Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.18.4.242-256
P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 18 Issue 4 October-December 2023

Assessment and Experimental Procedure Polycystic Ovary Syndrome (PCOS) Rat Model: A Review

E. Y. Kurniawati[1], N. Pramono[2], S. T. Hidayat[3], and E. Mahati[4]

[1] Doctoral Program Medical & Health Science, Medical Faculty, Diponegoro University
[2, 3] Departement Fertility & Endocrinology, Medical Faculty, Diponegoro University
[4] Departement Pharmacology & Therapy, Medical Faculty, Diponegoro University
Jl. Prof. Soedarto Street Semarang, Central Java, Indonesia
Corresponding Author: yovi.raharjanto@gmail.com
Revised: 2023-12-25, Accepted: 2023-12-27, Publish: 2023-12-30

ABSTRACT

A complicated endocrine condition that affects many women worldwide is called polycystic ovarian syndrome, or PCOS. The use of rat models has greatly aided research on many facets of PCOS. In addition to discussing the parameters, evaluation techniques, and indicators pertinent to PCOS research, this study evaluates the standard circumstances of PCOS rat models. Our knowledge of PCOS has improved due to investigating endocrine, hormonal, inflammatory, oxidative stress, metabolic, genetic, and microbiota-related factors in these models. The results highlight how important it is to use PCOS rat models to understand the complexities of this illness and offer possible treatment avenues.

Keywords: assessment, experimental procedure, polycystic ovary syndrome, rat model

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a severe and complex endocrine disorder that affects the health and quality of life of a large number of women worldwide. The condition is not only a significant female health problem but also a social and economic burden that demands serious attention(Wekker et al., 2020). PCOS has far-reaching, long-term impacts on physical and psychological health, and greater understanding and research are needed to address this challenge (Chaudhari et al., 2018).

In the quest to better understand PCOS, the role of animal models, particularly the PCOS mouse model, cannot be ignored. The PCOS mouse model provides an essential platform for controlled research and assessment of the pathological mechanisms of PCOS. With these models, researchers can approach fundamental questions such as what causes PCOS, how PCOS affects various organs, and how we can develop better intervention strategies. In-depth knowledge of assessment methods for using PCOS mouse models is also critical. Proper assessment is vital to ensuring that these models properly reflect the PCOS condition and provide valid insights (Koçak, 2021).

This review aims to provide a deeper understanding of the profile of mouse strains as PCOS experimental animals and the role played by PCOS mouse models in research. We will also discuss the parameters, models, or examination techniques and indicators of PCOS in mouse models, as in-depth knowledge of such methods is a crucial foundation for understanding PCOS in mouse models and driving progress in experimental animal research. Thus, this review will provide valuable guidance for researchers and clinicians, enabling improvements in understanding the rat as a PCOS model.

MATERIALS AND METHODS

The research methodology for this study involved an exhaustive search for research articles within a specified time frame. A systematic exploration of three major databases, namely PubMed, Elsevier, and Google Scholar, was conducted to acquire relevant data. The search encompassed a period of five years, from 2018 to 2023. Both Indonesian and English-language articles were considered in the search. To ensure a thorough search, articles were selected based on predefined keywords closely aligned with the study's core focus. These keywords included "Polycystic Ovary Syndrome (PCOS)", "Rat Models", "Experimental Procedures", and "Assessment". The choice of keywords adhered to the PICOT framework (population, intervention, comparators, outcome, time). In alignment with established guidelines, the quality of selected studies was assessed using the Joanna Briggs (JBI) Critical Assessment and PRISMA (Preferred Reporting Item for Systematic Reviews and Meta-Analyses)

guidelines, as outlined in Figure 1. This evaluation aimed to ensure the rigour and validity of the research included in the review. To prevent the inclusion of duplicate content, a meticulous check was conducted on the search results. Any duplicates identified were removed from consideration. Following this initial screening, a two-stage selection process was employed to refine the selection further. The first stage involved matching the titles and abstracts of the articles with the predefined inclusion criteria.

Literature that passed the initial selection stage underwent a second round of evaluation. It entailed a comprehensive analysis of the correspondence between the content of the articles and the established inclusion criteria. Independent reviewers performed both stages of literature selection, a method designed to enhance the accuracy of the results and minimize potential errors in the selection process. By implementing this systematic and robust methodology, this study aimed to ensure the inclusion of high-quality research articles and provide a comprehensive review of the assessment and experimental procedures associated with the PCOS rat model.

Table 1. Inclusion and Exclusion Criteria

Criteria	Inclusion	Exclusion
Population	PCOS rat models	Human subjects, other animal species, in vitro studies
Intervention	Various PCOS induction methods and assessments	PCOS rat models without specified procedures
Comparators	Control groups	PCOS rat models without control groups
Outcomes	Research on PCOS etiology, pathophysiology, and potential therapeutic strategies	Other unrelated research on PCOS rat models
Time	Studies published in the last five years	Studies published more than five years ago
Study design	Experimental research	Analytical observational research
Language	Articles in English and other relevant languages	Articles in unrelated languages or not specified

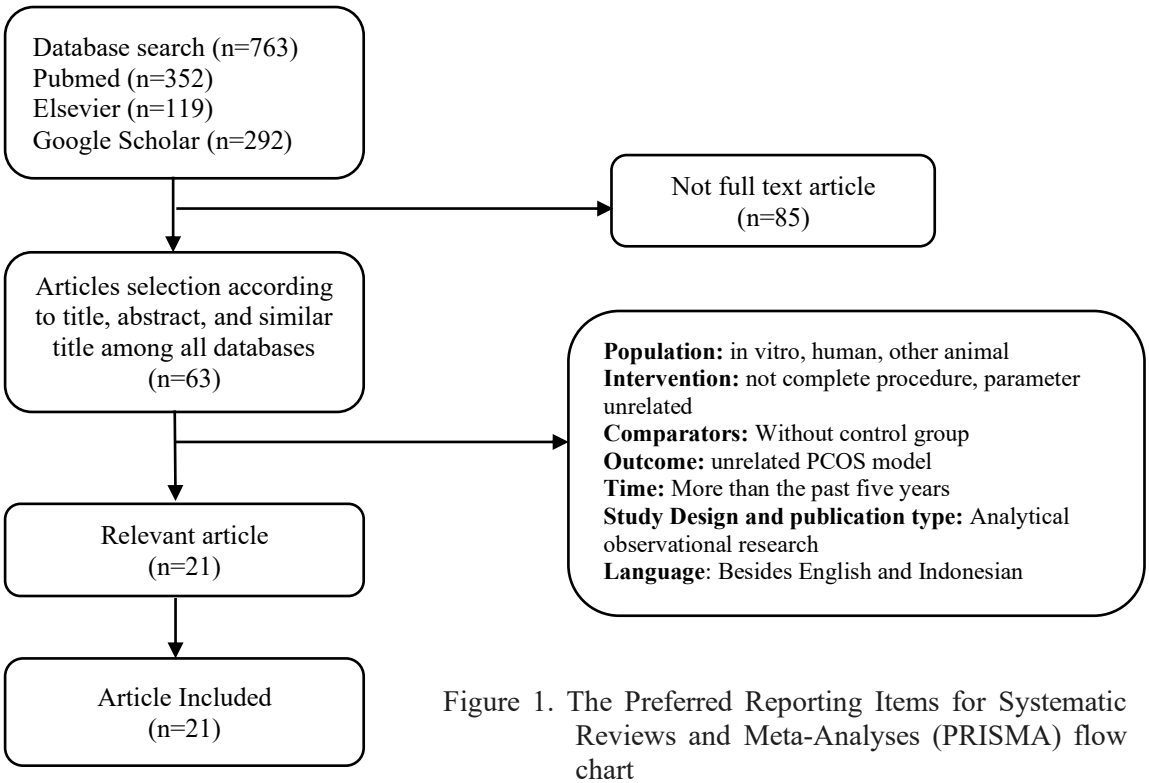

Figure 1. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart

RESULTS AND DISCUSSION

Sprague Dawley rats

The endocrine system of Sprague-Dawley rats is analogous to that of other rats and mammals. The endocrine system is a complex network of glands that cache hormones into the bloodstream, regulating colourful physiological processes and maintaining homoeostasis. The pituitary gland, frequently referred to as the" master gland," produces and releases a variety of hormones that control other endocrine glands and regulate colourful fleshly functions(Adelakun et al., 2022). The adrenal glands cache hormones like cortisol and adrenaline, which are involved in the body's response to stress and play roles in metabolism and vulnerable functions. The thyroid gland produces hormones that control metabolism and influence colourful physiological processes. The pancreas secretes insulin and glucagon, pivotal in regulating blood sugar levels. The reproductive glands (testes in males and ovaries in females) produce coitus hormones (testosterone and oestrogen, independently) that are involved in the development and regulation of the reproductive system (Chu et al., 2020).

Sprague-Dawley rats have a reproductive system similar to that of other mammals. The ovaries are the female reproductive organs that produce eggs and sex hormones such as oestrogen and progesterone. The folliculogenesis process Sprague-Dawley rats Prophase Meiosis I This process begins when the Sprague-Dawley rats are still in the womb. The ovum cells undergo the phase of prophase in meiosis I division, which means that the division of the ovum is delayed until adulthood. Pre-natal oogenesis, during the prenatal period (pre-birth), the ovum in the meiosis I prophase stage stops in the diplotene stage of the meiosis I prophase and is called the ovary. These diplotene eggs are stored in the ovaries of unborn mice. Postnatal oogenesis, after birth, and folliculogenesis continue (Adelakun et al., 2022). A previously stored diplotene ovum will undergo postnatal oogenesis when it reaches puberty. During this stage, several mature follicles will form in the female ovaries. Only a few follicles will grow and develop into a mature Graafian follicle containing the ovum ready to be excreted during ovulation. Once the Graafian follicle is ripe, ovulation occurs, in which the ripe ovum is released from the ovaries and enters the fallopian tube, ready for fertilisation in the event of an encounter with sperm (Modlinska and Pisula, 2020).

Wistar rats

The Wistar rat is one of the most commonly used laboratory rat strains in medical and scientific exploration. Wistar mice were chosen as model creatures due to their stable reproductive, health, and behavioural characteristics and ease of being kept in a laboratory environment. The endocrine system of Wistar rats involves the endocrine glands and hormones that regulate colourful body functions. The hypothalamus is the part of the brain that plays a central role in regulating the endocrine system. The hypothalamus produces a hormone that stimulates or inhibits the release of hormones from the pituitary gland. The pituitary gland is located beneath the brain and consists of two corridors: the anterior (adenohypophysis) and the posterior (neurohypophysis) (Kasyoki et al., 2022).

The pituitary gland produces a variety of hormones that regulate numerous body functions, such as growth, development, reduplication, and metabolism. The hormones of the anterior portion include growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormones (FSH), and prolactin hormone. The hormones of the posterior part include the hormone oxytocin and the antidiuretic hormone (ADH and vasopressin). Thyroid glands in the neck produce thyroid hormones, videlicet thyroxin(T4) and triiodothyronine(T3), which regulate the body's metabolism and growth. The adrenal glands are located above the feathers and produce the hormones cortisol (the stress hormone), adrenaline(adrenalin), and norepinephrine (noradrenalin), which are involved in the body's response to stress and blood pressure regulation. The pancreas produces insulin and glucagon hormones, which regulate blood glucose levels and carbohydrate metabolism(Gede et al., 2022).

In female Wistar rats, the ovaries produce the hormones oestrogen and progesterone, which are involved in the reproductive cycle and prepare the body for pregnancy. In male Wistar rats, the testes produce the hormone testosterone, which regulates the development and functioning of the male reproductive system (Pournaderi et al., 2017). The Wistar rat's endocrine system regulates various bodily functions, including growth,

metabolism, stress response, reproduction, and more. Since laboratory rats are often used as model animals in biomedical research, knowledge of the endocrine system of Wistar rats becomes essential in understanding how hormones and endocrine glands work together to maintain balance and health in the body (Mvondo et al., 2020).

The oestrus cycle in Wistar rats is a normal phase of the female reproductive cycle, during which they undergo hormonal and physical changes to prepare their bodies for reproduction(Pournaderi et al., 2017). The oestrus cycle consists of several periods of periodic repetition, and female rats, under normal conditions, will go through this cycle for most of their lives as long as they have not gone through menopause. Proestrus: this phase is the beginning of the oestrus cycle, during which the level of the hormone oestrogen begins to rise. Women in heat tend to be more active and can attract the attention of men, but they are not ready for marriage. The oestrus phase is when female rats are most energetic and ready to mate. Levels of the hormone oestrogen peak and are followed by the release of luteinizing hormone (LH) that triggers ovulation. The female rat will take over the male mouse and mate during oestrous. Metestrus: after oestrous, female rats will go through oestrous, during which the hormone oestrogen decreases and switches to the hormone progesterone. Without fertilisation, the female rat will enter this phase, and the oestrus cycle will continue(Mvondo et al., 2020; Pournaderi et al., 2017).

Oestrus is the final stage of the oestrus cycle, during which the hormone progesterone remains elevated. Without pregnancy, the female rat will stay in oestrus before returning to oestrus and starting the next oestrus cycle. The duration of the oestrus cycle in female Wistar rats is approximately 4-5 days. Still, it can vary from person to person and is influenced by age, environmental conditions, and temperature (Monima et al., 2019). Knowledge of the oestrus cycle in female Wistar rats is essential in biomedical, reproductive, and developmental studies and in determining the appropriate timing of marriage and animal-specific studies (Monima et al., 2019).

CD-1 rats

One of the laboratory rat strains frequently used in scientific studies is the CD-1 rat (Crl: CD1). The CD-1 rat strain is well-liked because it possesses specific traits, making it an excellent choice for various biological and scientific studies. These mice exhibit steady reproductive characteristics, regular female oestrous cycles, and high fertility. Additionally, these mice are reasonably simple to raise and breed in a scientific setting (Hedrich, 2000). Like all rats, CD-1 rats have an endocrine system that controls several bodily processes, including growth, metabolism, the reproductive system, and stress response. The hormones CD-1 mice's endocrine system produces control several biological procedures crucial to their survival and growth. Researchers frequently choose CD-1 mice because of their consistent biological traits, simplicity of care and reproduction, and availability as a standardised strain. Understanding various facets of biology and health has significantly benefited from the success of utilising CD-1 mice in numerous research (Koçak, 2021).

C57BL/6 rats

C57BL/6J mice and C57BL/6N mice are derived from the C57BL/6 strain, and the C57CL/6J type is the most commonly used in research. These mice were developed with changes in the nicotinamide nucleotide transhydrogenase (Nnt) gene, which can affect how the body reacts to oxidative stress and glucose metabolism. Different genetic changes in C57BL/6J, C57BL/6N mice, and C57B6J mice have mechanisms for achieving the condition of overweight or obesity. C57BL/6N is often used in genetic research because it is more consistent with the results. C57BL/6J mice often show behaviours associated with higher levels of fear. Considering substrains in the three variations of mice will determine the study's results and its compatibility with the research objectives and the desired disease model (Simon et al., 2013).

The endocrine and reproductive systems are the same as in other female rats. C57BL/6 mice are frequently used in studies related to diabetes and metabolic disorders. Researchers investigate these mice's insulin secretion, glucose metabolism, and sensitivity to understand better the mechanisms underlying type 2 diabetes and other metabolic conditions. The endocrine system of C57BL/6 mice is used to investigate reproductive endocrinology. It includes studies on hormones like estrogen, progesterone, and gonadotropins, which play vital roles in regulating the reproductive system. The hypothalamic-pituitary-adrenal (HPA) axis, a

vital part of the endocrine system, is studied in C57BL/6 mice to understand the stress response and its impact on health and behaviour. C57BL/6 mice have a propensity to develop obesity when fed a high-fat diet. Researchers use this strain to investigate the role of hormones like leptin in regulating appetite, body weight, and metabolism(Koçak, 2021). The well-characterized genetics and physiology of C57BL/6 mice make them an invaluable model for investigating various aspects of the endocrine system and its role in health and disease. Researchers can use genetically modified C57BL/6 mice to study specific genes, pathways, and hormones associated with endocrine function and related disorders.

Goto-Kakizaki rats

The Goto-Kakizaki (GK) rat is a specific strain of laboratory rats commonly used in research on type 2 diabetes and metabolic syndrome. These rats were developed through selective breeding and were derived initially from Wistar rats (Osuka et al., 2019). Goto-Kakizaki (GK) rats exhibit impaired glucose tolerance, insulin resistance, and elevated blood glucose levels, hallmarks of type 2 diabetes in humans. Unlike some other rat strains used in diabetes research, Goto-Kakizaki (GK) rats tend to be lean and do not become obese, even when fed a high-calorie diet. It is in contrast to many cases of type 2 diabetes in humans, where obesity is a common risk factor(Kondo et al., 2016). The development of diabetes in Goto-Kakizaki (GK) rats is believed to have a vital genetic component, and researchers have identified several genetic markers associated with diabetes susceptibility in this strain. Goto-Kakizaki (GK) rats are considered a polygenic model of type 2 diabetes, meaning that multiple genes are involved in developing the disease in this strain. It reflects the complex genetic basis of type 2 diabetes in humans. In addition to diabetes-like symptoms, Goto-Kakizaki (GK) rats may also exhibit other metabolic abnormalities, such as dyslipidemia (abnormal lipid profiles) and hypertension (high blood pressure), which are often seen in people with metabolic syndrome (Olson and Graham, 2014; Osuka et al., 2019).

Goto-Kakizaki (GK) rats are primarily used in research related to type 2 diabetes and metabolic syndrome PCOS; they can also be used to study aspects of the estrous cycle and folliculogenesis, especially when investigating reproductive health in the context of metabolic disorders (Osuka et al., 2019). The reproductive phenotyping shows that the Goto-Kakizaki (GK) rats have longer estrus cycles, and 75% are acyclic at six months. Moreover, they have an increased number of small antral follicles, high circulating testosterone, LH, and AMH, and increased ovarian mRNA expression of Amhr2. Thus, the Goto-Kakizaki (GK) rats model displays all three phenotypic features of human PCOS (Bourgneuf et al., 2021).

Rattus Norvegicus rats

Rattus norvegicus, commonly known as the Norway rat or brown rat, is one of the world's most well-known and extensively studied species of rats. These rats are often used in scientific research for various purposes, including studies related to physiology, genetics, behaviour, toxicology, and disease models. These rats have been used as models for various human diseases, including diabetes, hypertension, cardiovascular disease, cancer, and obesity(Hakam et al., 2022).

Reproduction in Rattus norvegicus rats is a well-documented process, and these rats are commonly used as model organisms in reproductive research due to their relatively large litter sizes, short reproductive cycles, and ease of maintenance in laboratory settings. Female rats have a bicornuate uterus (two uterine horns), and the ovaries are located near the kidneys. Female reproductive organs include the ovaries, oviducts (fallopian tubes), uterus, cervix, and vagina. Female rats undergo an estrous cycle of proestrus, estrus, metestrus, and diestrus (Talakua and Unitly, 2020). The estrous cycle typically lasts about 4-5 days, and female rats are only receptive to mating during estrus (referred to as being "in heat"). Mating behaviour in Rattus norvegicus involves a series of courtship behaviours, with males pursuing and courting females—successful copulation results in sperm transfer into the female's reproductive tract. Gestation in rats lasts approximately 21-23 days. After fertilization, the developing embryos move from the oviducts to the uteri, where they implant and develop. Rattus norvegicus rats are widely used in reproductive physiology, fertility, contraception, embryonic development, and reproductive toxicology studies. Researchers manipulate the reproductive system in rats to investigate specific questions and develop insights into human reproductive processes (Siahaan et al., 2022).

BALB/c Mice

Mus musculus (strain BALB/c) is a laboratory rat strain developed through selective laboratory breeding. It was first developed at the Jackson Institute for Genetic Research in 1913. Balb/c mice have a body size of about 15-20 cm (5,9–7,9 inci). BALB/c mice have a stable and consistent genetic background; some genetic characteristics are explicitly identified in this strain. Because of their standardised genetic properties, these mice are often used as models for genetic, immunological, and cancer research studies. BALB/c mice are widely used as laboratory animal models in a variety of biomedical and biological research, including research related to cancer, immunology, neurology, toxicology, and many other fields of research. Because of their genetic consistency, Balb/c rats are often used to isolate the effects of certain factors in research. Although Balb/c rats have many advantages as laboratory animal models, they also have some limitations, such as susceptibility to certain diseases and behavioural properties that may not be suitable for all types of research (Koçak, 2021).

Balb/c mice are used in reproductive research because of their regular estrus cycles. Research on the Balb/c rat estrus cycle can help understand the regulation of sex hormones such as oestrogen and progesterone. Balb/c mice are also used in fertility research to understand factors that affect reproductive ability in rats and models to understand fertility problems in humans. This research may involve observing the development of the ovum, reproductive cycles, and responses to the hormones involved in reproduction. The endocrine system in Balb/c rats will regulate the estrus cycle, sexual development, and overall reproductive function. Research on the endocrine system of Balb/c rats can help understand the hormonal regulation involved in the estrus cycle and reproductive problems in these animals and have relevance in broader biomedical research (Kim et al., 2018).

Table 2 provides a comprehensive overview of the various standardised conditions used in PCOS research using mouse models. These standardised conditions include mouse body weight, age, food type, water access, ambient temperature, lighting cycle, and reference to related studies. PCOS research in mouse models involves different types of mice that vary in body weight and age. It reflects the diversity of the mouse populations used in the studies. For example, the mice's body weight ranged from about 20g to 220g. The age of the mice also varied, ranging from three weeks to ten weeks. The relevance of this variation is that PCOS research should consider the role of mouse body weight and age in the study results. For example, PCOS in younger mice may have different characteristics than older mice. Variations in diet type and food access are essential factors in the development of PCOS in model mice. Different diets, including different compositions of protein, fat, carbohydrate, and fibre, may influence the development of PCOS. Therefore, diet selection is an essential factor in designing accurate PCOS studies.

For example, CD1 model mice are fed a diet of 16% protein and 4.0% fat, while Sprague-Dawley rats are fed an approximately 25% protein diet. Changes in diet might result in different PCOS characteristics. Maintaining controlled environmental conditions in PCOS research with mouse models is essential. Stable environmental temperatures and humidity directly influence the development of PCOS. For example, the Sprague-Dawley rat model requires an ambient temperature of 20±2°C and humidity of 60%±5%. Significant fluctuations in temperature or humidity may result in inconsistent research results. Therefore, environmental control is highly relevant in PCOS research (Walters et al., 2018).

Table 2. Standard Condition PCOS rat model

Type rat model	Body weight	Age	Food & Water	Temperature	Photocycle	References
BALB/c	20.0 ± 1.5 g	Eight weeks old	Food pellets (Livestock and Poultry Feed Company, Tehran, Iran) Free water access	(22°C), humidity of 55% ± 3%	12-hour light/12-hour dark	(Asghari et al., 2021)
CD1	-	Gestational mice	Water ad libitum Diet (16% protein, 4.0% fat, 48.5% carbohydrate, Teklad 2916 irradiated global rodent diet, Envigo)	(21-23 °C) and humidity (30%-70%)	12-hour light/12-hour dark	(Cara et al., 2023)
	20–21 g	Four-week-old	Free access to feed and water ad libitum	-	12 h light/dark cycle (7.00–19.00)	(Palmerini et al., 2023)
Sprague-Dawley	-	21 day old	Diet (protein 25% and lipids 5%) and drink *ad libitum*	20±2°C and a humidity of 60%±5%	12-h artificial light period	(Zhou et al., 2018)
		Pre-pubertal: 21 days old Post pubertal: 42 days old	Chow (21.68% protein, 4.6% fat, 2.45% fibres, 5.97 ash, and 2.5% minerals; water *ad libitum*	23 ± 2˚C and humidity of 55–65%	12 h light-dark cycle (lights on from 08:00 to 20:00)	(Kim et al., 2018)
	200–220 g	Six weeks old	Standard chow and water *ad libitum*	22–24˚C	12-h/12-h light/dark cycle	(Chu et al., 2020)
		Three weeks old,	free access to regular food and tap water.	25℃ clean environment with 50% humidity	12-h light/dark cycle	(Ding et al., 2019)
Wistar	200 ± 20 g	ten-week old	Standard pellets and drinking water ad libitum	22 ± 2 ˚C, 55 ± 5% humidity	12 hr light and dark cycle	(Ibrahim et al., 2022)
	-	-	*ad libitum* access to food and water	20 °C, 40% humidity	12:12 hour light: dark cycle	(Marshall et al., 2020)
	170-190 gr	75-85 days	Free access to food and water ad libitum	22 ± 3°C, relative humidity of 45-55%	12 hr light/dark cycle	(Sadeghian Bakhi et al., 2023)
	180-230 gr	12-18 weeks	standard pellet diet and water *ad libitum*	(23 ± 2ºC) and a relative humanity of 60% ± 10%	12/12 light/dark rhythm	(Sudhakar et al., 2019)
C57BL/6J	-	3 weeks old	Free access to rodent food and water	(22°C ± 3°C), stable humidity	12-h light/dark cycle	(Ullah et al., 2022; Wang et al., 2022)
		4 weeks old	Food and water available *ad libitum*	-	12:12 light-dark cycle	(Esparza et al., 2020)
C57BL/6 N	-	3 weeks of age	Food and water ad libitum	-	12:12 light-dark cycle	(Ryu et al., 2023)

Table 3. PCOS Rat Parameter and Measurement

Parameter	Indicator	Sample-Method	Measurement Tools	References
Endocrine	Testosterone	Serum-subxiphoid blood sample Serum- trunk blood sample Plasma-Abdominal aorta Serum- cardiac puncture Serum- rats' livers Serum-Tail-tip blood	Enzyme-linked immunosorbent assays (ELISA) Liquid chromatography-tandem mass spectrometry Radioimmunoassay Electrochemiluminescence immunoassay kit (ECLIA)	(Asghari et al., 2021; Zhou et al., 2018; Cara et al., 2023; Ullah et al., 2022; Esparza et al., 2020; Chu et al., 2020; Linares et al., 2019; Ryu et al., 2023; Wang et al., 2020, 2022; Ding et al., 2019)
	Luteinizing Hormone	Serum-retroorbital blood Serum-orbital venous blood	Enzyme-linked immunosorbent assays (ELISA) Liquid chromatography-tandem mass spectrometry Radioimmunoassay	(Asghari et al., 2021; Zhou et al., 2018; Cara et al., 2023; Ullah et al., 2022; Esparza et al., 2020; Chu et al., 2020; Ryu et al., 2023; Wang et al., 2020)
	Follicle Stimulating Hormone		Enzyme-linked immunosorbent assays (ELISA) Radioimmunoassay	(Asghari et al., 2021; Zhou et al., 2018; Ullah et al., 2022; Chu et al., 2020; Wang et al., 2020; Ding et al., 2019)
	Progesterone		Enzyme-linked immunosorbent assays (ELISA) Liquid chromatography-tandem mass spectrometry Radioimmunoassay	(Asghari et al., 2021; Cara et al., 2023; Ullah et al., 2022; Chu et al., 2020; Linares et al., 2019)
	Estradiol		Enzyme-linked immunosorbent assays (ELISA) Liquid chromatography-mass spectrometry (LCMS) assay Radioimmunoassay	(Asghari et al., 2021; Zhou et al., 2018; Chu et al., 2020; Esparza et al., 2020; Linares et al., 2019; Ryu et al., 2023)
	AMH		Enzyme-linked immunosorbent assays (ELISA)	(Cara et al., 2023; Chu et al., 2020; Wang et al., 2022)
	Thyroid-stimulating hormone (TSH)		Enzyme-linked immunosorbent assays (ELISA)	(Chu et al., 2020)
	Corticosterone		Enzyme-linked immunosorbent assays (ELISA)	(Esparza et al., 2020)
	Leptin		Enzyme-linked immunosorbent assays (ELISA)	(Chu et al., 2020)
	Insulin		Radioimmunoassay ELISA-micro plate reader at 450 nm ± 2 nm. (AUC- Insulin) using GraphPad Prism 6.0 software.	(Zhou et al., 2018; Ibrahim et al., 2022; Chu et al., 2020; Wang et al., 2020, 2022; Ding et al., 2019)
	Lactate dehydrogenase (LDH)		Enzyme-linked immunosorbent assays (ELISA)	(Ding et al., 2019)
Inflammatory cytokines	IL-1β, IL-6, and TNF-α	Serum-orbital venous blood	Enzyme-linked immunosorbent assays (ELISA) Spectrophotometer at 450 nm ± 2 nm	(Ibrahim et al., 2022)
	Ovarian level of IL-1β	Ovarian tissue		

Parameter	Indicator	Sample-Method	Measurement Tools	References
Oxidative stress markers	Malondialdehyde (MDA)	lipid peroxidation ovarian tissues	MDA kit Spectrophotometry 535 nm.	(Ibrahim et al., 2022; Ding et al., 2019)
	Nitric oxide (NO)	Ovarian tissues	Griess reagent- Spectrophotometry 540 nm	(Ibrahim et al., 2022)
	Superoxide dismutase (SOD)		SOD kit Spectrophotometry at 420 nm	(Ibrahim et al., 2022; Ding et al., 2019)
	Catalase		Spectrophotometry at 510 nm	(Ibrahim et al., 2022)
	Glutathione (GSH)		GSH kit	(Ding et al., 2019)
	ATP analysis		ATP assay kit	(Ding et al., 2019)
	Mitochondrial membrane potential (MMP)	Mitochondria protein	Jc-1 kit (cat. no. T4069, Sigma-Aldrich; Merck KGaA)	(Ding et al., 2019)
	(Reactive Oxygen Species) ROS levels		2',7'-dichloro-rodihydrofluorescein diacetate (H2dcFdA) DCF (cat. no. d6883; Sigma-Aldrich; Merck KGaA)	(Ding et al., 2019)
Antioxidant	Muscle and liver tissue		Hematoxylin and eosin (H&E)	(Ibrahim et al., 2022)
	Keap-1, Nrf2, and OH-1		Enzyme-linked immunosorbent assays (ELISA) Spectrophotometer at 450 nm	(Ibrahim et al., 2022)
	Antioxidant capacity (T-AOc)		Enzyme-linked immunosorbent assays (ELISA)	(Ding et al., 2019)
Metabolic Phenotype	Body weight	(straight line from nose to anus-	Digital scale (Japan 2J-V1000AMax1200 gr,	(Asghari et al., 2021; Kim et al., 2018;
	Body weight and length	Lee's index [Lee's=(weight×1000)^(1/3)/length]	accuracy 0.01 gr)	Ullah et al., 2022; Ibrahim et al., 2022; Ryu et al., 2023)
	Body composition	Fat mass and Lean mass	Nuclear magnetic resonance-based device	(Cara et al., 2023)
	Fasting glucose levels	Tail-tip blood	Accu-Chek glucometer	(Zhou et al., 2018; Cara et al., 2023;
	Fasting Plasma Glucose (FPG)	Tail vein blood	Portable Glucometer	Ibrahim et al., 2022; Chu et al., 2020;
	Intraperitoneal glucose	Abdominal aortic blood	Accu Chek Active glucometer	Sudhakar et al., 2019; Wang et al., 2020,
	tolerance test (IPGTT)	Orbital venous blood	Roche active blood glucose meter	2022; Ding et al., 2019)
	Oral glucose tolerance test OGTT		(AUC-Glucose)- GraphPad Prism 6.0 software	
	Lipid profile		Colourimetric	(Ibrahim et al., 2022; Sudhakar et al.,
	Total lipid, total cholesterol (TC), HDL (high-density lipoprotein), triglycerides (TGs), LDL and VLDL		Standard laboratory technique Enzyme-linked immunosorbent assays (ELISA)	2019; Wang et al., 2020, 2022)
	Insulin resistance	Serum Plasma	Homeostasis model assessment of insulin resistance (HOMA-IR) (mg/dl) × serum insulin (mIU/ml)/405 [fastingbloodglucose(mmol/L) × fasting insulin (mIU/L)]/22.5 HOMA-IR was >2.8	(Ibrahim et al., 2022; Chu et al., 2020; Wang et al., 2021; Ding et al., 2019)

Parameter	Indicator	Sample-Method	Measurement Tools	References
	β-cell function		Homeostasis assessment of β-cell function (HOMA-β) 20 × fasting insulin (FINS, mIU/L)/ (fasting plasma glucose (mmol/L) − 3.5) (%)	(Chu et al., 2020)
Reproductive Phenotype	Estrous cycles/ stage	Vaginal cytology	Wet smear method Exfoliative cystoscopy	(Cara et al., 2023; Palmerini et al., 2023; Kim et al., 2018; Ibrahim et al., 2022; Marshall et al., 2020; Ryu et al., 2023; Ding et al., 2019; Chu et al., 2020)
		Vaginal smear-epithelial, leukocytes, and cornified cells, keratinocytes	Giemsa Methylene blue	
	Sexual maturation	Anogenital distance (AGD) and anovaginal distance (AVD) Vaginal opening (VO)	Digital calipers Vernier caliper	(Cara et al., 2023; Sadeghian Bakhi et al., 2023)
Histology	Follicle morphology and morphometric	Adipose tissue-laparotomy (multilayered preantral 1–8 μm in diameter and antral follicles), corpus luteum Weight for ovaries and uteri; length for uteri), ovarian cyst-laparotomy Ovary tissue-ovarian cyst, atretic follicle, quantity follicular-ovariectomy Corpora lutea, healthy antral follicles and follicular cysts- ovariectomy Uterine horn, uterine tissue-microtome	Hematoxylin and eosin (H&E) Masson's trichrome (M.T.)	(Asghari et al., 2021; Zhou et al., 2018; Cara et al., 2023; Kim et al., 2018; Ullah et al., 2022; Palmerini et al., 2023; Ibrahim et al., 2022; Chu et al., 2020; Linares et al., 2019; Sadeghian Bakhi et al., 2023; Sudhakar et al., 2019; Wang et al., 2020; Ding et al., 2019)
Molecular	Gene expression	RT-qPCR (Reverse Transcription- Quantitative real-time polymerase chain reaction): Gene expression level (GDF9, BMP15, TGFB1, BMPR2, dan BMP6)- ovarian tissue	Tyramide Signal Amplification TSA	(Asghari et al., 2021)
		qPCR: mRNA hypothalamus and pituitary glands	CFX-384 Bio-Rad Real-Time PCR TaqMan Gene Expression MasterMix (Applied Biosystems)	(Cara et al., 2023)
		qRT-PCR: Liver, parametrial adipose tissue, and muscle	-	(Chu et al., 2020)
		qRT-PCR ovarian tissue DESeq	BIO-RAD, CFX Connect^{TM} Real-Time PCR system DESeq R package (1.10.1)	(Ullah et al., 2022)
		qRT-PCR: ovary, parametrial fat, and hypothalamus tissues	SYBR Green Supermix dari Bio-Rad Laboratories 2−△△Ct method	(Ryu et al., 2023)
		NOD-like receptor family protein 3 (NLRP3) and caspase-1 in ovarian tissue	Delta-delta threshold (ΔCt)	(Ibrahim et al., 2022)
		In situ hybridization (ISH)- ARC Kiss1, Pdyn, or Tac2 (NKB)	Software (GRAINS; Dr. Don Clifton, University of Washington)	(Chu et al., 2020)

Parameter	Indicator	Sample-Method	Measurement Tools	References
	Subcellular localization analysis	Immunofluorescence: Androgen Receptor Immunoreactivity (AR-ir) neuron Kiss1	TSA-biotinylated tyramide ProLong Gold Antifade mounting medium	(Cara et al., 2023)
		Fluorescent In Situ Hybridization (FISH): RNA gen Lepr, Ar, dan Kiss1 visualisation	Tyramide Signal Amplification TSA	(Cara et al., 2023)
	Immunohistochemical	MG-AGE and 4-HNE Western Blot in ovarian tissue	Image J 1.44p software (IHC profiler plugin)	(Palmerini et al., 2023; Ding et al., 2019)
		NFκB in the ovary	NFκB primary antibody (Invitrogen-Thermo Fisher Scientific) Mayer's hematoxylin	(Ibrahim et al., 2022)
		NPYARN (Neuropeptide Y/Agouti-Related Peptide Neurons): medial septum (MS), rostral preoptic area (rPOA), and anterior hypothalamic area (AHA)	Double-label fluorescent immunohistochemistry.	(Marshall et al., 2020)
	Microbial diversity	Microbial DNA Components analysis (PCoA) of the OTUs (Gut microbiota) Microbial differences Microbial composition Function analysis	QIAamp Fast DNA Stool Mini Kit version 3.1.0; R Foundation for Statistical Computing, Vienna, Austria LEfSe analysis coupled with the Kruskal-Wallis rank sum test Linear discriminant analysis (LDA) score PICRUSt algorithm based on the Kyoto Encyclopedia of Genes and Genomes Orthology (KO) classification	(Chu et al., 2020)
		Bacterial composition-fecal	16S rRNA gene sequencing	(Wang et al., 2022)

Various types of mice are used in Polycystic ovary syndrome (PCOS) research, and each type has different genetic and physiological characteristics. Selecting an appropriate mouse breed is an important step in designing a study. For example, PCOS research often uses C57BL/6J and C57BL/6N model mice. The selection of the appropriate mouse breed should consider the genetic and physiological characteristics of the mice. Polycystic ovary syndrome (PCOS) research with mouse models often involves mice in different age stages, such as pre-pubertal and post-pubertal. The hormonal and developmental influences that change during the life cycle of mice play a role in the pathogenesis of PCOS. Therefore, the relevance of age-based changes should be considered in PCOS research to understand the different effects on PCOS development. The standardised conditions listed in Table 2 significantly impact the results of PCOS research with mouse models. An in-depth understanding of the role of body weight, age, diet, environment, mouse breed, and changes based on age is crucial in designing accurate and meaningful studies. Researchers should carefully consider these factors to understand and address PCOS using mouse models. Continuity of research and consistency in standardised conditions are critical to better understanding PCOS (Leonie et al., 2012; Binder et al., 2023).

Polycystic ovary syndrome (PCOS) Rat Parameter and Measurement

In studies using Polycystic ovary syndrome (PCOS) mouse models, an in-depth understanding of the examination parameters and methods is essential to explain the development of PCOS and its impact on various aspects of health. Endocrine hormones, such as testosterone, LH, and FSH, play an essential role in the development of PCOS. Using PCOS mouse models allows for a more controlled study of these hormonal changes. In mouse model studies, the measurement of endocrine hormones can provide a more detailed picture of how hormonal changes are related to the development of PCOS. Methods such as ELISA, liquid chromatography-tandem mass spectrometry, and radioimmunoassay are used to measure these hormones. Besides sex hormones, other hormones such as progesterone, estradiol, and AMH are also the focus of research. Measuring these hormones helps understand the hormonal changes associated with PCOS in the mouse model (Walters et al., 2018).

Inflammation and oxidative stress are essential elements in the pathogenesis of PCOS. Research with PCOS model mice may allow a better understanding of how inflammation and oxidative stress contribute to developing PCOS. Levels of inflammatory cytokines such as IL-1β, IL-6, and TNF-α were evaluated using ELISA and spectrophotometric methods. These results may provide insight into the inflammation level associated with PCOS in model mice. Oxidative stress parameters, such as MDA, NO, SOD, and catalase, are also essential to study. These measurements may provide an understanding of the role of oxidative stress in PCOS at the cellular level. The results of these measurements may help design appropriate interventions to address these changes in PCOS model mice.

Metabolic changes and their impact on body weight and composition are relevant in studying PCOS. PCOS model mice are often used to understand how PCOS affects these parameters. These studies ' measurements of body weight, body composition, and blood glucose levels are essential components. Model mice undergoing these changes can provide a better understanding of the progression of PCOS and its impact on the body's health. Other measurements, such as lipid profile, insulin resistance, and beta cell function, are also relevant in PCOS model mice. The results of these measurements may help understand how PCOS affects the metabolic and reproductive systems of the model mice (Rakic et al., 2023).

Genetic studies and gut microbiota analysis are essential in understanding PCOS at the molecular and microbiological levels. PCOS model mice are used to evaluate genetic changes associated with PCOS. Methods such as RT-qPCR measure gene expression in model mouse tissues. Gut microbiota analysis is also of interest, as the role of gut microbiota in developing PCOS is not fully understood. Model mice are used to understand the changes in gut microbiota composition associated with PCOS. The 16S rRNA sequencing helped identify these changes (Zhang et al., 2022). An in-depth understanding of PCOS parameters and methods of examining them in the context of PCOS model mice is essential to detailing the progression of this disease and identifying potential relevant therapeutic targets. Continued research in this domain may provide better insights into the

mechanisms of PCOS and ways to address its impact on health.

CONCLUSION

PCOS mouse model research is essential in understanding Polycystic Ovary Syndrome (PCOS) disease. Using a wide array of endocrine, hormonal, inflammatory, oxidative stress, metabolic parameters, and genetic and microbiota analyses, these studies have provided deep insight into the pathophysiology of PCOS. The results of studies in PCOS mouse models have revealed the central role of hormonal changes and inflammation in developing this disease. In addition, the model mice also allow a better understanding of the impact on metabolism, reproduction, and associated genetic and microbiota factors. It may provide a solid basis for the development of more targeted therapies and further understanding of PCOS in humans.

REFERENCES

Adelakun, Sunday Aderemi, Olalekan Wasiu Akintunde, Grace Temitope Akingbade, and Oluwafemi Abidemi Adedotun. 2022. Bioactive component of aqueous extract of Solanum melongena ameliorate estradiol valerate induced ovarian-pituitary dysfunctions in female Sprague–Dawley rats: Histomorphological and biochemical evidence. Phytomedicine Plus 2(1).

Asghari, Reza, Vahid Shokri-Asl, Hanieh Rezaei, Mahmood Tavallaie, Mostafa Khafaei, Amir Abdolmaleki, and Abbas Majdi Seghinsara. 2021. Alteration of TGFB1, GDF9, and BMPR2 gene expression in preantral follicles of an estradiol valerate-induced polycystic ovary mouse model can lead to anovulation, polycystic morphology, obesity, and absence of hyperandrogenism. Clinical and experimental reproductive medicine 48(3), 245–54.

Binder, April K, Danielle L Peecher, Amanda J Qvigstad, Silvia D Gutierrez, Jennifer Magaña, David B Banks, and Kenneth S Korach. 2023. Differential Strain-dependent Ovarian and Metabolic Responses in a Mouse Model of PCOS. Endocrinology 164(4).

Bourgneuf, Camille, Danielle Bailbé, Antonin Lamazière, Charlotte Dupont, Marthe Moldes, Dominique Farabos, Natacha Roblot, Camille Gauthier, Emmanuelle Mathieu d'Argent, Joelle Cohen-Tannoudji, Danielle Monniaux, Bruno Fève, Jamileh Movassat, Nathalie di Clemente, and Chrystèle Racine. 2021. The Goto-Kakizaki rat is a spontaneous prototypical rodent model of polycystic ovary syndrome. Nature Communications 2021 12:1 12(1), 1–17.

Cara, Alexandra L, Laura L Burger, Bethany G Beekly, Susan J Allen, Emily L Henson, Richard J Auchus, Martin G Myers, Suzanne M Moenter, and Carol F Elias. 2023. Deletion of Androgen Receptor in LepRb Cells Improves Estrous Cycles in Prenatally Androgenized Mice. Endocrinology 164(3).

Chaudhari, Aditi P, Kaustubh Mazumdar, and Pooja Deepak Mehta. 2018. Anxiety, Depression, and Quality of Life in Women with Polycystic Ovarian Syndrome. . Indian journal of psychological medicine 40(3), 239–46.

Chu, Weiwei, Junyu Zhai, Jieying Xu, Shang Li, Weiping Li, Zi Jiang Chen, and Yanzhi Du. 2020. Continuous Light-Induced PCOS-Like Changes in Reproduction, Metabolism, and Gut Microbiota in Sprague-Dawley Rats. Frontiers in Microbiology 10(3145), 1–13.

Ding, Yu, Zhaochang Jiang, Bohou Xia, Lizong Zhang, Caijuan Zhang, and Jianhang Leng. 2019. Mitochondria-targeted antioxidant therapy for an animal model of PCOS-IR. International Journal of Molecular Medicine 43(1), 316–24.

Esparza, Lourdes A, Danielle Schafer, Brian S Ho, Varykina G Thackray, and Alexander S Kauffman. 2020. Hyperactive LH pulses and elevated kisspeptin and NKB gene expression in the arcuate nucleus of a PCOS mouse model. Endocrinology 161(4), bqaa018.

Gede Dian S, Ni Luh, Eviana Norahmawati, and Miftahul Jannah. 2022. Pengaruh Pemberian Ekstrak Etanol Biji Pepaya (Carica papaya L.) terhadap Jumlah Folikel Preantral, Antral dan Berat Ovarium Tikus Putih Betina (Rattus norvegicus) Galur Wistar . Journal of Issues in Midwifery 6(2), 63–75.

Hakam Maulidy, Wildan, Arifa Mustika, and Indri Safitri Mukono. 2022. The Effect of Butterfly Pea (Clitoria Ternatea) Extract on Reducing Total Cholesterol Levels in Rattus Norvegicus with the Hypercholesterolemia Model. International Journal of Research Publications 115(1).

Hedrich, Hans J. 2000. History, Strains and Models. The Laboratory Rat, 3–16.

Ibrahim, Yasmine F, Mohammed Alorabi, Walaa Yehia Abdelzaher, Nisreen DM Toni, Khaled Thabet, Abdel Rahman Hegazy, Haitham Ahmed Bahaa, Gaber El Saber Batiha, Nermeen N Welson, Mohamed A Morsy, Katharigatta N Venugopala, and Asmaa Mohamed Abdel-Aziz. 2022. Diacerein ameliorates letrozole-induced polycystic ovarian syndrome in rats. Biomedicine and Pharmacotherapy 149.

Kasyoki, Joseph, Catherine Kaingu, Christine Wangia, Peter J Kasyoki, Catherine K Kaingu, Christine O Wangia, and Faraj O Alkizim. 2022. Fertility Effects Of Grewia tenax Methanolic Root Extract On Female Wistar Rats. International Journal of Medicinal Plants. Photon 116, 992–1001.

Kim, Eun-Jeong, Minhee Jang, Jong Hee Choi, and Kyoung Sun Park. 2018. An Improved Dehydroepiandrosterone-Induced Rat Model of Polycystic Ovary Syndrome (PCOS): Post-pubertal Improve PCOS's Features. Frontiers in Endocrinology | www.frontiersin.org 9, 735.

Koçak, Seda. 2021. PCOS Animal Models: An Approach Induced By Dehydroepiandrosterone. Experimental and Applied Medical Science 2, 136–45.

Kondo, Mika, Satoko Osuka, Akira Iwase, Tatsuo Nakahara, Ai Saito, Bayasula, Tomoko Nakamura, Maki Goto, Tomomi Kotani, and Fumitaka Kikkawa. 2016. Increase of kisspeptin-positive cells in the hypothalamus of a rat model of polycystic ovary syndrome. Metabolic Brain Disease 31(3), 673–81.

Leonie, E, AF Van Houten, Piet Kramer, Anke McLuskey, Bas Karels, Axel PN Themmen, and Jenny A Visser. 2012. Reproductive and metabolic phenotype of a mouse model of PCOS. Endocrinology 153(6), 2861–69.

Linares, Rosa, Gabriela Rosas, Elizabeth Vieyra, Deyra A Ramírez, Daniel R Velázquez, Julieta A Espinoza, Carolina Morán, Roberto Domínguez, and Leticia Morales-Ledesma. 2019. In Adult Rats With Polycystic Ovarian Syndrome, Unilateral or Bilateral Vagotomy Modifies the Noradrenergic Concentration in the Ovaries and the Celiac Superior Mesenteric Ganglia in Different Ways—frontiers in Physiology 10.

Marshall, Christopher J, Melanie Prescott, and Rebecca E Campbell. 2020. Investigating the NPY/AgRP/GABA to GnRH neuron circuit in prenatally androgenized PCOS-like mice. Journal of the Endocrine Society 4(11), 1–12.

Modlinska, Klaudia, and Wojciech Pisula. 2020. The natural history of model organisms, the norway rat, from an obnoxious pest to a laboratory pet . eLife 9.

Monima, Lemuel Ann, Muhammad Buhari, Sodiq Lawal, Echoru Isaac, Ssempijja Fred, Owembabazi Elna, Bukenya Edmund, Mario Edgar Fernandez Diaz, Archibong Victor Bassey, and Kokas Ikwap. 2019. Effect of Cleome gynandra leaf extract on the estrous cycle and histology of the ovary and uterus of wistar albino rats . Anatomy Journal of Africa 8(1), 1385–94.

Mvondo, Marie Alfrede, Flavie Ingrid Mzemdem Tsoplfack, Charline Florence Awounfack, and Dieudonné Njamen. 2020. The leaf aqueous extract of Myrianthus arboreus P. Beauv. (Cecropiaceae) improved letrozole-induced polycystic ovarian syndrome associated conditions and infertility in female Wistar rats . BMC Complementary Medicine and Therapies 20(1), 1–13.

Olson, Erin, and Delyth Graham. 2014. Animal Models in Pharmacogenomics . Handbook of Pharmacogenomics and Stratified Medicine, 73–87.

Osuka, Satoko, Natsuki Nakanishi, Tomohiko Murase, Tomoko Nakamura, Maki Goto, Akira Iwase, and Fumitaka Kikkawa. 2019. Animal models of polycystic ovary syndrome: A review of hormone-induced rodent models focused on hypothalamus-pituitary-ovary axis and neuropeptides . Reproductive Medicine and Biology 18(2), 151–60.

Palmerini, Maria Grazia, Guido Macchiarelli, Domenica Cocciolone, Ilaria Antenisca Mascitti, Martina Placidi, Teresa Vergara, Giovanna Di Emidio, and Carla Tatone. 2023. Modulating Morphological and Redox/Glycative Alterations in the PCOS Uterus: Effects of Carnitines in PCOS Mice . Biomedicines 11(2).

Pournaderi, Parisa Sadat, Parichehreh Yaghmaei, Hamidreza Khodaei, Zahra Noormohammadi, and Seyed Hossein Hejazi. 2017. The effects of 6-Gingerol on reproductive improvement, liver functioning and Cyclooxygenase-2 gene expression in estradiol valerate – Induced polycystic ovary syndrome in Wistar rats . Biochemical and Biophysical Research Communications 484(2), 461–66.

Rakic, Dejana, Jovana Joksimovic Jovic, Vladimir Jakovljevic, Vladimir Zivkovic, Maja Nikolic, Jasmina Sretenovic, Marina Nikolic, Nikola Jovic, Marija Bicanin Ilic, Petar Arsenijevic, Aleksandra Dimitrijevic, Tatjana Vulovic, Natasa Ristic, Kristina Bulatovic, Sergej Bolevich, Lazar Stijak, and Suzana Pantovic. 2023. High Fat Diet Exaggerate Metabolic and Reproductive PCOS Features by Promoting Oxidative Stress: An Improved EV Model in Rats . Medicina 59, 1104.

Ryu, Ki Jin, Hyuntae Park, Young In Han, Hee Jung Lee, Seunghyun Nam, Hye Gyeong Jeong, and Tak Kim. 2023. Effects of time-restricted feeding on letrozole-induced mouse model of polycystic ovary syndrome . Scientific Reports 2023 13:1 13(1), 1–9.

Sadeghian Bakhi, Elahe, Nasim Hayati Roodbari, Morteza Anvari, and Fahimeh Ramezani Tehrani. 2023. Prenatal kisspeptin antagonist exposure prevents polycystic ovary syndrome development in prenatally-androgenized rats in adulthood: An experimental study. International journal of reproductive biomedicine 21(2), 99–110.

Siahaan, Salmon Charles PT, Budi Santoso, and Widjiati. 2022. Effectiveness of Moringa oleifera Leaves on TNF-α Expression, Insulin Levels, Glucose Levels and Follicle Count in Rattus norvegicus PCOS Model. Diabetes, Metabolic Syndrome and Obesity 15, 3255–70.

Simon, Michelle M, Simon Greenaway, Jacqueline K White, Helmut Fuchs, and Valérie Gailus-Durner. 2013. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. Genome Biology 14(7).

Sudhakar, P, R Kothai, and V Thenmozhi. 2019. Development of an animal model for Polycystic Ovarian Syndrome in relation to reproductive and metabolic phenotype. Journal of Pharmaceutical Sciences and Research 11(8), 2827–32.

Talakua, Fernanda Clara, and Adrien Jems Akiles Unitly. 2020. EFEK PEMBERIAN EKSTRAK ETANOL RUMPUT KEBAR (Bhiophytum petersianum Klotzsch) TERHADAP PENINGKATAN JUMLAH FOLIKEL PADA OVARIUM TIKUS Rattus norvegicus TERPAPAR ASAP ROKOK . Biofaal Journal 1(2), 74–84.

Ullah, Amin, Mei Jiao Wang, Jun Pu Yang, Enoch Appiah Adu-Gyamfi, Armin Czika, Sanjay Kumar Sah, Qian Feng, and Ying Xiong Wang. 2022. Ovarian inflammatory mRNA profiles of dehydroepiandrosterone plus high-fat diet-induced polycystic ovary syndrome mouse model. Reproductive BioMedicine Online 44(5), 791–802.

Walters, KA, MJ Bertoldo, and DJ Handelsman. 2018. Evidence from animal models on the pathogenesis of PCOS. Best Practice and Research: Clinical Endocrinology and Metabolism 32(3), 271–81.

Wang, Lan, Jing Zhou, Hans Jürgen Gober, Wing Ting Leung, Zengshu Huang, Xinyao Pan, Chuyu Li, Na Zhang, and Ling Wang. 2021. Alterations in the intestinal microbiome associated with PCOS affect the clinical phenotype. Biomedicine and Pharmacotherapy 133.

Wang, Ming Xing, Qian Yin, and Xin Xu. 2020. A rat model of polycystic ovary syndrome with insulin resistance induced by letrozole combined with a high-fat diet. Medical Science Monitor 26.

Wang, Xuejiao, Liping Gu, Yahui Zhang, Chuanhao Xiong, Yongde Peng, and Xiaoying Ding. 2022. Effects of dehydroepiandrosterone alone or in combination with a high-fat diet and antibiotic cocktail on the heterogeneous phenotypes of PCOS mouse models by regulating gut microbiota. Frontiers in endocrinology 13, 1030151.

Wekker, V, L Van Dammen, A Koning, KY Heida, RC Painter, J Limpens, JSE Laven, JE Roeters Van Lennep, TJ Roseboom, and A Hoek. 2020. Long-term cardiometabolic disease risk in women with PCOS: A systematic review and meta-analysis. Human Reproduction Update 26(6), 942–60.

Zhang, Yaling, Lei Wang, Yajing Weng, Daojuan Wang, Rong Wang, Hongwei Wang, Lihui Wang, Shanmei Shen, Hongwei Wang, Yan Li, and Yong Wang. 2022. Curcumin Inhibits Hyperandrogen-Induced IRE1 α - XBP1 Pathway Activation by Activating the PI3K/AKT Signaling in Ovarian Granulosa Cells of PCOS Model Rats. Oxidative Medicine and Cellular Longevity 2022.

Zhou, Dan ni, Sai jiao Li, Jin li Ding, Tai lang Yin, Jing Yang, and Hong Ye. 2018. MIF May Participate in Pathogenesis of Polycystic Ovary Syndrome in Rats through MAPK Signalling Pathway. Current Medical Science 38(5), 853–60.