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Development of Research on the Etiology and Pathogenesis of Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that affects 6-20% of women of childbearing age (Zafar U, Memon Z, Moin K, et al, 2019). It usually manifests as anovulation or rare ovulation, hirsutism, polycystic ovary changes, and quite common insulin resistance. Due to the heterogeneity of clinical manifestations, there is currently no unified international standard for the diagnosis of PCOS. The etiology and pathogenesis of this syndrome are not fully understood and are generally considered a multifactorial disease with various genetic, endocrine, and metabolic abnormalities.

Keywords: polycystic ovary syndrome, etiology and pathogenesis, review

1. Introduction

PCOS is one of the most common gynecologic endocrine diseases. Clinically, it is characterized by the clinical or biochemical manifestations of excessive androgen, persistent anovulation, and polycystic ovarian changes (polycystic ovarian morphology, PCOM), often accompanied by insulin resistance and obesity (Xie Xing, Kong Beihua & Duan Tao, 2022). According to statistics, 80–85% of women with clinical hyperandrogenism have PCOS (Azziz R, et al, 2009), Polyircentism is the most common symptom of hyperandrogenism, in about 60% of PCOS, and the patient develops hairy (Ferriman D & Gallwey JD, 1961). Approximately 50–70% of PCOS women and 95% of PCOS obese women are experiencing insulin resistance (DeUgarte CM, Bartolucci A A & Azziz R, 2005). The prevalence of PCOS in obese women was as high as 37% (Álvarez-Blasco F, Botella-Carretero JI, San Millán JL, et al, 2006). Nearly 40–70% of patients with PCOS are overweight or obese (Sanchez-Garrido MA & Tena-Sempere M, 2020). The prevalence of PCOS is similar in different populations around the world, and although the etiology of PCOS is not fully understood, PCOS is considered a multifactorial disease with genetic, endocrine and metabolic abnormalities.

2. Diagnosis of PCOS

In 1935, Stein and Leventhal first reported that "menstrual menstruation, infertility, male hairy and obesity" were associated with bilateral

polycystic ovary, so it was also called Stein-Leventhal syndrome (Stein IF & Leventhal ML, 1935). The diagnostic criteria for the diagnosis of PCOS differ due to the heterogeneity of the clinical manifestations.

2.1 The Diagnostic Criteria for PCOS

In 1990, the National Institutes of Health (National Institute of Health NIH) recommended that the main criteria for PCOS should include: sparse ovulation or anovulation, clinical and (or) biochemical manifestations of high androgen, and the exclusion of other known diseases that can cause ovulation disorders or high androgen, such as hyperprolactinemia, Cushing syndrome, congenital adrenocortical hyperplasia, etc. (Zawadski JK & Dunaif A, 1992). In 2003, the Rotterdam consensus expanded the diagnostic criteria to include at least two of the three characteristics: ① clinical and (or) biochemical hyperandrogenism; ② anovulation or anovulation; ③ PCOM: 10 mL (ovarian volume = 0.5 x length x width x thick), and > 12 follicles within 2 to 9 mm in one or both (or) ovary; 2 of 3 ④ and excluded related diseases (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). In 2006, the Society of Androandrogen and Polycystic Ovary Syndrome (Androgen Excess and PCOS Society, AES) recommended defining PCOS as clinical and (or) biochemical hyperandrogenism, including sparse ovulation and (or) PCOM, excluding related diseases (Azziz R. et al, 2006). The PCOS criteria of NIH and AES in 1990 emphasized the importance of high androgens for diagnosis, and the Rotterdam criteria are more suitable for PCOS diagnosis that does not show high androgen nationality clinically. In order to better adapt to the clinical practice in China, the former Ministry of Health issued PCOS Diagnosis (WS330-2011), loose menstruation, amenorrhea or irregular uterine bleeding are necessary for diagnosis; meanwhile, meeting one of the following 2 items, and excluding other diseases that may cause high androgen and ovulation abnormalities can be diagnosed as PCOS: ① high androgen clinical manifestations or hyperandrogenism; ② PCOM. In 2012, NIH issued a statement recommending the use of Rotterdam diagnostic criteria, and at present, the Rotterdam standard is still more used at home and abroad.

2.2 Ultround Diagnosis of PCOS

The ultrasound diagnostic criteria for PCOS were based on ultrasound suggesting 12 follicles of 2 to 9 mm in one or both ovarian diameter, and (or) ovarian volume of 10ml. Although more than 80% of women may have PCOM (Ardaens Y, Robert Y, Lemaitre L, et al, 1991). However, ultrasound detection PCOM still does not fully predict PCOS, because PCOM may occur in 20% to 30% of women of normal childbearing age (Endocrinology Group and Guidance Expert Group of the Obstetrics and Gynecology Branch of the Chinese Medical Association, 2018). The 2023 Chinese Guidelines for Diagnosis and Treatment state that ultrasound examination is not mandatory for adults who have existing menstrual abnormalities and hyperandrogenism and related manifestations (Expert consensus writing group for the diagnosis and treatment pathway of polycystic ovary syndrome, 2023). The 2023 Expert Consensus on the Pathway of PCOS suggested that the necessary conditions for the diagnosis of PCOS in puberty are hyperandrogenism and (or) hyperandrogen clinical manifestations and menstrual irregularities, as well as ovulation disorders, which was similar to the diagnosis of puberty in 2013. Both recommend no ultrasound finding of PCOM for adolescent women to diagnose PCOS. However, the 2013 PCOS diagnosis and treatment guidelines indicate that for perimenopausal women, PCOM is more supportive of PCOS diagnosis.

2.3 High Androgen Diagnosis of PCOS

Androgenexcess is the most typical hormonal alteration in PCOS. The 2018 PCOS China Diagnosis and Treatment Guidelines suggest that: for women of childbearing age, perimenopause and puberty, hyperandrogenemia should be evaluated first, and other diseases that may cause high androgen and ovulation disorders should be excluded one by one to clarify the diagnosis of PCOS. However, there is no international consensus on the evaluation of hyperandrogenism including the hormones and the numerical criteria (Teede HJ, Misso ML, Costello MF, et al., 2018; Endocrinology and Metabolism Branch of Chinese Physicians Association, 2018). The biochemical test is mainly total testosterone (total testosterone, TT), free testosterone (free testosterone, FT), dehydroepiandrosterone (dehydroepiandrosterone, DHEA), dehydroepiandrosterone sulfate

(dehydroepiandrosterone sulfate, DHEAS), 4-androstenedione (4-androstenedione, A4) as well as by calculating the free androgen index (FAI = (TT / SHGG) x100) (Cao Zhengruan, Xiang Yan, Zhai Yanhong, et al., 2023). At present, the measurement of serum TT level is the primary method for the increase of androgen in women, and the combination of anti-Miller tube hormone (anti-mullerian hormone AMH) and TT has high diagnostic value for PCOS (Zhao Li, Cai Zhaowei, Li Xueling, et al, 2020). The results show that elevated serum FT levels are the best indicator for the diagnosis of hyperandrogenemia (Escobar-Morreale HF, 2018). As a precursor of testosterone, A4 is important for determining the androgen levels in PCOS patients (Cao Zhengruan, Xiang Yan, Zhai Yanhong, et al, 2023). 2023 Expert Consensus on PCOS Androgen Mass spectrometry detection suggests that clinical attention should be paid to serum A4 detection (Niu Jingyun, Hou Lihui, Kou Lihui, et al, 2018). The detection of hyperandrogenism depends on the quality and type of androgen test used and the standard values of the control population. The simultaneous detection of TT, A4, DHEA-S and DHT by LC-M S/MS increases the detection rate of hyperandrogenism from 67.6% to 91.0% (Cao Z, Lu Y, Cong Y, Liu Y, et al, 2020).

2.4 Diagnosis of Ovulation Disorders in PCOS

Anovulatory menstrual cycles usually present as loose menstruation, secondary amenorrhea, or abnormal uterine bleeding, and frequent menstruation may occur in a few cases. Rare-onset ovulation is common in puberty, especially in the first year after menarche. The study found that the incidence of irregular menstrual cycles in adolescent PCOS varied greatly, with about 43% of less menorrhagia, 21% primary or secondary amenorrhea, 21% of regular menstrual cycles, 7% frequent menstruation, and 95% of adult women with PCOS having amenorrhea (Bekx MT, Connor EC & Allen DB, 2010). The 2018 Chinese Guidelines for the diagnosis and treatment of PCOS believe that the diagnosis of PCOS in adolescence should be based on all three criteria in Rotterdam, with a rare menstrual history of at least two years since menarche, and the diagnosis of PCOM by abdominal ultrasound should be based only on an increase in ovarian diameter (> 10ml). If the diagnosis cannot be confirmed, the patient should be carefully monitored until adulthood, and the diagnosis is reassessed if symptoms persist.

3. Possible Etiology of PCOS

3.1 The Disorder of the Hormone Levels

3.1.1 Increased Androgen Levels

Androgen excess is considered by some scholars to be essential for PCOS. A significant increase in testosterone levels is generally considered as a marker of hyperandrogenism in women with PCOS (Cao Zhengruan, Xiang Yan, Zhai Yanhong, et al, 2023). Androgens in women of childbearing age are produced together by the ovary and adrenal glands, and androgens in menopausal women are mainly derived from the adrenal gland. In physiological conditions, 50-60% of testosterone comes from A4, 25–30% is produced by the ovary and the rest by the adrenal cortex; A4 comes from the ovary and adrenal glands, the two organs each produce 50% A4 (Cao Zhengruan, Xiang Yan, Zhai Yanhong, et al, 2023). Eighty percent of circulating testosterone in women binds to sex hormone-binding globulin (sex hormone binding globulin, SHBG), 19% to albumin, only about 1% in the form of FT and plays the physiological role of androgen, and A4, DHEA and DHEAS exist in women (Lizneva D, Gavrilova-Jordan L, Walker W, et al, 2016).

In many women with PCOS, there is more than one source of androgen overproduction. DHEAS is the major marker of adrenal-derived androgens, and the elevated DHEAS in PCOS patients suggests that excessive androgens arise from the adrenal gland. Approximately 50% of PCOS women have elevated levels of DHEAS and 11-hydroxyandrostenedione in the circulation, both androgens secreted almost exclusively by the adrenal meshwork (FZ Stanczyk, L Chang, E Carmina, Z Putz, et al, 1991). KUMAR, A et al found that about 20–30% of human serum DHEAS was elevated in adult women with typical anovulatory PCOS (KUMAR A, WOODS KS, BARTOLUCCI AA, et al, 2005). The 2015 PCOS diagnosis and treatment guidelines indicated that 30% to 35% of PCOS patients had elevated DHEAS, and about 5% of PCOS patients only had elevated DHEAS as an androgen (GOODMAN NF, COBIN RH, FUTTERWEIT W, et al, 2015). It indicates that the increased androgens in PCOS patients are not only from the ovary, some patients have androgens from the adrenal gland, and even a few patients are only from the adrenal gland. The occurrence of this situation

does not rule out the unclear diagnosis of PCOS.

Among the most common androggenic PCOS, the ovary is the main source of androgen, and ovarian hyperandrogenemia is the main pathogenesis of this syndrome. The increase in androenedione and testosterone production by the ovaries is due not only to increased luteizing hormone (luteinising hormone LH) drive but also to increased primary androgen secretion by the follicle membrane cells (C GILLING-SMITH, H STORY, V ROGERS et al, 1997). In vitro studies show that the follicle membrane cells of the polycystic ovary can produce more A4 after both basal conditions and gonadotropin stimulation (C GILLING-SMITH, H STORY, V ROGERS et al, 1997). Hyperinsulinemia enhances androgen production stimulated by LH and insulin-like growth factor 1 (insulin like growth factor 1 IGF-1), and also enhances serum free testosterone levels by reducing hepatic SHBG production and enhances the biological activity of serum IGF-1 by inhibiting the production of IGF binding proteins (Goodarzi MO, Dumesic DA, Chazenbalk G, et al, 2011). Increased androgenesis combined decreased, resulting to increased free androgens is be the main cause of hyperandrogenism.

3.1.2 Steroid Hormone Production Pathway

Several lines of evidence suggest that disruption of the steroidogenic pathway caused by hyperandrogens or insulin resistance in PCOS women is a prominent factor in ovarian dysfunction and abnormal folliculogenesis (Catteau-Jonard S & Dewailly D, 2013). Steroid hormone-producing enzymes, mainly P450 side chain lyase (P450scc or Cyp11a1), cytochrome P450 aromatase (Cyp19a1), 3 β -hydroxysteroid dehydrogenase (3 β -HSD), and steroidogenic acute regulatory protein (StAR), play key roles in the regulation of the steroidogenic pathway (Mason HD, Willis DS, Beard RW, et al, 1994). Studies have shown that cholesterol is important in steroid hormone-producing cells (such as ovarian cells). P450scc and StAR are involved in transferring cholesterol from the outer mitochondrial membrane to the inner membrane and converting it to pregnenolone (Motta A, 2010). Pregnenolone acts as a substrate for progesterone synthesis through the mediated effects of the 3 β -HSD (Taira H & Beck M, 2006). Aromatase (Cyp19a1) is a key enzyme that plays a role in ovarian oestradiol production, converting androgens to oestradiol in granulosa cells (Motta A, 2010). D. Lin, S. M et al found that the 17,20-lyase activity of the $\triangle^4$ channel is almost completely absent in humans (D Lin, T Sugawara, JF Strauss 3rd, et al, 1995). In humans, almost all A4 is produced by the transformation of DHEA (Longcope C, 1986).

Altered steroidal enzymes and hormones in granulosa cells are important factors leading to follicle development and maturation and can lead to the disturbance of PCOS follicle development (Salilew-Wondim D, Wang Q, Tesfaye D, et al, 2015). Lee et al found that testosterone and androstadione significantly elevated 3 β -HSD and aromatase mRNA expression in human granulosa cells (Lee C-T, Wang J-Y, Chou K-Y, et al, 2014). Kozłowska Reported that the expression of P450scc, 3 β -HSD and STAR proteins involved in progesterone production and Cyp19a1 converting androgens to estradiol was also higher in PCOS women and animal models (Kozłowska, Majewski M & Jana B, 2009). Shabnam Bakhshalizadeh Reported that the steroidogenic enzymes, Cyp11a1, StAR, Cyp19a1, and 3 β -HSD were upregulated in the granule cells of PCOS mice induced by DHEA (Bakhshalizadeh S, Amidi F, Alleyassin A, et al, 2017). However, low aromatase activity in PCOS women, De Leo et al (VD Leo, MC Musacchio, V Cappelli, et al, 2016), Haq Shah reported that letrozole induced a decrease in Cyp11a1 and CYP19a1 expression and increased CYP17a1 expression in PCOS mice (Mohd Zahoor Ul Haq Shah, Vinoy Kumar Shrivastava & Manzoor Ahmad Mir Kehinde S Olaniyi, 2023). Although the activity of the enzyme varies in different reports, the disorder of the steroidogenesis pathway exists objectively, and the reason for this difference may be the different induction drugs and species used.

3.1.3 Imbalanced Hypothalamic-Pituitary-Ovarian (Hypothalamic-Pituitary-Ovarian HPO) Axis

The HPO axis imbalance is recognized as an important pathophysiological mechanism of PCOS (Liao B, Qiao J & Pang Y, 2021). Gonadotropin-releasing hormone (Gonadotropin releasing hormone GnRH) neurons reach the central hypothalamic bulge and pulses the GnRH peptide directly into the pituitary portal system, thereby driving the pituitary secretion of LH and FSH. GnRH stimulation causes excessive LH production in PCOS females (Moore AM & Campbell RE, 2017). This situation can be determined by the

higher frequency or amplitude of the GnRH (Esparza LA, Schafer D, Ho BS, et al, 2020). It is unclear whether the changes in the hypothalamic-pituitary axis in PCOS patients are primary or secondary to altered steroid hormone secretion. Hyperinsulinemia can promote the pituitary response to GnRH, leading to enhanced LH and androgen secretion, which in turn affects the function of the hypothalamic-pituitary-ovarian-gonadal axis (HPO axis) (Stepto NK, Cassar S, Joham AE, et al, 2013). PCOS causes increased secretion of GnRH and LH and a weaker response to exogenous estrogen and progesterone (Burt Solorzano CM, Beller JP, Abshire MY, et al, 2012), suggesting the impaired negative feedback effect of steroid hormones on GnRH neurons (Moore AM, Prescott M, Marshall CJ, et al, 2015). Now the neuropeptide treatment of PCOS is a hotspot and direction of research (Chen WH, Shi YC, Huang QY, et al, 2023).

Abnormal GNRH pulse release can result in an abnormal LH / FSH ratio. Abnormal gonadotropin secretion has been suggested to be the main cause of hyperandrogenism and anovulation (Diamanti-Kandarakis E & Dunaif A, 2012). Abnormal gonadotropin secretion patterns, including reduced or relative reduction in FSH production, increased LH production and increased LH / FSH ratio, are common hormonal features of PCOS. Excessive LH production during PCOS development will stimulate testosterone production and inhibit FSH production, leading to increased LH / FSH ratio and causing arrest of ovarian follicular esis (Franks S, Stark J & Hardy K, 2008). The elevated LH / FSH was mainly observed in lean women with PCOS, probably because obesity reduced the LH pulse amplitude and other LH pharmacokinetic structures (Pagán YL, et al, 2006). Some scholars believe that the elevated ratio of LH / FSH in the high proportion of PCOS women (55-75%) may be due to high LH levels rather than reduced FSH production (VD Leo, MC Musacchio, V Cappelli, et al, 2016). Thus, modulation of LH levels has become a target for PCOS therapy. Study reports suggest that treatment with inositol can significantly improve hormonal parameters by reducing LH levels and thus improve PCOS (Tessaro I, Modina SC, Franciosi F et al, 2015). Recent studies have shown that inositol and the first-line therapy metformin are equally beneficial in improving metabolic and hormonal parameters in patients with PCOS (Fatima K, Jamil Z, Faheem S, et al, 2023). Oral administration of FSH significantly reduced the severity of PCOS in mouse models by reducing the number of cystic follicles and restoring estradiol levels (Spritzer PM, Lecke SB, Satler F, et al, 2015).

3.2 Insulin Resistance (Insulin Resistance IR)

Hyperinsulinemia and IR promote each other, causing hyperandrogenism. In vitro studies have shown the presence of insulin receptors in the hypothalamus and pituitary, and insulin stimulates FSH and LH release through the receptor under basal conditions and after GnRH stimulation (Qiao J & Feng HL, 2011). Hyperinsulinemia is also thought to directly stimulate ovarian steroid hormone production by acting on the follicle membranes and granulosa cells, thus leading to hyperandrogenism. It has been shown that insulin stimulates membrane cell proliferation, increases the LH-mediated androgen secretion, and increases the cytochrome P450 expression of the LH and IGF-1 receptors (VD Leo, MC Musacchio, V Cappelli, et al, 2016). In vitro data obtained from a cell culture model suggest that co-incubation of insulin and FSH with bovine oocytes promotes upregulation of LH receptors on granulosa cells, which helps to block follicle growth, inhibit aromatase activity, and may trigger ovarian hyperandrogenism (VD Leo, MC Musacchio, V Cappelli, et al, 2016). Because the enzymes involved in ovarian steroidogenesis are similar to those of the adrenal gland, many studies suggest that insulin may directly stimulate adrenal steroidogenesis (Bremer AA & Miller WL, 2008). Metformin is an insulin-sensitizing drug that significantly reduces the production of 17 hydroxyprogesterone, T, and A4 in response to ACTH in women with PCOS (Bremer AA & Miller WL, 2008). In addition, insulin also affects hyperandrogenism by inhibiting the hepatic synthesis of SHBG 2 and IGFBP-1(Bremer AA & Miller WL, 2008). Many studies have shown that the IGF-1 / IGFBP-1 ratio is significantly increased in patients with PCOS (VD Leo, MC Musacchio, V Cappelli, et al, 2016). Increased availability of IGF-1 in membrane cells can induce increased androgen production. Furthermore, IGF-1 stimulates estrogen production in granulosa cells and cooperates with FSH and LH to regulate aromatase expression in granulosa cells. IGF-1, like insulin,

also indirectly controls ovarian steroidogenesis via the hypothalamic - pituitary axis. Indeed, it induces GnRH expression and gonadotropin release through the pituitary gland (Bremer AA & Miller WL, 2008). Insulin-sensitizing drug treatment increases IGFBP-1 levels, decreases the IGF-1 / IGFBP-1 ratio, and decreases the availability of IGF-1 in peripheral tissues (De Leo V, La Marca A, Orvieto R, et al, 2000).

Obesity is associated with IR, and approximately 30% of obese women with PCOS have impaired glucose tolerance. However, many studies have shown that IR is also present in many lean women with PCOS (Bremer AA & Miller WL, 2008). Mechanisms leading to IR include defects in insulin binding to its receptor or changes in insulin signalling (Bremer AA & Miller WL, 2008). However, the ovaries of these women maintained a roughly normal response to insulin. At increased insulin concentration, insulin acts on the ovary through the IGF-1 receptor. Furthermore, the action of insulin on the ovary uses the inositol glycan system as a signaling mediator, which is different from systems activated by receptor phosphorylation of tyrosine levels in other tissues (Nestler JE, Jakubowicz DJ, de Vargas AF, et al, 1998). Increased urinary clearance of inositol was observed in some American and Greek women with PCOS. It reduces the availability of inositol in the tissues. This mechanism may lead to the emergence of IR in PCOS women (Baillargeon JP, Nestler JE, Ostlund RE, et al, 2008).

3.3 Inflammatory Response and the Immune System

Patients with PCOS usually have low-grade chronic inflammation (Shorakae S, Teede H, de Courten B, et al, 2015). An increasing number of studies suggest that the metabolic effects of PCOS, including impaired glucose homeostasis, dyslipidemia, visceral obesity, and (potentially) cardiovascular disease, are closely associated with low-grade chronic inflammation combined with hyperandrogenism and hyperinsulinemia contributing to the development of PCOS (Shorakae S, Teede H, de Courten B, et al, 2015).

Increased pro-inflammatory cytokines and decreased anti-inflammatory factors have been implicated in the pathogenesis of PCOS, and this inflammatory state may impair insulin sensitivity and promote the development of PCOS (Bhatnager R, Jalthuria J, Sehrawat R, et al, 2019). Soysal C Found elevated proinflammatory cytokines and chemokines in granule cells, plasma, and monocytes in PCOS patients (Soysal C, Bıyık İ, İnce O, et al, 2002). TNF-a, IL-1b and IL-18 are 3 proinflammatory factors that drive inflammatory responses to various internal and external stimuli, and IL-10 is an anti-inflammatory factor that achieves its biological function by inhibiting the inflammatory response. TLR 4 also plays a key role in pro-inflammatory signaling, and SREBP1 is thought to be able to promote TLR 4-induced proinflammatory responses by reprogramming fatty acid metabolism (Dewailly D, Lujan ME, Carmina E, et al, 2013). We found that the levels of TNF-a, IL-1b and IL-18 were significantly increased in DHEA-induced rat ovarian tissues. EPA treatment could significantly reduce the inflammatory response in ovarian tissues of PCOS rats by inhibiting the SREBP 1 / TLR 4 pathway, and then improved the condition of PCOS (Wang YT, He JY & Yang J, 2018).

Oxygen radicals or reactive oxygen species act as mediators in various signaling pathways and also participate in inflammatory responses (Bremer AA & Miller WL, 2008). Lower mitochondrial O2 consumption and glutathione levels, together with an increased production of reactive oxygen species, explain the mitochondrial dysfunction observed in PCOS patients (Victor VM, Rocha M, Banuls C, et al, 2011). Monocytes in patients with PCOS are increased in this inflammatory state (Gonzalez F, Rote NS, Minium J, et al, 2006). This inflammatory state is more common in response to hyperglycemia and C-reactive proteins. Phyologic hyperglycemia increases monocytes producing reactive oxygen species, which then activates the release of TNF- α and increases the inflammatory transcription factor NF-KB. Thus, the known TNF- α concentration of IR media is further increased. The resulting oxidative stress creates an inflammatory environment that promotes IR and leads to hyperandrogenism (Costello MF, Shrestha B, Eden J, et al, 2007). Garruti, G et al. believe that PCOS-related inflammation may depend on visceral adipose tissue and that adipokines will also produce excessive local and systemic inflammatory responses, which will play a key role in the pathophysiology of PE / PIH (pre-eclampsia / pregnancy hypertension) and the birth of SGA infants (VD Leo, MC Musacchio, V Cappelli, et al, 2016).

Macrophages are also involved in the development of PCOS. Macrophages produce

cytokines, chemokines, and growth factors during the normal, inflammatory, and disease processes of the ovary and uterus. Ovarian macrophages can coordinate tissue remodeling and apoptosis, both of which are involved in folliculogenesis, ovulation, and luteal formation (Wu R, Van der Hoek KH, Ryan NK, et al, 2004). MCP-1 and macrophage inflammatory protein-1 α were elevated in PCOS patients (Glintborg D, Andersen M, Richelsen B, et al, 2009). The activity of transforming growth factor- β, nitric oxide synthase, and cyclooxygenase-2 was increased in the ovaries of PCOS patients (Hatzirodos N, Bayne RA, Irving-Rodgers HF, et al, 2011). Macrophages are one of the most abundant immune cells in uterine function (Lee SK, Kim CJ, Kim DJ, et al, 2015). Macrophages are scattered throughout the endometrium. They appear particularly around the glands (Song JY & Fraser IS, 1995). They are significantly increased in the inflamed endometrium, such as endometrial hyperplasia, endometrial cancer, the secretory phase (especially the premenstrual period), and implantation (Jiang XF, Tang QL, Li HG, et al, 2013). Placental macrophages may also promote inflammation within the placenta by secretion of proinflammatory cytokines such as IL-1, TNF- α, and IL-6 in cytotrophoblast and syncytiotrophoblast cells (Denison FC, Roberts KA, Barr SM, et al, 2010).

T cells also play an important role in PCOS. In the retroperitoneal or lumbar lymph node ovarian tissues and uterine tissues in DHEA-induced PCOS rats and mice (Denison FC, Roberts KA, Barr SM, et al, 2010) $CD4^+$ (auxiliary / induced) and $CD8^+$ (cytotoxic / inhibitory) increased. Metformin reduced the increase of $CD4^+$ number in the uterine tissues of PCOS mice (Jang M, Lee MJ, Lee JM, et al, 2014). Macrophages, $CD4^+$, and $CD8^+$ can release inflammatory mediators to modulate uterine function (Lee SK, Kim CJ, Kim DJ, et al, 2015). Endocrine disorders may be directly related to the differentiation and maturation of T lymphocytes. For example, estradiol and progesterone regulate the maturation of thymocytes, with autoimmune premature ovarian failure (Obradovic S, Vidic-Dankovic B, Pejcic-Karapetrovic B, et al, 2001). $CD8^+$ is increased in patients with premature ovarian failure (Yan G, Schoenfeld D, Penney C, et al, 2000), increased in the lymph node/ovarian tissues of PCOS rats induced by DHEA (Meikle AW, Dorchuck RW, Araneo BA, et al, 1992). The estrogen receptor α is a novel pathological marker expressed by follicular dendritic cells in lymph nodes (Sapino A, Cassoni P, Ferrero E, et al, 2003), estrogen deficiency after ooprectomy or menopause changes T lymphocyte differentiation (Safadi FF, Dissanayake IR, Goodman GG, et al, 2000). Serum estradiol also regulates T lymphocyte differentiation (Obradovic S, Vidic-Dankovic B, Pejcic-Karapetrovic B, et al, 2001; Yan G, Schoenfeld D, Penney C, et al, 2000). These results suggest that the immune system is involved in the pathogenesis of PCOS.

3.4 Vitamin D (Vitamin D VD) Deficiency

VD helps regulate calcium and phosphate levels in the body, maintain nutrients needed for bone, tooth, and muscle health, and helps prevent cancer, diabetes, migraine, and autoimmune diseases (Pittas AG, Dawson-Hughes B, Sheehan P, et al, 2019). VD deficiency has been demonstrated in patients with PCOS (THOMSON RL, SPEDDING S & BUCKLEY JD, 2012). Studies have suggested that VD deficiency may be associated with metabolic disorders. VD deficiency may lead to IR and inflammation, and studies have shown that VD improves glucose metabolism by increasing insulin production, insulin receptor expression and reducing pro-inflammatory cytokines. Therefore, therefore, some scholars believe that the effects of VD on metabolic and reproductive dysfunction associated with PCOS may be caused by IR (Mohan A, Haider R, Fakhor H, et al, 2023). VD deficiency may induce hyperandrogenism and IR in PCOS by reducing SHBG levels and the insulin receptor, respectively (Selimoglu H, Duran C, Kiyici S, et al, 2010). Using VD 3 as a supplement reduces IR in obese and IR patients with PCOS (Firouzabadi R, Aflatoonian A, Modarresi S, et al, 2012).

A role of VD deficiency in PCOS pathology is also demonstrated, which exacerbates the symptoms of PCOS (Rashidi B, Haghollahi F, Shariat M, et al, 2009). A comprehensive report of the role of VD in fertility disorders such as PCOS, premature ovarian failure, endometriosis, male infertility, and IVF techniques shows that VD plays an important role in the reproductive process. Despite the therapeutic effect of VD 3 on infertility, the mechanism is unclear (Pal L, Zhang H, Williams J, et al, 2016). VD receptors are expressed in follicle membrane cells,

granulosa cells, placenta, endometrium and pituitary (Luk J, Torrealday S, Perry GN, et al, 2012). VD 3 was found to regulate steroidogenesis in granule cells through AMPK (Bakhshalizadeh S, Amidi F, Shirazi R, et al, 2018). In the case of pregnenolone as a substrate, VD 3 attenuates progesterone production in granulosa cells by modulating the activity of the 3 β -HSD enzyme, and in the case of testosterone as a precursor substrate, VD 3-treated granulosa cells reduce 17 β -estradiol secretion by affecting aromatase activity. Thus, VD 3 plays a pivotal role in improving steroidogenesis and folliculogenesis in granulosa cells of patients with multiple PCOS. VD 3 also reduces abnormally elevated AMH receptor II (AMHR-II) and FSHRmRNA levels in PCOS patients (Merhi Z, Doswell A, Krebs K, et al, 2014). Furthermore, it was found that women with VD deficiency in PCOS, through calcium dysregulation, lead to impaired follicular development (THOMSON RL, SPEDDING S & BUCKLEY JD, 2012).

3.5 Obesity

The 2018 expert consensus stated that about 70% of PCOS patients had abnormal lipid metabolism (Endocrinology and Metabolism Branch of Chinese Physicians Association, 2018). Weight loss in women with PCOS was found to reduce circulating androgens and insulin levels, while improving hirsutism, menstrual and ovulation dysfunction, and dyslipidemia (Cena H, Chiovato L & Nappi RE, 2020).

Obesity is associated with the IR. Insulin can stimulate lipogenesis and lipogenesis, and can inhibit lipolysis (Shang Y, Zhou H, Hu M, et al, 2020), also plays a role in the dysregulation of adipokine secretion (Sadeghi HM, Adeli I, Calina D, et al, 2022). Adipokines are hormone-like mediators that control insulin sensitivity and energy balance, and excess adipose tissue accumulation in obesity is associated with the release of adipokines (Alenezi SA, Khan R, Snell L, et al, 2023). Paracrine dysregulation of cytokines secreted by macrophages to adipokines (e.g., adiponectin) facilitates the development of IR (Chazenbalk G, et al, 2010). Primary defects in insulin-mediated glucose transport, glucose transporter 4 production, and insulin-or adrenaline-regulated lipolysis in PCOS patients were reported (occasionally in myocytes and fibroblasts) (Ciaraldi TP, Aroda V, Mudaliar S, et al, 2009). Choe S. S suggests that the NLRP 3 inflammasome plays a key role in obesity-induced inflammation and IR (Choe SS, Huh JY, Hwang IJ, et al, 2016). Lukens J. R found that inhibition of the NLRP 3 inflammasome reduced obesity-associated inflammation and enhanced insulin sensitivity (Lukens JR, Dixit VD & Kanneganti TD, 2011). Evidence-based data clearly demonstrate the use of glucagon-like peptide 1 analogues in overweight / obese PCOS patients not only with weight loss but also improves insulin resistance and hyperandrogenemia (Baranowska-Bik A, 2022). Obesity and high androgens are also implicated.

Androgens can alter the fat distribution of PCOS, mainly in abdominal obesity (Sadeghi HM, Adeli I, Calina D, et al, 2022). It has been widely reported that abdominal obesity is associated with increased visceral adipose tissue, which is closely associated with disturbed adipokine secretion, proinflammatory activity, hyperinsulinemia, and insulin resistance. Kempegowda P Reported that thin women with PCOS, may also show changes in regional fat distribution due to hyperandrogenism (Kempegowda P, Melson E, Manolopoulos KN, et al, 2020). Zeng Xiying et al found that AMH was independently and negatively associated with central obesity, but had no significant association with general obesity (Zeng XY, HuangYX, ZhangML, ChenY & YeJW, 2022). However, the results of the MRI examination indicated that PCOS women exhibited overall obesity, rather than visceral fat predominance (Barber TM, Golding SJ, Alvey C, et al, 2008). Hyperandrogenism in PCOS leads to dysregulation of potentially harmful adipokine secretion (de Medeiros SF, Rodgers RJ, Norman RJ, 2021). Under physiological conditions, adiponectin inhibits androgen synthesis in ovarian follicle membrane cells, and studies have found that serum adiponectin levels are low in women with polycystic ovary syndrome (Lin K, Sun X, Wang X, et al, 2020). Obesity has also been associated with increased leptin secretion. High leptin levels affect aromatase activity in granulosa cells, which interrupting the conversion of androgen to estrogen (Zeng X, Xie YJ, Liu YT, et al, 2020).

3.6 Genetic Factors

3.6.1 Epigenetics

Epigenetics is a heritable phenomenon of altered gene expression levels due from non-DNA variation. Epigenetic changes in fetal life may be related to the developmental origin of PCOS.

Lambertini L et al made an analysis of global methylation in the cord blood of PCOS women and showed that intrauterine reprogramming and epigenetic features increase the risk of PCOS (Lambertini L, Saul SR, Copperman AB, et al, 2017). Increasing evidence suggests a role for epigenetic programming alterations in the etiology of PCOS. Genome-wide analysis of transcriptomic and DNA methylation status in adipose tissue and skeletal muscle revealed tissue-specific epigenetics, as well as transcriptomic differences between women with and without PCOS (Nilsson E, Benrick A, Kokosar M, et al, 2018). Danielle Hiam Found that genes regulating reproductive function are epigenetically reshaped in specific immune cells. Indicating a role of epigenetic reprogramming in the reproductive defects associated with PCOS (Hiam D, Simar D, Laker R, et al, 2019). Recent evidence suggests the presence of mitochondrial dysfunction and epigenomic dysregulation in PCOS pathophysiology. Jia and his group detected significant hypermethylation in the mitochondrial DNA-coding gene, the D loop, 12 S rRNA, 16 S rRNA, and ND 4 genes in gilt polycystic ovaries, resulting in poor oocyte quality and hyperhomocysteinemia in follicular fluid (Jia L, Li J, He B, et al, 2016). Communication between the mitochondria and the nucleus is essential for regulating the metabolic demands of the cells (Janssen JJE, Grefte S, Keijer J, et al, 2019). Methylation of nuclear-associated mitochondrial genes was also altered in PCOS. A study of leukocytes in PCOS females found that increased PGC-1 α promoter methylation was associated with reduced mtDNA copy number compared with control females (Zhao H, Zhao Y, Ren Y, et al, 2017).

3.6.2 Gene

Siddiqui S et al suggested that PCOS is a complex polygenic disease and that these genes affect ovarian function through various mechanisms of action and pathogenicity (Siddiqui S, Mateen S, Ahmad R, et al, 2022). Considering that inheritance is the main cause of PCOS, Bruni V et al (Bruni V, Capozzi A & Lello S, 2022). 20–40% of first-degree female relatives of women with PCOS are reported to be affected by the syndrome, and PCOS is more prevalent among family members compared to the general population（Kahsar-Miller MD, Nixon C, Boots LR, Go RC & Azziz R, 2001). Genes where PCOS have been shown to be involved in adrenal and ovarian steroidogenesis, as well as in hormonal responses to GNRH, androgens and insulin (Bruni V, Capozzi A & Lello S, 2022). The miRNA was shown to be a valid biomarker for PCOS diagnosis, where miR-222 was equally expressed in all PCOS cases, and other miRNA, such as miR-146a and miRNA-30c, were highly expressed (Throwba H, Unnikrishnan L, Pangath M, et al, 2022).

3.7 Disordered Intestinal Microflora

In 2012, Tremellen et al suggested that the pathogenesis of PCOS and the disordered intestinal flora were involved (Tremellen K & Pearce K, 2012). Intestinal flora is a symbiotic microorganism living in the human gut, composed of bacteria, viruses and fungi. According to the size of the flora, intestinal flora can be divided into main flora and subdominant flora. As the "second human genome", intestinal flora has become a hot spot in PCOS research in recent years.

The study found that the intestinal microbiota was associated with hyperandrogenism, inflammation, obesity, and IR. Kelley and Torres et al (Torres PJ, Ho BS, Arroyo P, et al, 2019; Kelley ST, Skarra DV, Rivera AJ, et al, 2016) found that the species and quantity of bacteria in the large intestine of letrozole-induced PCOS hyperandrogenism was decreased. Studies on the serum and follicular fluid components of PCOS patients found that intestinal microflora can mediate inflammatory response through LPS, branched-chain amino acids, short-chain fatty acids (a key factor causing obesity) and bile acids, thus affecting insulin sensitivity (An Jie, Zhou Qin & Xue Yifang, 2021). Ying Zhu et al found that Guizhi Fuling pill alleviated inflammation and improved insulin resistance in polycystic ovary syndrome by regulating intestinal flora (Zhu Y, Li Y, Liu M, Hu XD & Zhu HQ, 2020). The gut microbiota produces obesity by affecting the host diet and metabolism. The intestinal flora was found to increase host appetite by reducing G protein-coupled receptor 5 activation and 44 serotonin (Zhang H, Butoyi C, Yuan GY, et al, 2023). In addition, Cani found that the intestinal microflora can participate in the regulation of adipose tissue metabolism through the LPS-eCB regulation ring, leading to obesity and abdominal fat accumulation (Cani PD, Plovier H, Hul MV, et al, 2015). Many clinical studies have found that the gut microbiome composition varies between PCOS patients and healthy controls, and the regulation of intestinal

microbiota disorders may be a new direction for PCOS therapy.

4. Conclusion

PCOS has been associated with obesity, cardiovascular disease, sleep apnea, endometrial hyperplasia, and endometrial cancer. Although the pathogenesis of PCOS is not clear, hyperandrogenism, LH / FSH ratio, insulin resistance, obesity, genetic, confirmed in PCOS patients. Though the relationship between these factors is not completely clear, independent animal model suggests that androgen can independently induce PCOS status, and high androgen can lead to hormone production disorder, IR, obesity and other metabolic disorders. So we can guess the production of PCOS is due to excessive androgen cause ovarian lesions, leading to further production of high androgen, sustained high androgen stimulation lead to HPO axis disorder, due to the characteristics of the growth of the follicle growth, hormone levels persistent disorder, androgen can come from the ovary can also come from the adrenal gland and maternal, gene increased the susceptibility of specific population, obesity, IR and PCOS promote each other, can aggravate this process, lead to clinical symptoms, PCOS can also lead to the production of obesity, IR. But further research is still needed to improve this conjecture.

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