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Review Article

Experimental animal models for polycystic ovarian syndrome (methods, effects, and implications)

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Abstract

Many studies have replicated the clinical and genetic features of polycystic ovarian syndrome (PCOS) using a range of experimental animal models to improve treatment outcomes. This article aims to present an overview of the various experimental animal models that have been used in PCOS research. In this study, we conducted a systematic review of relevant research articles on the induced animal model PCOS. We searched research articles in Indonesian and English published over the last five years through three databases: PubMed, ScienceDirect, Google Scholar. We use established inclusion and exclusion criteria to select suitable articles. Out of 19 research articles included in our systematic review, we found the animal model PCOS based Rotterdam criteria, PCOS-IR model, PCOS-Inflammation model, PCOS-Gut microbiota model and PCOS-syndrome metabolic model. Androgen agents such as testosterone propionate, free testosterone, DHEA, and letrozole, as well as sodium valproate, are effective in the induction of PCOS phenotypes based on the Rotterdam criteria (oligo/amenorrhea, hyperandrogenic, and polycystic ovaries).

Keywords: Animal model; Induced methods; Polycystic ovarian syndrome

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is an endocrine disorder that can cause infertility in women, for which the specific etiology of the therapy for PCOS is currently unknown. PCOS is characterized by a combination of various symptoms, including hormonal disruption, ovulation disorders, hyperandrogenism, and insulin resistance [1]. This condition not only causes reproductive problems and the output of pregnancy but also increases the risk of long-term health complications, such as type 2 diabetes, heart disease, and other metabolic problems, as well as psychological disorders and quality of life [2–4]. To understand the pathophysiology of PCOS and develop more effective therapies, many studies have used a variety of experimental animal models to replicate the clinical and molecular characteristics of the disease. This approach provides valuable insights into the underlying mechanisms behind the development and progression of this polycystic ovarian syndrome.

This article aims to present an overview of the various experimental animal models that have been used in PCOS research This article analyzes the various animal species that have been used as PCOS induction models, as well as the clinical and molecular aspects that have been successfully replicated in each

model. It also discusses the results of studies using these models and their relevance for understanding human PCOS.

A better understanding of the biological mechanisms underlying PCOS through experimental animal modelling approaches can enhance efforts to develop more targeted and effective therapies to address the complex problems associated with this syndrome. It should be noted that although experimental animal models can provide valuable insights, there are also limitations and differences to be considered when interpreting the findings of this review in a human context; therefore, integrating data from animal models with human clinical data will be a critical step towards a comprehensive understanding of PCOS and its treatment. This article can be used as a useful reference for researchers and medical professionals interested in deepening their understanding of PCOS and developing more effective therapies to address the health challenges it poses.

MATERIALS AND METHODS

Research articles were searched using three databases, PubMed, ScienceDirect, Google Scholar in five years of publication (2019- 2023) that written in Indonesian and English. The keywords used in the article search have been matched to Medical Subject Titles (MeSH) including "PCOS model", "Animal Model", "PCOS (t/n: SOPK)", "Polycystic Ovary Syndrome (t/n: Sindrom Ovarium Polikistik)", "Hyperandrogenism", "Insulin Resistance", "Ovarian Dysfunction", "Induction", according to PICOTs (Population, Intervention, Comparators, Outcome, Time), see Table 1.

Guidelines for evaluating the quality of the study are Joanna Briggs (JBI) Critical Assessment and PRISMA guidelines (see Figure 1). Literature from the search results was checked for indications of duplication. Literature that did not have duplicates was then screened in two stages. The first stage of selection was carried out by determining the suitability of the title and abstract with the predetermined inclusion criteria. Literature that had passed the first stage of selection was selected again by analyzing the correspondence between the journal content and the predetermined inclusion criteria (Table 1). This literature selection was carried out by both reviewers to obtain accurate results and minimize errors. Risk of Bias (Figure 2 and 3) identified use Rob 2 by Revman 5.41 software[5,6].

RESULTS

Nineteen research articles were included studies. Table 2 summaries of each in this article in a systematic review of PCOS animal model induction consists of methods, effects, and implications. The induction methods, doses, duration, and PCOS phenotypes observed in the experimental models are presented in Table 3. Several induction methods, such as prenatal administration of Testosterone Propionate and Free Testosterone, successfully explored PCOS phenotypes including oligo-anovulation, hyperandrogenism, and polycystic ovaries[7–9]. DHEA and Letrozole are also effective in exploring various aspects of PCOS, including inflammation, hyperlipidemia, and insulin resistance [10–20].

Table 1. Inclusion and exclusion criteria

Criteria	Inclusion	Exclusion
Population	Mus musculus, rats, mice, murine, sheep	In vitro
Intervention	PCOS model induction using various methods (hormone, diet, drug, genetic manipulation)	Not focusing on PCOS model induction
Comparators	With or without control group	-
Outcomes	Study that relevant results related to PCOS phenotypes on models	Studies that do not provide sufficient information to be evaluated
Time	Within the past five years (2019-2023)	More than the past five years (> 2019)
Study design	Experimental research	Analytical observational research
Language	Indonesian, English	Besides Indonesian and English

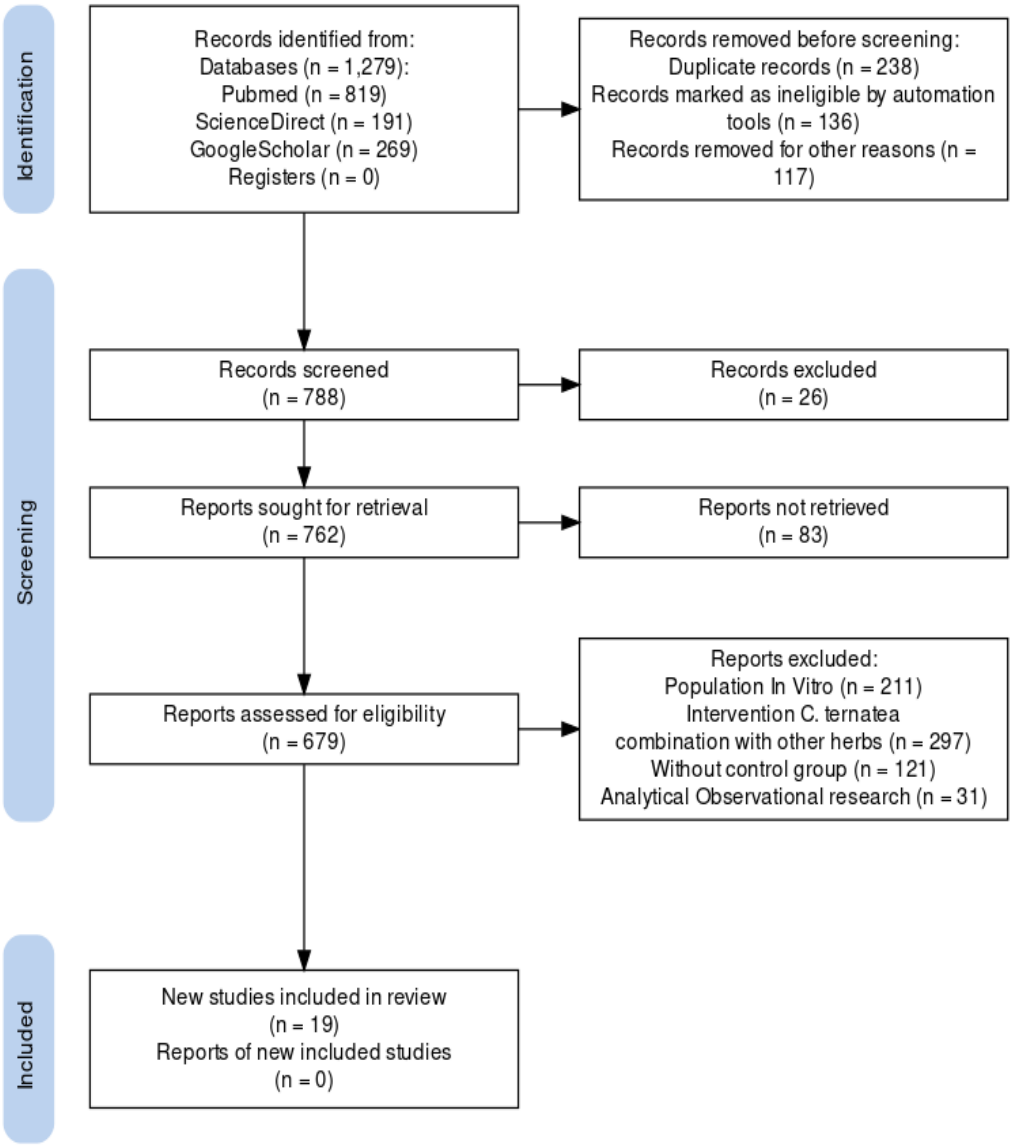

Figure 1. PRISMA Flow diagram of eligible studies

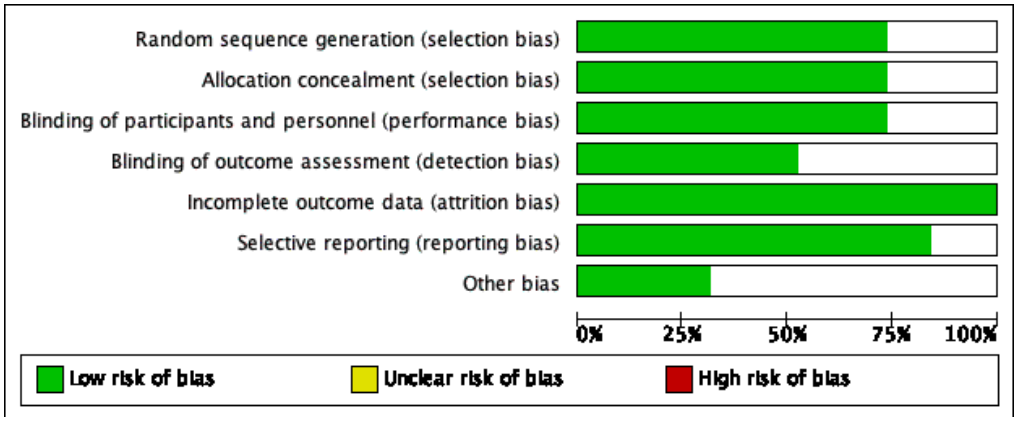

Figure 2. Risk Bias Graph of eligible studies

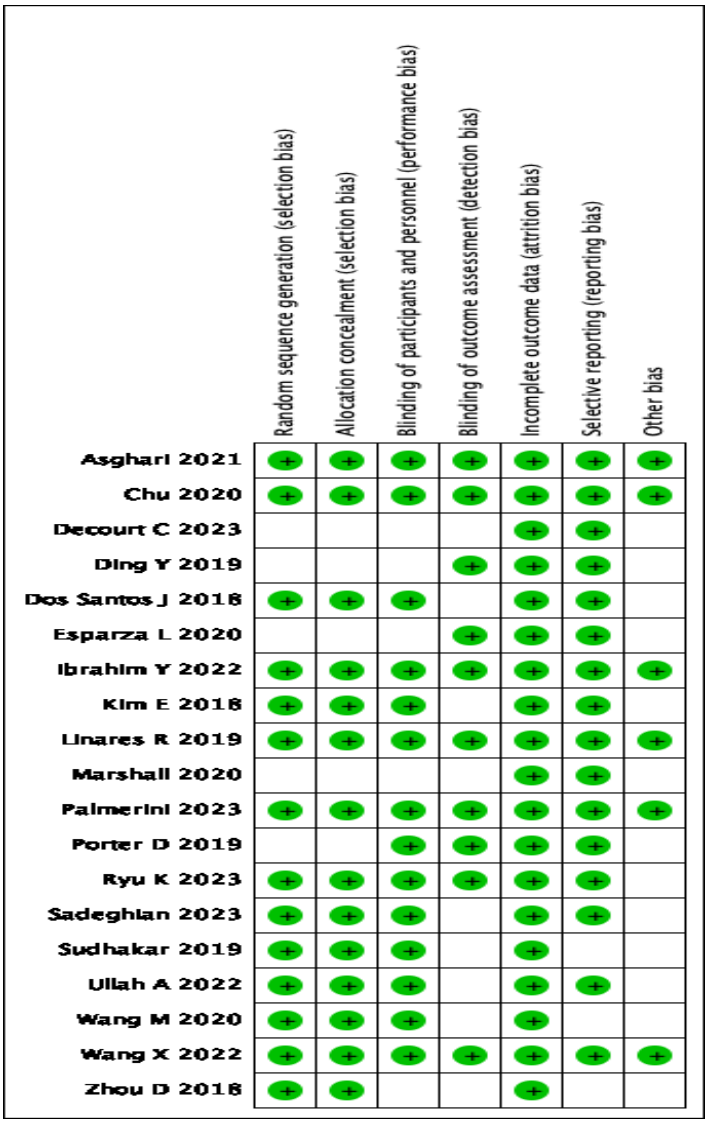

Figure 3. Risk Bias Summary of eligible studies

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
Ding *et al*., 2019 [25]	Female Sprague-Dawley (3 weeks old, 230-250g) Total: 30 (3 groups)	HOMA IR (FPG, FINS) Testosterone Lactate dehydrogenase (LH) FSH Histopathology MDA T-AOC, SOD, GSH	Control: (n=10) normal diet+injection olive oil 0.05 ml PCOS-IR: (n=10) 100 µg testosterone propionate dissolved in 0.05 ml corn oil subcutant injection + High fat Diet (54.2% standard diet, 16.8% lard, 15% sucrose, 9% casein, 1% minerals, 1% vitamins and 3% malt dextrin) Duration: 8 W	Significantly increased T, LH, LH/FSH, FIN and HOMA-IR levels PCOS-IR rats exhibited higher levels of cyto c and Bax and a higher ratio of Bax to Bcl-2, whereas the level of Bcl-xL was decreased. The rats in the PCOS-IR group had higher MDA levels and lower T-AOC, SOD and GSH levels, suggesting increased OS in PCOS-IR	Vaginal keratosis Abnormal Estrus cycle Insulin resistance Hyperandrogenic Inflammatory Polycystic ovary
Decourt et.al., 2023[15]	Female mice C57BL/6J (26 day of age) Total: 20 (2 groups)	Testosterone Histology Body weight Insulin	Control: placebo LET: subcutaneous implant letrozole 4.5 mg Duration: 90 day	LET treatment had a significantly higher plasma concentration of testosterone at 50 days. LET were in constant diestrus. LET mice presented haemorrhagic follicles in addition to cystic follicles.	Polycystic ovary Weight gain Abnormal Estrus cycle Insulin Resistance Hyperandrogenic

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Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
				LET treatment were significantly heavy LET was significantly smaller compared to control mice, indicating insulin resistance. The number of cystic follicles per ovary was significantly higher in LET mice	Irregular cycles and ovulatory disruption
Sadeghian Bhahi *et al*., 2023 [9]	Pregnant Wistar rats (170-190 g, 75-85 days of age) Total: 20 (2 groups)	Estrous cycle Histology Testosterone Body Weight	PCOS rats: (n=10) 5 mg free testosterone dissolved in a 500 µl sesame oil & benzyl benzoate in a 4:1 ratio (s.c) Control: (n=10) vehicle 500 µl solvent simultaneously (s.c) Time: 20th day of pregnancy period	Prenatal exposure can develop PCOS in prenatal androgenizing mice in adulthood. The group given testosterone experienced significant weight gain, have morphological changes that resemble male characteristics, such as increased lengths of anogenital distance (AGD) and anovaginal distance (AVD).	Polycystic ovary Estrus cycle disorder Hyperandrogenic Weight again
Palmerini *et al*., 2023 [11]	Female CD-1 mice (4w old, 20-21g) Total: 40 (4 groups) female offspring of PNA	Estrous cycle Histology (morpho-functional & molecular alterations uterus)	DHEA group (n=10): daily subcutaneous injection of DHEA (6 mg/100g BW) + sesame oil 100 µl with 10% of 95% ethanol	DHEA mice showed abnormal estrous cyclicity DHEA group, the endometrium appeared hyperplastic and thicker. the luminal epithelium thickness increased	Abnormal Estrus cycle Glandular Epithelial Proliferation

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Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
			3hr after testosterone inj-received 200 µl phosphate buffered saline (PBS) Vehicle control group (n=10): injected 0.09 ml sesame oil + 0.01 ml 95% ethanol daily Time: 20 days	and was invaginated in the underlying stroma, which showed lower cellularity. Increased presence of inflammatory cells (eosinophils, lymphocytes, and macrophages) The LC3II/LC3I ratio decreased significantly, p62 increased, indicating the decreased level of autophagy. 17 β-HSD4 expression increased expression in all the endometrial compartments, but also in the myometrium. The increased immunostaining for 4-HNE in the luminal and glandular endometrial epithelium, as well as in the myometrium. SIRT1 expression increased, SOD2 protein was significantly upregulated	Luminal and glandular epithelial hyperplasia MG-AGE accumulation
Ryu *et al*., 2023 [16]	Female mice C57BL/6N (3w old age)	LH pulse Testosterone	Chow Group: LET group, Con, LET-TRF	Hyperactive pulsatile LH secretion pattern	Hyperandrogenic Polycystic ovary

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
	Total: 60 (6 group)	Oestradiol Body Weight Estrus cycle Histology	HF Group (60% high-fat): LET-HF, Con-HF, LET-TRF HF Letrozole dose of 50 μg per day through subcutaneous implant installation (4 w age) Time: 60 days	LET mice had increased T levels, mirroring hyperandrogenaemia, E2 levels were not different LET mice showed a more rapid initial weight gain from 6 to 8 weeks of age The average adipocyte size of the LET mice was higher. Macrophage infiltration in adipose tissue sections appeared with higher frequency in LET mice Levels of inflammatory markers in adipocyte tissues, including interleukin 1 alpha (IL-1α), C–C Motif Chemokine Ligand 2 (CCL2), tumour necrosis factor alpha (TNF-α), and CD11c, were higher in LET and LET-TRF mice The ovaries of LET mice showed an enlarged polycystic morphology and a higher number of cystic follicles	Inflammation Abnormal Estrus cycle

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
				Rev-erbα decreased markedly in LET mice, Bmal1 expression was higher in LET , GLUT-4, a marker for insulin responsiveness decreased in LET mice , Kiss1r, Gnrh, and Pgr, were increased in LET mice	
Ullah *et al*., 2022 [10]	Female mice C57BL/6J Total: 55 (2 group)	Insulin LH FSH Testosterone Progesterone Histology Body weight	DHEA Group: 6 mg/100 g of weight, dissolved in 0.1 ml of ricin oil (subcutaneous injection) + high-fat diet (HFD) with 60% calorie fat. This high-fat diet has 5.24 kcal/g, with 20% protein (26 g%), 20% carbohydrate (26 g%) and 60% fat (35 g%) Time: 20 day	Significantly higher serum insulin levels, higher levels of serum LH, no significant difference in serum FSH levels, significantly higher serum testosterone levels, lower levels of serum progesterone. Ovaries significantly lower numbers of corpora lutea, follicular dysplasia and ovulation disorder, and significantly higher numbers of atretic and cystic.	Hyperandrogenic Abnormal estrous cycle Cystic follicle Weight again Insulin Resistance
Ibrahim *et al*., [17]	Female albino Wistar rats Total: 36 (6 group)	FBG Histology Lipid Profile (TC, HDL, TGs)	Control (2 group): 1% carboxy methylcellulose PCOS (4 group): Letrozole oral dose of 1 mg/kg	The body weight of the PCOS-induced rats was higher, higher ovarian weights cystic follicles predominated in the ovarian structure, with a limited	Inflammation Insulin Resistance Polycystic ovary Hyperandrogenic

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
		Inflammation profil (IL-1β, IL-6, TNFα) Antioxidant (MDA, NO, SOD, CAT)	Time: 21 days	number of growing follicles and corpus lutea. significantly higher FBG, higher serum insulin and HOMA/IR levels serum LH level was reported to be higher, testosterone level was significantly raised by letrozole (by 134%) The cholesterol and triglyceride concentrations were two-fold higher, lower HDL level Lower SOD activity level, lower catalase activity, higher MDA level, total nitrite level was significantly higher, higher level of total nitrite The protein level of the inflammatory cytokines IL-1β in ovarian tissues and IL-1β, IL- 6, and TNF-α higher in the serum	

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
Wang *et al*., 2022 [13]	Female C57BL/6J mice (6 weeks old) Total: 40 (4 group)	TC, LDL-C Testosterone FBG, FINS Estrus cycle Histology Microbiota	Control group: normal diet and injected daily with sesame oil. HFD group: (60% of energy provided by fat) DHEA group: subcutaneous injection of DHEA (6 mg/100 g of body weight) dissolved in 0.1 mL of sesame oil DHEA+HFD group Time: 5 weeks	The DHEA and DHEA + HFD groups stayed in the dioestrus stage, serum T levels were significantly higher Significantly increased TC and LDL-C levels Bifidobacterium and Lactobacillus, was significantly increased in the DHEA and DHEA+HFD groups. contained several antral follicles and no CL DHEA+HFD group showed extremely heterogeneous hyperinsulinemia and IR Hyperinsulinemia and insulin resistance in the DHEA group	Hyperandrogenic Insulin resistance Estrus disorder Ovary polycystic Dysbiosis Gut microbiota Metabolic disorder
Marshall *et al*., 2020 [22]	Adult female mice	NPY^{ARN} neurons Vaginal cytology	VEH control: 100 μL of sesame oil PNA: 250 μg of di-hydro testosterone (DHT) Time: days 16, 17, and 18 of pregnancy	PNA mice were acyclic, spending the majority of time in persistent diestrus ARN was significantly elevated in PNA mice. PR-ir was less abundant and less intense in PNA . The co-expression of	Estrus cycle disorder Hyperandrogenic Androgen receptor ↑

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
				ERα within NPYARN neurons extremely limited in PNA	Progesterone receptor, estrogen receptor alpha ↓
Wang *et al*., 2020 [20]	Female Sprague-Dawley rats (6 to 8 weeks old) Total: 48 (2 group)	FPG, FINS LH/ FSH Triglycerides Testosterone Triglyceride Body weight Histology	PCOS group: letrozole (1 mg/kg/day) + HFD Control group: 0.5% carboxy-methylcellulose (CMC)-Na (1 mL/100 g/day) HFD: 20% protein, 20% carbohydrate, and 60% fat Normal diet: 3.85 Kal/g, protein 20%, carbohydrate 70%, fat 10% Time: 30 days.	Letrozole combined with a high-fat diet for 21, 24, 27, and 30 days resulted in body weight and Lee's index of the rats in each model group to be significantly in- creased. ovarian volume of the rats was significantly increased, hawed polycystic changes and the number of follicles increased significantly the levels of LH/FSH, testosterone, and TG were significantly increased levels of FINS and HOMA-IR were significantly in- creased	Insulin resistance Hyperandrogenic Polycystic ovaries Metabolic disorder
Chu *et al*., 2020 [8]	Sprague-Dawley (SD) females (200-220 gr, aged 6 weeks) Total: 22 mice (2 group)	AMH Insulin Intestinal microbiota	The control group was under a periodic cycle (12-h/12-h light/dark cycle, L/D group) while the light group was	The phenotype induced by light exposure in SD rats included estrous cycle disorder, oligo/anovulation, ovulation dysfunction, elevated AMH	Estrus cycle disorder Insulin resistant Oligo/Anovulation

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
		Histology	exposed to continuous light (12-h/12-h light/light cycle, L/L group) for four consecutive weeks	level, glucose metabolic abnormality, abnormal gut profile resembling metabolic disorder, Gut microbiota profile shaped by continuous light exposure could be in close relevance with endocrine disturbance and glucose intolerance in PCOS.	Gut microbiota
Esparza *et al*., 2020 [19]	Female C57BL/6 mice Total: 16 mice	Vaginal smear GnRH/LH pulse Testosterone Oestradiol	LET group: LET (letrozole) of 50 μg/day (age of 4w) subcutaneous implantation. CON group: placebo Time: 60 days	Circulating T was significantly upregulated in LET females, BWs were also greater at both 3 and 5 weeks post-LET, serum E2 levels higher in control than in LET females LH for the entire sampling period was significantly higher, by 4-fold, in LET Kiss1 and Tac2 gene expression in the ARC were strongly upregulated in the LET condition, suggestive of a higher capacity for these ARC cells to make kisspeptin and NKB	Abnormal estrous cycle LH hypersecretion Neuroendocrine disorders Hyperandrogenic

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
Linares *et al*., 2019 [23]	Female CIIZ-V strain rats Total: 100 (2group)	Oestradiol Testosterone Progesterone Noradrenalin Histology	EV group: 2 mg EV (oestradiol valerate) dissolved in 0.1 ml of corn oil Control group: VH (vehicle) 0.1 ml of corn oil. Time: 76 days	The group induced with EV had higher concentrations of oestradiol, testosterone, progesterone in the serum. In the EV control group, the ovaries showed polycystic follicles and no corpus luteum.	Hyperandrogenic Polycystic ovaries
Porter *et al*., 2019 [7]	Suffolk sheep Total: 15 (2 group)	GABAergic synaptic inputs on GnRH and KNDy neurons	PCOS group (n=7): injected 100 mg testosterone propionate in cottonseed oil Control (n=8): untreated Time: 2 weeks (30-90 day gestation, of a 147 days pregnancy)	There was a significant decrease in the number of GABAergic synapses on GnRH neurons in the preoptic area (POA) and the medio basal hypothalamus (MBH), as well as a significant increase in the amount of GABAergic input on KNDy neurons.	GnRH/LH pulse frequency
Sudhakar *et al*., 2019 [18]	Wistar albino (12-18 weeks age, 180-230 gram) Total: 24 (4 group, n=6)	Estrus cycle FPG Lipid profile Testosterone Estrogen Progesterone Body weight	Group I: letrozole 400 µg/kg/day Group II: Diazepam 4 mg/kg/day Group III: Sodium valproate 300 mg/kg/day	Significant weight gain occurred after 21 days in all groups, higher difference in BW group I (32.8g). Abnormal changes in the estrus cycle with the dominance of the diestrous phase. Hyperglycaemia and glucose intolerance higher in group I and III. High testosterone, lower	Hyperandrogenic Polycystic ovaries Hyperglycaemia Glucose intolerance Hyperlipidaemia Weight again

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
		Ovary weight Histology	Group IV: carbamazepine 50 mg/kg/day Time: 35 day	progesterone group IV-III-I. High estrogen in group III-IV-I. There was a decrease in HDL levels and an increase in LDL and VLDL levels in all groups, lipid profiles most influential in groups I and III. Ovary weight higher in group I-IV-III. Increased size, thickened capsule, cystic graafian follicle, follicle cyst in group all group except diazepam group. Reduction of corpus luteum, changes in the granulose cells and an increase in the number of atretic follicles in group I, III and IV, the numbers of cysts are higher in sodium valproate and carbamazepine group. sodium valproate induced PCOS in similar to the human PCOS with the same metabolic and reproductive characteristic features.	Abnormal estrous cycle

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
Asghari *et al*., 2021 [24]	Balb/c female mice (20.0 ± 1.5 g, 8 weeks old) Total: 20 mice (2 group)	LH FSH Progesterone Oestradiol Testosterone Estrous cycle Gene expression Histology Body weight	PCOS group (n=10): intramuscular injections of oestradiol valerate (EV) 0.2 mL of EV dissolved in normal saline Control (n=10): injection of sesame oil (0.1 mL) Time: 56 days	The PCOS group of mice showed weight loss, a lower number of preantral and antral follicles, as well as lower levels of oestradiol, luteinizing hormone, testosterone, and follicle-stimulating hormone. In addition, there was a decrease in the expression of the genes TGFB1, GDF9, and BMPR2 in the preantral follicle. However, levels of the hormone progesterone in mice with PCOS were higher than in the control group.	Decrease follicle Cystic follicle Abnormal estrous cycle
Dos Santos *et al*., 2018 [21]	Corriedale sheep (3-5 years age), multiparous, good nutrition Total: 46 (2 group)	Estrous cycle Glucose tolerance Insulin Progesterone Testosterone Androstenedione Gene expression	Control group (n=24): no intervention Androgen group (n=22): Androgenol, 100 mg IM Time: biweekly to pregnant ewes during days 30 to 90 of gestation (26–28)	PA sheep became heavier compared to controls from 8 to 12 months of age. PA female lambs had a significantly increased anogenital distance compared to female controls. PA sheep showed a disruption of the ovarian cycle, characterized by the absence of a sustained elevation in serum	Insulin resistance Abnormal estrous cycle Hyperandrogenic

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
				progesterone. PA androgenized female sheep showed evidence of insulin resistance, as defined by a higher insulin to glucose ratio at +15 minutes during an intravenous glucose tolerance test. Significant increase in testosterone levels at 12 months of age in the prenatally androgenized (PA) group, no significant increase in androstenedione. no significant difference in gene expression	
Kim *et al*., 2018 [12]	Sprague Dawley (21-day-old and 42-day-old)	Body weight Histology Vaginal smears (estrous cycle)	Pre pubertal (n=56) Post pubertal (n=109) DHEA group: subcutaneously injected daily with DHEA (60 mg/kg BW dissolved in 0.2 mL sesame oil) Sham group: sesame oil without DHEA Time: 20 day	In pre-puberty rat models, only 33% of rats experienced significant weight gain after DHEA injections. Furthermore, about 72% of rats in the PCOS group showed an irregular estrus cycle. 50% of PCOS rats showed a significant increase in ovarian weight, increased ratio of rats with irregular	Weight gain Abnormal estrous cycle Polycystic ovary

Table 2. List of research articles for experimental animal models for polycystic ovarian syndrome

Author	Sample	Measured/ Parameter	Intervention (type, Dose, Duration)	Result	Effects
				estrus cycles (66.7–100%) and multi-cystic ovaries (75–100%).	
Zhou *et al*., 2018 [14]	Female Sprague-Dawley rats (21 day old)	Insulin Testosterone LH FSH Oestradiol Body weight Histology	Control: SC 0.2 mL castor oil PCOS: 6 mg/100 g body weight dissolved in 0.2 mL castor oil PCOS+HFD: SC DHEA+ HFD (protein 25% and lipids 15%) Time: 21 day	The weight gain in rats of the PCOS plus HFD group was significantly higher. The serum testosterone (T), fasting blood insulin (FIN), FSH, LH/ FSH ratio, and HOMA-IR index were significantly elevated in the PCOS group and the PCOS plus HFD. The levels of LH and fasting blood glucose (FBG) in the PCOS plus HFD group were slightly higher than those in the PCOS group. The number of follicular cysts in the PCOS group and the PCOS plus HFD group was greater than that in the control group, and the ratio of follicular cysts to normal follicles was significantly increased.	Hyperandrogenic Insulin resistance Polycystic ovary Weight gain

Table 3. Induction method, dose, duration, and phenotype of PCOS

Induction Method	Dose	Duration	PCOS Phenotype (Rotterdam Criteria)			Another PCOS effect
			Oligo-Anovulation	Hyper-androgenic	Ovary Polycystic	
Testosterone Propionate	100 μg + HFD	8 weeks	Yes	Yes	Yes	Insulin resistance Inflammation (MDA)
	100 mg	30–90-day gestation	-	-	-	Increased GnRH/LH pulse frequency
Free Testosterone	5 mg	Prenatal day 20	Yes	Yes	Yes	Weight again
Androgenol	100 mg	Prenatal day 30-90 (biweekly)	Yes	Yes	-	Insulin resistance
DHT (Dihydrotestosterone)	225 μg	Prenatal day 16-17-18	Yes	Yes	-	AR↑, PR & ERα↓
DHEA (Dehydroepiandrosterone)	6 mg/ 100 g BW	20 days	Yes	Yes	Yes	Inflammation (eosinophils, lymphocytes, and macrophages) Endometrium hyperplasia
		21 days	-	Yes	Yes	Insulin resistance
		5 weeks	Yes	Yes	Yes	Gut microbiota
	60 mg/kg BW	20 days	Yes	-	Yes	Weight again
	6 mg/ 100 g BW + HFD	20 days	Yes	Yes	Yes	Insulin resistance Weight again
		21 days	-	Yes	Yes	Insulin resistance Weight again
		5 weeks	Yes	Yes	Yes	Gut microbiota Glucose intolerant
Letrozole	4.5 mg	90 days	Yes	Yes	Yes	IR Weight again
	50 μg	60 days	Yes	Yes	Yes	Inflammation (TNF α, IL-1α) LH hypersecretion Neuroendocrine disorder

Table 3. Induction method, dose, duration, and phenotype of PCOS

Induction Method	Dose	Duration	PCOS Phenotype (Rotterdam Criteria)			Another PCOS effect
			Oligo-Anovulation	Hyper-androgenic	Ovary Polycystic	
	1 mg/ kg BW	21 days	-	Yes	Yes	Insulin resistance Inflammation (IL-1β, IL- 6, and TNF-α)
	1 mg/ kg BW + HFD	30 days	-	Yes	Yes	Insulin resistance Metabolic disorder
	400 μg/kg/day	35 days	Yes	Yes	Yes	Glucose intolerance Hyperlipidemia Weight again
Estradiol Valerate	2 mg	76 days	-	Yes	Yes	-
	0.2 ml	56 days	Yes	No	Yes	-
HFD	60% of energy provided by fat	5 weeks	No	Yes	No	Bacteroides and Alistipes Glucose intolerant
Light periodic cycle	12h/ 12h light/light	4 weeks	Yes	No	Yes	Insulin resistance Gut microbiota
Diazepam	4 mg/kg/day	35 days	Yes	Yes	No	Glucose intolerance Weight again
Sodium valproate	300 mg/kg/day	35 days	Yes	Yes	Yes	Hyperlipidemia Glucose intolerance Weight again
Carbamazepine	50 mg/kg/day	35 days	Yes	Yes	Yes	Glucose intolerance Weight again

However, some induction methods such as the use of Diazepam or Sodium Valproate show an incomplete PCOS phenotype, not covering all PCOS characteristics such as hyperandrogenism or polycystic ovaries[18]. In addition, some doses and durations of induction elicit a milder PCOS phenotype, as seen with a dose of 0.2 ml Estradiol Valerate for 56 days. Therefore, interpretation of the results should be done with caution, and selection of an appropriate induction method is key to achieving an accurate representation of the PCOS phenotype in experimental models[18].

Testosterone Propionate

Testosterone propionate is a synthetic form of testosterone that is exogenously administered and given from outside the body of an experimental animal. Increased testosterone levels are one of the main characteristics of PCOS in humans. Sheep induced with testosterone propionate experience reproductive disorders like women with PCOS, including increased frequency of the luteinizing hormone (LH) pulse due to steroid hormone feedback disorder, changes in sensitivity to the steroid hormone feedback, and reproductive abnormalities due to prenatal exposure to testosterone[7].

Prenatal exposure to testosterone (T) hormone resulted in changes in steroid hormone feedback on GnRH neurons and KNDy neurons (kisspeptin, neurokinin B, dynorphin) in the polycystic ovarian syndrome (PCOS) sheep model. In sheep exposed to prenatal T, there was a decrease and an increase in the number of GABAergic synapses in the preoptic (POA) and medio basal hypothalamic (MBH) neurons, respectively, increasing the number of GABAergic inputs on the KNDy neuron. GnRH and KNDy neurons are important in controlling the reproductive cycle, and alterations in the hormonal steroid feedback mechanism in these neurons may contribute to the occurrence of polycystic ovary syndrome (PCOS). GABAergic refers to the neurotransmitter gamma-aminobutyric acid (GABA), which acts as an inhibitor in the nervous system. Changes in the number of GABAergic synapses on POA and MBH neurons affect the activity and response of KNDy neurons. Increased GABAergic input on KNDy neurons can modulate the function of kisspeptin, neurokinin B, and dynorphin, all of which play a role in regulating the reproductive cycle [7].

Mice were injected with 100 μg of testosterone propionate and given a high-fat diet for 8 weeks to form a PCOS-IR mouse model, showing symptoms such as vaginal keratosis and abnormal estrus cycles, indicating significant increases in levels of the hormones testosterone (T), luteinizing hormone (LH), LH/FSH, insulin fasting (FIN), and HOMA-IR [8]

Free testosterone

The use of free testosterone in PCOS studies in experimental animals aims to investigate the pathophysiological mechanisms of PCOS, including how increased androgen influences the regulation of reproductive hormones and the development of ovarian follicles. High levels of testosterone or an increase in androgen can interfere with the regulation of reproductive hormones, ovulation, and ovarian follicle development. High androgen exposure during the developmental period can cause changes in the structure and function of the spinal cord in female rats; it can interfere with the process of folliculogenesis and cause an increase in the number of preantral and antral follicles, as well as a decrease in the amount of preovulatory follicle. In addition, PCOS rats also show irregular estrus cycles. High androgen exposure during prenatal periods can affect gonadotropin sensitivity to GnRH stimulation, which can lead to increased pulsed LH secretion and excess androgen. High androgen exposure can also interfere with ovarian granulocyte function and cause increased secretion of LH and testosterone. This may lead to enhanced cAMP activity and the formation of ovarian cysts [9].

Androgenol

Androgen-induced sheep showed changes in the diameter of fur fibers, like an increase in hair diameter in women with PCOS. The findings provide new insights into the influence of prenatal androgenization on hair development and its possible association

with PCOS. The research also shows that there are no differences in the expression of the AR, SRD5A1, and HSD17B2 genes between the group of sheep who underwent prenatal androgens and the control group. This research has some limitations, such as a limited number of animals and a limited time of research [21].

Dihydrotestosterone (DHT)

Prenatal androgen induced by DHT (dihydrotestosterone) produces phenotypic changes like polycystic ovarian syndrome (PCOS). Prenatal androgen excesses cause increased androgen sensitivity in the ARN NPY neurons (arcuate nucleus) and changes in the circuit of the GABA neuron (gamma-aminobutyric acid) associated with the GnRH neuron (gonadotropin-releasing hormone). However, there were no significant changes in the projection of NPY ARN neurons to GnRH neurons. Increased androgen sensitivity in NPY RNA neurons can interfere with the regulation of reproductive hormones, including the release of GNRH, which can cause changes in patterns of release of reproduction hormones that can further affect folliculogenesis or the formation of ovary follicles. Changes in the circuit of GABA neurons that regulate GnRh release can disrupt menstrual cycle regulation and folliculogenesis. Increased androgen sensitivity to NPY RNA neurons and changes in the circuit of GABA neurons associated with GnRH neurons can interfere with the complex hormonal regulation in the body, which is one of the characteristics of PCOS. It results in disturbances in folliculogenesis, ovulation, and menstrual cycles, all of which are characteristic of PCO. Changes in hormone regulation and nervous activity can contribute to PCOS pathogenesis and associated symptoms [22].

Dehydroepiandrosterone (DHEA)

DHEA (dehydroepiandrosterone) plays a role in inducing the PCOS phenotype in mice (Polycystic Ovary Syndrome) by increasing testosterone and lipid levels as well as lowering blood glucose levels. Mice given DHEA or DHEA and HFD had PCOS-like phenotypes, such as hyperandrogenism, anovulation, and polycystic ovaries. However, rats given DHEA and HFD also experienced more heterogeneous glucolipid metabolic disorders, hyperinsulinemia, and insulin resistance. In addition, the study also showed that the intestinal microbiota plays an important role in the heterogeneous phenotype of the PCOS mouse model. Better and more stable PCOS patterns can be induced with DHEA alone, not with a combination of DHEA and HFD. Antibiotic intervention (ABX) can improve glucose metabolic disorders and hyperinsulinemia but worsen hyperandrogenism and lipid metabolic disturbances in PCOS mice. The results show that the gut microbiome plays a major role in heterogeneous phenotypes in PCOS models. The findings provide new insights into the formation of a PCOS rat model and research into the pathophysiological mechanisms of PCSOS [13].

DHEA increases the proliferation of the glandular epithelium of the uterus, which can lead to the enlargement of glands and the thickening of the walls of the womb. Mice exposed to DHEA experience luminal and gland hyperplasia, with an increase in the number of single uteruses and a decrease in the number of uterine glands. This can affect endometrial receptivity in PCOS patients. DHEA raises the level of estrogen, which may shorten the implantation window. In pregnant mice, excess androgen caused by DHEA leads to decreased progesterone levels and increased estradiol levels. DHEA causes increased collagen deposition, which is an additional phenotype of the uterus. PCOS can contribute to uterine dysfunction and increase the risk of endometrial cancer. Mice exposed to DHEA experience uterine inflammation and fibrosis [11].

DHEA can affect ovary folliculogenesis and oocyte lipid metabolism. In a PCOS mouse model, postnatal DHEA treatment produces PCOS features such as polycystic ovaries, hyperandrogenism, and anovulation. In a related study, the expression of inflammatory genes in the ovary of a mouse model of PCOS induced by DHEA and HFD was studied using RNA sequencing technology. The results showed that differently expressed genes were associated

with inflammatory processes and homocysteine metabolism. Path analysis showed an abnormally expressed mRNA was linked to NF-kB signal pathways, tyrosine metabolic processes, and phenylalanine metabolization. These pathways are involved in chronic inflammation and PCOS. DHEA and HFD can affect PCOS phenotypes and folliculogenesis through mechanisms that involve inflammation and metabolism. The genes that are expressed differently in the ovary of a mouse model of PCOS can be potential therapeutic targets to cope with the inflammation that occurs in PCOS [10].

The pre-pubertal PCOS model in rats, induced by DHEA injection, exhibits irregular phenotypes. These include irregular estrous cycles, multi-cystic ovaries, and abnormal weight changes in the ovary and uterus. However, it should be noted that this model does not fully replicate the key features of PCOS, such as increased ovarian and uterine weight, and enhanced homogeneity of ovarian cyst formation. Therefore, while this model provides some insights into PCOS, it has limitations in accurately representing the disorder. The post-pubertal PCOS model shows improved phenotypes, including a stable increase in ovarian and uterine weight and size, as well as increased homogeneity of ovarian cyst formation and uterine abnormality. These features are key characteristics of PCOS. This new model provides a better understanding of the pathogenesis of PCOS and may contribute to the development of potential therapeutics [12].

Protein expressions of MIF, JNK, and P38 increased significantly in the PCOS and PCOS groups with high-fat diets (HFD) compared to the control groups. However, the difference between PCOS groups and PCOS with HFD is not significant. These results showed that MIF and MAPK signal pathways played a role in the pathogenesis of PCOS in the ovaries of mice. Mice with PCOS and PCOS with HFD had significant weight gains compared to the control group. In addition, the hormones testosterone, insulin, and FSH, the LH/FSH ratio, and the HOMA-IR index also increased significantly in the PCOS and PCOS groups with HFD [14].

Letrozole

The increased androgen signal through the androgen receptor (AR) plays an important role in the development and maintenance of PCOS features. The specific AR signal in the cells that produce kisspeptin also plays a critical role in mechanisms that cause hyperandrogenism to contribute to the development of the reproductive and metabolic features found in PCOS. Another mechanism involved with PCOS is the AR signal specific to cells producing kisspeptin, which plays a critical role in the mechanism that causes hyperandrogenism to contribute to the evolution of the reproductive and metabolic characteristics found in PCOS. Mice with androgen receptor removal are not fully protected from the hyperandrogenism effects induced by letrozole and still experience a decrease in the number of corpora luteum and increased weight gain. Letrozole induction has a significant impact on the development and characteristics of PCOS in mice. The use of letrozole in female rats' results in hyperandrogenism, weight gain, estrus cycle disruption, and insulin resistance. Letrozole also causes an increase in the number of cystic follicles and a decrease in the amount of corpus luteum in the rat's ovaries. Letrozole can be used as an effective PCOS mouse model to study the pathogenesis and treatment of PCOS in humans [15].

The results showed that letrozole-induced mice experienced various phenotypes of PCOS, including ovulation dysfunction, polycystic ovaries, and weight gain. However, after being given a time-limited diet (TRF) for 4 weeks, most of these phenotypes could recover to normal levels [16]. The use of letrozole in rats produced several characteristics of PCOS in rats, including hyperandrogenism, abnormal follicles, hyperglycemia, and oxidative stress. Letrozole also causes the inability of the ovaries to produce a regular ovarian cycle. In mice with PCOS induced by letrozole, there is a significant increase in ovarian weight due to the anabolic effect of letrozole, which causes the accumulation of ovary fat and the formation of many cysts. Increased testosterone levels in mice with PCOS are caused by increased LH pulse frequency [17].

Letrozole is an oral non-steroidal aromatase inhibitor used as an induction of hyperandrogenism in animal models for PCOS. Letrozole inhibits the conversion of androgen to estrogen. Letrozole administration to mice for 7–35 days at a dose of 400 μg/day produced characteristics like PCOS in females, mainly associated with artificial hyperandrogenemia, and did not help identify abnormalities that occurred prior to hyperandrogenemia. The mechanisms of letrozole's influence in inducing PCOS involve weight gain, obesity, estrus cycle changes, insulin resistance, and increased androgen levels. However, this model does not show an increase in the basal LH level that is characteristic of PCOS. Nonetheless, the model is still considered a good one for researching PCOS in humans [18].

Letrozole has a significant effect on the phenotype of PCOS in female rats. Females treated with letrozole showed increased circulatory androgen levels, which is characteristic of women with PCOS. Moreover, rats treated with Letrozole also showed an increase in the expression of the Kiss1, Tac2, and PDYN genes associated with reproductive regulation. This suggests that letrozole may affect the PCOS phenotype in female rats. Female mice treated with letrozole showed an increase in the number of polycystic follicles and a decrease in the amount of the corpus luteum, which indicates a disorder in ovulation. In addition, mice treated with letrozole also showed changes in the expression of the FSHR gene and the steroidogenic enzymes associated with the production of reproductive hormones. It suggests that letrozole may affect folliculogenesis in female rats with PCOS [19].

Letrozole and a high-fat diet (HFD) are used to create a PCOS-IR mouse model. letrozole inhibits the aromatase enzyme, which is responsible for converting androgen to estrogen. In the PCOS-IR mouse model, letrozole produced an increase in androgen levels, which led to ovulation disorders and polycystic characteristics in the ovaries. The high-fat diet (HFD) also plays a role in affecting the phenotype of mice. This diet can cause inflammation in the hypothalamus and disrupt glucose and lipid metabolism in the body, leading to obesity and insulin resistance. With the combination of letrozole and HFD, mice in the PCOS-IR model experienced weight gain, changes in ovarian morphology, increased levels of sex hormones such as LH/FSH and testosterone, as well as increased triglyceride levels, according to the pathological characteristics of PCOS-IR involving endocrine disorders and metabolism. The mechanisms of letrozole and HFD in affecting the PCOS-IR phenotype of mice involve increased levels of androgen, ovulation changes, inflammation of the hypothalamus, and disturbance of glucose and lipid metabolism [20].

Estradiol valerate

Giving EVs to mice aims to increase estrogen levels in the body significantly. This increase in estrogen is one of the main characteristics of PCOS, as in many cases, women with PCOS have higher levels of estrogen than they should have. Increased estrogen levels can interfere with hormone regulation in the body of mice and cause an increase in androgen levels, creating a condition known as hyperandrogenism, which is a major characteristic of PCOS. In PCOS, ovulation often does not occur regularly or does not happen at all; giving EV to mice aims to trigger a similar disorder. Giving EVs to mice can interfere with the development of ovarian follicles, creating conditions that resemble ovarian cysts in PCOS mice. Induction of estrogen valerate (EV) in mice indicates a change in noradrenergic activity in the ovaries. In the group of mice induced by EV, there is an increase in serum estradiol concentrations and a decrease in the concentration of norepinephrine in the upper mesenteric celiac ganglia (CSMG) and the ovary. Decreased concentrations of noradrenaline in the CSMG and ovaries may indicate that the noradrenergic nervous system is involved in the response to hormonal changes induction by EV. These findings show that EV administration in rats affects both the hormonal and nervous systems simultaneously. Changes in estrogen and norepinephrine levels can have an impact on menstrual cycle regulation, reproductive

organ development, and other functions in the reproductive system of rats [23].

Estradiol valerate affects the polycystic ovarian syndrome phenotype (PCOS) through several mechanisms. EVs cause increased ERK (extracellular signal-regulated kinase) activity involved in the extracellular pathway. EV induces an increase in PI3K-Akt (phosphatidylinositol 3-kinase-Act) involved in the intracellular pathway. Both pathways contribute to the hormonal and metabolic changes that occur in the ovaries affected by PCOS. The study investigated gene expression in the preantral follicle, and the results showed that the expression of the TGFB1, GDF9, and BMPR2 genes was significantly lower in the PCOS group compared to the control group. However, there were no significant differences in BMP6 or BMP15 expression between the two groups. These findings suggest that gene expression modifications in the preantral follicle can contribute to the pathological condition of PCOS. Animal models with EV injections do not fully replicate the clinical and molecular conditions of PCOS in humans [24].

High fat diet

After induction with a high-fat diet (HFD), mice showed more severe glucolipid metabolic disorders compared to mice not given HFD. They had higher blood glucose levels, worse insulin resistance, and higher cholesterol and triglyceride levels. HFD can also worsen lipid metabolic disorders and increase the risk of obesity in rats. The high-fat diet (HFD) has been shown to have an impact on the development and progression of polycystic ovary syndrome (PCOS). In PCOS mouse models, the HFD has been found to aggravate glucolipid metabolic disorders [13].

Light periodic cycle

Continuous light exposure can disrupt circadian rhythms and cause reproductive changes in mice, characterized by a disrupted oestrus cycle, a reduced number of antral follicles, an increased number of atretic follicles resembling PCOS characteristics, and elevated AMH levels, which is an important clinical parameter in PCOS patients. Mice exposed to continuous light showed increased blood glucose levels after meals and decreased pancreatic beta cell function. There were also changes in gene expression indicating impaired insulin signaling and decreased expression of GLUT4, which plays a role in glucose transport. Continuous light exposure can also affect the composition of the gut microbiota in mice. There was a difference in the diversity of gut microbiota between the group of rats exposed to continuous light and the control group. The dominant bacteria in the mouse gut microbiota were Bacteroidetes and Firmicutes. 4 weeks of continuous light exposure in mice resulted in changes in the phenotype of PCOS in mice, including reproductive and metabolic disorders, and there were changes in the composition of the gut microbiota of mice exposed to continuous light [8].

Diazepam

Diazepam (4,0 mg/kg/day) is used as one of the anti-epileptic drugs used to develop the animal model of PCOS. However, the results of the study show that diazepam use does not produce the same PCOS phenotype as the metabolic and reproductive abnormalities seen in other study groups. After administering diazepam to the rats, milder changes were observed in the morphology of the ovary compared to the other groups. The weight of the ovary did not show a significant increase, and there were fewer cysts present [18].

Sodium valproate

Administration of Sodium Valproate for 21 consecutive days caused weight gain, obesity, estrus cycle irregularities, insulin resistance, and hyperandrogenism in female rats. Sodium valproate also affects ovarian function and androgen synthesis, because of its effects on the spinal cord-hypothalamus-ovaries. The results of this study show that sodium valproate can induce PCOS with metabolic and reproductive properties like PCOS in humans [18].

Carbamazepine

Carbamazepine is one of the antiepileptic drugs used in the treatment of bipolar disorder. The use of carbamazepine may have adverse effects on the levels of reproductive

hormones and consequently on the hypothalamus-gonadal pituitary (HPG) and reproductive functions. Studies have shown that carbamazepine can cause menstrual abnormalities, polycystic ovarian syndrome (PCOS), and overall reproductive endocrine dysfunction in women with bipolar disorder [18].

CONCLUSION

Androgen agents such as testosterone propionate, free testosterone, DHEA, and letrozole, as well as sodium valproate, are effective in the induction of PCOS phenotypes based on the Rotterdam criteria (oligo/amenorrhea, hyperandrogenic, and polycystic ovaries). PCOS with Insulin Resistance models are effective inductions using testosterone propionate, DHEA, and letrozole. PCOS models are efficient inductions using testosterone propionate and DHEA. The PCOS with Gut Microbiota models are inductively effective metabolic syndromes using DHEA, letrozole, and HFD combinations. The use of the PCOS model is adapted to the desired etiopathogenesis, given that PCOS has multiple pathophysiological characteristics. The adjustment will determine the accuracy of using the PCOS model induction method.

CONFLICT OF INTEREST

The author wishes to state that there is no conflict of interest that may affect the outcome or interpretation in this article.

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