic changes in the estrous cycle due to the DHEA, HFD and DHEA+HFD treatment. The y-axis represents the different estrous stages (P, proestrus; E, estrus; M, metestrus; D, diestrus), and the x-axis represents the number of days. Each graph corresponds to one animal since the cycles were similar in all animals.

Table 1 — Biochemical markers in PCOS rat model

Groups	Blood sugar (mg/dl)	Lipid profile (mg/dl)		Renal function test (mg/dl)			Liver function test (IU/L)		
		Total cholesterol	Total lipids	Creatinine	Uric acid	Blood urea	Alkaline phosphate	SGPT	SGOT
Normal control	203.7±3.5	74.3±1.7	258.0±2.2	0.7±0.03	4.7±0.2	17.3±.9	101.7±1.7	43.7±1.2	26.5±1.0
Vehicle control	201.0±3.0	102.0± 1.6	279.3±2.5	0.8±0.01	3.9±0.2	17.5±1.1	98.7±1.2	45.4±1.7	25.7±1.7
DHEA 20	207.7±2.5	97± 1.6	277.0±2.2	0.8±0.02	5.1±0.1	17.2±.6	97.7±1.7	$51.0\pm0.8^{**}$	$35.0\pm2.2^{***}$
DHEA 30	$242.7\pm3.1^{\#\#}$	$129.3\pm2.1^{\#,**}$	$401.3\pm2.6^{\#\#,**}$	$0.9\pm0.02^{\#\#,*}$	$5.9\pm0.2^{\#\#}$	19.2±.6	108.3±1.2	$68.0\pm1.6^{\#\#,**}$	$64.3\pm1.2^{\#\#,***}$
HFD 20	223.0±2.6	$80.3\pm1.2^{**}$	$261.3\pm2.6^{*}$	0.7±0.01	5.3±0.2	17.0±.8	99.0±1.6	42.7±2.5	$34.7\pm2.1^{***}$
HFD30	$244.7\pm2.5^{\#\#}$	87.0±1.6	$310.0\pm2.4^{*}$	0.7±0.02	5.9±0.2	18.7±1.2	101.7±1.2	50.7±1.7	$52.7\pm2.1^{\#\#,***}$
DHEA+HFD 20	233.7±4	$135.0\pm3.7^{\#,**}$	$331.7\pm2.5^{**}$	0.7±0.01	$6.0\pm0.1^{***}$	$21.7\pm1.3^{\#,***}$	$122.7\pm2.1^{\#,**}$	$84.0\pm1.6^{\#,**}$	$58.3\pm1.2^{\#,***}$
DHEA+HFD 30	$247.7\pm2.5^{\#\#}$	$156.7\pm1.2^{\#\#,**}$	$449.7\pm2.9^{\#\#,**}$	$0.9\pm0.03^{\#\#,**}$	$9.9\pm0.3^{\#\#,***}$	$28.5\pm0.7^{\#\#,***}$	$146.3\pm2.5^{\#\#,**}$	$1101.7\pm1.7^{\#\#,**}$	$72.3\pm1.7^{\#\#,***}$

[Values are presented as means ± SD. *$P\leq 0.05$ (significant), **$P\leq 0.01$(highly significant) and ***$P\leq 0.001$ (extremely significant) represent differences within the same group when compared to day 20 and day 30; compared with the control group on day 20; #-highly significant difference ($P\leq 0.01$) as compared to control on day 20; ##- highly significant difference ($P\leq 0.01$) as compared to control on day 30]

group ($P\leq0.01$). Also, there was a sharp increase in the level in comparison to the alone groups (P=0.0010). Additionally, results showed a rise in SGPT levels in the DHEA and HFD 30-day group, whereas the increase was significant in the DHEA+HFD group on day 30 ($P\leq0.01$). The difference between day 30 in DHEA alone and day 20 & 30 in DHEA+HFD groups was also significant ($P\leq0.01$) ((P=0.0041). Further, SGOT levels progressively rose in every group when compared to the control. Nonetheless, a noticeable rise was noted in the day 30 model groups compared to the day 20 model groups ($P\leq0.001$). Still, this increase was high in the combination group ($P\leq0.01$; P=0.0024).), indicating that DHEA and HFD administration leads to metabolic dysregulation (Table 1).

HFD accelerated metabolic and endocrine deficits induced by DHEA administration

Fig. 3 illustrates the serum concentration of LH, FSH, estradiol, and testosterone. DHEA administration alone and in combination with HFD led to the elevation in LH, testosterone, and estrogen levels with no increase in FSH levels. FSH levels were markedly decreased in the DHEA, HFD and DHEA+HFD groups in comparison to the control ($P\leq0.01$), however, this decrease was profound in the day 30 DHEA+ HFD model group ($P\leq0.01$) (Fig. 3A; P=0.0001). While the FSH levels were down-regulated, the LH levels were elevated in the DHEA and DHEA +HFD groups in comparison to the control and an increase between day 20 and 30 was noted in the DHEA and DHEA + HFD group ($P\leq0.05$) (Fig. 3B; P=0.0018). The serum estrogen levels were also elevated in the 30-day treated DHEA+HFD group in comparison to the control ($P\leq0.01$; P=0.0011). Additionally, estrogen levels considerably surged in 30-day DHEA-treated groups

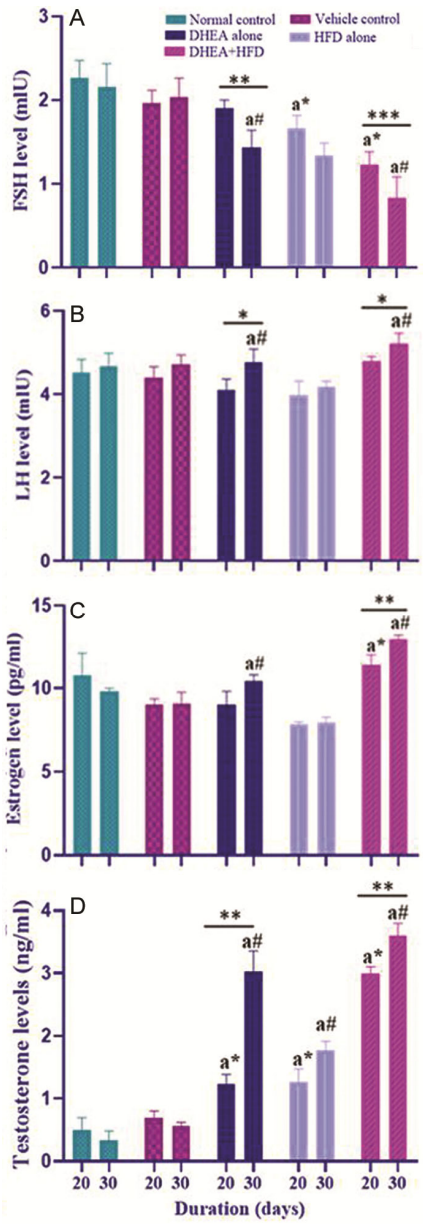


Fig. 3 — Serum hormonal levels in the rat. (A) FSH level; (B) LH level; (C) Estrogen level; (D) Testosterone level.

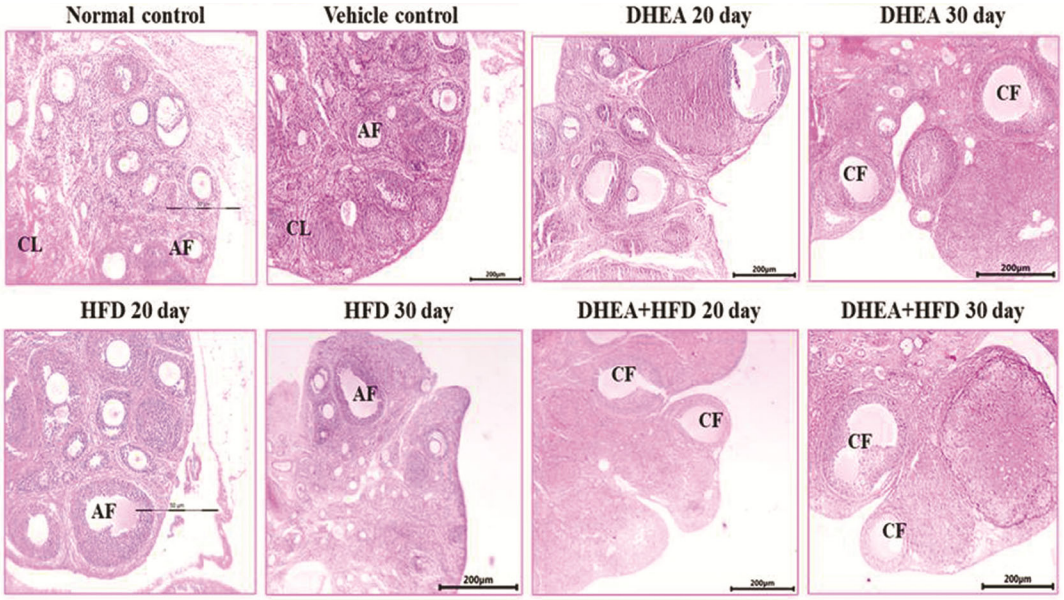


Fig. 4 — Representative images of histological sections of ovaries (CL: Corpus luteum, AF: Antral follicle, CF: Cystic follicle) (Magnification level ×10).

(Fig. 3C; P=0.0023). Moreover, the testosterone levels were significantly elevated in the DHEA, HFD and combination groups compared to the control. Specifically, the 30-day combination model group showed a predominance of higher values ($P \leq 0.01$) (Fig. 3D; P=0.0026). The reason can stem from DHEA's impact on hormone balance.

DHEA and HFD-induced ovarian morphological alterations

Control groups exhibited normal morphology, with intact theca and granulosa cells and follicles at various stages (Fig. 4A). In contrast, 30-day DHEA-treated rats showed a cyst-like appearance and few corpora lutea compared to the 20-day induced model. Cystic follicles are large, fluid-filled, and lack or reduced/thin granulosa cell layers, reflecting incomplete ovulation. In HFD alone, 20 and 30-day model groups' histological analysis displayed developing follicles at various stages alongside atretic follicles, which represent degenerating follicles marked by fragmented granulosa cells and apoptosis, arising from normal follicular atresia. However, it did not exhibit any cystic follicles as compared to the other induced groups, which may be due to the shorter period of induction of PCOS by 40% HFD. Further, DHEA+HFD 20 and 30-day model cohorts showed a higher number of cystic follicles characterised by diminished granulosa cell layers. They contained fluid-filled cysts (Fig. 4B-4D), compared to the control and other induced groups. Thus, findings imply that rats treated with DHEA and DHEA+HFD exhibited ovarian abnormalities, which worsened when HFD was supplemented.

Discussion

PCOS, with its dual challenges of metabolic and reproductive dysfunctions, profoundly impacts women's health and well-being, affecting over 80% of women[20]. Obesity is a common comorbidity of PCOS, as diagnosed patients often have a history of obesity. Further, at least two of the three cardinal symptoms oligo- or anovulation, hyperandrogenism, and polycystic ovaries (PCO) are obligatory for the diagnosis of PCOS[21]. This study presents a rat model that combines diet-induced obesity and DHEA administration, successfully replicating the complex metabolic and reproductive hallmarks of PCOS with unprecedented accuracy. Moreover, the ethical and logistical limitations greatly restrict the clinical trials essential to provide a conclusive understanding of the factors behind PCOS development. Thus, investigating the genesis and pathology of this enigmatic medical ailment requires animal models that mimic the features of PCOS. Over several decades, numerous animal models have been developed for PCOS, including models using sheep, monkeys, and rodents (mice and rats) through the perpetual prenatal and postnatal testosterone treatments which lead to the reprogramming of the

hypothalamic-pituitary-ovarian axis or by exposing them to excess hormones such as androgens or estrogens, or through genetic modifications that replicate human reproductive and metabolic dysfunctions[14]. Given that PCOS is a multifactorial illness, the ideal model would be one that could represent every symptom that the condition can cause but most of the models mentioned above cannot demonstrate the symptomatic characteristics of PCOS patients[22]. While creating an apt animal model for PCOS, factors like reproducing human PCOS characteristics, expense, specificity, precision, ease of induction, and duration are important considerations.

Among the drawbacks of the many animal models of PCOS is their inability to faithfully represent every aspect of the human state, especially the DHEA and HFD alone model. The DHEA alone model may not be able to accurately replicate insulin resistance or hyperinsulinemia while inducing a phenotype akin to PCOS. However, the reproductive characteristics of PCOS may not be fully captured by the HFD model on its own. Additionally, the ability of different animal models to precisely mimic the conditions of PCOS in humans may be limited because of variances in metabolic responses and phenotypic outcomes[14,23]. Given the strong association between obesity and PCOS, the significance of diet in reproductive health has been highlighted[24]. Perturbations in menstruation, hyperandrogenism, and hirsutism are more common in obese people. In light of this, nutrition is vital in determining the occurrence and severity of PCOS[25]. The adverse consequences of androgens, which cause insulin resistance and glucose intolerance, can be made worse by HFD. The current study's findings corroborate earlier research that showed DHEA and HFD considerably accelerated weight gain.

The substantial risk of metabolic problems associated with PCOS is most likely caused by its correlation with insulin resistance and obesity, particularly abdominal obesity[26]. The present investigation employed the DHEA model, which exhibited high stability and was consistently shown to exhibit disrupted cyclicity, hyperandrogenism, and polycystic ovaries. Overall, our results suggested that the metabolic and endocrine concerns caused by DHEA alone were made worse by adding 40% HFD, especially when combined with DHEA for 30 days. PCOS women frequently exhibit obesity and metabolic characteristics, and studies indicate a positive relationship between obesity and the magnitude of PCOS symptoms[23]. In prior research, HFDs have been employed to induce the metabolic characteristics of PCOS; however, achieving these changes necessitates an incubation period of around 15-16 weeks[27]. In this experiment, we implemented HFD and DHEA protocols for 20 and 30 days, both alone and in combination. The HFD alone group exhibited minimal metabolic changes due to the shorter induction period. Conversely, the combination group displayed notable rises in body weight, with these effects becoming more pronounced on day 30. Furthermore, the findings align with the prior studies that showed a correlation between PCOS and obesity[28,29]. The synergistic effect of DHEA+HFD demonstrated in this study accelerated PCOS development within a shorter time and accurately replicated both metabolic and reproductive features, addressing a critical gap in existing animal models. This approach provides a more time-efficient and comprehensive platform for investigating PCOS pathophysiology and potential therapeutic interventions, particularly highlighting the crucial role of dietary factors in disease progression.

Studies demonstrate that various metabolic markers, such as triglycerides, low-density lipoprotein, and homocysteine, only showed changes when HFD was administrated, highlighting the cumulative consequences on metabolism over DHEA alone[30]. Next, in the present study, the metabolic features including lipid profile, and liver and renal function tests were evaluated, and results revealed an elevated level in cholesterol and lipid levels in DHEA-treated groups, particularly when combined with HFD, indicating a profound effect on lipid metabolism. Abnormal blood lipid levels are thought to be present in 70% of PCOS patients and dyslipidemia is caused by insulin resistance, which also lowers lipoprotein lipase activity[31]. Moreover, this research lends credence to the idea that dyslipidaemia and PCOS are related and could be a factor in the elevated cardiovascular risk seen in PCOS-afflicted women. The alterations in liver and kidney function markers, particularly in the DHEA+HFD groups, suggest that these treatments may have systemic effects beyond the reproductive system. The increased levels of SGOT and SGPT indicate potential liver damage, while elevated blood urea and uric acid levels suggest renal impairment. Studies showed that metabolic disturbances are intricately intertwined with the aetiology and progression of chronic liver conditions in PCOS women[32]. The findings highlight the need for monitoring hepatic and

renal function in PCOS patients, especially those with concurrent metabolic syndrome.

The estrous cycle in rats parallels the human menstrual cycle in hormonal regulation and ovarian function, further providing insights into menstrual irregularities despite the cycle duration differences. Both cycles are regulated by estrogen and progesterone, influencing mood and physiological responses[33]. Moreover, estrous cycle disruption in DHEA and DHEA+HFD groups, typified by protracted or irregular periods, is consistent with the traditional manifestation of PCOS. Diestrus smears are consistently observed in the groups, indicating a severe impairment of ovarian function that is made worse by the inclusion of HFD and time duration. Studies show that increased steroidal hormone is associated with follicular cyst formation and altered ovarian steroidogenesis[34]. This is made worse by GnRH-dependent LH secretion, which stimulates androgen production and prevents follicular maturation and anovulation[35]. Further, imply that DHEA can alter the hypothalamic-pituitary-gonadal axis feedback mechanisms, which may decrease the release of FSH and increase the release of estrogen and LH. HFD in conjunction with DHEA may further have a detrimental impact on hormonal balance that could lead to fluctuation in hormonal levels.

These findings indicated that the PCOS rat model was effectively developed with DHEA treatment; however, the combination group experienced substantial metabolic issues brought on by HFD. The alterations exacerbated in combination groups suggest that dietary factors might be involved in modulating the severity of PCOS hormone abnormalities. Above that, the histological changes observed in the ovaries of DHEA and DHEA+HFD-treated rats, the presence of cyst-like follicles and reduced corpus luteum, closely mimic the ovarian morphology seen in PCOS patients whereas the HFD alone groups did not show any prominent changes in the hormonal and histological characteristics due to the short period of incubation i.e. 20 and 30 day. Additionally, the severe changes in the combination group underscore the potential synergistic effect of DHEA and HFD in promoting ovarian dysfunction.

Looking ahead, this model opens new avenues for investigating therapeutic interventions targeting both metabolic and reproductive aspects of PCOS. Future research should focus on elucidating the molecular mechanisms underlying the interaction between dietary factors and hormonal disturbances in PCOS development. Furthermore, studying the reversibility of PCOS features through dietary and therapeutic interventions using this model could provide valuable insights for clinical applications. This comprehensive model thus provides a robust platform for advancing our understanding of PCOS pathophysiology and developing more effective treatment strategies.

Conclusion

In conclusion, the present study establishes that a 20 and 30 day combined DHEA+HFD treatment successfully induces comprehensive PCOS-like features in rats, demonstrating significant metabolic alterations, including increased body weight, elevated blood glucose, and disturbed lipid profiles, particularly in the combination group compared to DHEA or HFD alone. Reproductive dysfunction was evidenced by irregular estrous cycles, predominantly characterised by persistent diestrus, while hormonal analysis revealed elevated testosterone and estrogen levels with suppressed FSH, especially in the 30 day DHEA+HFD group. Histopathological examination confirmed the presence of multiple cystic follicles in the ovaries of treated rats, alongside compromised hepatic and renal function markers. This DHEA+HFD model represents a significant advancement in PCOS research by successfully replicating the syndrome's metabolic and reproductive features within a shorter timeframe than traditional HFD models. The synergistic effect of DHEA and HFD demonstrates that dietary factors can substantially influence the severity of PCOS symptoms, providing valuable insights into the complex pathophysiology of this condition. The model's ability to induce both endocrine and metabolic disturbances makes it particularly relevant for studying the multifaceted nature of PCOS and its systemic implications.

Author contribution

Authors have made significant, direct, and intellectual contributions to this work, and have approved it for submission for publication. NN performed experiments, analysed the data, and wrote the manuscript draft. IS conceived the study and reviewed the final paper.

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Conflict of interest

The authors declare that they have no conflict of interest.

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