

# A promising animal model for Polycystic Ovary Syndrome: Borderline Hypertensive Rats

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## Research Article

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# Abstract

**Background:** Borderline Hypertensive Rat (BHR) is the offspring of female spontaneously hypertensive rats (SHR) and male Wistar-Kyoto (WKY) rats, that were used in studies of hypertension and cardiovascular disease. We found that the fertility rate of BHR decreased and the pregnancy rate was 60% (15/25) when BHR was prepared as a pregnant animal model of preeclampsia. We found that there was no study on the reproductive system of female BHR. The purpose of this study was to explore the reproductive phenotype of female BHR. The vaginal smears were performed to detect and evaluate the phases of the estrous cycle. According to the estrous cycle, BHR were divided into two groups: normal group and polycystic ovary syndrome (PCOS) group. The HE staining was used to observed the morphology of ovary and uterus, and serum sex hormone concentrations were measured.

**Result:** The results showed that there were 46 female BHR, 21 of them (45.7%) were found with PCOS. In PCOS group, the weight of ovary was increased, the estrous cycle was irregular, the cell layer of cystic follicles membrane was thickened, the endometrium was thickened, and the levels of serum testosterone, estrogen, luteinizing hormone (LH), LH / FSH (Follicle Stimulating Hormone) were increased. Polycystic ovaries were found in female BHR with PCOS. And the estrous cycle and sex hormone level of female BHR with PCOS were consistent with the characteristics of PCOS.

**Conclusions:** PCOS occurred in some female BHR, the pathophysiological changes were including the estrous cycle, the morphology of ovary and uterus, and the sex hormone levels.

## Background

Borderline Hypertensive Rat (BHR) is the first offspring of female spontaneously hypertensive rats (SHR) and male Wistar-Kyoto (WKY) rats[1]. The SHR and WKY were the inbred parent strains, the sympathetic nervous system of BHR was sensitive to the environmental pressure, and it has a significant impact on its hypertension and cardiovascular system[1, 2]. The BHR was an ideal and widely used animal model of hypertension, which was induced by environmental stress[3]. Because of the influence of sex hormone on hypertension[4], male BHR, as an animal model of environmental hypertension, was used to study the environmental genetic factors of hypertension[3, 4], which played an important role in mediating the effects of environmental stress on hypertension and cardiovascular[1, 5]. Until now, we have not found any research on the reproductive system of BHR.

We found that the reproductive function of BHR was decreased or even lost, when we used BHR as a preeclampsia animal model. The Hematoxylin-Eosin (HE) staining of ovarian morphology showed that there were no mature follicles in ovaries of BHR with infertility. On the contrary, there were multiple and enlarged polycystic follicles in ovaries. A combination of maternal hypertension gene and paternal normal blood pressure gene was inherited by BHR[1]. We hypothesized that the impact of the genetic of BHR first-degree relatives led to their polycystic ovary syndrome (PCOS) like changes. The reproductive phenotype of the BHR was described in this study.

# Results

## Estrous cycle in BHR

In this study, there were 46 female BHR. According to the Estrous cycle, the BHR were divided into normal group and PCOS group. There were 25 rats in the normal group and 21 in the PCOS group. The incidence of PCOS in BHR was 45.7%. Figure 1 is showing the estrous cycle of female BHR in two groups, which was representative. In the normal group, the estrus cycle was proestrus, estrus, metestrus, and diestrus. The estrus cycle of the normal group lasted for 4–5 days and regularly (Fig. 1a). In the PCOS group, the estrus cycle was irregular, there was no obvious the phase of proestrus and metestrus, the phase of diestrus was prolonged. And after the estrus period, it was directly the diestrus, the diestrus lasted for a period of time, and then changed to estrous. In the PCOS group, some BHR even lasted for diestrus, without other stages of oestrus cycle (Fig. 1b).

## Body Phenotype In Bhr

Table 1 is showing the characteristics of body phenotype in two groups. Compared with normal group, PCOS group had higher value of body length, body weight, BMI (Body Mass Index,  $\text{g}/\text{cm}^2$ ), clitoral length, anogenital distance, different shape of uterus, ovarian weight ( $P < 0.01$ ). There was no difference in uterine weight between 2 groups ( $P = 0.07$ ). Figure 2a is showing the comparison of body weight between two groups of BHR from 21 to 84 days after birth. Compared with normal group, PCOS group had higher value of weight gain from 21 to 84 days after birth ( $P < 0.05$ ). Figure 2b is showing the comparison of ovarian weight between two groups, and each ovary in PCOS group was heavier than that in normal group ( $P < 0.05$ ). Figure 2c is showing the comparison of clitoral length between two groups, and compared with the normal group, the clitoris length in group PCOS was longer ( $P < 0.05$ ).

Table 1  
Compared of body phenotype in the two groups.

| Phonotype   | Normal group<br>(n = 20) | PCOS group<br>(n = 21) | T/Z   | P     |
|---|--------------------------|------------------------|-------|-------|
| Body length (cm)  | 18.5 (18.3, 18.7)        | 19.3 (19.0, 19.9)      | 4.457 | 0.000 |
| Body weight (g)   | 164.0 (158.4, 167.1)     | 190.7 (184.4, 196.8)   | 5.79  | 0.000 |
| BMI (g/cm <sup>2</sup> )  | 4.9 (0.3)                | 5.1 (0.3)              | 3.173 | 0.003 |
| Clitoral length (cm)  | 0.5 (0.5, 0.6)           | 0.7 (0.7, 0.8)         | 5.673 | 0.000 |
| Anogenital distance (cm)  | 1.2 (1.0, 1.2)           | 1.4 (1.3, 1.5)         | 4.783 | 0.000 |
| Different shape of uterus (cm)  | 0.5 (0.4,0.5)            | 0.1 (0.0, 0.2)         | 5.891 | 0.000 |
| Uterine weight (mg/cm)  | 141.3 (10.7)             | 137.1 (13.5)           | 1.787 | 0.074 |
| Ovarian weight (mg/each)  | 104.8 (100.0, 110.0)     | 130.3 (126.4, 139.0)   | 7.897 | 0.000 |
| Note: The measurement data with normal distributed are shown as non-normal distributed measurement data are shown as median (P25, P75). P < 0.05 was considered to be statistically significant. Body Mass Index (BMI). |                          |                        |       |       |

## Ovarian And Uterine Morphology In Bhr

In the normal group, they had mature follicles in ovaries (Fig. 3a and Fig. 3c). In the PCOS group, multiple follicles appeared in ovaries, and cystic follicles have cystoid appearance and increased volume (Fig. 3b and Fig. 3d). In the follicle of the normal group, the boundary between the inner and outer of membrane cell layer was obvious, and granulosa cells were arranged orderly (Fig. 3e). In PCOS group, the membrane cell layer of the enlarged polycystic follicle was proliferative and thickened, and the boundary between the inner membrane and the outer membrane was not obvious, and the granulosa cells was reduced, and the apoptotic granulosa cells appeared in the follicle cavity (Fig. 3f). In the normal group, the epithelial layer of endometrium was composed with a simple layer of columnar epithelial, which were arranged in order (Fig. 3g). In the PCOS group, the epithelial layer was disorganized and thickened (Fig. 3h).

## Sex Hormone In Bhr

In the female BHR, the concentration of serum testosterone in normal group was  $1.4 \pm 0.8$  ng/ml, and in the PCOS group was  $3.2 \pm 0.4$  ng/ml (Fig. 4a). Compared to the normal group of BHR, the concentration of serum testosterone was more than 2-fold in the PCOS group (Fig. 4a). In normal group and PCOS group the concentration of serum estrone was 39.3 (26.4, 42.9) pg/ml and 48.3 (40.0, 56.2) pg/ml (Fig. 4b). In normal group and PCOS group the concentration of LH was 283.1 (236.9, 361.5) pg/ml and 235.6 (135.0, 268.8) pg/ml (Fig. 4c), and LH/FSH was  $33.9 \pm 3.4$  and  $52.9 \pm 3.8$  (Fig. 4d). Although FSH in

PCOS group was decreased compared with that in normal group, the difference was not statistically significant (Fig. 4e). There was no difference in progesterone between them (Fig. 4f).

## Fatty Tissue In Bhr

Compared with the normal group, the gonadal fatty cells and the peritoneal fatty cells were larger in the PCOS group (Fig. 5).

## Discussion

Polycystic ovary syndrome is an endocrine and metabolic disorder that is common in women of reproductive age, and the incidence is 8–13%[6]. Polycystic ovary syndrome leads to low fertility at reproductive age in women and is often associated with insulin resistance and obesity, metabolic syndrome, type 2 diabetes, gestational diabetes, and increased susceptibility to cardiovascular disease[7–10]. High concentrations of androgen secretion in the ovary was the pathophysiological basis of PCOS, which led to hyperandrogenemia and its related to hyperandrogenic symptoms .[11, 12]. In our study, the incidence of PCOS in BHR was 45.7%. Female BHR not only have hypertension, that the reproductive phenotype of BHR with PCOS was similar to that of PCOS animal model. The symptoms similar to those of PCOS include polycystic ovarian changes, hyperandrogenemia and high LH / FSH, weight gain and increase of gonadal fatty cells and peritoneal fatty cells.

The menstrual cycle of PCOS patients was sparse or amenorrhea[13]. There were more than 12 small follicles in one or both ovaries with a diameter of 2-9mm[14]. The estrous cycle of rats was similar to that of women, was also regulated by Hypothalamus-Pituitary ovary-Gonad axis[15, 16]. The basic characteristic of PCOS models of rodent animal was that the estrous cycle was irregular, and multiple cystic follicles in the ovary[17]. The estrous cycle of BHR was shown that the ovaries were anovulatory, and normal and PCOS BHR could be distinguished before HE staining of ovarian pathology by estrous cycle. We found that the estrous cycle of BHR with PCOS was irregular, and the diestrus stage was prolonged, or even other stages of oestrus cycle were absent, which persisted in the diestrus stage. The estrous cycle of BHR with PCOS was similar to that of androgen and letrozole induced PCOS animal model[15, 18].

This study was observed with Hematoxylin and Eosin staining, that Contrary to mature follicles, they were replaced by multiple, enlarged cystic follicles and atretic follicles in BHR with PCOS. The ovarian morphology of BHR with PCOS was consistent with the clinical feature of human PCOS patients[14], and the ovarian phenotype for animal models[15, 18, 19]. The theca cells of the enlarged cystic follicle in the ovaries of BHR with PCOS was thickened, and the granules decreased. This phenomenon was consistent with the results of animal models induced by androgen induced PCOS animal model in rats or mice[19]. About 33% of the testosterone was produced by the theca cells of ovaries[20]. The thickened theca cells was associated with hyperandrogenemia and high LH level, which led to PCOS[21]. Granulosa cell reduction was associated with low level of FSH, and the follicular growth could be inhibited by low level

of FSH and hyperandrogenemia, which lead to increased atresia follicles [12]. The endometrium of BHR with PCOS was thickened, which was consistent with PCOS animal model[22].

The increase of serum testosterone, estrone, LH and LH / FSH in BHR with PCOS was also consistent with the characteristics of PCOS animal model[19, 23]. The animal models of PCOS established by letrozole showed an increase in serum testosterone, LH and LH/FSH [15, 24]. The changes of serum sex hormone in these animal models were consistent with the BHR with PCOS. The female offspring of sheep and rhesus monkeys showed the consistent clinical characteristics of PCOS, and the change of sex hormone level was similar to that of the BHR with PCOS, when the sheep and rhesus monkeys were exposed to excessive androgen during pregnancy[25, 26].

Hypertension was associated with hyperandrogenemia, and the patients with PCOS has a high prevalence risk of hypertension[27]. The relationship between hypertension and PCOS was not clear. Hypertension was a symptom of PCOS patients, or hypertension was a risk factor of PCOS? The systolic blood pressure and the diastolic blood pressure of BHR was  $137.97 \pm 7.12$  mmHg and  $95.01 \pm 6.43$  mmHg, respectively[28]. The systolic blood pressure and the diastolic blood pressure of SHR was  $183.63 \pm 7.02$  mmHg and  $127.06 \pm 7.38$  mmHg[28]. We found that the reproductive phenotype of some BHR was similar to that of animal model of PCOS, but it did not happen in female SHR.

The BHR inherited the genetic gene of hypertension, and it was often used as an animal model of chronic stress induced hypertension[3, 5]. The BHR was the offspring of female SHR and male WKY, with hypertension genetically[1]. The hypothalamus pituitary adrenal axis of BHR was sensitive to environmental pressure[29]. Women with PCOS usually had high blood pressure in their mothers and normal blood pressure in their fathers[30], which was similar to the family spectrum of BHR. The female BHR might become a new naturally animal model of PCOS, its reproductive phenotype was similar to other animal models of PCOS, and its maternal hypertension family spectrum was similar to human being.

This study found that the performance of animal models resembling PCOS in some female BHR was spontaneous. However, other PCOS animal models were prepared by exposure to excessive androgen, which were not consistent with the onset of human PCOS. In this study, the incidence of PCOS in female BHR was 45.7%, which was lower. We hypothesized that BHR is sensitive to environmental stress, and whether environmental stress has a role in inducing higher PCOS in female BHR. And the metabolic changes of female BHR with PCOS are the direction of our future research.

In summary, this study described the reproductive related physical performance of female BHR, Some female BHR suffered from a PCOS like condition, that was necessary to consider the impact of PCOS on low fertility and experimental results in the female BHR.

## Conclusions

The physical reproduction was similar to PCOS animal model in some female BHR, the changes were including the weight gain, the estrous cycle, the morphology of ovarian and uterine, and the sex hormone levels.

## Methods

### Experimental animals

All procedures and methods that have been used in this study were performed according to the National Institute of Health guidelines on the care and use of animals. The experimental protocol was approved by the Ethics Committee of Hebei General Hospital Ethics Committee, China (approval number 201806). Forty-six BHR were used in this study. Clean grade of female SHR and male WKY were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (SCXK 2016-0006, Jing, China). Eight female SHR, 9–10 weeks old, weighing 153.6–159.0 g. Four male WKY, 10–12 weeks old, weighing 224.2–226.4 g. The rats were housed in the clinical research center of Hebei General Hospital (SYXK 2015-0065, Ji, China). The ratio of female SHR to male WKY was 4:2, they were housed in a suitable environment with a temperature (22–24°C) and relative humidity (45%–55%), and the room was in a 12-h light/12-h dark cycle. The rats were fed with common diet and drank tap water. In order to make the rats adapt to the environment, they were fed for about one week before mating. The female SHR and male WKY mated in 2:1, and fed in separate cages after having vaginal suppository. The experimental animals were the BHR of the offspring of female SHR and male WKY.

### Main Reagents

The reagents of Rapid Gram Stain and HE Stain were purchased from Zhuhai BASO Company (419041, 715073). Rat Testosterone ELISA (Enzyme linked immunosorbent assay) Kit was purchased from Wuhan CUSABIO Company (CSB-05100r). Rat Estrone ELISA Kit and Rat Progesterone ELISA Kit were purchased from Wuhan EIAab Company (E2070Ge, E0459Ge). Rat Luteinizing Hormone ELISA Kit and Rat Follicle Stimulating Hormone ELISA Kit were purchased from Wuhan Cloud-Clone Corp (CEA441Ra, CEA830Ra).

### Vaginal Smears

The estrous cycle of BHR was determined by rapid Gram staining on vaginal smear of rats from 11 weeks old to the end of the experiment. The morphology of vaginal exfoliated cells of rats was observed under the microscope. In the stage of proestrus, nucleated cells and keratinocytes could be observed, mainly in nucleated cells. During estrus, only keratinocytes can be observed. There were keratinocytes and leukocytes in the stage of metestrus, and the number of keratinocytes were more than leukocytes. In the stage of diestrus, three kinds of cells could be observed, and a large number of leukocytes could be observed under the microscope. According to the estrous cycle, the 46 BHR were divided into two groups: normal group and PCOS group.

# Histological Examination

The rats ovaries and uterus were excised after anesthesia using pentobarbital (25 mg/kg) in the stage of diestrus at 12–14 weeks old. The morphology of ovary and uterus of BHR were observed. The tissues of ovary and uterus were fixed by Formalin solution for 24–48 hours, dehydrated by gradient alcohol, cleared in xylene, dipped and embedded in wax. The thickness of the sections were 4  $\mu\text{m}$ , HE staining was carried out, image of it was obtained with DPTO digital optical microscope systems (Olympus, Japan).

# Hormone Measurements

The concentration of serum testosterone, estrone, progesterone, LH and FSH was measured by rat ELISA kit according to the instructions. The range of testosterone, estrone, progesterone, LH and FSH measured by ELISA was: 0.13–25.6 ng/ml, 15.6–1000 pg/ml, 0.156–10 ng/ml, 98.77–8000 pg/ml, 2.47–200 ng/ml. The optical density value of each hole was measured at 450 nm using the 80416-kbhl automatic enzyme labeling detector (VersaMax, United States), the standard curve was drawn by ELISA analyzed software (SOFTmax PRO 4.3 LS) according to the concentration of serum samples.

# Statistical analysis

All data were analyzed by SPSS Version 17.0. The data were test for normality using Kolmogorov Smirnov. The data with normal distributed were compared using independent sample T test between two groups, and homogeneity of variance was tested using Leven, t value and P value were used, if the variance was homogeneous. Non-normal distributed data were tested using Mann Whitney U test. All statistical tests were two-tailed, and  $P < 0.05$  was considered to be statistically significant.

# Abbreviations

BHR: Borderline Hypertensive Rat; SHR: Spontaneously Hypertensive Rats; WKY: Wistar-Kyoto; PCOS: Polycystic Ovary Syndrome; LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; HE: Hematoxylin-Eosic; BMI: Body Mass Index.

# Declarations

## Acknowledgments

Not applicable.

## Authors' contributions

ZT and WL conceived and designed the experiments. ZT and XJF performed the experiments and collected the data. XJF and ENT. M analyzed the data. ZT, CL and WL wrote the paper and edited the

manuscript. WL supervised the study. All authors have read and approved the final version of the manuscript, and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

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## Availability of data

All data generated or analysed during this study are included in this published article.

## Ethics approval and consent to participate

All procedures and methods that have been used in this study were performed according to the Animal Ethics Procedures and Guidelines of the People's Republic of China. The experimental protocol was approved by the Ethics Committee of Hebei General hospital Ethics Committee, China (approval number 201806). The study was also carried out with the relevant guidelines and regulations stipulated in the ARRIVE guidelines.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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## References

1. Zemancíková A, Török J: **Comparison Of Cardiovascular Characteristics In Normotensive And Hypertensive Rat Strains**. Indian journal of physiology and pharmacology 2015, **59(4)**:361–368.
2. Zhou JJ, Shao JY, Chen SR, Li DP, Pan HL: **α2δ-1-Dependent NMDA Receptor Activity in the Hypothalamus Is an Effector of Genetic-Environment Interactions That Drive Persistent Hypertension**.

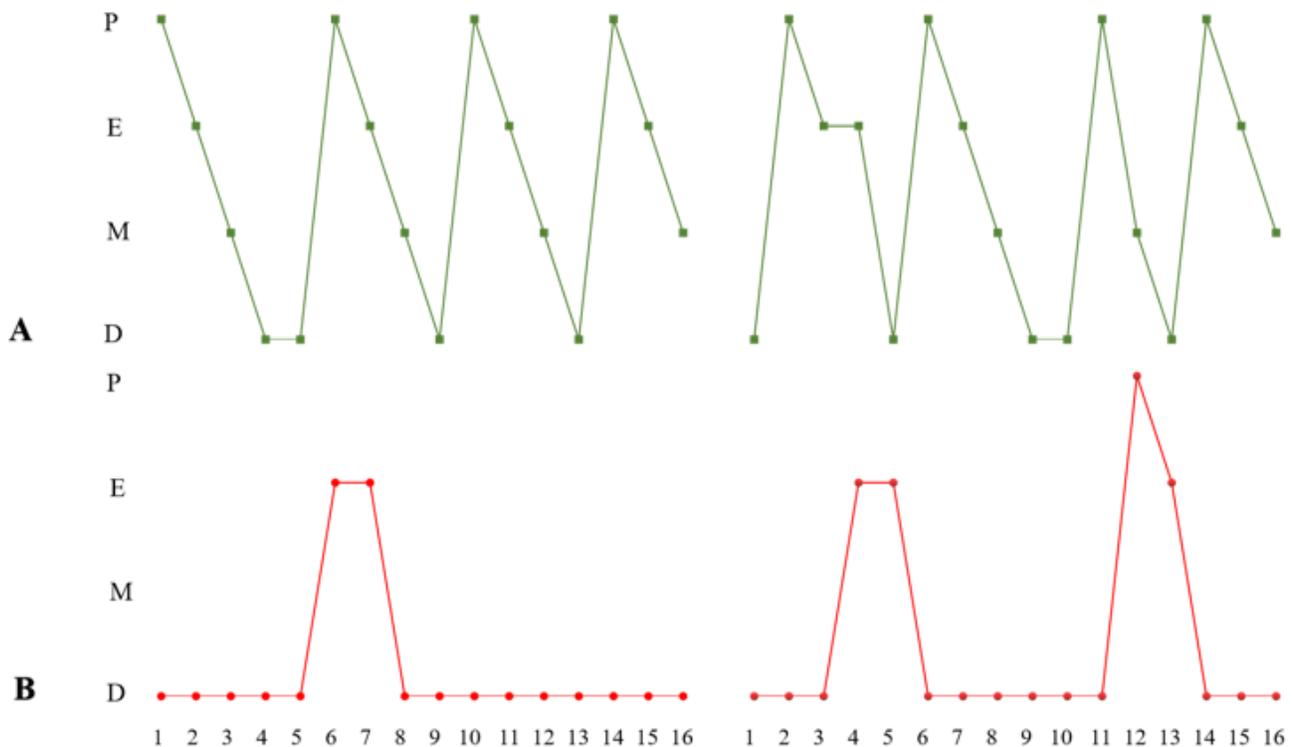
- The Journal of neuroscience: the official journal of the Society for Neuroscience 2021, **41(30)**:6551–6563.
3. Savić B, Martin A, Mecawi AS, Bukumirić Z, Antunes-Rodrigues J, Murphy D, Šarenac O, Japundžić-Žigon N: **Vasopressin and v1br gene expression is increased in the hypothalamic pvn of borderline hypertensive rats.** Hypertension research: official journal of the Japanese Society of Hypertension 2020, **43(11)**:1165–1174.
  4. Horvathova M, Zitnanova I, Kralovicova Z, Balis P, Puzserova A, Muchova J, Kluknavsky M, Durackova Z, Bernatova I: **Sex differences in the blood antioxidant defense system in juvenile rats with various genetic predispositions to hypertension.** Hypertension research: official journal of the Japanese Society of Hypertension 2016, **39(2)**:64–69.
  5. Bernatova I, Puzserova A, Balis P, Sestakova N, Horvathova M, Kralovicova Z, Zitnanova I: **Chronic Stress Produces Persistent Increases in Plasma Corticosterone, Reductions in Brain and Cardiac Nitric Oxide Production, and Delayed Alterations in Endothelial Function in Young Prehypertensive Rats.** Frontiers in physiology 2018, **9**:1179.
  6. Skiba MA, Islam RM, Bell RJ, Davis SR: **Understanding variation in prevalence estimates of polycystic ovary syndrome: a systematic review and meta-analysis.** Human reproduction update 2018, **24(6)**:694–709.
  7. Barber TM, Franks S: **Obesity and polycystic ovary syndrome.** Clinical endocrinology 2021, **95(4)**:531–541.
  8. Pandurevic S, Macut D, Fanelli F, Pagotto U, Gambineri A: **Biomediators in Polycystic Ovary Syndrome and Cardiovascular Risk.** Biomolecules 2021, **11(9)**.
  9. Zehravi M, Maqbool M, Ara I: **Polycystic ovary syndrome and reproductive health of women: a curious association.** International journal of adolescent medicine and health 2021, **33(6)**:333–337.
  10. Livadas S, Anagnostis P, Bosdou JK, Bantouna D, Papanicolaou R: **Polycystic ovary syndrome and type 2 diabetes mellitus: A state-of-the-art review.** World journal of diabetes 2022, **13(1)**:5–26.
  11. Rodriguez Paris V, Bertoldo MJ: **The Mechanism of Androgen Actions in PCOS Etiology.** Medical sciences (Basel, Switzerland) 2019, **7(9)**.
  12. Walters KA, Rodriguez Paris V, Aflatounian A, Handelsman DJ: **Androgens and ovarian function: translation from basic discovery research to clinical impact.** The Journal of endocrinology 2019, **242(2)**:R23-r50.
  13. Ezeh U, Pisarska MD, Azziz R: **Association of severity of menstrual dysfunction with hyperinsulinemia and dysglycemia in polycystic ovary syndrome.** Human reproduction (Oxford, England) 2022.
  14. Escobar-Morreale HF: **Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment.** Nature reviews Endocrinology 2018, **14(5)**:270–284.
  15. Bries AE, Webb JL, Vogel B, Carrillo C, Keating AF, Pritchard SK, Roslan G, Miller JW, Schalinske KL: **Letrozole-Induced Polycystic Ovary Syndrome Attenuates Cystathionine-β Synthase mRNA and**

- Protein Abundance in the Ovaries of Female Sprague Dawley Rats.** *The Journal of nutrition* 2021, **151(6)**:1407–1415.
16. Benevides ROA, Vale CC, Fontelles JLL, França LM, Teófilo TS, Silva SN, Paes AMA, Gaspar RS: **Syzygium cumini (L.) Skeels improves metabolic and ovarian parameters in female obese rats with malfunctioning hypothalamus-pituitary-gonadal axis.** *Journal of ovarian research* 2019, **12(1)**:13.
  17. Ryu Y, Kim SW, Kim YY, Ku SY: **Animal Models for Human Polycystic Ovary Syndrome (PCOS) Focused on the Use of Indirect Hormonal Perturbations: A Review of the Literature.** *International journal of molecular sciences* 2019, **20(11)**.
  18. Wang Z, Yang L, Dong H, Dong H, Cheng L, Yi P, Huang D: **Effect of electroacupuncture on the kisspeptin system in a pubertal rat model of polycystic ovary syndrome.** *Acupuncture in medicine: journal of the British Medical Acupuncture Society* 2021, **39(5)**:491–500.
  19. Corrie L, Gulati M, Singh SK, Kapoor B, Khursheed R, Awasthi A, Vishwas S, Chellappan DK, Gupta G, Jha NK *et al*: **Recent updates on animal models for understanding the etiopathogenesis of polycystic ovarian syndrome.** *Life sciences* 2021, **280**:119753.
  20. Sharma A, Welt CK: **Practical Approach to Hyperandrogenism in Women.** *The Medical clinics of North America* 2021, **105(6)**:1099–1116.
  21. Chaudhary H, Patel J, Jain NK, Joshi R: **The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis.** *Journal of ovarian research* 2021, **14(1)**:125.
  22. Kuyucu Y, Çelik LS, Kendirlian Ö, Tap Ö, Mete U: **Investigation of the uterine structural changes in the experimental model with polycystic ovary syndrome and effects of vitamin D treatment: An ultrastructural and immunohistochemical study.** *Reproductive biology* 2018, **18(1)**:53–59.
  23. Stener-Victorin E, Padmanabhan V, Walters KA, Campbell RE, Benrick A, Giacobini P, Dumesic DA, Abbott DH: **Animal Models to Understand the Etiology and Pathophysiology of Polycystic Ovary Syndrome.** *Endocrine reviews* 2020, **41(4)**.
  24. Fu LL, Xu Y, Li DD, Dai XW, Xu X, Zhang JS, Ming H, Zhang XY, Zhang GQ, Ma YL *et al*: **Expression profiles of mRNA and long noncoding RNA in the ovaries of letrozole-induced polycystic ovary syndrome rat model through deep sequencing.** *Gene* 2018, **657**:19–29.
  25. Tonello Dos Santos J, Escarião da Nóbrega J, Jr., Serrano Mujica LK, Dos Santos Amaral C, Machado FA, Manta MW, Rizzetti TM, Zanella R, Figuera R, Antoniazzi AQ *et al*: **Prenatal Androgenization of Ewes as a Model of Hirsutism in Polycystic Ovary Syndrome.** *Endocrinology* 2018, **159(12)**:4056–4064.
  26. Bishop CV, Takahashi D, Mishler E, Slayden OD, Roberts CT, Hennebold J, True C: **Individual and combined effects of 5-year exposure to hyperandrogenemia and Western-style diet on metabolism and reproduction in female rhesus macaques.** *Human reproduction (Oxford, England)* 2021, **36(2)**:444–454.
  27. Özkan S, Yılmaz Ö, Yavuz B: **Increased masked hypertension prevalence in patients with polycystic ovary syndrome (PCOS).** *Clinical and experimental hypertension (New York, NY)* 1993

2020, 42(8):681–684.

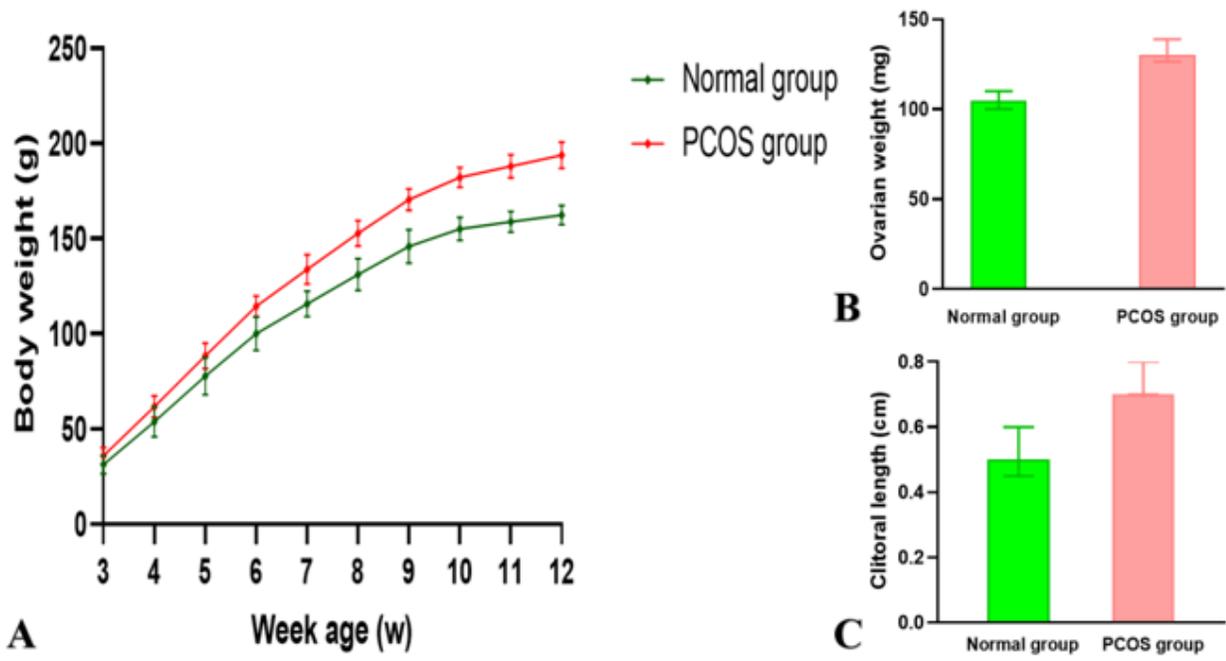
28. François M, Fernández-Gayol O, Zeltser LM: **A Framework for Developing Translationally Relevant Animal Models of Stress-Induced Changes in Eating Behavior.** *Biological psychiatry* 2021.
29. Šarenac O, Lozić M, Drakulić S, Bajić D, Paton JF, Murphy D, Japundžić-Žigon N: **Autonomic mechanisms underpinning the stress response in borderline hypertensive rats.** *Experimental physiology* 2011, **96(6)**:574–589.
30. Yilmaz B, Vellanki P, Ata B, Yildiz BO: **Metabolic syndrome, hypertension, and hyperlipidemia in mothers, fathers, sisters, and brothers of women with polycystic ovary syndrome: a systematic review and meta-analysis.** *Fertility and sterility* 2018, **109(2)**:356–364.e332.

## Figures



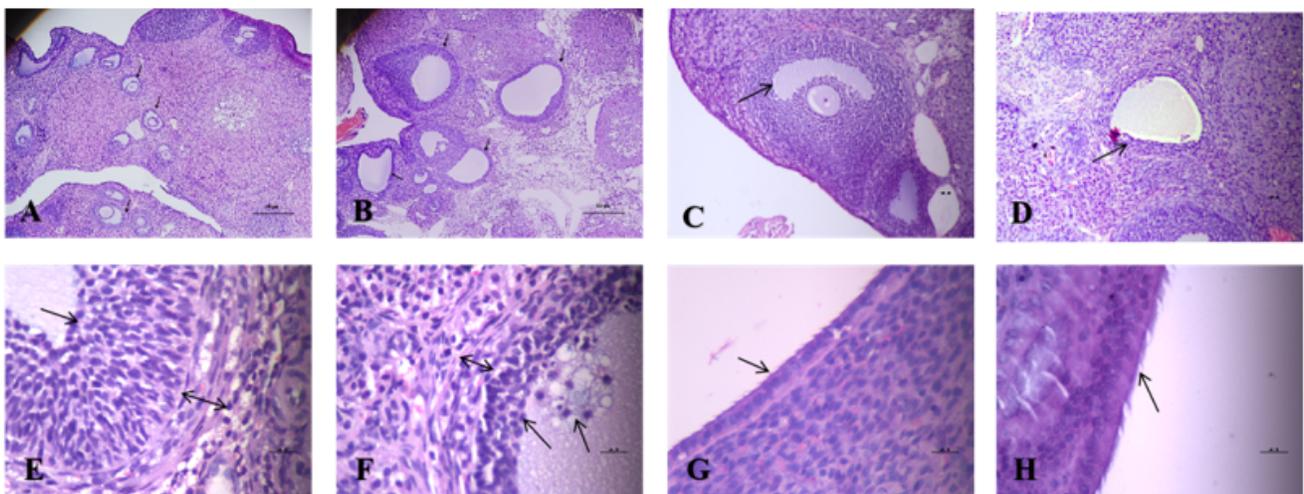
**Figure 1**

Estrous cycle stage classification of female BHR at 77–92 d of age. a: Estrous cycle of the normal BHR. b: Estrous cycle of the female BHR with PCOS. P, proestrus; E, estrus; M, metestrus; D, diestrus.



**Figure 2**

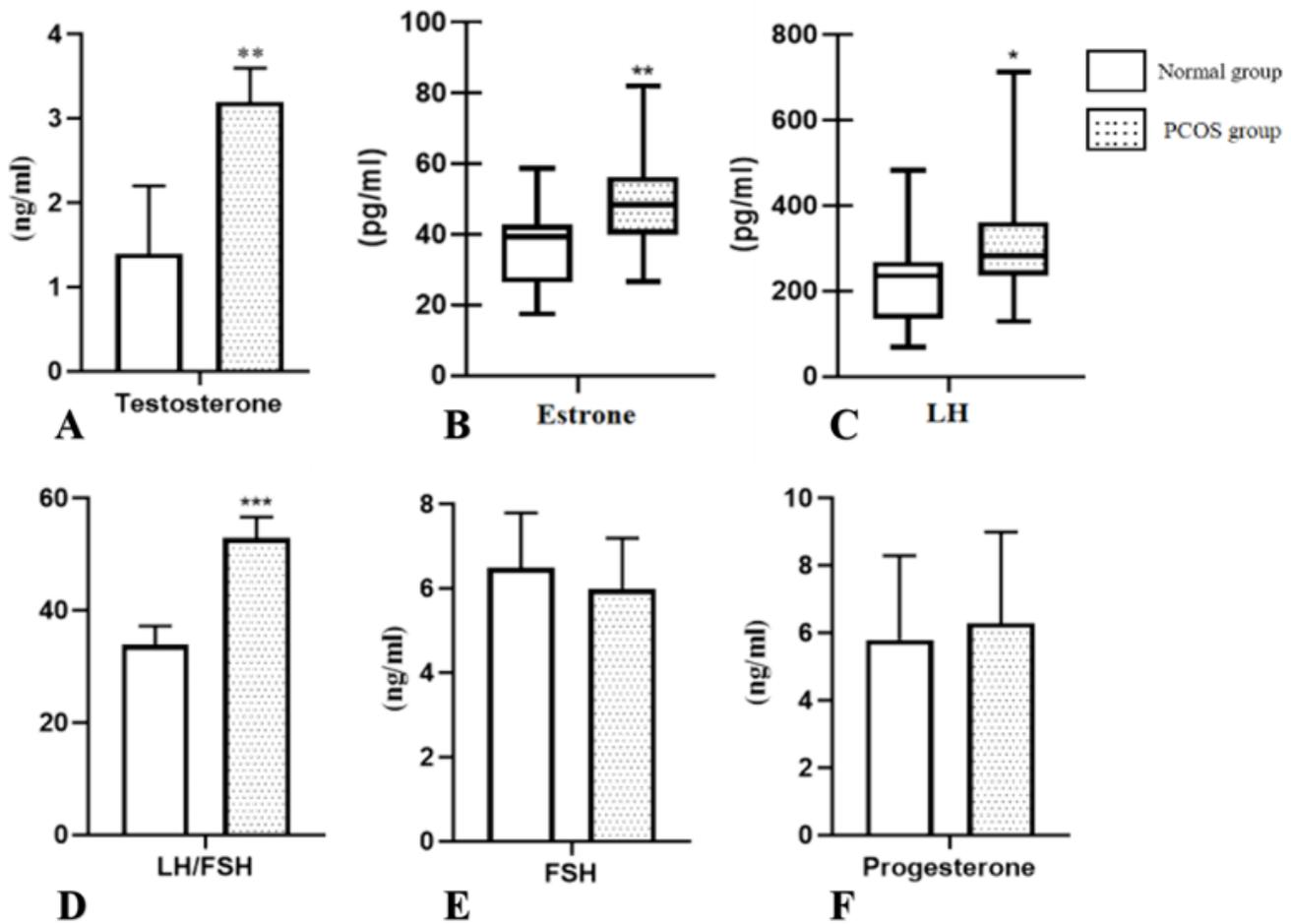
Phenotypic differences between two groups of BHR. a: Body weight between two groups from 3 to 12 weeks after birth. b: Ovarian weight between two groups. c: Clitoral length between two groups.



**Figure 3**

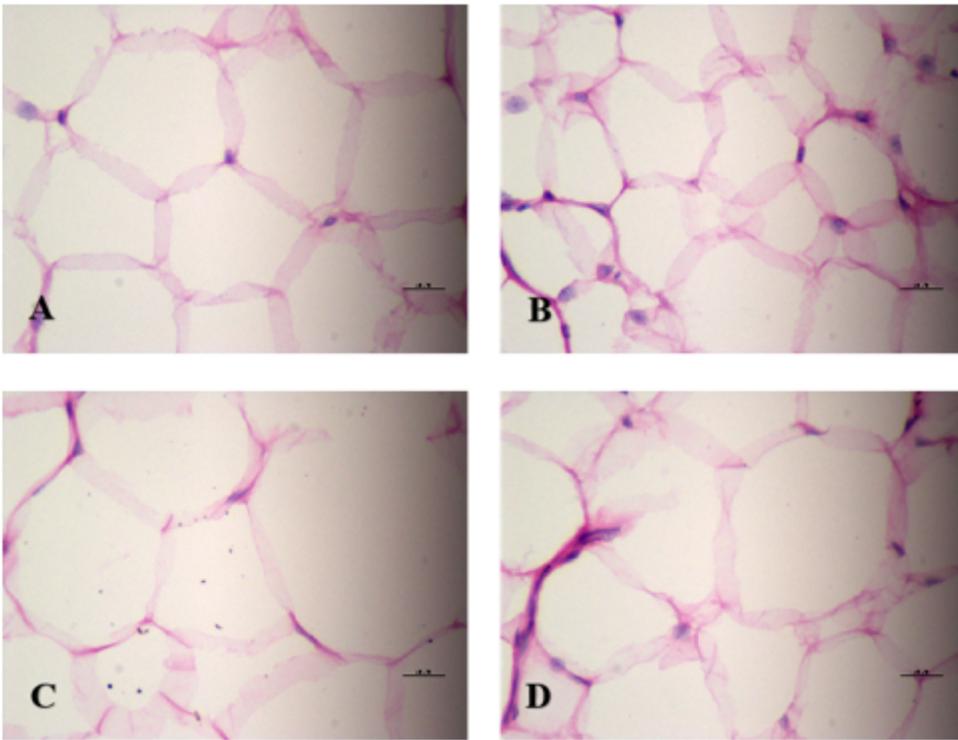
The ovarian morphology and the uterine morphology of two groups by HE staining. The magnification is 4x in image a to b, and 10x in image c to d, and 40x in image e to h. a and c: The ovary from a normal BHR showing healthy growing follicles (black arrows). b and d: The ovary from a BHR with PCOS

showing enlarged cystic follicles (black arrow). e: Normal membrane cell layer (black double-side arrow) and granulosa cell layer (black arrows). f: Enlarged cyst-like follicle, the boundary of membrane cell layer is not clear and widened (black double-side arrow), the granules decreased and the apoptotic granulosa cells appeared (black arrow). g: Uterine from a normal BHR, the epithelial layer of endometrium is composed with a simple layer of columnar epithelial with uniform size and arrangement (black arrow). h: Uterine from a BHR with PCOS is the epithelial layer was arranged in disorder, and thickened (black arrow).



**Figure 4**

The sex hormone concentrations of female BHR. a-f, showing testosterone, estrone, progesterone, LH, LH/FSH of BHR from normal group and PCOS group. LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.



**Figure 5**

Peritoneal fatty tissue and gonadal fatty tissue in BHR. a: HE-stained of peritoneal fatty tissue in normal group. b: HE-stained of gonadal fatty tissue in normal group. c: HE-stained of peritoneal fatty tissue in PCOS group. d: HE-stained of gonadal fatty tissue in PCOS group.