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Review

Hormonal and Physiological Manipulation Methods to Induce Polycystic Ovary in Rodents: A Review of the New Findings

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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy causing non-ovulation infertility in women. Women with PCOS have higher levels of luteinizing hormone (LH), testosterone, cholesterol and triglycerides but, in contrast, lower levels of follicular stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) compared with healthy counterparts. Because of the limitations of human studies, animal models of PCOS have been developed to identify appropriate therapeutics and to explore their mechanisms of action. This study aimed to review the methods of PCOS induction in animal models. This systematic review used the keywords of PCOS, induction methods and animal models. The literature search was performed in PubMed, ScienceDirect and Scopus databases to recruit studies published from 1900 to 2023. The titles and abstracts were read to eliminate unrelated studies. There are two types of hormonal and non-hormonal PCOS animal models. These

animal models (i.e. female rats) present similarities to human PCOS regarding inflammatory and pathogenic pathways. The hormonal and metabolic functions of the hypothalamic-pituitary-ovarian axis, which plays a vital role in the development of the ovary can be modulated by these pathways. In this review study, various PCOS induction methods in animal models, including the use of dehydroepiandrosteron, dihydrotestosterone, testosterone, human chorionic gonadotropin, estradiol valerate, letrozole, RU486 (mifepristone), and adenocorticotropin, belong long-term use of light is mentioned along with the effect mechanism and their advantages and disadvantages. Some PCOS animal models are considered the first generation, which present advantages and disadvantages compared with second-generation PCOS animal models. These animal models can be developed based on the researcher's purpose. Considering their advantages and disadvantages, different types of PCOS animal models may be used for clinical research.

Keywords
Polycystic ovary syndrome; animal models; PCOS induction

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine, metabolic and genetic disorder characterized by non-ovulation, polycystic ovary, and biochemical and clinical signs of hyperandrogenesis. PCOS is associated with adrenal-pituitary-hypothalamic dysregulations and is observed in about 4 to 18% of women at fertility age [1]. Women with PCOS have higher levels of luteinizing hormone (LH), testosterone, cholesterol and triglycerides but, in contrast, lower levels of follicular stimulating hormone (FSH), sex hormone-binding globulin (SHBG), and high-density lipoproteins (HDLs) compared with healthy counterparts [2]. The increased LH/FSH ratio leads ovaries to produce androgens preferentially. The synthesis of androgens is further promoted by the action of insulin and insulin-like factors, which are also elevated in women with PCOS. According to the Rotterdam criteria, PCOS is diagnosed when two of the three following criteria are met: 1) Oligo- and/or anovulation, 2) Clinical and/or biochemical signs of hyperandrogenism, and 3) Polycystic ovaries [3].

Along with an-ovulation, type 2 diabetes, and cardiovascular diseases, menstrual disorders, which can lead to infertility if they remain untreated, are the most common complications of PCOS. PCOS can trigger acne, obesity, hirsutism, and alopecia. Nevertheless, environmental risk factors and complications of PCOS vary in societies regarding different lifestyles. It is, therefore, critical to early diagnose and treat PCOS to prevent its complications, particularly cardiovascular diseases and infertility [4]. Although various genetic and environmental factors, hormonal disorders, chronic inflammation, oxidative stress, and inappropriate lifestyle can facilitate the development of PCOS, the exact pathogenic mechanisms remain unclear [5].

Sheep and non-humane primates with some reproductive and metabolic features of PCOS (such as polyfollicular ovaries, excessive LH secretion, increased androgen production, elevated mass of visceral fat, and IR) have already been introduced. Nevertheless, using these models has some limitations, such as high costs, long periods of reproduction and gestation, and difficulty in

performing genetic manipulation. Because of this, many researchers prefer to use rodents as reasonable models of PCOS. Rodents present many advantages such as small size, anatomical, physiological, and genetic features, relatively short reproduction period, high rate of reproduction, and ease of use and maintenance. In comparison to mice, rats have several advantages, including larger sizes of animals and limbs (which may facilitate some surgical procedures and laboratory manipulations) and larger blood volume, which provides more specimens to measure various parameters. In addition, rats are more resistant to multiple diseases. In order to better understand these mechanisms, this review addresses the methods of PCOS induction in animal models.

2. Materials and Methods

2.1 Search Strategy and Study Framework

This review recruited related articles from PubMed, SID, Springer, Medlib, and ScienceDirect databases without any language restrictions from 1900 to 2023. The keywords included PCOS, animal model, and PCOS induction. In the primary search, 460 related articles were found, of which 342 unrelated studies were eliminated after reading the titles and abstracts. Finally, 118 articles were selected, categorized and reviewed (Figure 1). In this study, the literature search was conducted using MeSH keywords, including "female infertility," "PCOS," "dehydroepiandrosteron," "dihydrotestosterone," "human chorionic gonadotropin", "testosterone," "letrozole", "RU486 (mifepristone)," and "adenocorticotropin."

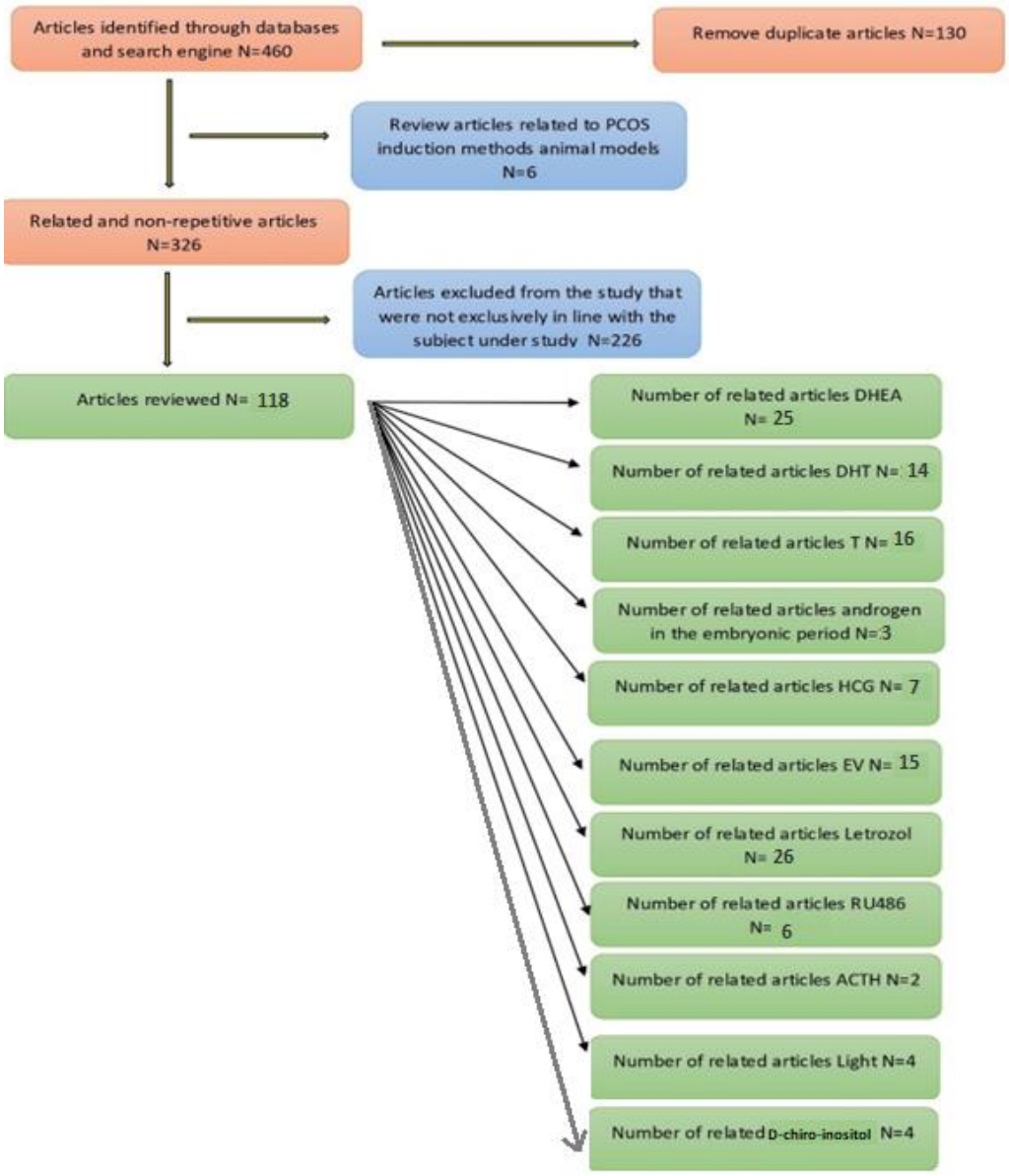

Figure 1 Flowchart steps of selecting information from different articles.

This article does not contain any studies with human and laboratory animals participants performed by any of the authors.

2.2 Inclusion Criteria

English-language articles evaluating the effects of methods on PCOS induction and their different mechanisms in animals were selected. Overall, 98 studies on animal models investigating the positive impact of different methods on PCOS induction in animals were selected.

2.3 Exclusion Criteria

Non-English language papers, those with insufficient data, studies with poor methodology, those assessing indirect effects of agents/drugs, and studies reporting ineffective (negative) effects on PCOS induction were excluded from the analysis.

3. Results

In this study, all the methods of PCOS induction in animal models were investigated. Generally, PCOS models are developed using either chemical compounds or long-term light irradiation. The animals are exposed to the chemicals by either intramuscular, subcutaneous, or oral administration for a specific period. The commonly used chemicals include androgens (dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), testosterone, and androgen), human chorionic gonadotropin (HCG), aromatase inhibitor (letrozole), estrogen (estradiol valerate), ACTH (Adenocorticotropin), D-chiro-inositol and progesterone antagonist (RU486) (Table 1). These chemicals are selected based on researchers' needs and may be administrated during embryogenesis (e.g. androgens).

Table 1 PCOS induction methods.

Reference	PCOS inducer	Animal	Administration method	Duration of injection	Dose
Demacopulo, 2019 [6] Maurya, 2014 [7] Wang, 2013 [8]	DHEA	rat	SC	20 days	6 mg/100 g BW
Li ,2016 [9] Wang,2019 [10] Rencber ,2018 [11] Huang ,2015 [12] Jang, 2014 [13] Zhu, 2009 [14] Utkan Korun, 2024 [15]		mice			
Wang, 2018 [16]		rat		35 days	
Mahmoud, 2018[17]		rat	Oral	2 months	150 mg/kg BW
Xia, 2017 [18]			SC	3 weeks	60 mg/kg/day
Singh, 2019 [19]		mice	SC	25 days	6 mg/100 g BW
Zhang, 2016 [20]		rat	SC	from 27 to day 46 day after gestation	6 mg/100 g BW
Ikeda, 2014 [21]				7, 15, and 30 days after gestation	
Honnma et al. 2006 [22]				22 days	
Zhang, 2019 [23] Zheng, 2021 [24]	DHT	rat	SC	75 days	15 mg
Hu, 2018 [25]		mice	SC	16^{th} to 18^{th} days after gestation	250 µg/body/d

Tang, 2015 [26] Nikolic, 2014 [27] Tepavčević, 2014 [28] Masszi, 2013 [29] Benrick, 2013 [30] Feng, 2012 [31] Mannera˚s, 2009 [32]		mice rat	SC	90 days	7.5 mg of DHT (daily dose, 83.3 µg)
Tehrani, 2019 [33]	T	rat	SC	gestational day 20	5 mg
Kalhori, 2018 [34]		mice	SC	5 weeks	1 mg/100 g
Abbott, 2018 [35]			SC	15– 35 consecutive days	10 mg
Chaudhari NK, 2017 [36]		rat	SC	35 days	10 mg/kg body
Noroozzadeh, 2015 [37]		rat	SC	on the gestational day 20	5 mg in 500 µl of sesame oil and benzyl benzoate
Hu, 2015 [38]			SC	On the gestational day 15–19	0.5 mg·kg·d
Amalfi, 2012 [39]			SC	from day 16 to day 19 of pregnancy	2 mg or 5 mg
Beloosesky, 2004 [40]		rat	SC	35 days	1 mg/100 g
Ota, 1983 [41]		rat	SC	In 5-day-old	1.25 mg
Miao, 2019 [42] Yang, 2022 [43]	HCG	rat	SC	21 days	DHEA 6 mg/100 g and 1.5 IU HCG
Lima, 2006 [44]		rat		22 days	3 IU
Ota, 1986 [45]				80 days	10 IU
Shao, 2019 [46] Hong, 2018 [47]	Letrozole	rat	SC	2 weeks	1 mg/kg

Karateke, 2018 [48] Patel, 2018 [49] Areloegbe, 2022 [50]				21 days	
Jahan, 2018 [51]			Oral	36 days	
Fu, 2018 [52]				23 days	
Rajan, 2017 [53] Lee, 2018 [54]			SC	60 days	1.8 mg/pellet
Yang, 2018 [55]				2 weeks	0.1 mg/kg/day
Pandey, 2017 [56]				7, 15 and 21 days	3 mg/kg
Kabel, 2017 [57]				36 days	1 mg/kg
Ullah, 2017 [58]					
Li, 2017 [59] Mihanfar, 2021 [60] Cao, 2017 [61] Aliabadi, 2017 [62] Wang, 2017 [63] Lian, 2016 [64] Rezvanfar, 2015[65]			oral	21 days	1 mg/kg
Kauffman, 2015 [66]		mice	SC	60 days	50 µg/day
Maliqueo, 2015 [67]		rat	SC	90 days	18.0 mg (daily dose, 200 µg)
Kelley, 2015 [68]		mice	SC	at 4 weeks of age	50 µg/day
Maharjan, 2010 [69] Kafali H, 2004 [70]		rat	Oral	21 days	0.5 mg/kg BW 1 mg\kg BW
Jin, 2019 [71]	EV	mice	SC	3 days	20 µg/day
Pournaderi, 2017 [72] Azin, 2022 [73]		rat	SC	40 days	0.4 ml
Barzegar, 2017 [74]			IM	60 days	2 ml/day

Mirabolghasemi,2017 [75] Ghafurniyan, 2015 [76] Karimzadeh, 2013 [77]			SC	60 days	2 mg
Li, 2017 [78] Miri, 2014 [79] Gode, 2011 [80]			IM	60 days	4 mg
Daneasa, 2016 [81]			IM	60 days	5 mg
Mesbah, 2015 [82]			IM	12 weeks	4 mg
Deshpande, 1999 [83]		mice	SC	4 days	20 mg
Kondo, 2018 [84] Priyadarshani, 2022 [85]	RU486	rat	surgically implanted under the back skin	between 8 and 9 weeks-of-age on the estrous day	4 mg/body
Priyadarshani, 2009 [86]			Oral	13 days	20 mg\kg \day
Ruiz, 1996 [87]			SC	8 consecutive days, starting on the day 1 of the estrous cycle.	4 mg/0.2 ml oil
Sánchez-Criado JE1993 [88]			SC	1-2 weeks	2-4 mg/100 g BW
Park, 2017 [89] Baravalle, 2007 [90] He, 2024 [91]	ACTH	rat	SC	18 days	20 UI/kg (200 µg/kg)
Nooranizadeh,2018 [92]	Light	rat	SC	18 days	24 hours per day fluorescent light with 350 lux intensity to 1 m2 on floor
Kang, 2015[93]			-	90 days	Continuous ight environment (L/L, lights on 24 hourse everyday)

Takahashi, 1977 [94]			-	16 weeks	light-proof box in which continuous lighting was supplied by fluorescent bulbs
Singh B, 2019 [95]			-	least 30 days	constant light
Yang et al., 2022 [96]	D-chiro-inositol	rat	Oral	2 weeks	50 mg/kg/BW/orally
Bevilacqua et al., 2021 [97]		C57BL/6N female mice	Oral	21 days	20 mg/BW/orally

*SC: Subcutaneous, IM: Intramuscular, IP: Intraperitoneal

4. Discussion

4.1 Dehydroepiandrosterone (DHEA)

DHEA is an androgen that is mainly produced by the adrenal gland. Women with PCOS have high levels of DHEA. In experimental studies, DHEA is used to develop animal models of PCOS. Roy et al. showed that DHEA administration promoted ovulation and cyst formation in the ovary [42]. In addition, subcutaneous injection of DHEA (dose of 6 g/100 mg body weight daily) over 20 days induced PCOS in rats [98-100]. DHEA increases the levels of prolactin hormone, which in turn converts DHEA into estrogen and subsequently increases the estrogen level in ovaries [101].

4.1.1 Ovarian Morphology

In postnatal induction, there was an increase in ovarian weight due to a rise in the number of cystic follicles and a thickened theca layer.

4.1.2 Sex Steroid Hormone and Gonadotropin

In animals induced by DHEA, low LH serum levels but high T and elevated LH/FSH ratio were seen. This was while E2 levels did not change.

4.2 Dihydrotestosterone (DHT)

DHT is a non-aromatizing androgen that has been used for the induction of PCOS in animal models. In one study, rats continuously treated with DHT (7.5 mg for 90 days) showed impaired estrous cycle and reduced ovarian weight [29]. In this model of PCOS, the most common clinical manifestations included increased body fat and weight, elevated glucose and leptin levels, insulin resistance, and increased systolic and diastolic blood pressures [102].

4.2.1 Ovarian Morphology

In both prenatal and postnatal inductions, there was an increase in the number of cystic atretic follicles. Also, there were decreases in antral follicles and corpus lutea.

4.2.2 Sex Steroid Hormone and Gonadotropin

In prenatally induction, T, LH, E2, and P4 levels increased, but postnatal induction increased T and decreased P4 levels, with no significant changes in other hormones (Table 2).

4.3 Testosterone

Testosterone is one of the androgenic hormones. Exposure to testosterone before and after birth can cause hyperandrogenesis in rats. In addition, embryos exposed to testosterone during the fetal period have shown developmental and morphological abnormalities in their reproductive systems [103]. Immature rats that received testosterone for 8 weeks showed increased testosterone levels, LH, LH/FSH ratio, increased body weight, and decreased insulin sensitivity [104]. In a study by John Nesar et al., PCOS was induced by daily subcutaneous injections of testosterone propionate (dose

of 1 mg per 100 g body weight) in 21-day-old rats. Ovarian histological analysis clearly showed the presence of cystic follicles, increased pre-antral follicles, and a lack of ovulation and yellow bodies. The number of primary follicles was remarkably higher. In comparison, the number of pre-antral and antral follicles was significantly lower in rats exposed to testosterone for four weeks compared with those treated for one week and control animals [32].

4.3.1 Ovarian Morphology

In prenatal induction of PCOS, there was an increase in the number of antral follicles, while there were decreases in pre-antral and preovulatory follicles and corpus lutea. Also, following this method, cystic follicles were observed, which showed successful induction of PCOS. On the other hand, in postnatal induction of PCOS, there were increases in the preantral and atretic follicles.

4.3.2 Sex Steroid Hormone and Gonadotropin

In rats with prenatally-induced PCOS, the levels of T and LH increased, but no prominent changes were observed in FSH, E2, and P4 levels. T and LH levels increased in rats with postnatally-induced PCOS, and E2 and P4 levels decreased.

4.4 Androgen Treatment during Embryonic Period

Early embryonic stages are crucial developmental phases that can be easily irritated by any chemical or hormonal insult, leading to long-term developmental problems. In particular, an imbalanced concentration of androgens in the early embryonic stages can cause PCOS (33, 35). In their study, Wu et al. subcutaneously administrated testosterone (3 mg) and DHT (3 mg) to mice at the days of 16 to 19 of gestation. In both groups, the number of pre-antral follicles increased. The biochemical analysis also showed an elevation of gonadotropins, indicating PCOS induction. Increased LH levels led to the elevation of androgens in this method [104].

4.5 Human Chorionic Gonadotropin (HCG)

The placenta produces HCG during pregnancy. Ota et al. subcutaneously administrated HCG (10 IU for 80 days) to create rat models of PCOS. As mentioned earlier, prolactin stimulates the production of adrenal androgens, which in turn lead to PCOS. In addition to the induction of prolactin, HCG administration also inhibits the production of progesterone [105, 106].

4.5.1 Ovarian Morphology

An increase in cystic follicles was seen.

4.5.2 Sex Steroid Hormone and Gonadotropin

Continuous stimulation with HCG initially increased estrogen secretion in the ovary. The high levels of estrogen subsequently modulate the function of the hypothalamus-pituitary axis and inhibit dopamine production, leading to prolactin secretion [107] and subsequently suppression of the pulsatile output of gonadotropins and LH in the middle of the menstrual cycle. These events finally result in the cease or severe reduction of ovulation.

4.6 Estradiol Valerate (EV)

EV disrupts the ovarian cycle by activating the ovary's sympathetic nerve and increasing intra-ovarian norepinephrine's expression. Silva et al., showed that a single-dose intramuscular injection of EV (two mg dissolved in 0.2 mm corn oil) induced PCOS in rats [106]. EV decreases the secretion of tyrosine kinase, which is a restriction enzyme for norepinephrine in the ovaries of PCOS rats. Therefore, EV-induced elevation of norepinephrine interferes with the ovarian cycle and ceases ovulation [108, 109].

4.6.1 Ovarian Morphology

There were decreases in primary, antral, and Corpus lutea follicles while the number of cystic follicles increased. The number of atretic follicles increased 5 to 16 days after the injection.

4.6.2 Sex Steroid Hormone and Gonadotropin

Serum levels of LH and FSH decreased. The levels of T and E2 increased after the injection of 2 mg, but after 4 mg injection, the levels of LH and P4 decreased.

4.7 Letrozole

Letrozole is a non-steroidal inhibitor of aromatase. The inhibition of aromatase in granulosa cells prevents the conversion of androgens to estrogen, which can lead to PCOS. Manneras et al. investigated ovarian morphology, body weight, body and mesenteric fats, and insulin resistance in rats treated with subcutaneous letrozole (36 mg for 90 days). They observed that cystic follicles and testosterone levels were increased in the experimental group [32]. In another study, administration of letrozole (1 mg/kg for 21-23 days) induced PCOS in rats [18]. In a recent study, the most characteristic features of PCOS included ovarian weight gain, atretic follicles, decreased luteinization, decreased or no ovulation, increased insulin levels, and progression of insulin resistance [65].

4.7.1 Ovarian Morphology

An increase in cystic follicles was seen.

4.7.2 Sex Steroid Hormone and Gonadotropin

The levels of T, LH, FSH and E2 increased while that of P4 decreased.

4.8 RU486 (Mifepristone)

The RU486 is one of the most common drugs used to terminate pregnancy. This is an industrial drug that acts as a P4 antagonist and results in severe depletion of progesterone receptors. The administration of RU486 increases the levels of gonadotropins, leading to an excess in the secretion of LH and testosterone, which ultimately triggers PCOS development [110].

4.8.1 Ovarian Morphology

The growth of ovarian follicles halted. On the other hand, the number of cystic follicles increased in the animals.

4.8.2 Sex Steroid Hormone and Gonadotropin

The levels of T, LH, FSH, P4, and E2 increased.

4.9 Adenocorticotropin (ACTH)

ACTH stimulates the secretion of adrenal cortex hormones, which induce the release of weak androgens such as dihydroepiandrosterone (DHEA). The DHEA is then converted to testosterone in both sexes. Increased androgen levels subsequently trigger PCOS, evidenced by ovarian morphological changes and clinical symptoms. Baravalle et al. induced PCOS in rats by subcutaneously injecting ACTH (dose of 20 UI/kg) for 18 days [89].

4.9.1 Ovarian Morphology

There were elevations in atretic and cystic follicles.

4.9.2 Sex Steroid Hormone and Gonadotropin

The levels of T, LH, and FSH declined.

4.10 Long-Term Use of Light

In studies on animal models, PCOS has also been induced by prolonged continuous light exposure. In a study, PCOS was induced by exposing mice to light for 90 days. Continuous exposure to light can disrupt the estrous cycle and increase estrogen levels. The morphological features of ovaries in this model appeared similar to those found in human PCOS [90].

4.10.1 Ovarian Morphology

Increased numbers of atretic and cystic follicles were observed.

4.10.2 Sex Steroid Hormone and Gonadotropin

While serum T level increased, no changes were seen in LH and FSH levels.

Experimental models of PCOS induction are essential in understanding the disease's underlying physiopathology. Hormonal imbalance during prenatal or postnatal periods may trigger the syndrome. Table 2 shows the changes in the levels of hormones produced by ovaries and follicles in PCOS induced by different experimental methods. In the testosterone-induced method, PCOS can be developed both prenatal and postnatal; however, the syndrome is only inducible postnatal in other methods.

Table 2 Hormonal and follicular changes in different PCOS induction methods.

Category	Regent	Prenatal/ Postnatal	Ovarian morphology	Sex steroid hormone	Gonadotropin	Reference
Androgen	**TP**	Prenatal	Preantral ↓, Antral ↑, Pre-ov ↓, CL ↑	T → or ↑	LH → or ↑, FSH →	[111]
			Polycystic	T ↑, E_2 →, P_4 →	N/A	[107]
		Postnatal	Polycystic	T ↑, E_2 ↓, P_4 ↓	LH ↑	[41]
			Preantral ↑ Atretic ↑	T ↑, E_2 ↑, P_4 →	N/A	[112]
	DHEA	Postnatal	Cyctic FC ↑, CL ↓	T ↑, E_2 →	LH/FSH ↑	[95, 99]
	DHT	Prenatal	Antral ↓, Pre-ov ↓, Atretic cyst-like ↑	T → or ↑ E_2 → or ↑, P_4 ↑ or →	LH ↑	[100, 105]
		Postnatal	Antral ↓, Cyctic FC ↑, CL ↓	T → or ↑, E_2 →, P_4 ↓	LH →	[113]
Aromatase inhibitor	**Letrozole**	Postnatal	Cystic FC ↑	T ↑, E_2 →, or ↑, P_4 ↓ or →	LH ↑ FSH ↑ or →	[114, 115]
Progesterone antagonist	**RU486**	Postnatal	Atretic FC ↑	T ↑, E_2 ↑, T/E_2 ↑	LH ↑	[84, 86, 87]
Estrogen	**Estradiol valerate**	Postnatal	Primodial ↑, Primary ↓, Antral ↓, CL ↓, Cystic FC ↑	T ↑, E_2 ↑	LH ↓, FSH ↓	[116]
			Atretic FC ↓, CL ↓	T ↑, E_2 →, P_4 ↓	LH ↑ FSH →	[82, 117, 118]
	ACTH	Postnatal	Atretic FC ↑, Cystic FC ↑	T ↓	LH ↓, FSH ↓	[88, 89]
	Light	Postnatal	Atretic FC ↑, Cystic FC ↑	T ↑	LH →, FSH →	[90-104]

*↑: increased, →: no change, ↓: decreased, FC: follicle, pre-ov: preovulatory, N/A: not available, CL: corpus luteum, E_2: estradiol, P_4: progesterone.

5. Conclusions

There are various methods to induce PCOS in animal models. The animal models developed by these methods mimic the hormonal profile of human PCOS and, therefore, are suitable for studying the pathogenesis of this syndrome in humans. Appropriate methods can be exploited depending on the researcher's needs and intended hormonal features. Among various PCOS induction methods, those developed by the infusion of estrogen and testosterone is considered first-generation because they induce PCOS by emulating hormonal profiles. However, these animal models have some limitations compared with second-generation PCOS animal models, which are developed by administrating letrozole and DHT. In testosterone-induced PCOS, this hormone is transformed into its metabolites in the animal's body. This is while DHT undergoes no such transformations (e.g. conversion to estradiol). On the other hand, letrozole can increase internal testosterone in PCOS

animal models. The effects of androgens on the reproductive system are highly dependent on the time of exposure to these compounds. According to this study, we can conclude that the time of exposure to hormones may play an essential role in the development of PCOS. In comparison to postnatal-induced PCOS, the models created by prenatal exposure to androgen seem to be more persistent and similar to human PCOS. This may be due to higher sensitivities of the reproductive and nervous systems to hormonal changes during their development and at the time of the formation and differentiation of the organs. Therefore, prenatal induction of PCOS requires lower doses and shorter times of exposure to hormones. In addition, prenatal hormone-induced PCOS persistently represents the features of PCOS. In contrast, in most animal models of postnatal-induced PCOS, these features may be transient and dependent on hormone exposure. They may not be re-established after the withdrawal of the hormone. Since human studies are associated with ethical limitations and other problems, studies on PCOS animal models can provide critical new insights into the pathophysiology of PCOS in humans.

Author Contributions

All authors participated in the performance of the research, data analysis, research design and writing of the paper.

Competing Interests

The authors declare that there is no conflict of interest.

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