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Exploring the Current Renews on Numerous Animal Models of PCOS: An Overview

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Abstract: A hormonal disorder prevalent among women during their childbearing years, is polycystic ovarian syndrome (PCOS). Recently the number of women with reproductive age who had PCOS increased to 82.44 per 100,000. The widespread presence of PCOS in India is between 3.7 and 22.5%. In PCOS, levels of hormones such as luteinizing hormone (LH), follicle stimulating hormone (FSH), and progesterone are significantly impacted. It also reduces the susceptibility of the hypothalamus's to progesterone's negative feedback regulation. Theca cells create more testosterone because of these interferences, which leads to anovulation, cysts in the ovary, hirsutism, and acne -- all of which are frequent in PCOS. These pathological factors can be utilized in the design of appropriate animal model of PCOS. Currently, tamoxifen, metformin, and clomiphene citrate are the most frequently given drugs for PCOS. Considering these drugs' negative effects and relative efficacy in treating PCOS, it is imperative to discover and develop alternatives. This review provides an overview of current hormone induced PCOS studies on rat models

Keywords: Polycystic Ovarian Syndrome, Prevalence, Progesterone, Ovarian cysts, Hormonal disorder

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Introduction

PCOS, the most common endocrine disorder in women of reproductive age is affecting over 116 million globally per recent WHO data (Ndefo *et al.*, 2013). Its incidence rate among women of childbearing age was 82.44 per 100,000 in 2017

(Liu *et al.*, 2021). In India it is showing a high incidence ranging from 3.7% to 22.5% (Ganie *et al.*, 2019). Environmental, genetic factors, poor lifestyle, nutrition and infections contribute to PCOS risk. Insulin resistance elevates androgen

levels, disrupts ovarian function, affecting prolactin, luteinizing hormone, follicle-stimulating hormone, and gonadotropin-releasing hormone levels (Diamanti-Kandarakis *et al.*, 2006). PCOS symptoms include infertility, pregnancy issues, type II diabetes, insulin resistance, depression, anxiety, and others (Hoeger *et al.*, 2021). Modern endocrine and metabolic illnesses can often be prevented through lifestyle changes (Aly and Decherney, 2021). Scientific interest is rising in exploring the benefits of using vitamins, minerals, and complementary medicines to improve PCOS outcomes (Günalan *et al.*, 2018). Many with PCOS choose these supplements, especially if dissatisfied with conventional treatments like fertility medicines and oral contraceptives, seeing them as natural and low-risk additions (Sills *et al.*, 2001). Commonly prescribed PCOS medications are clomiphene citrate, metformin, and tamoxifen (Marx and Mehta, 2003). Clomiphene usage may cause adverse effects such as headache, vertigo, gynecomastia, psychiatric exacerbations, testicular tumors, cardiovascular flushing, gastrointestinal issues, and mastalgia (Wheeler *et al.*, 2019). Digestive symptoms like anorexia, abdominal discomfort, diarrhea, flatulence, metallic taste, and vomiting are primary adverse effects of metformin medication (Nestler, 2008). Identifying alternative PCOS medications without these drawbacks is crucial due to limited efficacy (Marx and Mehta, 2003). Researchers seek affordable, easily handled animal models with stable genetic characteristics for drug screening (van Houten and Visser, 2014). Rodents are preferred for PCOS research due to size, lifespan, reproductive rate, and genetic diversity. Induction methods include pharmaceutical treatment, continuous light exposure, and transgenic techniques, yet limitations exist in studying cardiometabolic features (Shi and Vine, 2012). Different changes that take place during PCOS pathology are depicted in Figure 1. While valuable for understanding PCOS, no animal model fully replicates human conditions (Singh *et al.*, 2023). This review outlines advantages and disadvantages of various PCOS-induced murine

models, aiding preclinical evaluations of potential treatments.

Hormones induced PCOS in rats:

Animal PCOS models use hormonal inducers like letrozole, prenatal androgens, estrogen, and antiproggestins (Table 1). Alterations include reduced testosterone, hyperandrogenism and elevated fasting glycemia.

(i) Prenatal androgen induced PCOS model:

Research on a rodent model of PCOS induced in prenatal stage, faithfully replicating associated ovarian and endocrine problems while preserving normal reproductive structure, is lacking (Rajashekar *et al.*, 2022). Earlier rat model studies explaining PCOS causation or male pseudo-hermaphroditism had limitations (Gharai *et al.*, 2021). The ongoing study aims to develop an improved rodent model demonstrating PCOS-like endocrine or ovarian problems while maintaining regular reproductive system morphology in adulthood. Rats were exposed to a single testosterone administration during crucial fetal development (Walters *et al.*, 2012). Female adult Wistar rats (n = 20), weighing 170-190 g and aged 75-95 days, were cohabitated overnight with a male in a polypropylene enclosure (43 cm x 30 cm x 15 cm) under standard animal housing conditions (Tehrani *et al.*, 2014). After mating, the first day of pregnancy was determined by the presence of a vaginal plug. Daily vaginal smears were collected between 8 and 12 for 20 days from all female progeny (aged 70 to 90 days) to monitor estrous cyclicity. Vaginal samples were obtained by gently inserting a sterilized cotton swab into the vagina and rolling it onto a glass slide. Smears were preserved with 70% ethanol, stained with Giemsa, and examined under a light microscope to calculate ratios of leukocytes, epithelial, and cornified cells, which vary during the estrous cycle phases: estrus, diestrus 1 (metestrus), diestrus 2, and proestrus, lasting four days (Marcondes *et al.*, 2002). Daily vaginal smear examinations for 20 days assessed oestrous cycle length and regularity in adult offspring of prenatally androgenized

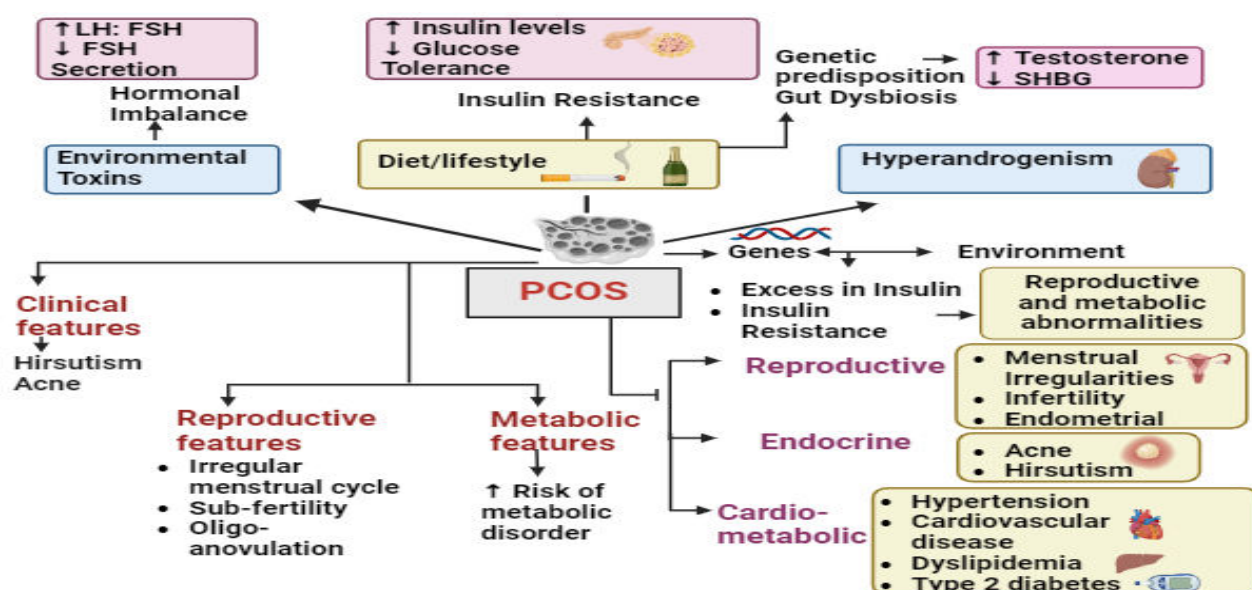


Fig. 1: The traits and suggested pathogenesis of PCOS. PCOS and hormonal disorder in women, disrupts ovaries, irregular periods along with high male hormones that causes acne, excess hair loss, insulin resistance, diabetes risk. Their management can be done with diet, medication, medical supervision that prevents infertility, obesity and endometrial cancer.

Table 1: Treatments with hormones and the characteristics of rodents PCOS models

S. No.	Category	Weight and age	Genus	Estrous cyclicity	Prenatal/ Postnatal	Testing agent	References
1.	Androgen	170-190 g 75-90 days	Rat	Irregular (Longer)	Prenatal	TP	Tehrani <i>et al.</i> (2014)
2.	Androgen	21-42 days	Rat	Irregular (Mainly estrous)	Postnatal	DHEA	Abramovich <i>et al.</i> (2012)
3.	Aromatase inhibitor	68-90 g 3 weeks	Rat	Acyclic or Irregular	Postnatal	Letrozole	Baravalle <i>et al.</i> (2006)
4.	Antiprogesterin	BW 200 + 15 g	Rat	Acyclic (Estrous)	Postnatal	RU486	Kondo <i>et al.</i> (2016)
5.	Estrogen	newborn female	Rat	Acyclic (Estrous)	Postnatal	EV	Brawer <i>et al.</i> (1986)
6.	PNA	21 days old	Rat	Irregular	Postnatal	DHEA	Ren <i>et al.</i> (2022)
7.	Ovarian and metabolic	21 days old	Rat	Acyclic or Irregular	Postnatal	DHT and Letrozole	Manneras <i>et al.</i> (2007)

AN- Androgen, AI- Aromatase Inhibitor, AP- Anti-progestin, ES- Estrogen, PNA- Peptide Nucleic acid mouse polycystic ovary syndrome ,OM- Ovarian and Metabolic.

animals. Prenatal testosterone exposure at a critical fetal development stage can establish a functional rat PCOS model with minimal morphological abnormalities (Tehrani *et al.*, 2014). Prenatal exposure to appropriate testosterone dosage during critical fetal growth enables practical rat PCOS model development with limited morphological abnormalities and enduring signs into maturity.

(ii) *Dehydroepiandrosterone (DHEA) nduced PCOS model:*

PCOS rat model: DHEA injections (6 mg/100 g body weight in sesame oil) were given to pre-pubertal 22-day-old rats for 20-27 days. High DHEA levels found in 25% of PCOS women produces PCOS phenotype. Female Sprague Dawley rats were individually housed at 21 and 42 days old. Roy *et al.* (1962) studied dams and their 8-10 pups under stable conditions: temperatures 23-25°C, humidity 55-65%, with 12 h light/dark cycles (lights on 8:00-20:00). Rats had unlimited access to standard commercial chow diet (21.68% protein, 4.6% fat, 2.45% fiber, 5.97% ash, 2.5% minerals) and water. In the pre-pubertal trial, 21-day-old females (n=56) were divided into sham and DHEA groups, while post-pubertal females (n=109) were separated similarly. Daily vaginal smear examinations began on day ten (31 days postnatal) for pre-pubertal rats and on day zero (42 days postnatal) for post-pubertal rats, collected using dampened cotton swabs inserted into the vaginal canal (Roy *et al.*, 1962). Cellular material from the swab was air-dried on a glass slide. Smears were fixed in pure methanol twice, air-dried, and stained with Giemsa for 20 min (1:20 dilution). After rinsing in buffer, slides were air-dried. Estrous stage was determined by major cell type: pro-estrus had nucleated epithelial cells, estrous had anucleated cornified cells, and metestrus showed equal amounts of leukocytes, cornified epithelial cells, or nucleated epithelial cells, with leukocytes predominating in diestrus (Landis *et al.*, 2012). It is concluded that Rat PCOS models aid understanding causative factors, therapy development. Each has pros and

cons, can not fully represent PCOS alone. Post-pubertal DHEA exposure increases PCOS induction, cystic ovaries, uterine malformation - valuable insights for research as shown in Figure 2.

(iii) *Letrozole induced PCOS model:*

Letrozole, aromatase inhibitor, elevates androgen levels inhibiting testosterone-estradiol conversion, inducing PCOS-like changes: increased ovarian size, thickened theca interna, anovulation. No metabolic abnormalities observed: obesity, insulin sensitivity, dyslipidemia. (Richardson *et al.*, 2007; Jang *et al.*, 2014). Zurvarra *et al.* (2009) studied 24 female weaning Sprague Dawley rats (3 weeks old, weighing 68-90 g) housed in groups of 6 under regulated conditions (21-22°C, 55-65% humidity, reversed 12 h light-dark cycle), with unlimited access to standard food and water. Rats were divided into 4 groups, each undergoing a 12-week therapy phase. After 8 weeks of gavage administration, vaginal smears were collected, fixed in 10% formaldehyde, and stained with hematoxylin and eosin. Estrus cycle variations were examined for 10 consecutive days under a light microscope. Ovaries were preserved in 4% formaldehyde, processed for histological analysis, and stained with hematoxylin and eosin. Follicles were counted using a light microscope equipped with cross-polarizing filters. Studies suggest that continuous letrozole administration, with a high-fat diet, induces PCOS-like phenotypic and metabolic disturbances in rodents, possibly via insulin signaling disruption, warranting further investigation with metformin and alternative insulin sensitizers (Manneras *et al.*, 2007). Letrozole + high-fat diet induces PCOS, metabolic issues, insulin disruption in rodents. Confirmation with metformin, diabetes drugs in PCOS rats is necessary. The letrozole model for PCOS induction has been depicted in Figure 3.

(iv) *Anti-progesterone Roussel-Uclaf (RU486) Induced PCOS Model:*

RU486 in rats induced ovulatory failure, persistent cornification, ovarian cysts, altered FSH/LH, high T/E2, enlarged pituitary, increased prolactin

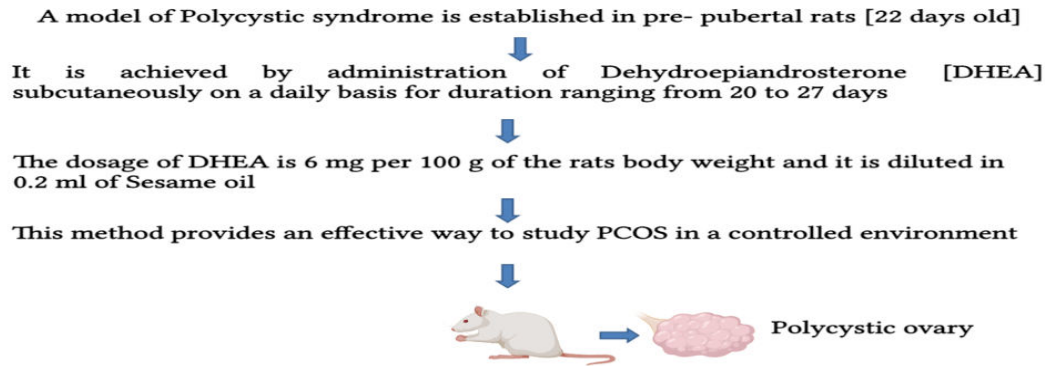


Fig. 2: The process of PCOS induction using DHEA in rats. This figure depicts the pre-pubertal rat PCOS model with daily subcutaneous administration of DHEA at 6 mg per 100 g body weight diluted in 0.2 ml sesame oil for 20-27 days.

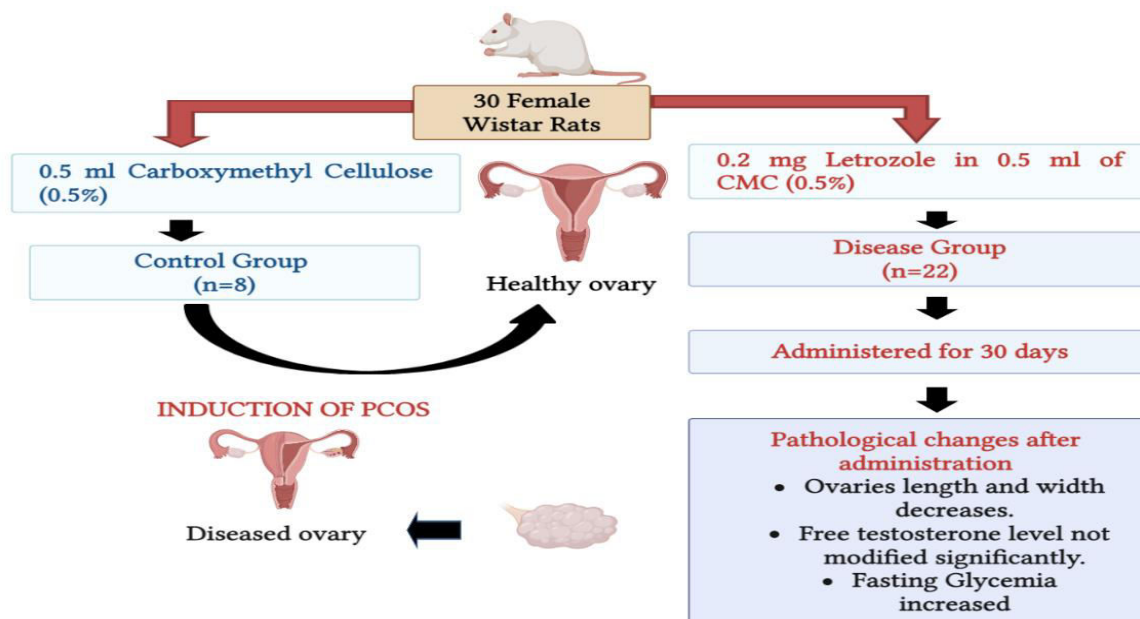


Fig. 3: Induction of PCOS by using Letrozole as inducing agent. In this study groups were divided in two distinct groups as Control (0.5% carboxymethyl cellulose vehicle), Disease (0.2 mg letrozole in vehicle) for inducing PCOS and the parameters were compared.

(Sanchez-Criado *et al.*, 1993). Sanchez Criado *et al.* (1993) conducted a study on Wistar mature female rats weighing 200 ± 15 g, housed in groups of 4 or 5 at a temperature of 20–22°C with a daily light cycle from 5:00 am to 7:00 pm. Rats had *ad libitum* access to standard feed and water. Daily vaginal smears were examined, and rats displaying at least two successive 4-day estrous cycles were selected. Vaginal smears were collected daily, including the day of sacrifice. Rats

received daily subcutaneous injections of mifepristone (RU486) at 4 mg/0.2 ml in oil for 8 consecutive days, with subcutaneous injections of 1 mg LHRH on Days 5 and 7 to achieve maximum LH serum level suppression (Manneras *et al.*, 2009). Ovaries and pituitaries removed, weighed postmortem. Ovaries dehydrated, fixed, embedded, sectioned, stained. Class 1 follicles ($>275 \mu\text{m}$), corpora lutea ($>700 \mu\text{m}$) quantified. Follicular growth assessed by percentage of

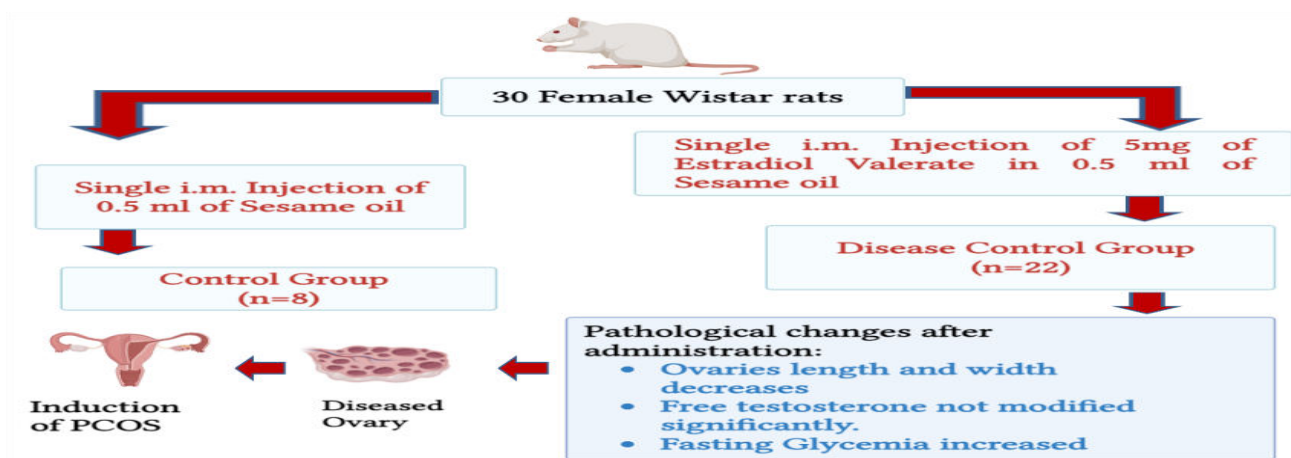


Fig. 4: Depiction of PCOS induction by Estradiol Valerate in animals. In this PCOS model study, animals were divided into two groups: Control and Disease control. The Control group received a single intramuscular injection of 0.5 ml sesame oil, while the Disease control group received 5 mg Estradiol Valerate in 0.5 ml sesame oil, leading to pathological changes and PCOS development.

follicles >350µm. Atresia in class 1 follicles determined follicular development (Furat Rencher *et al.*, 2018). RU486 in cyclic rats caused anovulatory cystic disease like PCO due to lack of progesterone. Without FSH/LH treatment, cystic condition persisted (Xu *et al.*, 2020). The finalized decision deducted anatomical and physiological similarities between animal models and human PCOS should be the primary criteria for assessing model accuracy. Studies confirm that the anti-steroid RU486 induces an anovulatory cystic state in rats over two estrous cycles. RU486 treatment did not impact adrenal function despite its anti-glucocorticoid activity.

(v) *Estradiol induced polycystic ovary syndrome model:*

Previous studies found sympathetic overactivity and ovarian nerve damage in EV (estradiol valerate) induced rat PCOS models (Fig. 4). Bilateral SON transection restored ovulation in EV rats, while unilateral transection mainly restored ovulation in denervated ovary. Lower norepinephrine in ovaries lacking intact nerves. This study investigates blocking ovarian adrenergic receptors to restore function in EV-induced PCOS rat model. Brawer *et al.* (1986), housed female newborn rats of the CII-ZV strain with other females under a 14 h light cycle (5 am

to 7 pm) until weaning, with *ad libitum* access to food and water. At 10 days old, female pups received intramuscular injections of 2.0 mg 17β-estradiol in 0.1 mL sesame oil, while the vehicle group (Vh) received sesame oil alone as shown in Figure 4 (Morales-Ledesma *et al.*, 2010). Daily vaginal smears began after vaginal opening. At 60 days old, rats in estrus were randomly assigned to four groups. Group Vh (n=10) received sesame oil and were sacrificed during estrus. Group Vh + propranolol (n=10) received propranolol with sesame oil. In the EV group (n=8), rats received EV treatment and were sacrificed on estrus day. The Propranolol + EV group (n=9) received propranolol and EV treatment. Propranolol (104 M) in 0.9% saline was injected into ovarian bursas of rats in the propranolol groups. Ovaries were fixed in paraformaldehyde, rinsed in saline, and stored in phosphate-buffered saline with 30% sucrose for histological analysis. Fresh CL were identified by healthy cells with large nuclei and blood vessels (Brawer *et al.*, 1986). Oocytes fixed with paraformaldehyde were sectioned and stained with hematoxylin-eosin. Stained sections were examined for corpora lutea and follicular cysts using a Leica DM750 binocular microscope and Leica ICC50 HD camera. Results indicate that acute ovarian ADRB inhibition improves ovulation rate, reduces testosterone levels, and promotes

follicular growth by attenuating ovarian noradrenergic system hyperactivity in rats with EV-induced PCOS.

Recent animal models of PCOS:

(a) Spontaneous prototypical rodent model or impaired insulin secretion and glucose tolerance in GK rats:

The GK rat strain develops lean non-obese type 2 diabetes mellitus via selective breeding, causing impaired glucose homeostasis without concurrent adiposity. However, high-calorie, fat-enriched diets can induce overweight/obese states in this genetically modified line. Unlike other genetic diabetes models, GK rodent diabetes is polygenic, akin to T2DM models. Wistar rats chosen for this strain display glucose intolerance. GK rats exhibit fetal reduction of pancreatic beta cell mass at 16.5 days of gestation and subsequently develop insulin resistance and late-life diabetic complications, including cardiovascular disease (Bourgneuf *et al.*, 2021).

Portha *et al.* (2012) demonstrated that GK rodents, a spontaneous type II diabetes model, exhibit PCOS-like phenotypic traits, encompassing metabolic and reproductive disorders. Wistar rats intolerant to glucose were selectively bred to establish the polygenic GK rat strain (Portha *et al.*, 2012). Confirming prior findings, GK rats exhibited elevated non-fasting glucose levels compared to age-matched Wistar controls at 3 and 6 months postnatal, indicating spontaneous type II diabetes mellitus onset in this model. Initially elevated insulin levels in GK rats normalized by six months, signifying a transition from insulin resistance to an insulin-deficient phenotype, accentuated by eighteen months. Clamp studies consistently identified hepatic insulin resistance and decreased peripheral tissue insulin sensitivity in GK rats, suggestive of insulin resistance. GK rat embryos show reduced pancreatic β -cell mass, while adults exhibit decreased insulin production, insulin resistance, dyslipidemia, hepatic steatosis, and adipocyte hypertrophy despite lower body weight. Further research is needed to clarify mechanisms underlying fat accumulation.

Reproductive phenotyping revealed that 75% of 6-month-old GK rats were acyclic with prolonged estrus cycle (Macut *et al.*, 2017). The conclusion implies that dysfunction in pancreatic beta cell populations regulating insulin secretion dynamics observed in the GK rodent model may contribute to similar clinical manifestations seen in second-generation female offspring susceptible to or exhibiting PCOS endocrinopathy during puberty onset.

(b) James C Russell corpulent (JCR: LA-cp) rodent model of PCOS:

JCR: LA-cp mice harbor a mutation in the obR gene, leading to leptin receptor dysfunction (Wu-Peng *et al.*, 1997). Male JCR: LA-cp mice with the cp genotype spontaneously develop visceral adiposity, insulin resistance, and dyslipidemia. Shi *et al.* (2009) found that male JCR: LA-cp mice also display spontaneous hyperandrogenemia (serum T) and cardiovascular anomalies similar to the female phenotype. Specifically, these mice develop oligo-ovulation polycystic ovaries and increased androgen biosynthesis by reproductive maturity without the need for additional experimental manipulation (O'Brien *et al.*, 2000). To facilitate nocturnal experiments, breeding colonies were maintained under 12 h light/dark cycles. Age-matched female lean control and cp/cp animals (n=9 per genotype) were used after weaning at 3 weeks old. Housing conditions post-weaning remained consistent with the breeding colony to minimize stress. Rats from separate breeding colony areas were transferred to individually ventilated cages as per protocol. Animals had *ad libitum* access to water and standard laboratory rat food. Experimental protocols and animal care procedures were followed (Shi *et al.*, 2009). Rodents were assessed longitudinally from peri-puberty to adulthood (6 to 12 weeks postnatal). Parameters measured at both adolescent (6 weeks) and young adult (12 weeks) stages included total body weight, visceral adiposity (peri-metrial and peri-renal fat depot weights), fasting serum metabolic analytes, and circulating reproductive hormones. This design tracked

phenotypic progression in JCR: LA-cp PCOS-like and age-matched control rodents. A meal tolerance test (MTT) assessed postprandial insulin/glucose metabolism between 6 and 12 weeks. Blood samples were obtained via tail vein extraction. Animals were euthanized at 12 weeks. Liver triglyceride (TG) levels were measured, and Oil Red-O lipid staining was performed. Ovaries were rapidly explanted, weighed, and preserved in 10% neutral buffered formaldehyde. Vaginal lavages were conducted daily from 9 weeks for 20 days to characterize cycle dynamics. Evans blue staining categorized estrous cycle stages - proestrus, estrus, metestrus, or diestrus - based on cytological profiles. This analysis examined reproductive cyclicity and cycle phase duration differences between JCR:LA-cp and control animals in relation to PCOS-like physiology (Russell et al., 1987). It is concluded that at six weeks of age, cp/cp rats exhibit reduced postprandial insulin responsiveness despite maintaining normal blood glucose levels.

(c) High-Fat Diet induced metabolic disorders in PCOS:

We posited that combining DHEA with a high-fat diet (HFD) could impact mice's reproductive and metabolic traits, mimicking PCOS features (Manneras et al., 2007). Han et al. (2023) studied the impact of high-fat feeding and androgenization on ovarian morphology and physiology in juvenile female C57BL/6 mice (21 days old). Mice were bred locally and housed under standard lighting conditions with *ad libitum* access to food and water. At postnatal day 25, mice weighing 7.3-10.3 g were randomly assigned to four groups (n=8 per group): Group 1 received standard food and sesame oil injections (s.c., 0.1 ml/100g body weight) as controls. Group 2 received high-fat food (60% calories from fats) and sesame oil injections. Group 3 received standard food and dehydroepiandrosterone injections dissolved in sesame oil (6 mg/100g body weight). Group 4 received both high-fat food and daily dehydroepiandrosterone injections. Injections were administered daily via the intra-peritoneal

route throughout the study. Parameters were compared between groups to assess the effects of diet and androgen excess (Han et al., 2023). Following a 20-day treatment period, metabolic and reproductive characteristics were evaluated, with treatments maintained until the study's conclusion. The results reveal distinct metabolic profiles in mice with DHEA-induced PCOS and HFD. Combining dehydroepiandrosterone with HFD presents an optimized preclinical model to explore causal pathways underlying endocrine and metabolic perturbations, including liver steatosis, in PCOS (Lai et al., 2014). The conclusion highlights metabolic disruptions in C57BL/6 mice with DHEA and a 60% HFD. Differences and similarities among mice exposed to HFD, DHEA, and the DHEA-HFD combination offer insights into treatment effects.

Other rodent models of PCOS:

Rodents provide practical PCOS-like models, but differ physiologically from humans. Additional rodent models with genetic modifications or continuous illumination exist, but extrapolating rodent ovarian function to humans has limitations. Rodents have rapid 4-5 day estrous cycles and poly-follicular ovaries, unlike humans. Androgenic signal pathway regulation differs across tissues; in primates, ovarian androgens stimulate folliculogenesis, but rodents show opposite responses. Animal models shed light on PCOS origins and metabolic dysregulation, but none fully replicate human pathology. Prenatally androgenized rhesus monkeys and ewes aim to improve human congruence. Researchers should cautiously interpret findings, considering inherent reproductive and endocrine differences compared to women (Sen et al., 2011). In summary, reliable animal models are vital for studying the cardiovascular risk and metabolic syndrome link in PCOS. Rat models, treated with testosterone and aromatase antagonists, mimic polycystic ovary traits but show reversible PCOS-like phenotypes. Single gene mutations or steroid administration start some aspects but miss the complex pathophysiology and heterogeneous presentation.

Enhanced translational relevance needs improved models starting in critical developmental windows. Preclinical models showing metabolic syndrome traits could help understand PCOS development and its cardiometabolic links (Manneras *et al.*, 2009).

Conclusion

Choosing the right PCOS models depends on research goals and hypotheses. The Peptide Nucleic Acid mouse model replicates PCOS features induced by hormones, aiding our understanding of its development. Administering dihydrotestosterone in rodents shows similar genetic transcript expression to human PCOS cases, suggesting potential protective factors in the hypothalamus. Studying insulin metabolism changes in rodent models, regardless of induced hyperandrogenemia, is important given the varied clinical manifestations of PCOS. Different animal models can deepen our understanding of PCOS pathophysiology and its associated cardio-metabolic risks. The effects of high-fat diets on DHEA-induced PCOS are still uncertain. The GK rat model naturally displays traits akin to PCOS, offering valuable insights into both reproductive and metabolic aspects, including ovarian hyperstimulation syndrome. Utilizing a range of animal models is crucial for gaining a comprehensive understanding of early PCOS development and its implications for cardiovascular and metabolic health.

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