Indian Journal of Experimental Biology
Vol. 64, May 2026, pp. 452-459
DOI: 10.56042/ijeb.v64i05.24929

Gut microbiome alterations in dehydroepiandrosterone and high-fat diet-induced PCOS in rats: A shift from beneficial to harmful bacterial taxa

Kajal Rawat[1], Arushi Sandhu[1], Anil Kumar[1], Lekha Saha[1]* & Alka Bhatia[2]

[1]Department of Pharmacology, [2]Department of Experimental Medicine and Biotechnology, Research Block B, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh 160012, India

Received 18 October 2025; revised 19 January 2026

Polycystic ovary syndrome (PCOS), a common endocrine disorder in women, involves complex interactions between hormonal imbalances, metabolic dysfunction, and gut microbiome dysbiosis. While hyperandrogenism and high-fat diets independently alter gut microbiota, their combined effects in PCOS remain unclear, limiting development of microbiome-targeted interventions. This study investigated how high-fat diet (HFD) and dehydroepiandrosterone (DHEA) affect gut microbiome and metabolic health in a PCOS rat model. We used immunoassays and other colorimetric assays to estimate various hormonal levels and metabolic parameters. Real time PCR was performed to quantify the relative abundance of the bacterial species using genus specific 16S rDNA primers and universal primer sequence was used as internal control. We found that DHEA alone reduced microbial diversity, while HFD plus DHEA exacerbated dysbiosis, increasing pathogenic bacteria linked to systemic inflammation. We also performed correlation analysis and it highlighted the significant impact of dietary and hormonal factors on PCOS pathophysiology. The comparison of gut microbiome composition between high-fat diet plus DHEA-fed PCOS rats and DHEA-only treated rats highlights the significant impact of diet on the gut microbiome and its subsequent influence on metabolic health in PCOS.

Keywords: Gut dysbiosis, Diet, Animal model

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women of reproductive age, characterized by hyperandrogenism, chronic anovulation, and polycystic ovaries[1]. Beyond reproductive dysfunction, PCOS is associated with significant metabolic complications including insulin resistance (50-70% of patients), type 2 diabetes, dyslipidemia, obesity, and cardiovascular disease[2]. Despite its high prevalence, PCOS pathophysiology remains incompletely understood, and current treatments are largely symptomatic[3]. The gut microbiome, a complex community of microorganisms residing in the gastrointestinal tract, has gained recognition as a crucial factor in various metabolic and endocrine disorders, including PCOS[4]. It plays a pivotal role in maintaining metabolic homeostasis. Recent studies suggest that the gut microbiome's composition and function can significantly influence the onset and progression of PCOS[5–7]. These alterations correlate with androgen levels, insulin resistance, and inflammatory markers, suggesting mechanistic involvement in PCOS pathophysiology[8]. Dietary patterns powerfully modulate gut microbiome composition, with potential to promote either eubiosis or dysbiosis[9]. High-fat diet (HFD) consistently induce gut dysbiosis- reducing microbial diversity, depleting SCFA-producing bacteria, increasing gut permeability, and promoting metabolic endotoxemia[10–12]. Women with PCOS frequently exhibit poor dietary habits with higher saturated fat and lower fiber intake[13]. Dietary factors and hormonal treatments can modulate the gut microbiome, thereby impacting metabolic outcomes in PCOS. In this briefing, we will compare the gut microbiome composition in HFD plus dehydroepiand rosterone (DHEA)-fed PCOS rat models to those treated with DHEA alone.

Material and Methods

DHEA induced prepubertal PCOS model

Female wistar rat pups of post-natal day (PND) 21 were procured and housed in polymer cages in a temperature-controlled animal room at 24ºC with a normal 12-hr light: 12-hr dark cycle and a relative humidity of 70±10%. From PND 21 to PND 46 the pups were administered with dehydroepiandrosterone, DHEA (60 mg/kg) dissolved in 0.2 ml corn oil, subcutaneously once a day and DHEA+ HFD rats were fed on HFD alongside DHEA throughout the study. The control rats were treated with 0.2 ml corn oil, subcutaneously once a day from PND 21 to PND 46. Experimental design is illustrated in Fig 1A. Each experimental group included at least 4 rats.

*Correspondence:
E-mail:lekhasaha@rediffmail.com

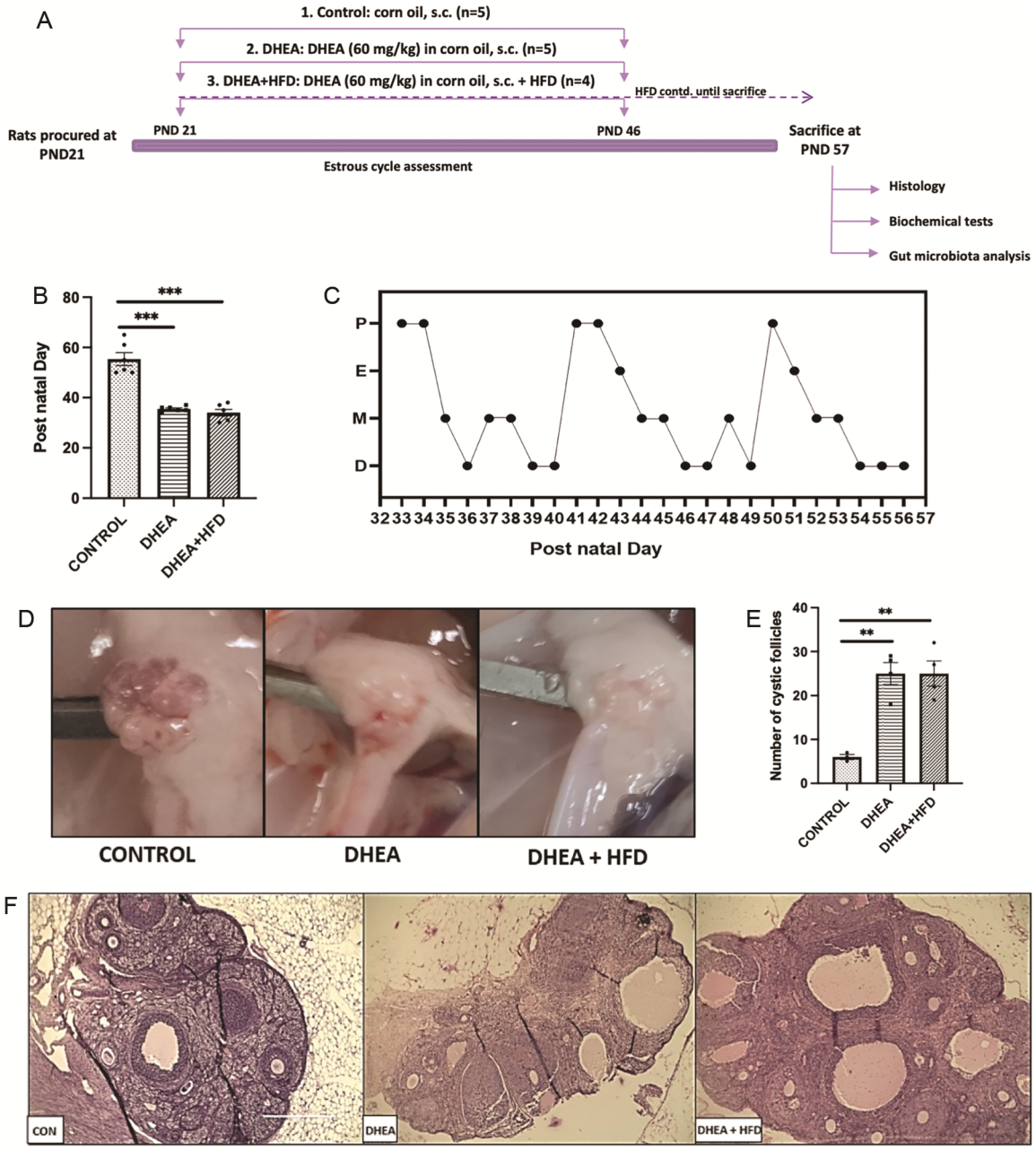

Fig. 1 — Experimental study design and PCOS phenotypes in different experimental groups. (A) Experimental study design; (B) Vaginal opening in control rats, DHEA treated rats and DHEA+HFD treated rats; (C) Representative estrous cycle of single rat from DHEA+HFD treated group; (D) Macroscopic pictorial representation of ovarian tissue isolated post sacrifice of all the experimental groups; (E) The number of cystic follicles from each group; (F) Representative picture of H and E ovarian sections; All the groups were analysed using one-way ANOVA for normally distributed data and Kruskal Wallis test for non-normal data. Data are reported as mean±SEM. ***P<0.001 and **P<0.01, P-value<0.05 was considered significant.

Ethical statement

All animal procedures used in this study were performed according to protocols approved by the Institutional Animal Ethics Committee of Postgraduate Institute of Medical Education and Research Chandigarh (IAEC no. 886/124). The study was conducted in accordance with CCSEA guidelines.

Estrous Cycle assessment

To determine the estrous stage, vaginal smear test was performed daily beginning since the onset of vaginal opening. A moistened cotton bud swab was inserted into the vagina. Cells from the vaginal lumen and walls were gently removed and transferred to a glass slide and air-dried. The air-

Table 1 — Primer sequences used in real time PCR

Gene	Forward (5' to 3')	Reverse (5' to 3')
Bifidobacterium spp.	CTCCTGGAAACGGGTGG	GGTGTTCTTCCCGATATCTACA
Lactobacillus spp.	TGGAAACAGRTGCTAATACCG	GTCCATTGTGGAAGATTCCC
Faecalibacterium spp.	GATGGCCTCGCGTCCGATTAG	CCGAAGACCTTCTTCCTCC
Akkermansia spp.	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT
Blautia spp.	CGGTACCTGACTAAGAAGC	GTTCCTCCTAATATCTACGC
Prevotella spp.	CACRGTAAACGATGGATGCC	GGTCGGGTTGCAGACC
Clostridium spp.	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAA
Universal	TCCTACGGGAGGCAGCAGT	GGACTACCAGGGTATCTAATCCTGTT

dried smears were then fixed by dipping the smears briefly (two dips) in a Coplin jar containing absolute methanol and air dried. The air-dried smears were then stained with diluted Giemsa stain (1:20, v/v) for 20 min. The stained smears were then washed and air dried[14]. The stage of the estrus cycle was then determined by the main cell types in vaginal smears: proestrus (round, nucleated epithelial cells), estrus (cornified squamous epithelial cells), metestrus (cornified squamous epithelial cells and leukocytes), and diestrus (nucleated epithelial cells and leukocytes).

Assessment of metabolic parameters

The animals were kept for 8 to 12 hrs. of overnight fasting and blood samples were collected next day in the morning by retro orbital plexus of rats under light anaesthesia in centrifuge tubes. The blood samples were immediately centrifuged at 5000 rpm at 4°C for 10 minutes to separate serum/plasma. Estimations for fasting plasma glucose, albumin, total cholesterol, high density lipoprotein (HDL) and low-density lipoprotein (LDL) was be carried out as per the manufacturer's instructions provided with commercially available kits (Autozyme STAT glucose kit #GU-2; Erba Cholesterol kit #120194; Erba Albumin kit #120223; LDL Direct reagent CliniQuant #LDLLFSR-02; HDL Direct Reagent CliniQuant #HDLFSR-01).

Assessment of hormonal levels

Hormones including testosterone (T), progesterone levels, follicle-stimulating hormone (FSH), luteinizing hormone (LH) were determined using enzyme linked immunosorbent assay (ELISA) with the help of commercially available kits (Elabscience® Rat Testosterone kit #E-EL-0155; Elabscience® Rat Progesterone kit #E-EL-0154; Elabscience® Rat FSH kit #E-EL-R0391; Elabscience® Rat LH kit #E-EL-R0026).

Hematoxylin and eosin (H&E) staining

On PND 57, following 8-12 hours of fasting, rats were euthanized by intraperitoneal injection of ketamine (150 mg/kg) and xylazine (20 mg/kg). Following collections of blood samples, the ovarian tissues were harvested for histology and fecal samples for microbiota analysis.A whole intact ovarian tissue were kept in 10% formalin solution. The tissue was then be processed and 4 μm thin sections were sliced using microtome. The sections were then stained with hematoxylin and eosin. Large fluid filled cystic follicles were numbered for each section from every group.

Gut microbiota analysis

Gut microbiome analysis of *Bifidobacterium spp., Lactobacillus spp., Faecalibacterium spp., Akkermansia spp., Blautia spp., Prevotella spp*. and *Clostridium spp*. was done by real time PCR method[15]. Fecal DNA was extracted using phenol-chloroform-isoamyl alcohol method. 10 ng DNA was further used for quantitative analysis. The bacterial relative amounts in fecal samples was estimated using the genus specific 16S rDNA primers and universal primer sequence was used as internal control. Primer sequences used are given in Table 1.

Statistical Analysis

Data are presented as mean ± SEM and were analyzed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). For datasets with a Gaussian distribution across multiple groups, statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for intergroup comparisons. Datasets that did not pass the Shapiro-Wilk normality test were analyzed using the Kruskal-Wallis test, followed by Dunn's post hoc analysis. Correlation among bacterial taxa and PCOS phenotype was estimated using Pearson's correlation and heatmap was generated using GraphPad Prism 8. A P-value of <0.05 was considered statistically significant.

Results and Discussion

DHEA, a testosterone precursor, is commonly used to induce PCOS in rodent models. In rats, DHEA administration mimics the hyperandrogenic state observed in human PCOS, leading to the development of ovarian cysts, irregular estrous cycles, and metabolic disturbances[16]. DHEA-induced PCOS models are widely used to explore the pathophysiology of PCOS and to assess the impact of various interventions[17,18]. Conversely, HFD is known to induce obesity, insulin resistance, and dyslipidemia, conditions frequently associated with PCOS. HFD also alters the gut microbiome by reducing microbial diversity and promoting the growth of pathogenic bacteria, resulting in a pro-inflammatory state[10–12]. The combination of HFD and DHEA treatment in PCOS models may exacerbate metabolic disturbances and further disrupt the gut microbiome. This study investigated the interactive effects of hyperandrogenism and HFD on gut microbiome composition in a prepubertal rat model of PCOS. We compared the effects of prepubertal DHEA administration alone versus DHEA+HFD in 21-day-old female Wistar rats. The DHEA-only group received DHEA (60 mg/kg/day, subcutaneously) for 25 days, while the DHEA+HFD group received the same DHEA treatment for 25 days (from PND 21 to PND 46) plus HFD for 37 days (Fig 1A). During treatment, rats were monitored for vaginal opening and estrous cycle changes, and were sacrificed on postnatal day (PND) 57. Results showed that DHEA led to early vaginal opening (PND 32-35) and irregular estrous cycles, with distorted ovarian structures in DHEA only and DHEA+HFD treated groups (Fig 1 B-D). Cystic follicles were observed in both DHEA and DHEA+HFD groups (Fig E and F). Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were elevated (Fig 2A-C), and progesterone levels were reduced in DHEA only and DHEA+HFD treated rats compared to controls (Fig 2D). Although there were no significant differences in hormone levels between the DHEA-only and DHEA+HFD groups, metabolic parameters were significantly altered in the DHEA+HFD group. Metabolic parameters, including HDL, LDL, total cholesterol, albumin and fasting blood glucose, were significantly higher in the DHEA+HFD group compared to DHEA-only and control groups (Fig 2 E-I).Altogether, DHEA administration successfully induced a hyperandrogenic PCOS phenotype characterized by early vaginal opening, irregular estrous cycles, ovarian cyst formation, and elevated androgen levels, consistent with established PCOS models[16–18].

Microbial diversity is a key indicator of a healthy gut microbiome, associated with enhanced metabolic flexibility, resilience to infections, and overall better health outcomes[19]. Studies have shown that DHEA treatment alone can reduce gut microbial diversity in PCOS rats[20–22]. This reduction is often linked to an overrepresentation of bacterial taxa associated with inflammation and metabolic dysregulation. In our study, DHEA-only treated rats showed a slight decrease in beneficial bacterial genera such as *Bifidobacterium, Lactobacillus, Akkermansia, Fecalibacterium,* and*Blautia,* and a slight increase in harmful species like *Prevotella* and *Clostridium* (Fig 3). The addition of HFD to DHEA treatment further reduced microbial diversity compared to DHEA treatment alone. HFD intensified DHEA-induced dysbiosis, leading to a more pronounced imbalance in the gut microbiome. This reduction in diversity is accompanied by an increase in pathogenic bacteria such as *Clostridium*, which are associated with systemic inflammation and insulin resistance. The combination of HFD and DHEA treatment may thus create a more severe metabolic and inflammatory environment, potentially worsening PCOS symptoms. We also observed a decrease in beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, in the DHEA+HFD group (Fig 3A and B). These bacteria are essential for maintaining gut barrier integrity and modulating immune responses[23]. HFD promotes the growth of pro-inflammatory bacteria such as *Prevotella* and reduces the abundance of beneficial bacteria like *Akkermansiamuciniphila*, which is associated with improved metabolic health. The increase in *Prevotella* and *Clostridium*, markers of dysbiosis, is often linked to increased gut permeability and systemic inflammation[24]. The reduction in *Akkermansiamuciniphila* may further exacerbate insulin resistance and metabolic dysfunction in PCOS[25].Theenhanced dysbiosis in the DHEA+HFD group was accompanied by significantly worse metabolic parameters including elevated total cholesterol, LDL, HDL, albumin, and fasting glucose. The absence of differential hormonal effects between DHEA and DHEA+HFD groups, despite dramatically different metabolic outcomes, suggests that gut microbiome alterations may mediate diet-induced

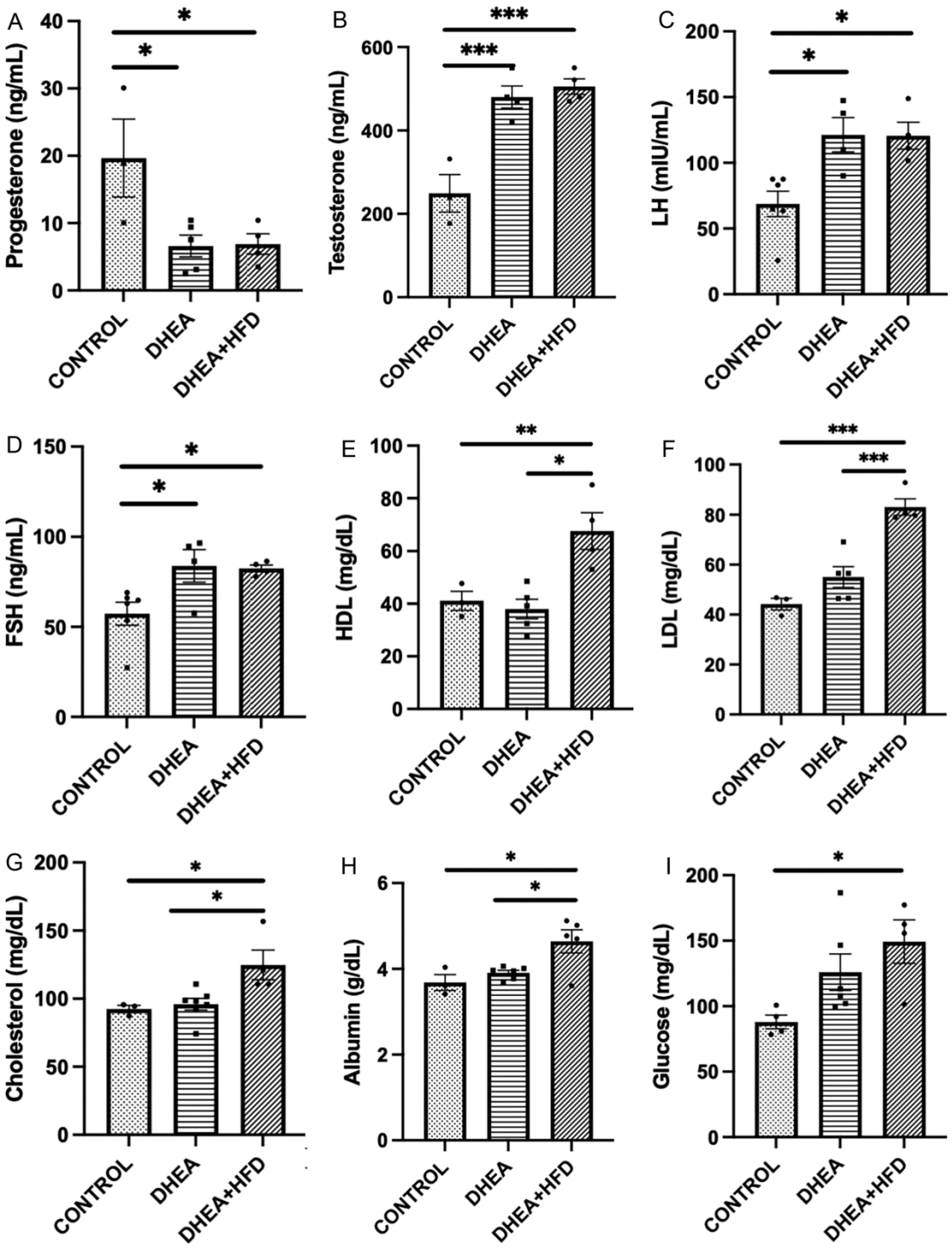

Fig. 2 — Various biochemical parameters assessed in control rats, DHEA treated and DHEA+HFD treated rats. (A) Progesterone levels, (B) Testosterone levels, (C) Luteinizing hormone (LH) levels, (D) Follicle stimulating hormone (FSH) levels, (E) High density lipoprotein (HDL) levels, (F) Low density lipoprotien (LDL) levels, (G) Cholesterol levels, (H) Albumin levels, (I) Glucose levels. All the groups were analysed using one-way ANOVA for normally distributed data and Kruskal Wallis test for non-normal data. Data are reported as mean±SEM. ***P<0.001, **P<0.01 and *P<0.05, P-value <0.05 was considered significant.

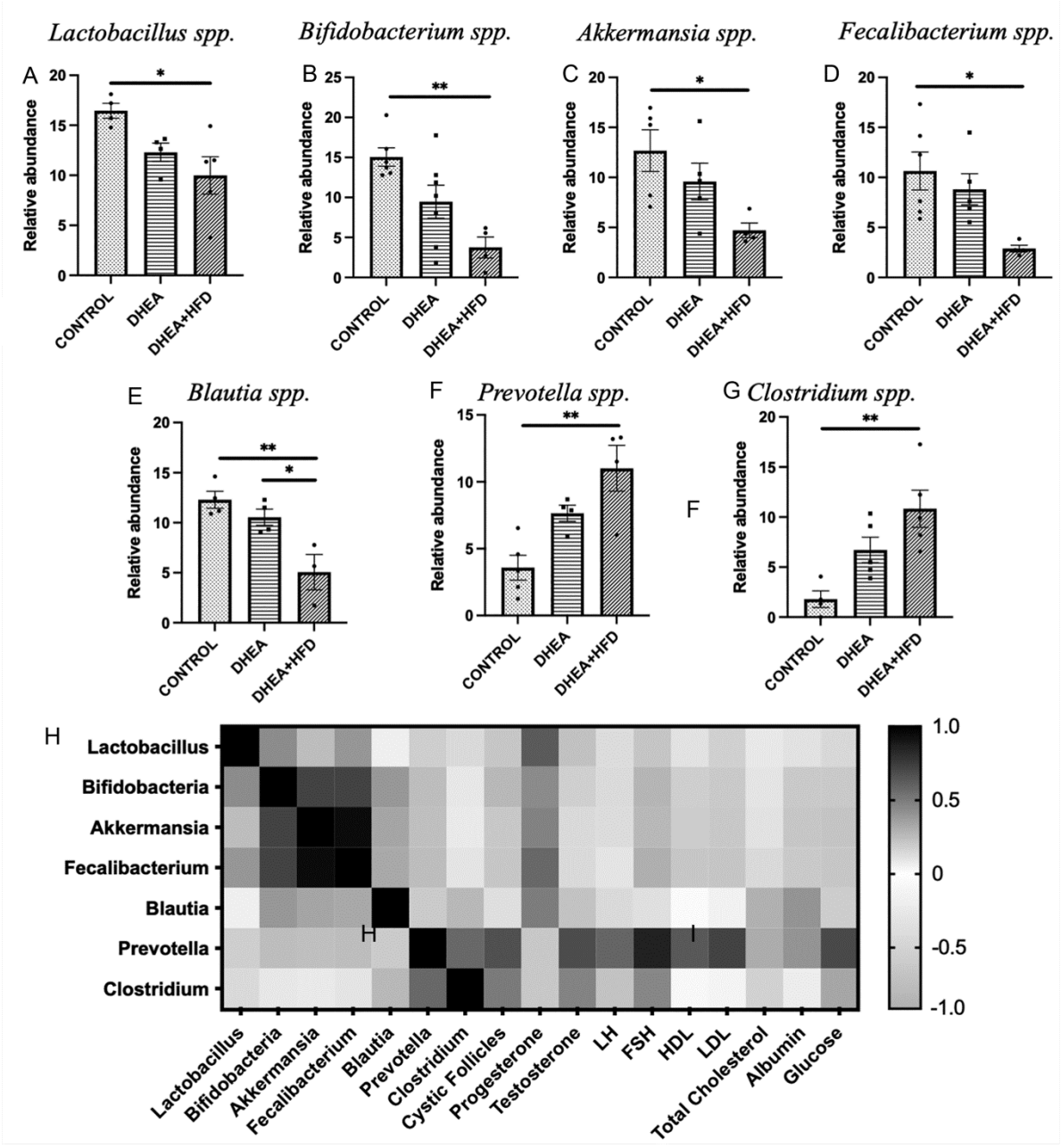

Fig. 3 — Gut bacterial abundance in the fecal samples of the rats from all groups. (A) Lactobacillus; (B) Bifidobacterium; (C) Akkermansia; (D) Fecalibacterium; (E) Blautia; (F) Prevotella; (G) Clostridium; (H) Heatmap indicating the correlation between relative abundance of gut bacterial species and PCOS phenotype. All the groups were analysed using one-way ANOVA for normally distributed data and Kruskal Wallis test for non-normal data. Data are reported as mean±SEM. $^{**}P<0.01$ and $^{*}P<0.05$, P-value<0.05 was considered significant.

metabolic dysfunction in PCOS independently of androgen levels. These findings suggest that PCOS women with poor dietary habits may experience disproportionately severe metabolic complications not solely attributable to obesity or androgen excess, but mediated through gut microbiome dysfunction. This highlights the gut microbiome as a potential therapeutic target for metabolic management in PCOS.

We also evaluated the effect of gut dysbiosis on the PCOS phenotype. Using Pearson's correlation, we investigated the potential correlations between the relative abundance of bacterial species and specific PCOS phenotypes (Fig 3C-H). We found that the

relative abundance of *Bifidobacterium, Lactobacillus, Akkermansia,* and *Fecalibacterium* was negatively correlated with testosterone, LH, and FSH levels. These findings suggest that a decrease in these beneficial bacteria may be linked to the hormonal imbalances characteristic of PCOS. However, while correlation analysis can indicate associations, it does not establish causality, leaving open the question of whether changes in gut microbiota drive hormonal changes or vice versa. Conversely, the abundance of *Prevotella* and *Clostridium* was positively correlated with androgen levels, suggesting a possible causal relationship.The relative abundance of beneficial bacterial taxa was also negatively correlated with the number of cystic follicles, further supporting that gut dysbiosis may contribute to ovarian dysfunction. However, it is important to consider that these relationship could be bidirectional, with hormonal changes also influencing microbial composition. Additionally, the relative abundance of beneficial bacterial taxa, particularly *Bifidobacteria*, *Akkermansia*, and *Fecalibacterium*, was negatively correlated with metabolic parameters (HDL, LDL, and fasting blood glucose levels), while the relative abundance of *Prevotella* and *Clostridium* was positively correlated with these metabolic markers. This suggests that gut microbiota may play a significant role in modulating metabolic health in PCOS, but further studies are needed to unravel the complex interactions between these bacteria and metabolic processes. The correlation analysis also indicated a negative correlation between the abundance of beneficial and harmful bacteria, underscoring the importance of maintaining a balanced gut microbiome for metabolic and reproductive health.

Taken together, the comparison of gut microbiome composition between HFD plus DHEA-fed PCOS rats and DHEA-only treated rats highlights the significant impact of diet on the gut microbiome and its subsequent influence on metabolic health in PCOS. While DHEA treatment alone induces changes in gut microbiome composition associated with inflammation and metabolic disturbances, the addition of a HFD exacerbates these effects, leading to more pronounced dysbiosis and metabolic dysfunction. However, it is important to acknowledge certain limitations of the present study. The number of animals used was relatively small, as each experimental group included at least four rats. Additionally, the findings are based on relative bacterial abundance quantified by 16S rDNA PCR, which does not capture species-level diversity or functional metabolic activity. Future studies employing metagenomic sequencing, larger sample sizes, and integrated multi-omics approaches are warranted to confirm and expand these findings and to provide deeper insights into the gut microbiome's role in PCOS pathophysiology.

Conclusion

In conclusion, our study demonstrates that high-fat diet substantially exacerbates DHEA-induced gut microbiome dysbiosis in a rat model of PCOS, with pronounced shifts from beneficial to pathogenic bacterial taxa. Critically, these microbiome alterations correlate strongly with metabolic dysfunction independent of further hormonal changes, suggesting that gut dysbiosis may mediate diet-induced metabolic complications in PCOS through mechanisms distinct from androgen excess. The combination of hyperandrogenism and poor dietary quality creates a particularly detrimental microbial environment that may amplify PCOS pathophysiology. These findings highlight the gut microbiome as a promising therapeutic target and support the rationale for microbiome-directed interventions including dietary modification, probiotics, and prebiotics, to improve metabolic outcomes in PCOS patients. Understanding and modulating the gut-ovary-metabolism axis represents a novel frontier in PCOS management that warrants intensive investigation in both preclinical and clinical settings.

Conflict of interest

The authors declare there are no conflict of interest

Author contribution statement

Conceptualization: LS and KR; Data curation: KR, AS and AK; Formal analysis: KR and AS; Funding acquisition: LS; Investigation: KR; Methodology: KR and AS; Resources: LS and AB; Supervision: LS; Validation: AB and LS; Writing – original draft: KR; Writing – review & editing: LS, AS and AK

Funding

The authors declare no specific funding for this work.

References

1 Norman RJ, Dewailly D, Legro RS & Hickey TE. Polycystic ovary syndrome. *Lancet*, 370 (2007) 685.

2 Deswal R, Narwal V, Dang A & Pundir CS. The prevalence of polycystic ovary syndrome: a brief systematic review. *J Hum Reprod Sci*, 13 (2020) 261.

3 Zeng X, Xie Y, Liu Y, Long S & Mo Z. Polycystic ovarian syndrome: correlation between hyperandrogenism, insulin resistance and obesity. *Clinicachimica acta*, 502 (2020) 214.

4 Giampaolino P, Foreste V, Di Filippo C, Gallo A, Mercorio A, Serafino P, Improda FP, Verrazzo P, Zara G, Buonfantino C, Borgo M, Riemma G, Angelis C, Zizolfi B, Bifulco G & Della Corte L. Microbiome and PCOS: state-of-art and future aspects. *Int J Mol Sci*, 22 (2021) 2048.

5 Zeng B, Lai Z, Sun L, Zhang Z, Yang J, Li Z, Lin J & Zhang Z. Structural and functional profiles of the gut microbial community in polycystic ovary syndrome with insulin resistance (IR-PCOS): a pilot study. *Res Microbiol*, 170 (2019) 43.

6 Min Q, Geng H, Gao Q & Xu M. The association between gut microbiome and PCOS: evidence from meta-analysis and two-sample mendelian randomization. *Front Microbiol*, 14 (2023) 1203902.

7 Zou Y, Liao R, Cheng R, Chung H, Zhu H & Huang Y. Alterations of gut microbiota biodiversity and relative abundance in women with PCOS: A systematic review and meta-analysis. *MicrobPathog*, (2023) 106370.

8 Mukherjee AG, Wanjari UR, Kannampuzha S, Murali R, Namachivayam A, Ganesan R, Dey A, Babu A, Renu K, Vellingiri B, Ramanathan G, Priya Doss C G, Elsherbiny N, Elsherbini AM, Alsamman AM, Zayed H & Gopalakrishnan AV.The implication of mechanistic approaches and the role of the microbiome in polycystic ovary syndrome (PCOS): a review. *Metabolites*, 13 (2023) 129.

9 Shang Z, Pai L & Patil S. Unveiling the dynamics of gut microbial interactions: a review of dietary impact and precision nutrition in gastrointestinal health. *Front Nutr*, 11 (2024) 1395664.

10 Zheng YH, Xu Y, Ma H-X, Liang CJ & Yang T. Effect of High-Fat Diet on the Intestinal Flora in Letrozole-Induced Polycystic Ovary Syndrome Rats. *Evid-Based Complement and Alternat Medic*, 2021 (2021) 6674965.

11 Lai H, Jia X, Yu Q, Zhang C, Qiao J, Guan Y & Kang J. High-fat diet induces significant metabolic disorders in a mouse model of polycystic ovary syndrome. *BiolReprod*, 91 (2014) 121.

12 Ressler IB, Grayson BE, Ulrich-Lai YM & Seeley RJ. Diet-induced obesity exacerbates metabolic and behavioral effects of polycystic ovary syndrome in a rodent model. *Am J Physiol Endocrinol Metab*, 308 (2015) E1076.

13 Barrea L, Marzullo P, Muscogiuri G, Di Somma C, Scacchi M, Orio F, Aimaretti G, Colao A & Savastano S. Source and amount of carbohydrate in the diet and inflammation in women with polycystic ovary syndrome. *Nutr Res Rev*, 31 (2018) 291.

14 Cora MC, Kooistra L & Travlos G. Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears. *Toxicol Pathol*, 43 (2015) 776.

15 Delroisse J-M, Boulvin A-L, Parmentier I, Dauphin RD, Vandenbol M & Portetelle D. Quantification of Bifidobacterium spp. and Lactobacillus spp. in rat fecal samples by real-time PCR. *Microbiol,* 162 (2008) 663.

16 Corrie L, Gulati M, Singh SK, Kapoor B, Khursheed R, Awasthi A, Vishwas S, Chellappan DK, Gupta G, Jha NK, Anand K & Dua K. Recent updates on animal models for understanding the etiopathogenesis of polycystic ovarian syndrome. *Life Sci*, 280 (2021) 119753.

17 Kim E-J, Jang M, Choi JH, Park KS & Cho I-H. An improved dehydroepiandrosterone-induced rat model of polycystic ovary syndrome (PCOS): Post-pubertal improve PCOS's features. *Front Endocrinol (Lausanne)*, 9 (2018) 735.

18 Xie Q, Xiong X, Xiao N, He K, Chen M, Peng J, Su X, Mei H, Dai Y, Wei D, Lin G & Cheng L. Mesenchymal stem cells alleviate DHEA-induced polycystic ovary syndrome (PCOS) by inhibiting inflammation in mice. *Stem Cells Int*, 2019 (2019) 9782373.

19 Ha CWY, Lam YY & Holmes AJ. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. *World J Gastroentero,* 20 (2014) 16498.

20 Shen H-R, Xu X, Ye D & Li X-L. Berberine Improves the Symptoms of DHEA-Induced PCOS Rats by Regulating Gut Microbiotas and Metabolites. *GynecolObstet Invest*, 86 (2021) 388.

21 Li T, Zhang T, Gao H, Liu R, Gu M, Yang Y, Cui T, Lu Z & Yin C. Tempol ameliorates polycystic ovary syndrome through attenuating intestinal oxidative stress and modulating of gut microbiota composition-serum metabolites interaction. *Redox Biol,* 41 (2021) 101886.

22 Han Q, Wang J, Li W, Chen Z-J & Du Y. Androgen-induced gut dysbiosis disrupts glucolipid metabolism and endocrinal functions in polycystic ovary syndrome. *Microbiome,* 9 (2021) 101.

23 Hiippala K, Jouhten H, Ronkainen A, Hartikainen A, Kainulainen V, Jalanka J & Satokari R. The potential of gut commensals in reinforcing intestinal barrier function and alleviating inflammation. *Nutrients*, 10 (2018) 988.

24 Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J & Liu Y. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep*, 5 (2015) 8096.

25 Duan L, An X, Zhang Y, Jin D, Zhao S, Zhou R, Duan Y, Zhang Y, Liu X & Lian F. Gut microbiota as the critical correlation of polycystic ovary syndrome and type 2 diabetes mellitus. *Biomedicine & Pharmacotherapy*, 142 (2021) 112094.