Factors Aiding in the Diagnosis of Polycystic Ovarian Syndrome

Lojain Alsolaiman[1]*,Walid Khaddam[1], Youssef Al Hussein[2]

[1]Department of Biochemistry and Microbiology, Faculty of Pharmacy, Al Baath University.

[2]Department of Obstetrics and Gynecology, Faculty of Human Medicine, Al-Baath University.

*Corresponding Author:

E-mail address: alsolaimanlojain@gmail.com , **Tel/Fax**: 0937007910 .

Abstract:

Polycystic ovarian syndrome (PCOS) is one of the most common issues facing women of reproductive age today. It has multiple health implications relating to reproduction, obesity and metabolic syndrome-related diseases, and is one of the most important endocrine disorders that doctors seek to treat.

Since PCOS is a multifactorial disease, diagnosis is complicated by the fact that some of the global criteria used are no longer adequate due to defects that have emerged, as well as the overlap between the physiological development of adolescents and some of the criteria used for adults. This makes diagnosis difficult. Therefore, this review focuses on some of the helpful diagnostic methods that can be used to confirm a PCOS diagnosis.

This review focused on the role of anti-Müllerian hormone (AMH) in the diagnosis of PCOS. AMH testing may have additional advantages over other international criteria, the most important of which is that it relies on a simple blood test that provides accurate results quickly and does not interfere with other tests.

1.Introduction:

Polycystic Ovarian Syndrome (PCOS) is of great interest worldwide as it is one of the most common endocrine disorders in women of reproductive age, often associated with enlarged and dysfunctional ovaries, elevated androgen levels and insulin resistance. It is estimated that approximately one in 10 women suffer from PCOS and its complications.

The exact pathogenesis of PCOS is not fully understood, although a high ratio of luteinising hormone (LH) to follicle-stimulating hormone (FSH) and increased frequency of gonadotropin-releasing hormone (GnRH) are known to be the underlying causes of PCOS, and studies suggest the role of various external and internal factors, including insulin resistance (IR), hyperandrogenism (HA), environmental and genetic factors, and it should be noted that PCOS increases the risk of other complications such as cardiovascular disease, type 2 diabetes, metabolic syndrome, depression and anxiety.

The diagnosis of PCOS according to the 2003 Rotterdam criteria is based on the presence of two out of three features: oligomenorrhoea/amenorrhoea, clinical/biochemical hyperandrogenism, and polycystic ovarian appearance on ultrasound after exclusion of secondary causes.

As PCOS is a complex, multi-system (hypothalamic-hypothalamic-pituitary-endocrine), heterogeneous disorder with long-term health complications, it is important to focus on the main factors contributing to this complexity, namely the diagnostic criteria for PCOS, which have evolved over time. As the diagnostic features of PCOS can vary significantly across the reproductive age range, based on BMI, ethnicity and other reasons, our study aimed to find a new and effective diagnostic method that would help to make a correct diagnosis as early as possible to avoid subsequent complications and start early treatment, namely to investigate the role of anti-Müllerian hormone in the diagnosis of PCOS and the role of anti-Müllerian hormone in the diagnosis of polycystic ovarian syndrome (PCOS).

2. Polycystic ovary syndrome:

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, with a prevalence of (8 -13%) in adult women and (3.4 - 19%) in adolescent girls **(1)**,first described by Dr Stein and Dr Leventhal in 1935 **(2)**. According to a recent

study, the cost of diagnosing and treating PCOS in the United States is more than $8 billion per year, not including the cost of serious comorbidities associated with PCOS **(3)**.

PCOS is often characterised by irregular menstrual cycles, hyperandrogenism and characteristic features on ovarian ultrasound. The confusion surrounding the diagnosis of PCOS stems from the heterogeneity of symptoms experienced by women with PCOS, and several health complications have been associated with PCOS, such as infertility, metabolic syndrome, obesity, impaired glucose tolerance, diabetes mellitus type 2 (DM-2), cardiovascular complications, depression, obstructive sleep apnoea (OSA), endometrial cancer, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH) and increased risk of pregnancy complications **(4)**. In addition, the clinical manifestations and complications of PCOS may vary with a woman's age **(5)**.

1.2 The role of genetics in PCOS:

PCOS is a multifactorial condition. More than 20 genetic loci contributing to the pathophysiology of PCOS have been identified in genome-wide association studies in different ethnic groups of women **(6)**, and one study showed that daughters of women with PCOS have a fivefold increased risk of developing PCOS **(7)**. These genes are involved in different levels of steroidogenesis and androgen pathways, some studies have estimated that around 70% of the disease is genetically caused, and the environment is a key factor in the expression of these genes and the development and progression of the disease **(8)**. One study found that girls with a genetic predisposition and exposure to certain environmental factors are more likely to express PCOS characteristics, and the most common environmental factors include obesity, insulin resistance and fetal exposure to androgens **(9)**.

2.2 History of diagnostic criteria for PCOS

Many of the global diagnostic criteria for PCOS originated decades ago when the pathogenesis of PCOS was not fully understood and the diagnosis was considered difficult and indirect. At the National Institutes of Health (NIH) National Conference on Polycystic Ovary Syndrome (PCOS) in 1990, hyperandrogenism and chronic anovulation were agreed as potential diagnostic features of PCOS, excluding other causes of hyperandrogenism and oligomenorrhoea **(10)** (Table 1), The ultrasound appearance of polycystic ovaries was added to the above criteria at the Rotterdam meeting in 2003, taking into account the heterogeneous nature of PCOS for early diagnosis **(11)**, and in 2006 the Androgen Excess and PCOS Society defined the AE-

PCOS criteria as a disorder of androgen excess and {polymenorrhea (or) polycystic ovaries (or) both} **(12)**.

PCOS is diagnosed by exclusion, meaning that disorders with similar clinical features, such as thyroid disease, Cushing's syndrome, hyperprolactinemia, congenital adrenal hyperplasia (CAH) and primary ovarian insufficiency, must be ruled out.

Table 1: Current diagnostic criteria for polycystic ovary syndrome **(13)**

International Diagnostic Standards	hyperandrogenism	Ovulation dysfunction	Appearance of polycystic ovary
NIH	Both criteria are required		
Rotterdam	Two out of three of the criteria		
AE-PCOS Society	Verified standard	One or both of these criteria	

3.2 PCOS phenotypes:

Four phenotypes of PCOS have been identified based on the syndrome's three main features: anovulation, hyperandrogenism and polycystic ovary appearance (see Table 2). These range from the most severe (phenotype A) to the least severe (phenotype D) **(14).**

Table 2: The four phenotypes of polycystic ovary syndrome (PCOS) **(14)**

Features	Phenotype A	Phenotype B	Phenotype C	Phenotype D
Clinical/Biochemical Hyperandrogenism	+	+	+	-
Ovulation dysfunction	+	+	-	+
Appearance of polycystic ovary	+	-	+	+

{(+) indicates the presence of the criterion, (-) indicates the absence of the criterion}

4.2 Pathophysiology of PCOS:

In order to understand the pathophysiology of PCOS, it is important to understand the anatomy and function of the ovary.

1.4.2 Ovarian structure:

The ovaries are located at the end of the fallopian tubes within the ovarian cavity, which is formed by the bifurcation of the external and internal iliac arteries. The anterior part of the ovary is connected to the medial umbilical ligament, while the posterior part is connected to the ureter and the internal iliac artery. The ovary sits above the uterine tube funnel along with the pelvic funnel ligament. The ovary is connected to the abdominal wall by the suspensory ligament of the ovary. This ligament carries both the ovarian artery and vein (Figure 1)**(15).**

A normal ovary is approximately 2 cm wide, 3.5 cm long and 1 cm thick — roughly the size of a golf ball. One study found that 69% of changes in ovarian size are due to age alone. The average ovarian size is 0.7 ml at two years of age, peaks at 7.7 ml by age 20, and then slowly decreases until menopause, when the average size is 2.8 ml **(16).**
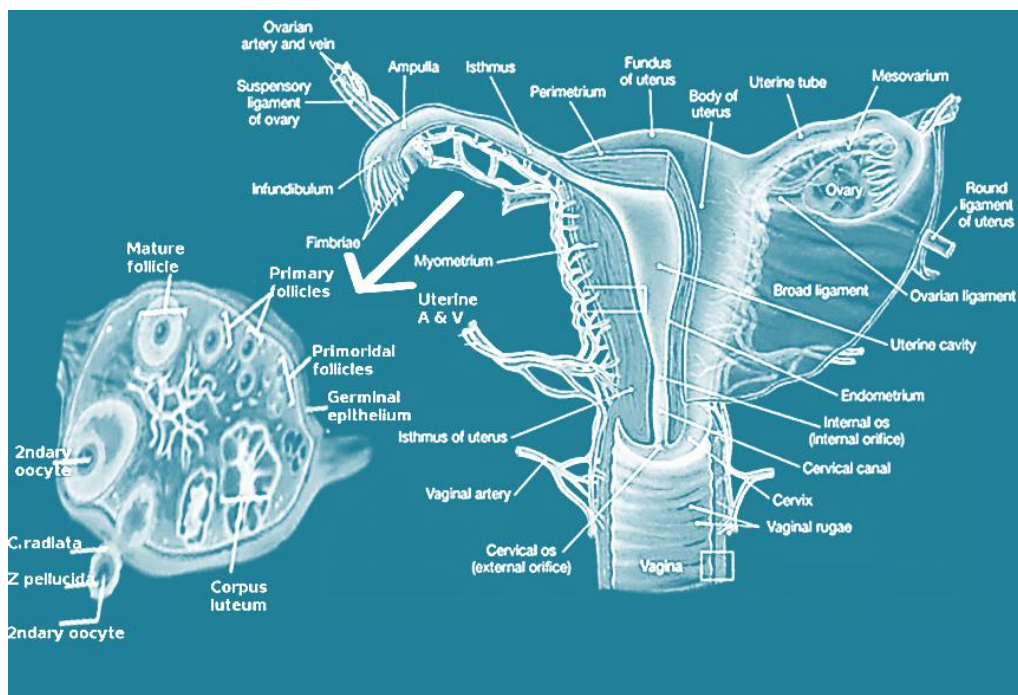

Figure 1: Anatomical structure of the ovary (22)

2.4.2 Ovarian function:

The ovary has two main functions: First, they produce hormones, especially during puberty when they begin to secrete increased levels of oestrogen, testosterone and progesterone in response to high levels of GnRH. GnRH also acts on cells in the anterior pituitary gland, which then produces FSH and LH. Second, they produce follicle-stimulating hormone (FSH) and luteinising hormone (LH). Secondly, the ovary contains follicles that grow temporarily and then stop growing until puberty. At puberty, these follicles resume growth, reaching an average diameter of 2–9 mm. During the menstrual cycle, these antral follicles enlarge until the dominant follicle is formed, while the others regress. The dominant follicle continues to grow until the egg matures and is released when LH is secreted by the pituitary gland, marking the stage of ovulation **(16).**
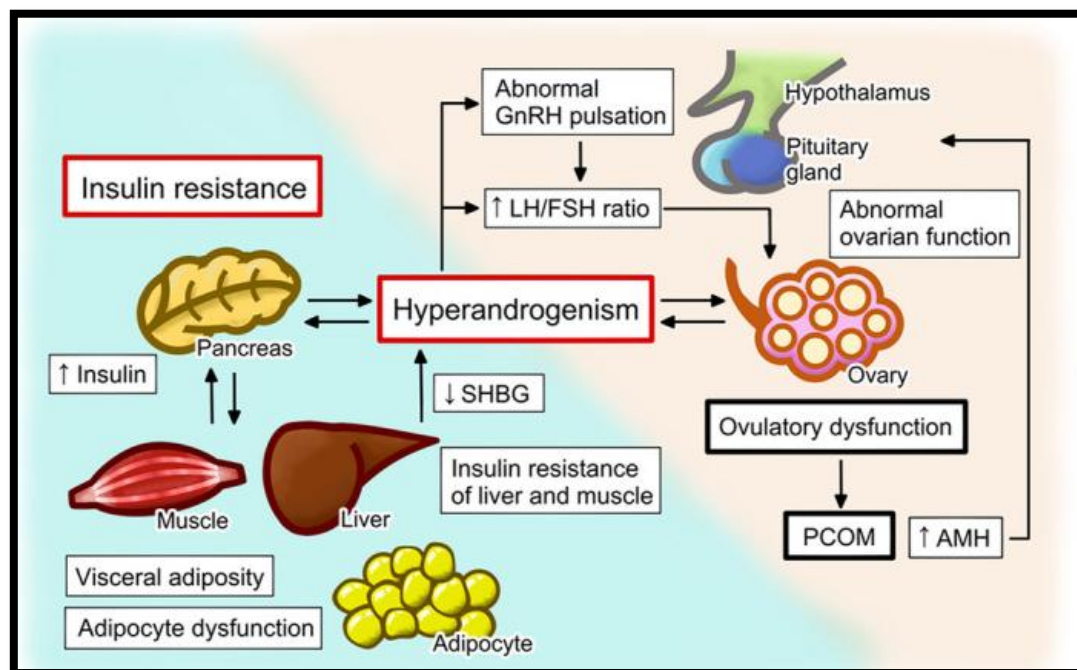

Figure 2: Pathophysiology of PCOS

Hyperandrogenism with ovulatory dysfunction, abnormal GnRH pulsatile secretion, and insulin resistance forms the defective loop that supports the pathophysiology of PCOS **(18).**

5.2 Key diagnostic criteria for PCOS:

PCOS is diagnosed in adult women when at least two of the three Rotterdam criteria are present. For adolescent girls, the World Health Organization initially defined

adolescence as the period between 10 and 19 years of age, during which critical changes in growth, puberty and development occur. The physiological changes involved, including menstrual irregularities, hyperandrogenism and polycystic ovary morphology (PCOM), overlap with the diagnostic criteria for PCOS in adults. This makes diagnosis during adolescence difficult and controversial **(19).** The Rotterdam criteria are not recommended for diagnosing PCOS in adolescents because they rely on the presence of two of the aforementioned features. Furthermore, PCOM should not be used in adolescents **(11).** Therefore, an accurate diagnostic approach and criteria are needed to provide a proper diagnosis during adolescence. Early diagnosis will enable the management of lifelong comorbidities associated with PCOS.

Diagnostic criteria should avoid overdiagnosis, which can cause unnecessary concerns about future fertility and other complications. At the same time, they should highlight the need for follow-up care for adolescent girls at risk of PCOS who do not meet the diagnostic criteria (Figure 4). Quality of life and healthcare improvement studies have emphasised the importance of educating people about the PCOS diagnostic criteria.

1.5.2 Menstrual irregularities and ovulatory dysfunction:

The first diagnostic criteria for PCOS (the NIH criteria) required oligomenorrhoea/amenorrhoea and anovulation for a PCOS diagnosis in adult women. However, some studies have shown that different criteria should be used for menstrual irregularity in adolescent girls. This is because menstrual irregularity is considered a physiological event that occurs in some menstrual cycles in the early years after the onset of menstruation, as regular ovulation may take time to establish itself in the years following the onset of menstruation. The gonadal axis is activated during puberty in a progressive manner, and menstruation does not necessarily indicate the full maturation of the hypothalamic–pituitary–ovarian axis hormonal feedback mechanism **(20).**

One study assessing ovulation in young, healthy women aged 16–24 years showed that up to one-third of cycles may be anovulatory, so determining ovulation by measuring serum progesterone levels in a single menstrual cycle — a method that has also been used to diagnose PCOS in adult women — is not recommended for adolescents.

A Dutch study emphasised the significance of menstrual irregularity during adolescence as an indicator of potential PCOS risk in a long-term study of adolescent girls **(21).** Consequently, adolescents exhibiting irregular periods or those unable to be categorised as such based on time since menarche, accompanied by

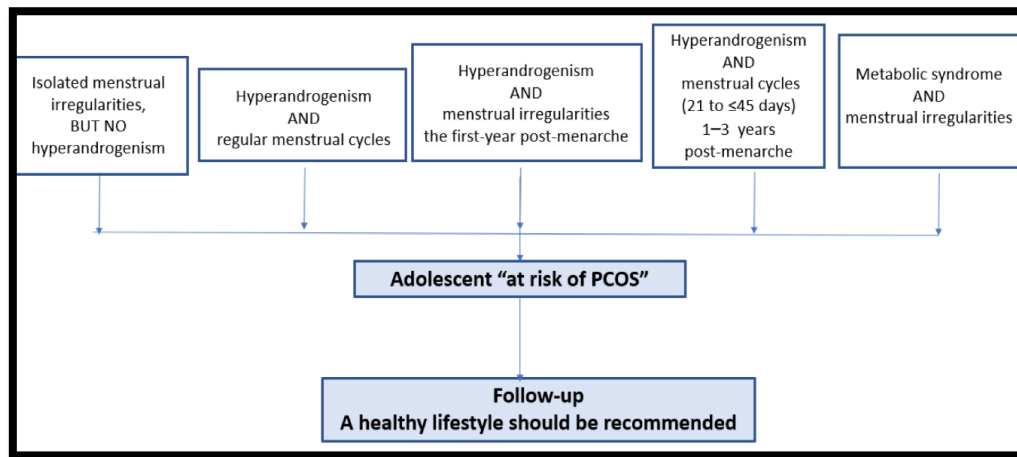hyperandrogenism, can be categorised as 'at risk of PCOS' and require monitoring (Figure 3).

Figure 3: Risk factors for PCOS in adolescent girls.

(From left to right): 1) Single menstrual irregularity without hyperandrogenism,

2) Hyperandrogen and regular menstrual cycle, 3) Hyperandrogenism with irregular menstrual cycle within the first year after menarche, 4) Hyperandrogenism and menstrual cycle (21 days or ≥45 days) within 1 to 3 years after menarche, 5) Metabolic syndrome with menstrual irregularities (21)

2.5.2 Hyperandrogenism:

Hyperandrogenism is categorised as either clinical or biochemical. Hirsutism, acne and alopecia are manifestations of clinical hyperandrogenism that require a thorough physical examination. The Ferriman-Gallwey scale is a point system that can be used to describe the prevalence of hirsutism in adult women, but it must take into account whether hair removal methods have been used, as the score will be affected if lasers, waxing or shaving have been used in the past three months **(23).**

Many adolescent girls develop mild features of clinical hyperandrogenism during puberty, which can make diagnosing PCOS difficult, as hirsutism and acne are the most common skin manifestations of PCOS in adolescent girls **(22).** The modified Ferriman-Gallwey (mFG) scale involves assessing nine body regions (upper lip, chin and neck, chest, upper and lower abdomen, upper arms, thighs, and upper and lower back) (Figure 4). These are categorised from 0 (no terminal hair growth) to 4 (extensive hair growth) depending on the extent of terminal hair growth at each site. solid hairs longer than 5 mm). For example, a score of 8–15 indicates moderate hirsutism, while a score greater than 15 indicates severe hirsutism. However, it should be noted that there are no studies confirming the optimal threshold for hirsutism in women of different ethnicities. Mild hirsutism may reflect racial variation or normal pubertal progression rather than indicating hyperandrogenism during adolescence **(24).**
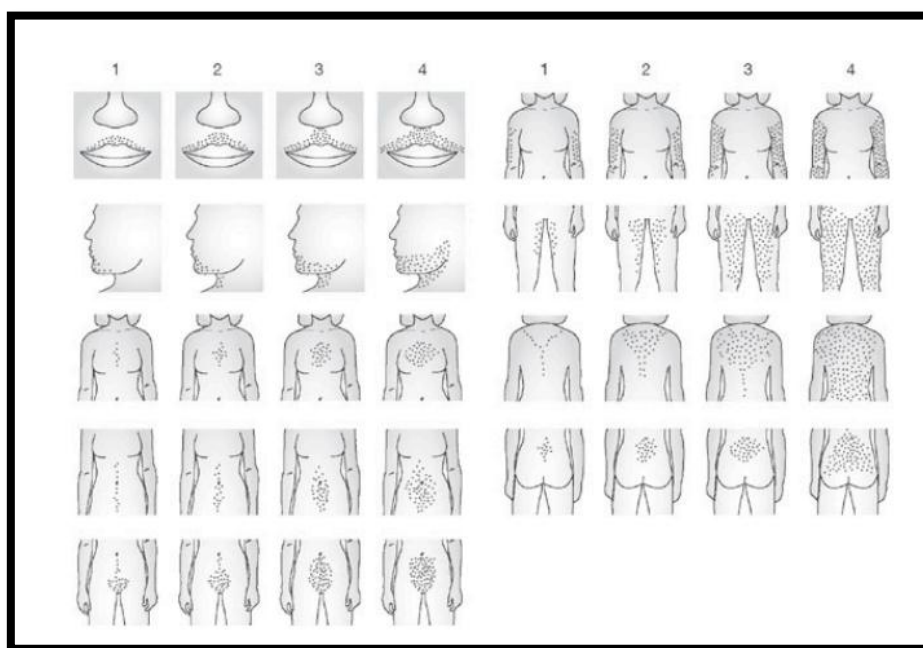

Figure 4: Hirsutism distribution areas (25)

Hirsutism must also be distinguished from hypertrichosis, which is defined as excessive growth of coarse hair in an androgenic pattern, often centred on the forearms or lower legs. Hyperandrogenism does not cause hypertrichosis, which may be hereditary and is common in girls of Mediterranean or Middle Eastern ethnicity. It may also result from malnutrition or certain medications, such as phenytoin or cyclosporine **(26).** At present, clinical or biochemical hyperandrogenism may not be accurately diagnosed, and it has not been determined whether the

prevalence of phenotypes A to D among PCOS patients varies by ethnicity. It is possible that clinical hyperandrogenism is often overlooked in East Asia because women have less dense villous hair. It has not yet been established whether the Ferriman–Gallwey (FG) scale can be used to diagnose hirsutism in ethnic groups with less dense villous hair **(24).** Another feature of clinical hyperandrogenism is androgenetic alopecia: diffuse thinning of the scalp hair around the crown that affects 28% of women with PCOS **(28).**

Although there is no consensus on a single scoring system for assessing recurrence severity, girls with more comedonal lesions that resist topical treatment and cause scarring generally experience more severe recurrence. A recent study also showed that women with PCOS have a higher prevalence of recurrence than women without PCOS **(27).**

Adolescents with isolated hyperandrogenism (clinical and/or biochemical) and regular menstrual cycles should not be diagnosed with PCOS, but they may be considered 'at risk' of developing it (Figure 3).

3.5.2 Clinical appearance of PCOM:

PCOM forms part of the Rotterdam diagnostic criteria for PCOS in adult women and is defined as the presence of 12 or more follicles per ovary, each measuring between 2 and 9 mm, and/or increased ovarian volume (>10 ml) **(29).** This threshold has evolved alongside advances in ultrasound imaging techniques, with the current criteria requiring the presence of 19 or 25 follicles per ovary **(30).**

International guidelines recommend against using pelvic ultrasound to diagnose PCOS in girls under 8 years old. There are two main reasons to avoid using pelvic ultrasound during adolescence: firstly, the majority of ultrasounds are performed transabdominally rather than transvaginally, which affects the accuracy of the results; and secondly, PCOM can be a transient condition in healthy adolescents, causing overlap between patients and healthy individuals **(31).**

4.5.2 Other features not included in the routine diagnostic criteria for PCOS:

This includes other tests that are useful for ruling out conditions that cause menstrual irregularities, hyperandrogenism and comorbidities associated with PCOS. These tests include blood tests, anti-Mullerian hormone (AMH) tests and tests for insulin resistance.

Blood tests:

Certain blood tests are necessary to diagnose PCOS in adult women and girls, and to rule out other disorders that can cause irregular menstrual cycles and/or hyperandrogenism. These tests include: HCG (if sexually active), LH, FSH, midnight

salivary cortisol, and 17-hydroxyprogesterone (17-OHP). Demirci and other researchers investigated whether any other marker could distinguish PCOS from congenital adrenal hyperplasia, finding that measurement of 17-OHP was necessary for this distinction **(32).** However, androstenedione may also be elevated in adrenal hyperplasia and mildly elevated DHEAS may be seen in adolescent girls with PCOS. Nevertheless, very high DHEAS levels are more likely to indicate the presence of an androgen-secreting tumour **(33).**

6.2 Some issues with the current diagnostic criteria:

The existence of different sets of criteria causes confusion for both physicians and patients. There are also multiple challenges with each of the three diagnostic elements of PCOS: Hyperandrogenism, oligomenorrhoea and the appearance of PCOS.

1) Hyperandrogenism can present clinically as hirsutism, hair loss, or increased hair growth (counting). Counting can only be considered a diagnostic criterion if it is severe. Modified Ferriman-Gallwey (mFG) visual scoring systems are used to assess the severity of hirsutism; however, no such system is available for counting **(23).**

2) Hirsutism manifests differently in different ethnic groups, so the values used to determine hirsutism must be reconsidered for different ethnicities. Self-managed hirsutism can also complicate clinical assessment **(34).**

3) Several biochemical issues arise with the laboratory tests available to measure free and total testosterone concentrations. Approximately 99% of testosterone in the blood is bound to proteins such as sex hormone-binding globulin (SHBG) and albumin. Only 1% circulates in the free, unbound form — the active form of testosterone. However, the most accurate method, equilibrium dialysis, is not widely available in many laboratories due to its high cost. Commonly available immunoassays that directly measure free testosterone levels are less accurate. SHBG levels can be low in women with PCOS, which may affect total serum testosterone concentration and Free Androgen Index (FAI) calculations, as well as the estimation of functionally effective testosterone **(35).**

4) A history of regular menstrual cycles does not rule out oligomenorrhoea, as it is a physiological condition that occurs around the time of the onset of menstruation and its definition varies with age. Girls who have menstrual cycles of less than 21 or more than 45 days one to three years after menarche are considered oligomenorrheic. After three years from menarche until menopause, adults who have periods of less than 21 or more than 35 days, or less than eight cycles per year, are considered oligomenorrheic **(36).**

5) The polycystic ovary appearance criterion cannot be used in women younger than eight years of age or premenopausal women. A transducer with a frequency higher than 8 MHz may be required to detect 20 or more follicles per ovary, although transvaginal ultrasound accurately measures ovarian size and antral follicle count. However, it is not suitable for virgin girls and can be difficult to use to accurately detect antral follicles, especially in obese women. Therefore, the search for a better diagnostic test or alternative standard is ongoing **(13).**

7.2 Treatment of PCOS:

Treatment for PCOS should be tailored to the specific needs of each patient. Goals of treatment may include alleviating symptoms of hyperandrogenism, stimulating ovulation, regulating the menstrual cycle and preventing cardiac complications. Menstrual irregularities, hirsutism and infertility are the most troublesome factors for women with PCOS and can cause significant social and psychological distress **(37).** Treatment is personalised based on the prevailing signs and symptoms, and due to the complex aetiology of the syndrome, it is rarely monotherapy, so several complementary therapies have been proposed for the management and treatment of PCOS.

1.7.2 Lifestyle modifications:

Dietary and lifestyle changes form the basis of PCOS management. As more than half of PCOS patients are overweight or obese, they are primarily advised to lose weight by following a balanced diet and exercising regularly, which increases their metabolism and improves their insulin sensitivity **(38).**

3.7.2 Metformin:

An essential component of the effective management of PCOS is a therapeutic approach that alleviates insulin resistance. Metformin, a biguanide, has been shown to be both safe and effective. It has long been used to treat type 2 diabetes and is one of the most commonly used insulin sensitizers in PCOS treatment. **(39).**

3 Anti-Mullerian hormone :

1.3 Molecular and genetic structure of AMH:

AMH, also known as Müllerian Inhibitory Substance (MIS), belongs to the transforming growth factor-β superfamily. It is a 140kDa homogeneous glycoprotein consisting of two identical glycoprotein subunits linked by disulfide bonds. In humans, the AMH gene, which is located on chromosome 19, encodes AMH. The AMH gene consists of five exons and is 275 amino acids in length.

The AMH gene encodes a protein consisting of 560 amino acid residues (pre-proAMH), which is then cleaved to produce proAMH (AMH25-560). ProAMH does not bind to the AMH receptor and undergoes further proteolytic cleavage by proto-oncoprotein subtyrosine/kexin convertase, resulting in the biologically active form, AMH N,C. AMH N,C is a complex consisting of an N-terminal (AMH N) and a C-terminal (AMH C) flange that are non-covalently linked. The AMHN flange is a 110-kDa homodimer composed of two 57-kDa subunits, while the AMHC flange is a 25-kDa homodimer composed of two 12.5-kDa subunits. Only the AMH N,C and AMHC isoforms bind to the AMH receptor (Figure 5). ProAMH and AMH N,C are both mobile forms that can be detected in varying proportions in the blood, while free AMHC and AMHN are undetectable in circulation under physiological conditions. Current commercially available immunoassays detect both pro-AMH and AMH-N,C; the reported values are a combination of both. The physiological role of pro-AMH in the circulation is currently unclear **(40).**
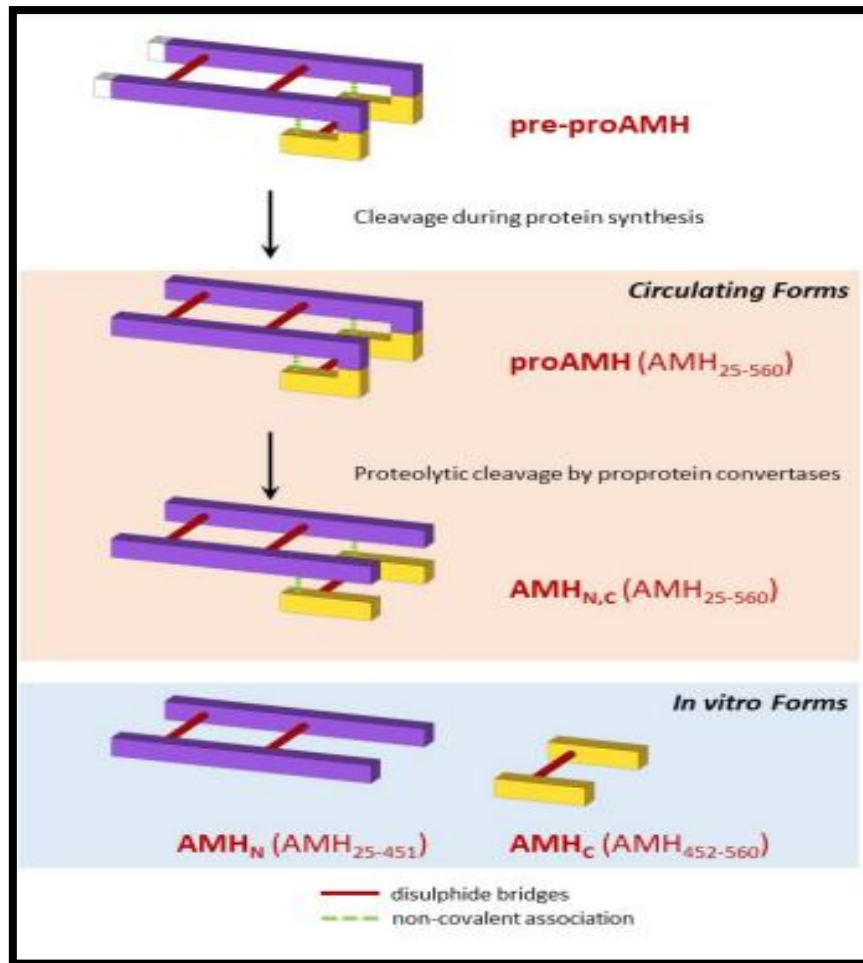
Figure 5: AMH polymorphisms resulting from cleavage

2.3 AMH physiology

Two types of anti-Mullerian hormone receptor (AMHR-I and AMHR-II) and SMAD proteins are involved in AMH signal transduction. AMH binds with great affinity to AMHR-II through its C-terminal. SMAD proteins are unique transcriptional molecules that transmit intracellular signals to promote nuclear and physiological effects **(41).**

Secretion of AMH by granulosa cells in the preantral and antral follicles of the ovary in females begins at 36 weeks of gestation. After a transient peak at birth, levels remain low until puberty, peaking at around 25 years of age before declining to undetectable levels at menopause (Figure 7) **(42).** While AMH secretion in males begins around the eighth week of pregnancy, it decreases in the first week after birth. It then increases rapidly during the first month, peaking at around six months of age. It subsequently decreases during childhood and declines to low levels at puberty. After sexual maturity, it continues to decline with age, mainly due to elevated testosterone levels (Figure 6). In males, AMH is secreted by immature

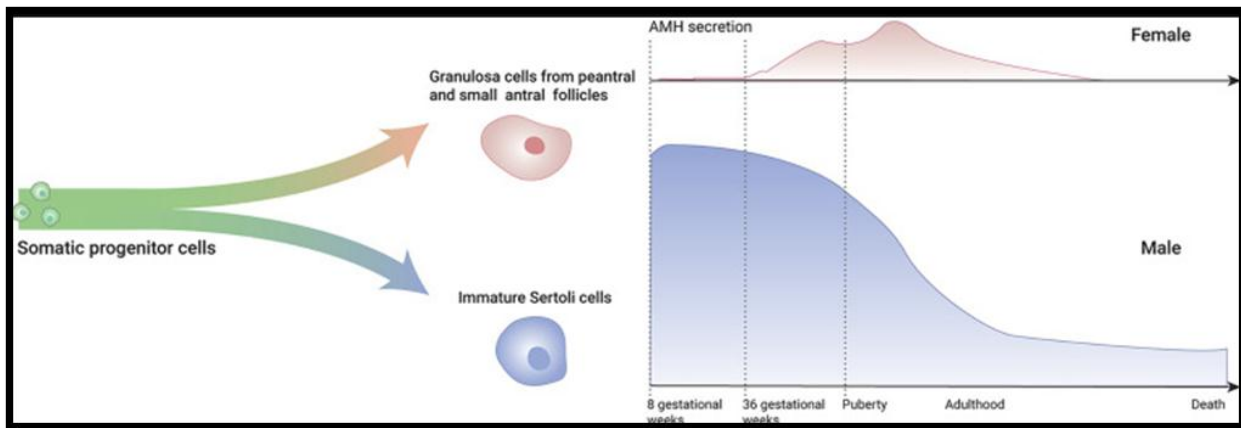Sertoli cells (SCs) in the testes, and its concentrations provide a valuable marker for these cells **(42).**

Figure 6: AMH production and level throughout life in both males and females (42)

AMH plays a critical role in prenatal sexual differentiation, suppressing the development of the Müllerian ducts in males. If a male foetus has low levels of AMH, or none at all, this can result in the simultaneous development of male and female reproductive organs. AMH is involved in ovarian follicle development and influences the hypothalamic–pituitary–ovarian (HPO) axis. At different stages of development, meanwhile, the regulation of AMH production may depend on a number of intracellular and extracellular signals. One such signal may come from the oocytes, which stimulate AMH secretion by releasing growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15). However, follicle-stimulating hormone (FSH) opposes the effects of GDF9 and BMP15, thus inhibiting AMH secretion **(41).**

The process of folliculogenesis involves two stages: initial recruitment and cyclic recruitment. AMH inhibits initial recruitment by preventing the transition of follicles from the initial follicle pool to the primary follicle stage. In cyclic recruitment, AMH reduces follicle sensitivity to follicle-stimulating hormone (FSH), and inhibits FSH-induced CYP19a1 expression. The inhibitory effect of AMH on FSH-induced CYP19a1 expression leads to lower levels of oestradiol (E2). In a study conducted in mice, it was shown that, in the absence of AMH, primordial follicles are recruited at a faster

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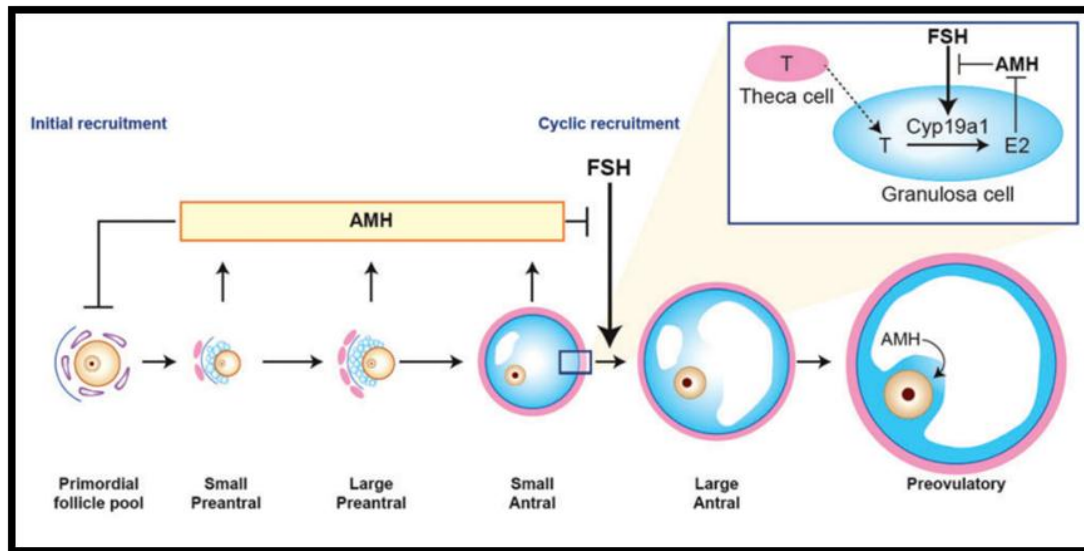rate, leading to the depletion of the primordial follicle pool at a younger age (Figure 7) **(43).**

Figure 7: Schematic model of the role of AMH in the ovary(43)

3.3 The relationship between AMH and AFC:

AMH is predominantly produced by the granulosa cells of follicles measuring between 2 and 9 mm (60%). Serum AMH levels are closely related to the number of developing follicles and can therefore be considered an indirect reflection of ovarian reserve. Thus, by assessing AMH levels, both ovarian reserve and PCOS can be predicted. There is a strong correlation between serum AMH levels and antral follicle count (AFC) or follicle number per ovary (FNPO). Several studies have examined the use of serum AMH levels versus FNPO for assessing ovarian reserve. The AMH test has been found to be more sensitive and specific than FNPO because it reflects the number of preantral and antral follicles measuring less than 2 mm, which are difficult to see on ultrasound. Therefore, serum AMH provides a more detailed assessment of the developing follicle pool than FNPO (Figure 8), and can be used as an alternative to FNPO **(44).**
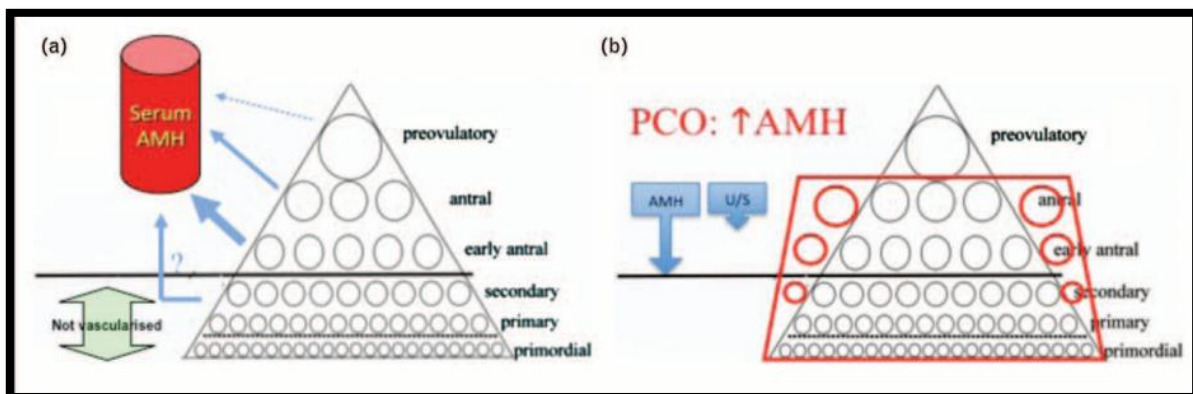
Figure 8: Using the AMH assay as a probe for PCOM appearance

a) Growing follicles secrete AMH, but serum AMH only reflects the secretion of larger follicles in contact with the vascular bed. In other words, AMH reflects the population of growing follicles, rather than the number of primitive follicles which do not secrete AMH.

b) In PCO, the number of growing follicles and the AMH concentration increase, meaning that AMH is a more sensitive indicator of follicular augmentation than AFC determined by ultrasound (44).

4.3 Factors affecting serum AMH:

To properly interpret serum AMH levels, it is critical to understand the factors that influence them.

Age: serum AMH levels are negatively correlated with age in adult women. However, studies have shown that this correlation depends on the age group analysed. It has been demonstrated that AMH levels are positively correlated with age until approximately 16 years of age. This positive correlation may reflect the increased rate of follicular recruitment. AMH levels continue to rise gradually until they peak at 25 years of age. From this point onwards, AMH levels begin to decline, reaching undetectable levels by the time of menopause. Therefore, from 25 years of age, an inverse relationship can be observed between AMH levels and age. This change appears consistent across different ethnicities. Studies have indicated significant differences in AMH levels between ethnic groups, such as white and African women. Similar variations have been observed in antral follicle count (AFC). However, significant variation in AMH levels also exists between individuals of the same age. Therefore, race and age must be considered when interpreting AMH values **(45).**

5.3 The Role of AMH in the Pathogenesis of PCOS:

AMH concentrations are normal in the physiological state. In the FSH-independent phase, AMH expression is lower in active primordial follicles and higher in small antral and preantral follicles. In the FSH-dependent phase, AMH expression is restricted; therefore, the absence of AMH leads to an increased level of oestradiol. Serum AMH levels are elevated in patients with PCOS, as shown in Figure 9. The elevated AMH level is 2–4 times higher in women with PCOS than in healthy women (63, 64) and has been observed in all patient groups. This increase is mainly observed in preantral and small antral follicles, which form due to an increased number of small follicles and increased secretion within each of them **(46).**

Initially, the increased AMH concentration in women with PCOS was thought to be due solely to the greater number of preantral and antral follicles. However, it was later discovered that AMH production in vitro was 75-fold higher in PCOS patients with anovulation and 20-fold higher in PCOS patients with normal ovulation than in healthy women **(46).**
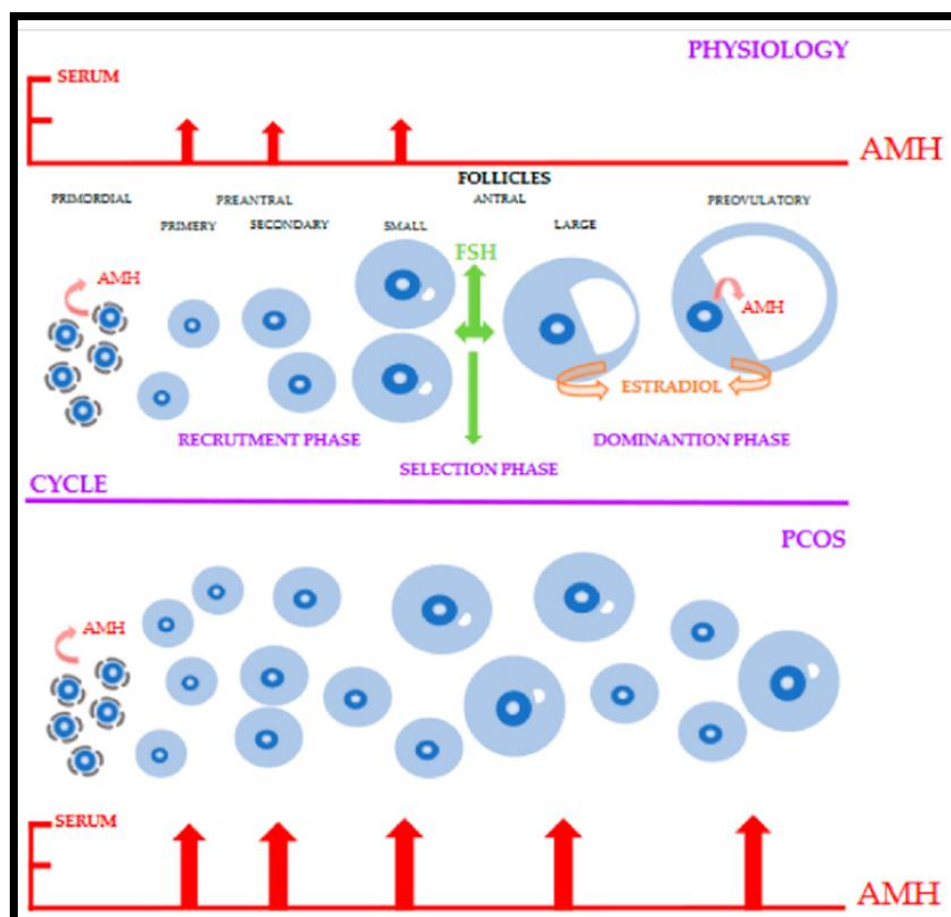

Figure 9: The role of AMH in physiological and pathological states.

In a physiological state, AMH expression is lower in active primordial follicles and higher in small antral and preantral follicles. In PCOS, AMH expression increases twofold, leading to an increased number of recruited follicles. Additionally, this elevated AMH level reduces FSH expression and oestrogen synthesis, thereby preventing the selection phase and follicle formation prior to ovulation .

The cause of this overproduction of AMH is still unknown, but some studies suggest that androgens play a role in this process due to the intrinsic dysregulation of granulosa cells, where an increase in AMH type II receptors has been observed. This excess AMH has been shown to play a key role in the follicular arrest characteristics of PCOS by adversely affecting aromatase expression and FSH action. The effect of FSH on serum AMH production can be illustrated as follows: In small antral follicles, FSH can stimulate AMH production directly. However, in large follicles, FSH increases the production of oestradiol (E2), which inhibits AMH expression via a negative feedback mechanism. This explains why AMH expression and levels of AMH in follicular fluid are reduced in FSH-dependent follicles in women with normal ovulation. This mechanism is disrupted in PCOS patients, in whom the simultaneous increase in E2 levels is absent. Consequently, AMH levels remain elevated in PCOS patients **(47).**

Clinical studies have demonstrated the stimulatory role of LH in AMH production. Both granulosa cells and box cells of antral follicles express higher levels of LH receptors in women with PCOS compared to healthy women. This stimulates elevated LH, which increases the production of ovarian androgens **(48).** Furthermore, some studies suggest that AMH may contribute to neuroendocrine dysregulation in PCOS. This hypothesis is based on the identification of AMHR2 expression in hypothalamic GnRH afferent neurons in both rodents and humans, indicating a potential positive feedback loop involving AMH, GnRH, and LH in PCOS **(49).**

6.3 The Role of Anti-Müllerian Hormone (AMH) in Diagnosing PCOS:

AMH has been suggested as a single diagnostic test for PCOS, or even as an alternative to antral follicle count (AFC), due to the strong correlation between circulating AMH levels and the number of antral follicles calculated by ultrasound. However, the results of studies based on these suggestions have been inconsistent, so identifying a specific AMH threshold for diagnosing PCOS or as an alternative to AFC is a major challenge **(50).** Disagreement among researchers is related to the age groups and phenotypes studied. In addition, technical issues such as different

thresholds and assay devices lead to heterogeneous results. Differences also arise from the inclusion or exclusion of study samples.

For these reasons, serum AMH levels should not be used as a single test to diagnose PCOS. However, it is likely that, with improved standardisation of laboratory assays and cut-off levels, AMH will become an accurate diagnostic test for PCOS.

As can be seen from the above, AMH concentration is significantly higher in PCOS patients than in healthy women. Therefore, it is clear that AMH is a valuable tool for diagnosing PCOS. AMH levels can be particularly useful when it is difficult to evaluate the ovaries during ultrasound examinations, for example in obese patients and virgins.

7. Conclusion:

Although several studies have observed elevated levels of anti-Mullerian hormone (AMH) in patients with polycystic ovary syndrome (PCOS), the World Health Organization (WHO) does not permit its use as a stand alone diagnostic tool. However, AMH can be used alongside other criteria to increase diagnostic certainty.

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