Endocrine and Metabolic Science 14 (2024) 100159

Contents lists available at ScienceDirect

Endocrine and Metabolic Science

journal homepage: www.elsevier.com/locate/endmts

Review

When one size does not fit all: Reconsidering PCOS etiology, diagnosis, clinical subgroups, and subgroup-specific treatments

V. Unfer [a,b,*], E. Kandaraki [a,c], L. Pkhaladze [a,d], S. Roseff [a,e], M.H. Vazquez-Levin [a,f], A.S. Laganà [a,g], C. Shiao-Yng [a,h], M.I.M. Yap-Garcia [a,i], N.D.E. Greene [a,j], C.O. Soulage [a,k], A. Bevilacqua [a,l], S. Benvenga [a,m], D. Barbaro [a,n], B. Pintaudi [a,o], A. Wdowiak [a,p], C. Aragona [a,q], Z. Kamenov [a,r], M. Appetecchia [a,s], G. Porcaro [a,t], I. Hernandez Marin [a,u], F. Facchinetti [a,v], T. Chiu [a,w], O. Pustotina [a,x], O. Papalou [a,c], M. Nordio [a,y], T. Cantelmi [a,z], P. Cavalli [a,aa], I. Vucenik [a,ab], R. D'Anna [a,ac], V.R. Unfer [a,ad], S. Dinicola [a,q], S. Salehpour [a,ae], A. Stringaro [a,af], M. Montaninno Oliva [a,ag], M. Tugushev [a,ah], N. Prapas [a,ai], M. Bizzarri [a,q,aj], M.S.B. Espinola [a,q], C. Di Lorenzo [a,ak], A.C. Ozay [a,al], J. Nestler [a,am]

[a] *The Experts Group on Inositol in Basic and Clinical Research (EGOI), 00161 Rome, Italy*
[b] *UniCamillus-Saint Camillus International University of Health Sciences, 00156 Rome, Italy*
[c] *Department of Endocrinology and Diabetes, HYGEIA Hospital, Marousi, Athens, Greece*
[d] *Ioseb Zhordania Institute of Reproductology, Tbilisi, Georgia*
[e] *Reproductive Endocrinology and Infertility, South Florida Institute for Reproductive Medicine (IVFMD), FL, USA*
[f] *National Council of Scientific and Technical Research, Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires, Argentina*
[g] *Unit of Gynecologic Oncology, ARNAS "Civico – Di Cristina – Benfratelli", Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, 90127 Palermo, Italy*
[h] *Department of Obstetrics and Gynecology, Yong Loo Lin School of Medicine, National University of Singapore, and the Singapore Institute for Clinical Sciences, A*STAR, Singapore*
[i] *St. Luke's Medical Center College of Medicine, William H. Quasha Memorial, Philippines*
[j] *Newlife Birth Defects Research Centre and Developmental Biology and Cancer Program, Institute of Child Health, University College London, London, UK*
[k] *University of Lyon, INSERM U1060, CarMeN, INSA de Lyon, Université Claude Bernard Lyon 1, Villeurbanne, France*
[l] *Department of Dynamic and Clinical Psychology, Sapienza University, Rome, Italy*
[m] *Department of Clinical and Experimental Medicine, University of Messina, Italy*
[n] *Director of U.O. Endocrinology in Livorno Hospital, USL Nordovest Toscana, Italy*
[o] *ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy*
[p] *Diagnostic Techniques Unit, Medical University of Lublin, Poland*
[q] *Systems Biology Laboratory, Department of Experimental Medicine, Sapienza University, Rome, Italy*
[r] *Department of Internal Medicine, Medical University of Sofia, Sofia, Bulgaria*
[s] *Oncological Endocrinology Unit, Regina Elena National Cancer Institute, IRCCS, Rome, Italy*
[t] *Women's Health Center, USL Umbria, 2, Terni, Italy*
[u] *Human Reproduction Department, Hospital Juárez de México, and Universidad Nacional Autónoma de México (UNAM), México City, Mexico*
[v] *Mother-Infant Department, University of Modena and Reggio Emilia, Modena, Italy*
[w] *IVF Centre, Hong Kong, China*
[x] *Sechenov Moscow Medical University and Post-graduation in Obstetrics & Gynecology at Scientific Center for Obstetrics, Gynecology, and Perinatology, Moscow, Russia*
[y] *A.S.L. RMF, Civitavecchia, Italy*

* Corresponding author at: UniCamillus-Saint Camillus International University of Health Sciences, 00156 Rome, Italy.
E-mail addresses: vunfer@gmail.com (V. Unfer), elenkand@gmail.com (E. Kandaraki), lpkhaladze@yahoo.com (L. Pkhaladze), dr.roseff@ivfmd.com (S. Roseff), mhvazl@gmail.com (M.H. Vazquez-Levin), antoniosimone.lagana@unipa.it (A.S. Laganà), obgchan@nus.edu.sg (C. Shiao-Yng), marisyap@hotmail.com (M.I.M. Yap-Garcia), n.greene@ucl.ac.uk (N.D.E. Greene), christophe.soulage@univ-lyon1.fr (C.O. Soulage), arturo.bevilacqua@uniroma1.it (A. Bevilacqua), s.benvenga@live.it (S. Benvenga), danielebarbaro1970@libero.it (D. Barbaro), basilio.pintaudi@ospedaleniguarda.it (B. Pintaudi), wdowiakartur@gmail.com (A. Wdowiak), aragonacesare@gmail.com (C. Aragona), zkamenov@hotmail.com (Z. Kamenov), marialuisa.appetecchia@ifo.it (M. Appetecchia), giusy.porcaro@gmail.com (G. Porcaro), marime64@hotmail.com (I. Hernandez Marin), fabio.facchinetti@unimore.it (F. Facchinetti), Tony.Chiu@ivf.hk (T. Chiu), pustotina@gmail.com (O. Pustotina), olinpap@hotmail.com (O. Papalou), maurizionordio1@gmail.com (M. Nordio), toninocantelmi@tiscali.it (T. Cantelmi), pietro.cavalli@gmail.com (P. Cavalli), ivucenik@som.umaryland.edu (I. Vucenik), rdanna@unime.it (R. D'Anna), virginia.unfer@icloud.com (V.R. Unfer), simonadinicola.sd@gmail.com (S. Dinicola), saghar.salehpour2014@gmail.com (S. Salehpour), annarita.stringaro@iss.it (A. Stringaro), dr.montanino@gmail.com (M. Montaninno Oliva), m.tugushev@yahoo.com (M. Tugushev), nikos@iakentro.gr (N. Prapas), mariano.bizzarri@uniroma1.it (M. Bizzarri), espinolasalome@gmail.com (M.S.B. Espinola), cherub@inwind.it (C. Di Lorenzo), dr.alicenk@gmail.com (A.C. Ozay), john.nestler@vcuhealth.org (J. Nestler).

https://doi.org/10.1016/j.endmts.2024.100159
Received 27 September 2023; Received in revised form 4 January 2024; Accepted 13 January 2024
Available online 15 January 2024

[z] Psychopathology, Institute of Psychology of the Gregorian University, Rome, Italy
[aa] Humanitas Research Hospital, Rozzano, Milan, Italy
[ab] Department of Medical & Research Technology and Pathology, University of Maryland School of Medicine in Baltimore, MD, USA
[ac] Department of Human Pathology, University of Messina, Messina, Italy
[ad] A.G.Un.Co. Obstetrics and Gynaecology Center, Rome, Italy
[ae] IVF Center at Ayatollah Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[af] National Center for Drug Research and Evaluation, Italian National Institute of Health, Rome, Italy
[ag] Department of Obstetrics and Gynecology, Santo Spirito Hospital, Rome, Italy
[ah] Department of Reproductive Medicine, Clinical Embryology and Genetics of Samara State Medical University, Russia
[ai] Third Department of OB-GYNAE, Aristotle University of Thessaloniki, and IVF Laboratory, IAKENTRO Fertility Centre, Thessaloniki, Greece
[aj] Department of Experimental Medicine, University La Sapienza, Via A. Scarpa 16, 00160 Rome, Italy
[ak] Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome Polo Pontino, Latina, Italy
[al] Obstetrics and Gynecology at Cyprus International University - Department of Obstetrics and Gynecology, Nicosia, Cyprus
[am] Division of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA, USA

ARTICLE INFO

Keywords:
Polycystic Ovary Syndrome
Phenotypes
Rotterdam Criteria
Insulin-resistance
Hyperandrogenism
Endometrial thickness

ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder that affects a large proportion of women. Due to its heterogeneity, the best diagnostic strategy has been a matter of contention. Since 1990 scientific societies in the field of human reproduction have tried to define the pivotal criteria for the diagnosis of PCOS. The consensus Rotterdam diagnostic criteria included the presence of hyperandrogenism, oligo/anovulation, and polycystic ovarian morphology (PCOM), and have now been updated to evidence based diagnostic criteria in the 2018 and 2023 International Guideline diagnostic criteria endorsed by 39 societies internationally. Within the Rotterdam Criteria, at least two out of three of the above-mentioned features are required to be present to diagnose PCOS, resulting in four phenotypes being identified: phenotype A, characterized by the presence of all the features, phenotype B, exhibiting hyperandrogenism and oligo-anovulation, phenotype C, presenting as hyperandrogenism and PCOM and finally the phenotype D that is characterized by oligo-anovulation and PCOM, lacking the hyperandrogenic component. However, it is the hypothesis of the EGOI group that the Rotterdam phenotypes A, B, and C have a different underlying causality to phenotype D. Recent studies have highlighted the strong correlation between insulin resistance and hyperandrogenism, and the pivotal role of these factors in driving ovarian alterations, such as oligo-anovulation and follicular functional cyst formation. This new understanding of PCOS pathogenesis has led the authors to hypothesis that phenotypes A, B, and C are endocrine-metabolic syndromes with a metabolic clinical onset. Conversely, the absence of hyperandrogenism and metabolic disturbances in phenotype D suggests a different origin of this condition, and point towards novel pathophysiological mechanisms; however, these are still not fully understood. Further questions have been raised regarding the suitability of the "phenotypes" described by the Rotterdam Criteria by the publication by recent GWAS studies, which demonstrated that these phenotypes should be considered clinical subtypes as they are not reflected in the genetic picture. Hence, by capturing the heterogeneity of this complex disorder, current diagnostic criteria may benefit from a reassessment and the evaluation of additional parameters such as insulin resistance and endometrial thickness, with the purpose of not only improving their diagnostic accuracy but also of assigning an appropriate and personalized treatment. In this framework, the present overview aims to analyze the diagnostic criteria currently recognized by the scientific community and assess the suitability of their application in clinical practice in light of the newly emerging evidence.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a complex disorder affecting the 10–13 % of women of reproductive age (Teede et al., 2023; Joham et al., 2022), and is routinely described as the most common endocrinological disorder of women in this age group. Stein and Leventhal initially defined PCOS in 1935 as a condition associated with clinical evidence of bilateral polycystic ovaries, amenorrhea or oligomenorrhea, obesity, hirsutism, and acne (Stein and Leventhal, 1935). The heterogeneity of PCOS, diversity of its clinical manifestation, uncertainty about its root causes, and a history of misdiagnosis have characterized the condition since its discovery and continue to be a point of debate (Stein and Leventhal, 1935; Gibson-Helm et al., 2017).

In 1990, the National Institutes of Health (NIH) laid the groundwork for the diagnostic criteria of PCOS, establishing oligo-anovulation and hyperandrogenism as diagnostic features in exclusion of other causes of androgen excess such as 21-hydroxylase deficiency, Cushing's Syndrome, thyroid dysfunction, and hyperprolactinaemia (*Hum. Reprod.*, 2004).

In 2003 the American Society for Reproductive Medicine (ASRM) and the European Society for Human Reproduction and Embryology (ESHRE), reaffirmed PCOS as "a syndrome", but expanded its definition to three diagnostic features: (i) oligo-anovulation, (ii) hyperandrogenism (clinical and/or biochemical), and (iii) polycystic ovaries detected *via* ultrasound examination. Since the presence of two out of the three criteria is required for PCOS diagnosis, the Rotterdam criteria resulted in four phenotypes (Fig. 1) (Gibson-Helm et al., 2017).

A recent international epidemiological survey indicated the prevalence of each phenotype. Specifically, phenotype A showed higher prevalence in all areas 44.8 %, the other phenotypes were reported as phenotype B 14.9 %, phenotype C 16.2 %, and phenotype D 19.5 % (Chiaffarino et al., 2022); however, this varies depending on region, for example hyperandrogenism rates are lower in East Asia resulting in a larger percentage of women with phenotype D (Cao et al., 2019; Lee et al., 2022).

In 2006, the expert committee of Androgen Excess and PCOS Society (AE-PCOS) suggested a more restrictive way for diagnosis of PCOS, considering only the first three phenotypes, and thus implying the presence of hyperandrogenism as a fundamental hallmark of the disease (Azziz et al., 2006).

Subsequently, several other organizations provided updated guidelines for PCOS diagnosis such as the National Institutes of Health (NIH) in 2012 (NIH, 2012), the American Association of Clinical Endocrinologists (AACE) in 2015 (Goodman et al., 2015), and the International evidence-based Guidelines (written by a consortium of 39 global societies) in 2018 and 2023 (Teede et al., 2023; Teede et al., 2018). Below

are reported some of the notable recommendations and findings from the 2018 and 2023 International evidence-based Guidelines:

- The necessity of improved diagnostic techniques with a focus on improving the accuracy of diagnosis.
- The need for larger cohort studies.
- Updated therapy recommendations.
- Updated details on the parameters examined for the assessment of PCOS, adding Anti-Müllerian Hormone (AMH) levels to the diagnostic criteria.
- Details on adolescent PCOS management.
- The need for evidence-based medical therapy.

Notably, insulin resistance (IR) is not included in the 2023 version of the International evidence-based Guidelines, based on insufficient evidence to date. Recently the characterization of the clinical presentations as "phenotypes" has been questioned, as the Rotterdam classification is not reflected within the genetic picture according to GWAS studies, with the authors stating that the Rotterdam "phenotypes" are rather clinical subgroups (Dapas et al., 2020; Dapas and Dunaif, 2022). To reflect these findings more accurately, from hereout, this review refers to the phenotypes as clinical subgroups. In this context, the individual diagnostic criteria described by Rotterdam have not been demonstrated to be directly heritable, with the Rotterdam clinical subgroups not showing predictive occurrence within family groups. Further analysis of the GWAS data revealed two genetically distinct groups: a metabolic group characterized by higher BMI, glucose, and insulin levels in addition to lower SHBG and LH levels, and a reproductive group characterized by higher LH, SHBH levels coupled with lower BMI and insulin levels, further study is required to see how this can influence clinical practice (Dapas et al., 2020).

Recently, the Experts Group on Inositol in Basic and Clinical Research (EGOI) hypothesized that the diagnostic criteria for assessing PCOS should take into account metabolic irregulates that are commonly observed in PCOS (Unfer et al., 2023a), as much evidence has demonstrated a correlation between PCOS and various metabolic issues such as dyslipidemia and altered insulin status (Chen and Pang, 2021; Anagnostis et al., 2018). The EGOI suggested the consideration of the inclusion of IR, as a diagnostic marker, since IR affects around 75 % of PCOS patients and can cause hyperandrogenism (Cassar et al., 2016; Tosi et al., 2021). Although published literature indicates that IR is often observed in patients with PCOS (Diamanti-Kandarakis and Dunaif, 2012; Moghetti, 2016), this has not led to its inclusion in the current diagnostic criteria. To highlight the relevance of IR in the pathogenesis of PCOS, Unfer and colleagues considered a redefinition of the Rotterdam clinical subgroups A, B, C which are characterized by hyperandrogenism, as endocrine-metabolic syndromes (Unfer et al., 2023a). On the other hand, the clinical subgroup D was classified as an ovarian PCOS, presenting as a gynecological issue, typically in the absence of metabolic issues (Unfer et al., 2023a). Since within the clinical subtype D the development of ovarian cysts cannot be explained by androgen excess or a metabolic disorder, an alternative mechanism would appear to be responsible. Defective signaling of the insulin-like growth factor-1 (IGF-1), recently described by Dai and colleagues, was proposed as a possible explanation for the arrest of follicular growth and maturation. Specially it was reported that an excessive concentration of IGF-1 triggers the arrest of follicle growth, leading to the formation of follicular ovarian cysts (Dai et al., 2022).

Taking the above into consideration, this review reexamines the historical and current diagnostic criteria, in light of the current literature and evaluates whether the inclusion of other diagnostic factors, such as insulin resistance, may be valuable to guide treatment and improve patient care, in order to spark further study and discussion. Furthermore, the authors consider whether a separation between the hyperandrogenic and normoandrogenic subgroups may be appropriate, with questions raised over whether they share a common etiopathogenesis, and how this may affect therapy choice going forward.

2. Clinical subtypes A, B, C of PCOS: the endocrine-metabolic PCOS

2.1. Insulin resistance

IR is defined as an impaired biological response to insulin stimulation in various tissues such as liver, muscle, and fat, affecting glucose metabolism and resulting in a compensatory increase in insulin secretion by the pancreatic islet cells to maintain glucose homeostasis, thus leading to hyperinsulinemia.

During systemic IR the ovaries remain sensitive to insulin signaling due to phenomenon known as the ovarian paradox (Carlomagno et al., 2011). Insulin has a direct stimulatory effect on steroidogenesis in the ovary, due to the presence of the insulin receptor on theca cell membranes (Munir et al., 2004). The intracellular signaling pathway triggered by the insulin receptor, stimulates 17-α-hydroxylase activity,

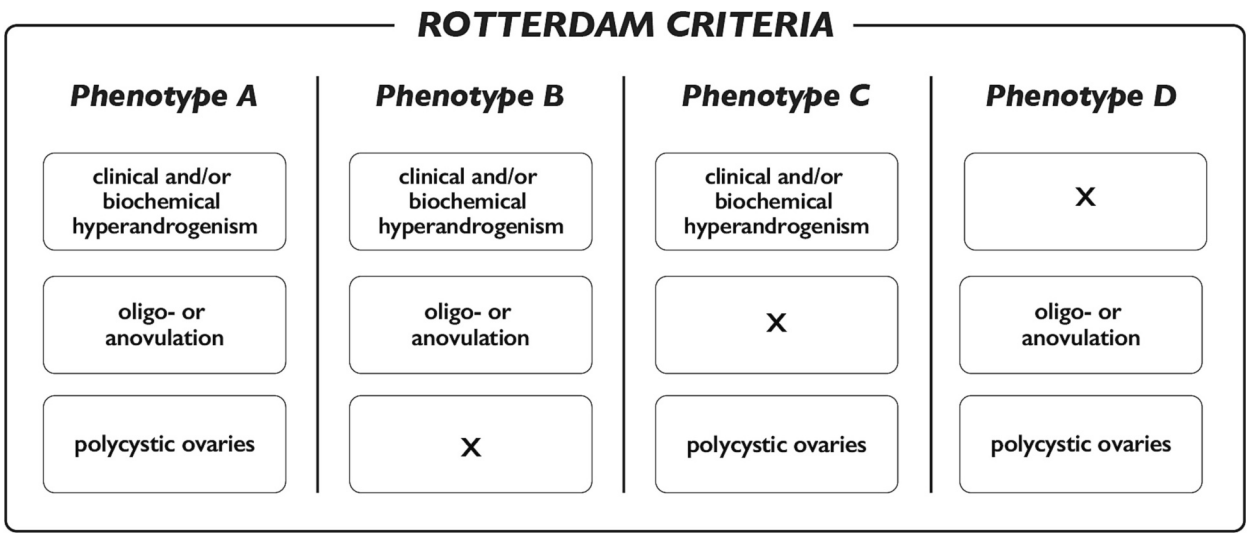

Fig. 1. Rotterdam criteria. According to the Rotterdam consensus, Polycystic Ovary Syndrome (PCOS) is diagnosed with the presence of at least two out of three of the following features: evidence of oligo- or anovulation, biochemical or clinical hyperandrogenism, and presence of polycystic ovaries. The four different combinations of these features reflect four different possible phenotypes.

which activates ovarian testosterone biosynthesis (Nestler et al., 1998). In this context, hyperinsulinemia due to the systemic IR may alter androgen steroidogenesis both directly and indirectly (Unluhizarci et al., 2021).

In the context of PCOS, insulin triggers other relevant biological processes, such as the stimulation of epimerase activity. A specific epimerase is responsible for the conversion of the predominant naturally occurring stereoisomer of inositol, myo-inositol (myo-Ins), into its stereoisomer D-chiro-inositol (D-chiro-Ins), and regulates the tissue-specific intracellular myo-Ins:D-chiro-Ins ratio, thereby modulating different metabolic processes (Carlomagno et al., 2011; Unfer et al., 2014; Heimark et al., 2014; Larner et al., 2010). In patients with hyperinsulinemia, epimerization of myo-Ins to D-chiro-Ins is exacerbated, resulting in a downregulation of aromatase-mediated androgens to estrogen conversion, and an increase of relative androgen levels (Fig. 2) (Monastra et al., 2021). In addition, myo-Ins is recognized as a secondary messenger in follicle stimulating hormone (FSH) signaling (Thomas et al., 2011). Due to this, excessive myo-Ins to D-chiro-Ins conversion can lead to a deficiency of myo-Ins, that may lead to oocyte maturation failure, anovulation, and a decrease of oocyte quality (Laganà et al., 2018). Insulin also stimulates the expression of luteinizing hormone (LH) receptors in the ovarian theca cells, thus potentiating the LH response in androgen production (Teede et al., 2023; Rosenfield and Ehrmann, 2016; Morley et al., 1989). Finally, insulin acts on the liver by stimulating the production of sex hormone binding globulin (SHBG), which is responsible for the transport of testosterone (Rajala et al., 2007). However, as the liver is not responsive to insulin signaling in patients with IR, SHBG synthesis is impaired, resulting in an increase in bioavailable free testosterone blood levels (Wallace et al., 2013).

IR is thought to contribute to the hyperandrogenism observed in the clinical subtypes A, B and, C. An interesting study conducted by Moghetti and coworkers measured IR with a glucose clamp assay in order to compare insulin sensitivity in women with different PCOS phenotypes with that of healthy women (Moghetti et al., 2013). They observed that insulin sensitivity was reduced in clinical subtypes A and C, but not in D where it was comparable to that of healthy women, potentially indicating inherent differences between the PCOS clinical subtypes in regards to IR. A more recent trial, conducted by Unfer et al. (Unfer et al., 2023b), clustered the clinical subtypes A, B, and C apart D, whereby they observed significant differences in metabolic indices at baseline. Specifically, insulin and glucose levels were elevated in clinical subtypes A, B, and C, compared with D. Considering the prevalence of IR in women with PCOS, treatment with insulin-sensitizing agents, such as metformin and inositol, are widely utilized in clinical practice (Fedeli et al., 2023), regardless of the presented clinical subtype. With an aim to evaluate the efficacy of this approach, Unfer et al. (Unfer et al., 2014) administered inositol to patients with different clinical subtypes of PCOS, whereby they observed a variable response. Indeed, the improvement of

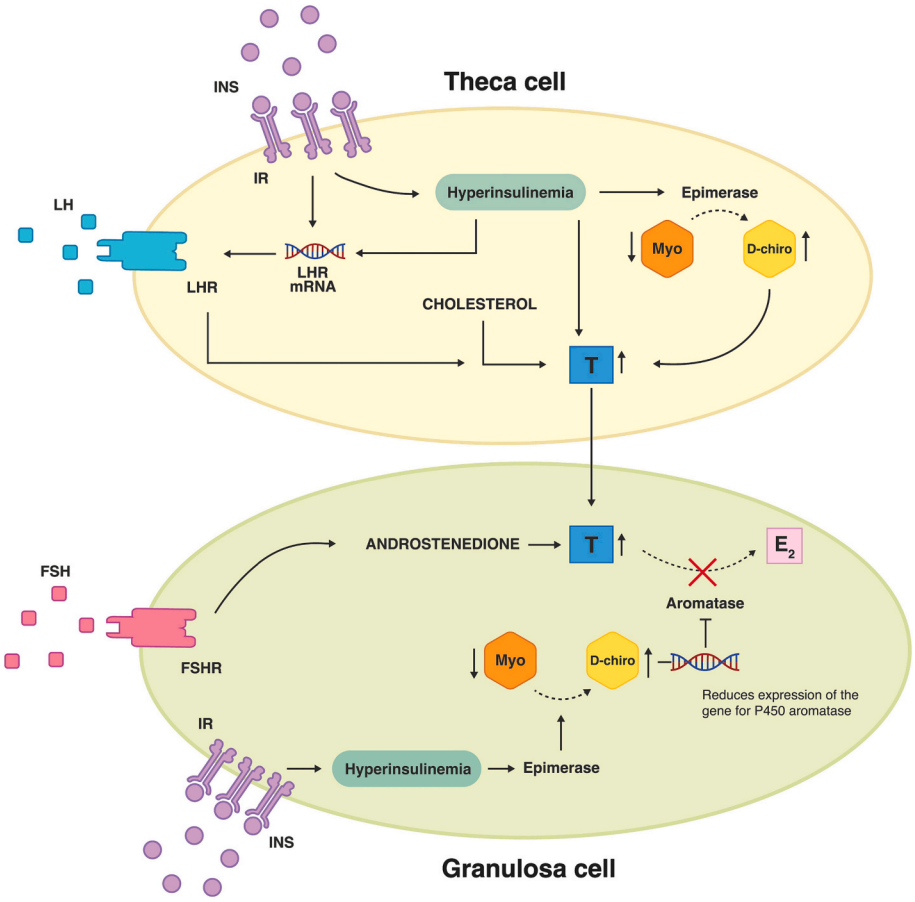

Fig. 2. Theca and granulosa cellular response to hyperinsulinemia. Schematic representation of ovarian follicle made up of theca and granulosa cells. Upon insulin stimulus in the event of hyperinsulinemia, theca cells demonstrate an increase in testosterone biosynthesis, in combination with the cellular response of LH binding to its receptor cause further androgen production. Moreover, insulin signaling upregulates LHR mRNA transcription, thus amplifying the cellular response to LH. In granulosa cells, FSH signaling increases the bioavailability of testosterone, which together with testosterone transport from the theca cell results in increasing testosterone concentration within the granulosa cell. In both theca and granulosa cells, hyperinsulinemia sustains epimerase activity, which converts myo-ins to D-chiro-ins. In granulosa cells this leads to a reduction of aromatase transcription, and a subsequent disruption of androgens to estrogens conversion. Abbreviations: D-chiro-inositol (D-chiro), follicle-stimulating hormone (FSH); follicle-stimulating hormone receptor (FSHR); insulin (INS), insulin receptor (IR); myo-inositol (Myo) luteinizing hormone (LH); luteinizing hormone receptor (LHR); testosterone (T), estradiol (E_2).

hormonal and metabolic parameters were recorded only in women with PCOS clinical subtypes A, B, and C, while only a minor effect was observed in women with clinical subtype D. As stated above, despite its crucial role in PCOS, IR has never been considered as a clear symptom or diagnostic criterion until the recent hypothesized classification posited by the EGOI, which suggested the inclusion of this further diagnostic criterion (Unfer et al., 2023a).

From a diagnostic point of view, the gold standard for measuring IR is the euglycemic hyperinsulinemic clamp. This assay; however, has limited clinical applicability, due to cost and complexity; therefore, several surrogates have been developed and validated. In clinical practice HOMA index represents a simpler and cheaper alternative to the glucose clamp, with both techniques often yielding comparable results (Bonora et al., 2000; So et al., 2020). HOMA index is based on measurement of fasting glucose (G) and insulin (I) values and calculated with the following formula:

$$(\text{I mIU/mL} \times \text{G mg/dL})/405 \text{ or } (\text{I } \mu\text{IU/mL} \times \text{G mmol/L})/22.5.$$

A HOMA index >2.5 is commonly associated with IR and can aid a subsequent diagnosis (Matthews et al., 1985).

However, for a PCOS diagnosis, an evaluation for IR should be supported by an assessment of androgen excess through hormonal assays or evaluation of dermatologic symptoms of clinical hyperandrogenism.

2.2. Hyperandrogenism

Androgens are produced by the theca cells of antral follicles, under LH stimulation. LH receptors are found in theca cells but also appear in the granulosa cells of mature follicles, such as the antral follicle that most likely will undergo ovulation. Androgens, predominantly androstenedione and testosterone, diffuse across the basal lamina of the follicle from the theca cell and pass into the granulosa cell layer where, under the control of FSH, they undergo aromatase-mediated conversion to estrogens (Franks and Hardy, 2018). The production of androgens also occurs in the adrenal gland under the stimulus of adrenocorticotropic hormone (ACTH) (Rege et al., 2013). Defects in both ovarian and adrenal steroidogenesis induce alterations of circulating androgen levels, which may lead to hyperandrogenism. Typically, androgen levels are measured clinically as total, free (or unbound) testosterone; although also other androgens such as androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulphate (DHEAS), are occasionally included in routine analysis. Moreover, hyperandrogenism presents specific clinical features including hirsutism, acne, and androgenic alopecia (Franks and Hardy, 2018). Hereafter, we briefly describe the biochemical and clinical parameters of hyperandrogenism.

Of note, a diagnosis of PCOS should exclude other causes of hyperandrogenism including hormonally active tumors, hypothyroidism, hyperprolactinemia, non-classical congenital adrenal hyperplasia, Cushing's syndrome, and acromegaly (Rosenfield and Ehrmann, 2016).

2.2.1. Biochemical parameters

Testosterone is the most important androgen in women and is typically bound to SHBG and other proteins such as albumin; however, free testosterone is required for cellular entry. Therefore, the measurement of free testosterone represents a better indicator of bioavailable testosterone and subsequently hyperandrogenism than the measurement of total testosterone (Khattak et al., 2021). However, the measurement of total *vs* free testosterone presents several limitations, with biochemical assays either due to limited accuracy and variability or to high cost and technical complexity (Rao and Bhide, 2020). A comprise between these limitations can be found with the measurement of the free androgen index (FAI), which describes the ratio between total testosterone and SHBG levels, allows for an accurate calculation of bioavailable testosterone levels (Xu et al., 2022). Androstenedione represents a possible alternative to measuring testosterone to identify hyperandrogenism in PCOS patients. For instance, in a study conducted by Knochenhauer et al., 9 % of PCOS patients were identified by evaluation of androstenedione levels (Knochenhauer et al., 1998). In a different study conducted by O'Reilly et al., 25 % of women with clinical signs of hyperandrogenism displayed elevated levels of androstenedione but not testosterone (O'Reilly et al., 2014). DHEA is primarily secreted by the adrenal cortex, and represents an additional potential biomarker for hyperandrogenism since heightened levels have been identified in PCOS patients (Azziz et al., 1998). However, DHEA has limited diagnostic use due to a low systemic concentration and significant diurnal variations. In contrast, its primary metabolite, DHEAS, is also altered in about 25 % to 35 % of women with PCOS and may represent the only elevated circulating androgen in approximately 10 % of women with PCOS (Azziz et al., 1998; Goodarzi et al., 2015). DHEAS and in general androgen levels are not always associated with an altered status of adrenocortical steroidogenesis, meaning that DHEAS is unsuitable as a sole criterion for the diagnosis of PCOS. The current 2023 guidelines do not currently recommend the use of DHEAS and androstenedione as diagnostic criteria to due to a lack sensitivity and specificity in the required assays (Teede et al., 2023).

Androgens assessment is frequently coupled with gonadotropin evaluation (LH and FSH), due to their physiological role in the regulation of ovarian steroidogenesis. Women with PCOS often exhibit increased secretion of LH and normal or decreased levels of FSH. Indeed, the increased secretion of LH and hence an increased LH to FSH ratio during the follicular phase of the menstrual cycle, is considered as a marker of PCOS (LH:FSH ratio >2) (Esmaeilzadeh et al., 2014; Saadia, 2020). It seems also that lean PCOS women are more commonly characterized by an increase in LH pulse amplitude. This aspect could partially justify the finding that obese women with PCOS frequently exhibit normal LH levels and a consequently normal LH:FSH ratio (Azziz et al., 2009).

2.2.2. Clinical signs

Hirsutism, defined as the presence of dark hairs in women in a male pattern (upper lip, chin, chest, upper back, lower back, upper and lower abdomen, upper arm, and thigh), is an characteristic feature of clinical subtypes A, B, and C of PCOS, affecting approximately 65 % to 75 % of patients (Azziz et al., 2009).

Acne vulgaris (acne) is a multifactorial inflammatory skin disease associated with hyperandrogenism, resulting in increased sebum production. Since hyperandrogenism represents one of the main features of PCOS, it is unsurprising that acne is one of the main cutaneous manifestations of this syndrome (Carmina, 2020), affecting almost half of women with PCOS as described in a meta-analysis by Tehrani et al. (Ramezani Tehrani et al., 2021). Hyperandrogenism can also contribute to the occurrence of seborrhea, another cutaneous manifestation frequently retrieved in PCOS patients. Seborrhea appears as follicular acneiform papules, pustules, and comedones, appearing diffusely on the face, upper trunk, forearms, buttocks, and thighs. Excessive activity of the sebaceous gland increases sebum excretion, resulting in cutaneous manifestations (Baroud et al., 2021).

Androgenic alopecia describes scalp hair loss, usually due to the pilosebaceous unit's response to androgen excess. The pattern of hair loss in PCOS generally involves thinning of the crown with preservation of the anterior hairline and is observed in 3.2 % to 34.8 % of the patients depending on the PCOS population (Quinn et al., 2014).

Of note, this recognized diagnostic criterion concerns clinical subtypes A, B, and C but not D, as these patients do not exhibit an altered endocrinological and metabolic profile.

2.3. Polycystic ovarian morphology (PCOM)

Androgen excess can directly impair folliculogenesis, as high androgen concentrations in ovarian theca cells can cause hyperplasia and block the development of follicles at the preantral and antral stages.

Consequently, an accumulation of cyst-like follicular structures may occur in the periphery of the ovary, classically resembling a string of pearls (Nisenblat and Norman, 2009). In the case of clinical subtype D, alternative – although yet unknown – mechanisms may lead to cyst formation in the absence of androgen excess.

In routine clinical practice, transabdominal or transvaginal sonography is widely used in the assessment of the ovarian status, allowing evaluation of both external general morphological features including diameter, volume, and area, and internal or structural features, such as the number of follicles, stromal volume, and echogenicity.

It should be noted that the inclusion of PCOM is not currently recommended in adolescent PCOS patients according to the 2018 and 2023 guidelines (Teede et al., 2023), as the PCOM morphology is routinely observed as a part of a normal pubertal transition; furthermore, concerns have been raised on the use of ultrasound on adolescent patients (Peña et al., 2020).

2.3.1. External features

- Ovarian volume is assessed by measuring the ovary's maximum diameter on the longitudinal, transverse, and anteroposterior planes. Considering its irregular shape, the ovary is classified as an ellipsoid and its volume is calculated using the following formula:

$\pi/6 \times$ maximal longitudinal $\times$ anteroposterior $\times$ transverse diameters or $\pi/6 \times$ length $\times$ width $\times$ thickness.

- Several studies have reported a mean ovarian volume for polycystic ovaries as greater than that of non-PCOS ovaries, and current guidelines describe an ovarian volume $\geq$10 ml as the threshold for PCOM (Teede et al., 2023).

2.3.2. Internal features

- Follicle number, size and location are visualized by scanning the longitudinal cross-section from the internal to the external edge. To diagnose PCOS according to the Rotterdam criteria, the ovary should contain at least 12 follicles measuring 2 to 9 mm in diameter, in addition to other clinical and/or biochemical features. These parameters can help distinguish PCOM from multi-cystic ovaries; a transient condition observed during puberty and in women recovering from hypothalamic amenorrhea. Multi-cystic ovaries exhibit a number of follicles $\geq$6, with diameters ranging between 4 and 10 mm (Balen et al., 2003). These criteria were subsequently updated and the cut off for number of follicles for PCOM was increased to 20 (Goodman et al., 2015; Teede et al., 2018).
- Stromal volume and echogenicity. The echo density of the stroma is an important histological feature, as increased stromal density is typical of polycystic ovaries. The stromal echogenicity is evaluated on a 1 to 3 scale, which correspond to normal, moderately increased, and frankly increased. Usually, the assessment of ovarian stroma can be replaced by the ovarian volume for a diagnosis of PCOS and as a reliable marker of ovarian function (Balen et al., 2003).

According to the Rotterdam classification, this criterion of PCOM applies for clinical subtypes A, C, and D but not for B, which is the only clinical subtype lacking the appearance of ovarian cysts. This consideration leads to further reflection about the current suitability of including, in the definition of PCOS, a clinical pattern excluding PCOM as in clinical subtype B. Moreover, the use of the term "polycystic" appears to be rather inappropriate as it refers to arrested follicles rather than cysts (Norman et al., 2023).

2.4. Ovulatory and menstrual dysfunctions

The exposure to androgen excess not only disrupts the growth and maturation of follicles but also inhibits ovulation and causes menstrual cycle alterations. Clinically, ovulatory dysfunction results in a disruption of the menstrual flow pattern, often resulting in oligo-amenorrhea (<6 cycles/year) or abnormal uterine bleeding. Menstrual dysfunction can be observed in the majority (approximately 75 % to 85 %) of PCOS patients. However, ovulatory dysfunction can also occur sub-clinically, with no disruption in the regularity of vaginal bleeding. Indeed, a history of apparent eumenorrhea (cycles every 27–34 days) does not exclude the presence of ovulatory dysfunction, especially in patients with hyperandrogenism (So et al., 2020). Furthermore, of interest to couples attempting to conceive, ovarian dysfunctions are strongly associated with of infertility issues and affect 70–80 % of all patients with PCOS (Shilpa et al., 2023).

AMH also plays a central role in the regulation of the ovulatory function and is highly expressed in the majority of PCOS cases even if normal AMH levels have been reported in a subset of women (Palomaki et al., 2020). The increased levels of AMH in preantral and small antral follicles inhibits the recruitment of primordial follicles from the oocyte pool in the ovary, and may also suppress the signaling of FSH, thus contributing to ovulatory disturbances (Rudnicka et al., 2021). These findings helped build the rationale that resulting in the measurement of AMH levels for being included for PCOS diagnosis in adults in the 2023 PCOS International evidence-based Guidelines (Teede et al., 2023).

3. PCOS clinical subtype D: do ovarian molecular alterations underpin its etiology?

According to the Rotterdam classification, clinical subtype D is the normo-androgenic PCOS characterized by the presence of PCOM and menstrual cycle alterations. Lacking a hyperandrogenic profile, the occurrence of these features likely does not originate from metabolic factors but may rather depend on alternative factors, one recently posited explanation is this clinical subtype may be caused by excessive levels of IGF-1. Indeed, increased IGF-1 concentration in granulosa cells affects the expression of three genes, namely CYP11A1, HSD3B and CYP19A1, thus enhancing 17-β-estradiol production (Dai et al., 2022; Mani et al., 2010). Recent literature in mice, has demonstrated that an increase of local estrogen levels may lead to an arrest in follicle growth, that in turn could potentially induce the formation of ovarian cysts (Mason et al., 1993). Moreover, high concentration of estrogens can affect the luteal phase of the menstrual cycle, resulting in a lower production of progesterone. Progesterone stimulates the production of IGF binding protein-1 (IGFBP-1) in the endometrium, which binds IGF-1 and inhibits the protein-protein interaction with the IGF-1 receptor (IGF-1R) (Young et al., 2021). Thus, low levels of progesterone reduce the IGFBP-1 level which in turn increases IGF-1 bioavailability and activity. In this context, clinical subtype D may exhibit an alteration in the physiology of the ovarian networks, meaning it may represent a gynecological condition of ovarian origin, as was recently hypothesized by Unfer and colleagues (Unfer et al., 2023a). The reported evidence opens discussion on the possible and alternative causes that might explain the occurrence of the PCOS condition in patients with clinical subtype D. The absence of

hyperandrogenism allows the likely exclusion of a condition originating from mutations of CYP21A2 gene, commonly observed in subjects affected by non-classic congenital adrenal hyperplasia (NCAH). This common autosomal recessive disorder is due to P450c21 (21-hydroxylase deficiency) and is characterized by alterations in ovarian and gonadotropic function, associated with the occurrence of a polycystic ovary-like phenotype, also favoring clinical features derived by androgen excess (Witchel and Azziz, 2010).

An additional hypothesis about the origin of clinical subtype D, was also formulated by Gleicher and colleagues in 2018 (Gleicher et al., 2018). They suggested that lean PCOS patients exhibit features of hyperandrogenism in younger ages, but during their late 20s to mid-30s a strong reduction in adrenal androgen production appears to occur, which significantly decreases the level of circulating androgens and their negative impact in the ovary. An autoimmune trigger is given as a possible cause of the decline in the adrenal androgen secretion. Additionally, in these patients, androgens exhibit a more rapid decrease compared to AMH, thus resulting in relatively high AMH levels with respect to androgens (and often FSH). This hypothesis indicates that phenotype D may represent an adrenal rather than an ovarian condition.

Notwithstanding the possible variation in etiopathogenesis of PCOS, the criteria applied for the diagnosis of clinical subtype D are much like those used for A, B, and C. However, assuming a diverse etiology, it may be speculated that PCOM may appear differently in terms of the number and size of follicles in different women. Therefore, further diagnostic criteria may be required to correctly differentiate clinical subtype D patients, and in this view endometrial thickness could represent one possible measurable parameter as discussed below.

4. Endometrial thickness

Several endometrial abnormalities have been reported in women with PCOS; however, to date, no common screening protocols or recommendations are in place. Frequently in women with PCOS, especially among clinical subtype A, B and D, the endometrium is exposed to prolonged stimulatory effects of unopposed estrogen due to chronic anovulation. A recent study by Unfer et al., observed a significant difference in endometrial thickness between these hyperandrogenic clinical subtype (A, B and C) and the normoandrogenic clinical subtype D. Accordingly, from day 1 to 6 of the menstrual cycle, patients with clinical subtype D demonstrated an endometrial thickness >7 mm, while in clinical subtypes A, B and C this was <7, potentially due to heightened levels of androgens inhibiting the proliferative effect of estrogens (Unfer et al., 2023b). Additionally, women with endometrial thickness >7 mm have a higher risk of developing endometrial hyperplasia (Unfer et al., 2023a; Giudice, 2006; Xu et al., 2021), which has been reported to affect 35 % of women with PCOS (Giudice, 2006). Since, endometrial thickness is also associated with infertility (Unfer et al., 2023a), impaired fertility in PCOS patients may be dependent on not only to anovulation, but also to endometrial dysfunction (Xu et al., 2021).

Various studies have shown in patients affected by PCOS an higher risk to develop not only hyperplasia, but also endometrial cancer (Meczekalski et al., 2020; Yin et al., 2019). Although patients affected by PCOS have an increased risk of endometrial cancer (by 3–4 times depending on different studies), the direct causality has not yet been confirmed. The risk of developing endometrial cancer may be related to several factors such as both metabolic syndrome and obesity (frequent in patients affected by PCOS with clinical subtype A, B or C), or to other pathogenetic factors such as hyperestrogenism. To date it has not been established if the risk of developing endometrial cancer may vary with phenotype. Further studies may be well-served to investigate the histological and cytological compositions of the endometrium in order to better understand whether morphological differences also exist among the PCOS clinical subtypes.

5. Current therapy for PCOS. Limitation of inositol use

The variation of symptoms and clinical manifestations of women with PCOS, may suggest different clinical conditions, which to date have been aggregated into the same syndrome. Indeed, even if PCOS encompasses different clinical subtypes and genetic phenotypes, the therapeutical approach to date has mostly consisted of a uniform therapy applied to all patients regardless of their clinical subtype (Gleicher et al., 2022).

Among the various recommendations for PCOS treatment, insulin sensitizing agents, such as metformin and inositols, are frequently considered as they have been demonstrated to significantly improvement various parameters of PCOS (Zhao et al., 2021; Fatima et al., 2023). Inositols are natural molecules present within human cells, and correct levels of these compounds are essential for the normal, physiological function of the female reproductive system (Facchinetti et al., 2020). Since women with PCOS often exhibit an imbalance of inositols, their oral administration represents a valid approach to treat the condition (Laganà et al., 2022; Nordio et al., 2019).

Inositols, particularly myo-Ins, mediate hormonal and endocrine activities, including FSH and insulin signaling in the ovaries, and sustain follicular development (Greff et al., 2023). Specifically, myo-Ins plays a pivotal role in ovarian granulosa cells as a molecular enhancer of FSH signaling, favoring the correct tuning of gonadotropin-mediated development of follicles (Tabatabaie et al., 2022). Moreover, it acts as a secondary messenger of insulin, influencing glucose metabolism and consumption in the maturing oocytes. Of note, an imbalance of myo-Ins levels in the ovary may reduce the overall oocyte quality and significantly impair the physiological ovulation process (Akbari Sene et al., 2019).

Additionally, D-chiro-Ins, a stereoisomer of myo-Ins, is also involved in the treatment of PCOS with limited application. Indeed, in overweight-obese women with PCOS, small amounts of D-chiro-Ins administered with myo-Ins in a physiological ratio of 40:1 (myo-Ins/D-chiro-Ins) seems effective at restoring ovulation and improving the metabolic parameters of these patients (Nordio et al., 2019; Artini et al., 2021).

Several clinical trials have demonstrated that inositol supplementation represents an effective therapeutic approach in women with PCOS, by improving metabolic and endocrine alterations, optimizing insulin and FSH signaling in the ovary, and reducing the presence of hyperandrogenism (Pkhaladze et al., 2021; Jethaliya et al., 2022). However, the different clinical subtypes of PCOS assessed by the Rotterdam criteria clearly indicate the diversity of the pathological features observed in PCOS patients. To this end, in a retrospective study, Unfer et al. investigated the effects of myo-Ins administration for a period of six months in different PCOS clinical subtypes. They observed a significant improvement in endocrine and metabolic parameters in patients with PCOS clinical subtypes A, B, and C, while this effect was not replicated in women with clinical subtype D. In particular, the treatment with myo-Ins improved BMI, insulin, and cholesterol levels in hyperandrogenic patients, in addition to the HOMA index, glycemia, LH/FSH ratio, and testosterone levels, with a prominent effect compared to the PCOS patients with clinical subtype D group (Unfer et al., 2023b).

The findings from this trial may change the evaluation of inositol's therapeutical utility in women with PCOS clinical subtype D, who are not usually affected by hyperandrogenism nor dysmetabolic phenomena (Moghetti et al., 2013) (Fig. 3). Hence, even if several authors endorsed inositol administration as beneficial treatment for patients with PCOS (Dinicola et al., 2021), new evidence based on the above-mentioned differences in response rates suggest that it might not be effective in all PCOS patients.

With this premise, clinical subtype D may also differentially respond to metformin treatment since the use of insulin-sensitizer does not find a therapeutical rationale in this case. However, studies investigating this hypothesis are not present in the literature to date.

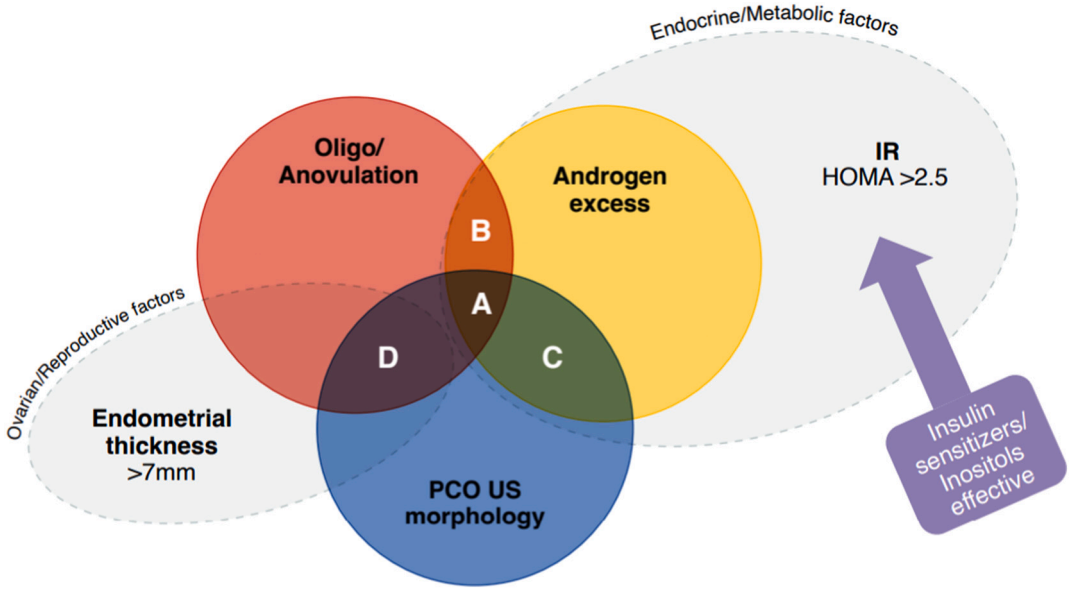

Fig. 3. Potential update to clinical guidelines. In red, yellow, and blue, are the clinical manifestations that make up the Rotterdam criteria and the present International evidence-based Guidelines, and the resulting clinical subtypes (A-D) are shown in white. In grey potential additions to the current guidelines as described in this review are shown, these are compared by use of the Venn diagram to display how these criteria overlap. Abbreviations: A: Classic PCOS, B: Hyperandrogenic anovulatory PCOS, C: Hyperandrogenic ovulatory PCOS, D: Normo-androgenic PCOS, PCO US morphology: polycystic ovarian ultrasound morphology, PCOS: Polycystic Ovarian Syndrome. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This data, while preliminary and in need of further study, supports the idea that it is inappropriate to search for a generic "one-size-fits-all" therapy, but rather the best approach should be investigated considering the characteristics of each patient. The increased understanding of PCOS and of the differences existing between its clinical phenotypes, should change the therapeutical rationale, highlighting the urgency for the correct evaluation of the clinical picture of each patient before recommending the appropriate therapy.

Of note, new therapeutical strategies should be tailored for patients with PCOS clinical subtype D, since these patients may be affected by a different type of condition. For instance, if the etiopathogenesis associated with the unbalancing of IGF-1 was to be confirmed, an innovative clinical approach properly targeting IGF-1 expression could be pursued for the treatment of this particular subgroup of patients.

6. Conclusions

PCOS has been long understood to be associated with potentially serious medical and metabolic abnormalities, in constant need of revisiting as new information comes to the fore. Despite the unanimous adoption of the Rotterdam criteria and the subsequent International evidence-based Guidelines, PCOS patients are rarely specifically clustered according to their clinical subtypes. In this context, the classification posited by the EGOI group may offer a new clinical approach (NIH, 2012). Furthermore, it has been suggested that changes in insulin signaling are partially involved in increasing ovarian androgen concentration, in addition to the subsequent formation of ovarian cysts and menstrual dysfunction in clinical subtypes A, B and C. Given the substantial role that IR plays in hyperandrogenic PCOS, it is thought by the authors these clinical subtypes may be better classified as parts of an endocrine-metabolic syndrome. Clinical subtypes, A, B, and C frequently display metabolic dysfunction and gynecological issues that may be potentially considered a side effect of an underlying metabolic disorder. For this reason, the diagnostic criteria would greatly benefit from the assessment of metabolic alterations such as IR, in addition, to those already encompassed by the International evidence-based Guidelines, to further addressing the needs of a diverse patient population. To formally recommend the inclusion of IR within the clinical guidelines a more robust evaluation of the literature is needed, with input from stakeholders and public consultation, for example through a GRADE recommendation process. Currently, there is not sufficient literature to complete such work, this review aims to prompt further critical discussion around the topic.

In contrast, a gynecological disease which may characterized by an ovarian etiology, could be more appropriate for women with clinical subtype D, where dysregulation of GH-IGF1 axis may offer a potential explanation for follicle growth arrest, ovarian cysts, and the resulting menstrual cycle perturbances as evidenced *in vitro*. Given the preliminary findings on the differential thickness of endometrium in normoandrogenic PCOS, it further warrants investigation and could potentially be included among the diagnostic criteria in the future to provide a complete clinical picture for PCOS patients. In light of the diverse etiopathogenesis that may exist between PCOS clinical subtypes A, B, C, and D, a more tailored clinical approach may be required that takes into account the individual needs of each patient based on the clinical presentation (NIH, 2012). While the use of insulin sensitizing molecules such as metformin and inositol are well documented for the treatment of the endocrine-metabolic alterations of clinical subtypes A, B, C (Heimark et al., 2014), for D more work is still required to clarify its etiopathogenesis and develop a more precise and effective therapeutic approach to rescue the altered ovarian status seen in this specific subpopulation. In summary, it is the intention of this article to support a consideration of the refinement of diagnostic criteria, supported by a reconsideration of clinical subgroup pathophysiology to facilitate targeted treatment of the existing clinical subgroups, most notably clinical subgroup D.

Funding

This research received no external funding.

Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

CRediT authorship contribution statement

V. Unfer: Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **E. Kandaraki:** Writing – review & editing, Visualization. **L. Pkhaladze:** Writing – review & editing, Conceptualization. **S. Roseff:** Writing – review & editing. **M.H. Vazquez-Levin:** Writing – review & editing. **A.S. Laganà:** Writing – review & editing, Conceptualization. **C. Shiao-Yng:** Writing – review & editing, Conceptualization. **M.I.M. Yap-Garcia:** Writing – review & editing. **N.D.E. Greene:** Writing – review & editing. **C.O. Soulage:** Writing – review & editing. **A. Bevilacqua:** Writing – review & editing. **S. Benvenga:** Writing – review & editing. **D. Barbaro:** Writing – review & editing. **B. Pintaudi:** Writing – review & editing. **A. Wdowiak:** Writing – review & editing. **C. Aragona:** Writing – review & editing. **Z. Kamenov:** Writing – review & editing, Visualization. **M. Appetecchia:** Writing – review & editing. **G. Porcaro:** Writing – review & editing. **I. Hernandez Marin:** Writing – review & editing. **F. Facchinetti:** Writing – review & editing. **T. Chiu:** Writing – review & editing. **O. Pustotina:** Writing – review & editing. **O. Papalou:** Writing – review & editing. **M. Nordio:** Writing – review & editing. **T. Cantelmi:** Writing – review & editing. **P. Cavalli:** Writing – review & editing. **I. Vucenik:** Writing – review & editing. **R. D'Anna:** Writing – review & editing. **V.R. Unfer:** Writing – review & editing. **S. Dinicola:** Writing – review & editing, Writing – original draft. **S. Salehpour:** Writing – review & editing. **A. Stringaro:** Writing – review & editing. **M. Montaninno Oliva:** Writing – review & editing. **M. Tugushev:** Writing – review & editing. **N. Prapas:** Writing – review & editing. **M. Bizzarri:** Writing – review & editing. **M.S.B. Espinola:** Writing – review & editing. **C. Di Lorenzo:** Writing – review & editing. **A.C. Ozay:** Writing – review & editing. **J. Nestler:** Writing – review & editing, Supervision.

Declaration of competing interest

Vittorio Unfer is employed at Lo.Li. Pharma srl, Rome, Italy. The other authors declare no conflicts of interest.

Data availability

No data was used for the research described in the article.

Acknowledgments

We would like to thank Dr. Michele Russo, Dr. Benedetta Manca, and Dr. Samuel H. Myers for the collaboration and contribution in the preparation and editing of the present review.

References

Akbari Sene, A., Tabatabaie, A., Nikniaz, H., Alizadeh, A., Sheibani, K., Mortezapour Alisaraie, M., Tabatabaie, M., Ashrafi, M., Amjadi, F., 2019. The myo-inositol effect on the oocyte quality and fertilization rate among women with polycystic ovary syndrome undergoing assisted reproductive technology cycles: a randomized clinical trial. Arch. Gynecol. Obstet. 299 (6), 1701–1707.

Anagnostis, P., Tarlatzis, B.C., Kauffman, R.P., 2018. Polycystic ovarian syndrome (PCOS): long-term metabolic consequences. Metabolism 86, 33–43.

Artini, P.G., Malacarne, E., Tomatis, V., Genazzani, A.D., 2021. The relevance of inositols treatment for PCOS before and during ART. Eur. Rev. Med. Pharmacol. Sci. 25 (14), 4799–4809.

Azziz, R., Black, V., Hines, G.A., Fox, L.M., Boots, L.R., 1998. Adrenal androgen excess in the polycystic ovary syndrome: sensitivity and responsivity of the hypothalamic-pituitary-adrenal axis. J. Clin. Endocrinol. Metab. 83 (7), 2317–2323.

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H.F., Futterweit, W., Janssen, O.E., Legro, R.S., Norman, R.J., Taylor, A.E., Witchel, S.F., 2006. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J. Clin. Endocrinol. Metab. 91 (11), 4237–4245.

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H.F., Futterweit, W., Janssen, O.E., Legro, R.S., Norman, R.J., Taylor, A.E., Witchel, S.F., 2009. The androgen excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil. Steril. 91 (2), 456–488.

Balen, A.H., Laven, J.S., Tan, S.L., Dewailly, D., 2003. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum. Reprod. Update 9 (6), 505–514.

Baroud, S., Wu, J., Zouboulis, C.C., 2021. Acne syndromes and mosaicism. Biomedicines 9.

Bonora, E., Targher, G., Alberiche, M., Bonadonna, R.C., Saggiani, F., Zenere, M.B., Monauni, T., Muggeo, M., 2000. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23 (1), 57–63.

Cao, N.T., Le, M.T., Nguyen, V.Q.H., Pilgrim, J., Le, V.N.S., Le, D.D., Pham, C.K., Aharon, D., Hill, M.J., 2019. Defining polycystic ovary syndrome phenotype in Vietnamese women. J. Obstet. Gynaecol. Res. 45 (11), 2209–2219.

Carlomagno, G., Unfer, V., Roseff, S., 2011. The D-chiro-inositol paradox in the ovary. Fertil. Steril. 95 (8), 2515–2516.

Carmina, E., 2020. Cutaneous manifestations of polycystic ovary syndrome. Curr. Opin. Endocr. Metab. Res. 12, 49–52.

Cassar, S., Misso, M.L., Hopkins, W.G., Shaw, C.S., Teede, H.J., Stepto, N.K., 2016. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. Hum. Reprod. 31 (11), 2619–2631.

Chen, W., Pang, Y., 2021. Metabolic syndrome and PCOS: pathogenesis and the role of metabolites. Metabolites 11 (12).

Chiaffarino, F., Cipriani, S., Dalmartello, M., Ricci, E., Esposito, G., Fedele, F., La Vecchia, C., Negri, E., Parazzini, F., 2022. Prevalence of polycystic ovary syndrome in European countries and USA: a systematic review and meta-analysis. Eur. J. Obstet. Gynecol. Reprod. Biol. 279, 159–170.

Dai, S., Zhang, H., Yang, F., Shang, W., Zeng, S., 2022. Effects of IGF-1 on the three-dimensional culture of ovarian preantral follicles and superovulation rates in mice. Biology (Basel) 11 (6).

Dapas, M., Dunaif, A., 2022. Deconstructing a syndrome: genomic insights into PCOS causal mechanisms and classification. Endocr. Rev. 43 (6), 927–965.

Dapas, M., Lin, F.T.J., Nadkarni, G.N., Sisk, R., Legro, R.S., Urbanek, M., Hayes, M.G., Dunaif, A., 2020. Distinct subtypes of polycystic ovary syndrome with novel genetic associations: an unsupervised, phenotypic clustering analysis. PLoS Med. 17 (6), e1003132.

Diamanti-Kandarakis, E., Dunaif, A., 2012. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr. Rev. 33 (6), 981–1030.

Dinicola, S., Unfer, V., Facchinetti, F., Soulage, C.O., Greene, N.D., Bizzarri, M., Laganà, A.S., Chan, S.Y., Bevilacqua, A., Pkhaladze, L., Benvenga, S., Stringaro, A., Barbaro, D., Appetecchia, M., Aragona, C., Bezerra Espinola, M.S., Cantelmi, T., Cavalli, P., Chiu, T.T., Copp, A.J., D'Anna, R., Dewailly, D., Di Lorenzo, C., Diamanti-Kandarakis, E., Hernández Marín, I., Hod, M., Kamenov, Z., Kandaraki, E., Monastra, G., Montanino Oliva, M., Nestler, J.E., Nordio, M., Ozay, A.C., Papalou, O., Porcaro, G., Prapas, N., Roseff, S., Vazquez-Levin, M., Vucenik, I., Wdowiak, A., 2021. Inositols: from established knowledge to novel approaches. Int. J. Mol. Sci. 22 (19).

Esmaeilzadeh, S., Andarieh, M.G., Ghadimi, R., Delavar, M.A., 2014. Body mass index and gonadotropin hormones (LH & FSH) associate with clinical symptoms among women with polycystic ovary syndrome. Glob. J. Health Sci. 7 (2), 101–106.

Facchinetti, F., Unfer, V., Dewailly, D., Kamenov, Z.A., Diamanti-Kandarakis, E., Laganà, A.S., Nestler, J.E., Soulage, C.O., 2020. Inositols in polycystic ovary syndrome: an overview on the advances. Trends Endocrinol. Metab. 31 (6), 435–447.

Fatima, K., Jamil, Z., Faheem, S., Adnan, A., Javaid, S.S., Naeem, H., Mohiuddin, N., Sajid, A., Ochani, S., 2023. Effects of myo-inositol vs. metformin on hormonal and metabolic parameters in women with PCOS: a meta-analysis. Ir. J. Med. Sci. 192 (6), 2801–2808.

Fedeli, V., Catizone, A., Querqui, A., Unfer, V., Bizzarri, M., 2023. The role of inositols in the hyperandrogenic phenotypes of PCOS: a re-reading of Larner's results. Int. J. Mol. Sci. 24 (7).

Franks, S., Hardy, K., 2018. Androgen action in the ovary. Front. Endocrinol. (Lausanne) 9, 452.

Gibson-Helm, M., Teede, H., Dunaif, A., Dokras, A., 2017. Delayed diagnosis and a lack of information associated with dissatisfaction in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 102 (2), 604–612.

Giudice, L.C., 2006. Endometrium in PCOS: implantation and predisposition to endocrine CA. Best Pract. Res. Clin. Endocrinol. Metab. 20 (2), 235–244.

Gleicher, N., Kushnir, V.A., Darmon, S.K., Wang, Q., Zhang, L., Albertini, D.F., Barad, D. H., 2018. Suspected ontogeny of a recently described hypo-androgenic PCOS-like phenotype with advancing age. Endocrine 59 (3), 661–676.

Gleicher, N., Darmon, S., Patrizio, P., Barad, D.H., 2022. Reconsidering the polycystic ovary syndrome (PCOS). Biomedicines 10, 7.

Goodarzi, M.O., Carmina, E., Azziz, R., 2015. DHEA, DHEAS and PCOS. J. Steroid Biochem. Mol. Biol. 145, 213–225.

Goodman, N.F., Cobin, R.H., Futterweit, W., Glueck, J.S., Legro, R.S., Carmina, E., 2015. American Association of Clinical Endocrinologists, American College of Endocrinology, and androgen excess and pcos society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome—part 1. Endocr. Pract. 21 (11), 1291–1300.

Greff, D., Juhász, A.E., Váncsa, S., Váradi, A., Sipos, Z., Szinte, J., Park, S., Hegyi, P., Nyirády, P., Ács, N., Várbíró, S., Horváth, E.M., 2023. Inositol is an effective and safe

treatment in polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. Reprod. Biol. Endocrinol. 21, (1), 10.
Heimark, D., McAllister, J., Larner, J., 2014. Decreased myo-inositol to chiro-inositol (M/C) ratios and increased M/C epimerase activity in PCOS theca cells demonstrate increased insulin sensitivity compared to controls. Endocr. J. 61 (2), 111–117.
Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum. Reprod. 19 (1), 2004, 41–47.
Jethaliya, H., Gajjar, N., Patel, V., Deshpande, S., Patel, R., 2022. Efficacy of myo-inositol on anthropometric, metabolic, and endocrine outcomes in PCOS patients: a meta-analysis of randomized controlled trial. Reprod. Sci. 29 (8), 2282–2298.
Joham, A.E., Norman, R.J., Stener-Victorin, E., Legro, R.S., Franks, S., Moran, L.J., Boyle, J., Teede, H.J., 2022. Polycystic ovary syndrome. Lancet Diabetes Endocrinol. 10 (9), 668–680.
Khattak, M., Usman, R., Sultana, N., Khattak, A., 2021. Comparison of free androgen index in polycystic ovary syndrome and non-polycystic ovary syndrome infertile patients. J. Ayub Med. Coll. Abbottabad 33 (4), 577–581.
Knochenhauer, E.S., Key, T.J., Kahsar-Miller, M., Waggoner, W., Boots, L.R., Azziz, R., 1998. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J. Clin. Endocrinol. Metab. 83 (9), 3078–3082.
Laganà, A.S., Garzon, S., Casarin, J., Franchi, M., Ghezzi, F., 2018. Inositol in polycystic ovary syndrome: restoring fertility through a pathophysiology-based approach. Trends Endocrinol. Metab. 29 (11), 768–780.
Laganà, A.S., Forte, G., Bizzarri, M., Kamenov, Z.A., Bianco, B., Kaya, C., Gitas, G., Alkatout, I., Terzic, M., Unfer, V., 2022. Inositols in the ovaries: activities and potential therapeutic applications. Expert Opin. Drug Metab. Toxicol. 18 (2), 123–133.
Larner, J., Brautigan, D.L., Thorner, M.O., 2010. D-chiro-inositol glycans in insulin signaling and insulin resistance. Mol. Med. 16 (11−12), 543–552.
Lee, H.J., Jo, H.N., Noh, H.K., Kim, S.H., Joo, J.K., 2022. Is there association between thyroid stimulating hormone levels and the four phenotypes in polycystic ovary syndrome? Ginekol. Pol. 94 (3), 203–210.
Mani, A.M., Fenwick, M.A., Cheng, Z., Sharma, M.K., Singh, D., Wathes, D.C., 2010. IGF1 induces up-regulation of steroidogenic and apoptotic regulatory genes via activation of phosphatidylinositol-dependent kinase/AKT in bovine granulosa cells. Reproduction 139 (1), 139–151.
Mason, H.D., Margara, R., Winston, R.M., Seppala, M., Koistinen, R., Franks, S., 1993. Insulin-like growth factor-I (IGF-I) inhibits production of IGF-binding protein-1 while stimulating estradiol secretion in granulosa cells from normal and polycystic human ovaries. J. Clin. Endocrinol. Metab. 76 (5), 1275–1279.
Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 (7), 412–419.
Meczekalski, B., Pérez-Roncero, G.R., López-Baena, M.T., Chedraui, P., Pérez-López, F.R., 2020. The polycystic ovary syndrome and gynecological cancer risk. Gynecol. Endocrinol. 36 (4), 289–293.
Moghetti, P., 2016. Insulin resistance and polycystic ovary syndrome. Curr. Pharm. Des. 22 (36), 5526–5534.
Moghetti, P., Tosi, F., Bonin, C., Di Sarra, D., Fiers, T., Kaufman, J.M., Giagulli, V.A., Signori, C., Zambotti, F., Dall'Alda, M., Spiazzi, G., Zanolin, M.E., Bonora, E., 2013. Divergences in insulin resistance between the different phenotypes of the polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 98 (4), E628–E637.
Monastra, G., Vazquez-Levin, M., Bezerra Espinola, M.S., Bilotta, G., Laganà, A.S., Unfer, V., 2021. D-chiro-inositol, an aromatase down-modulator, increases androgens and reduces estrogens in male volunteers: a pilot study. Basic Clin. Androl. 31 (1), 13.
Morley, P., Calaresu, F.R., Barbe, G.J., Armstrong, D.T., 1989. Insulin enhances luteinizing hormone-stimulated steroidogenesis by porcine theca cells. Biol. Reprod. 40 (4), 735–743.
Munir, I., Yen, H.W., Geller, D.H., Torbati, D., Bierden, R.M., Weitsman, S.R., Agarwal, S. K., Magoffin, D.A., 2004. Insulin augmentation of 17alpha-hydroxylase activity is mediated by phosphatidyl inositol 3-kinase but not extracellular signal-regulated kinase-1/2 in human ovarian theca cells. Endocrinology 145 (1), 175–183.
Nestler, J.E., Jakubowicz, D.J., de Vargas, A.F., Brik, C., Quintero, N., Medina, F., 1998. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J. Clin. Endocrinol. Metab. 83 (6), 2001–2005.
NIH, 2012. Evidence-based Methodology Workshop on Polycystic Ovary Syndrome. Presented at: 2012-EXECUTIVE SUMMARY (Final Report). December 3–5.
Nisenblat, V., Norman, R.J., 2009. Androgens and polycystic ovary syndrome. Curr. Opin. Endocrinol. Diabetes Obes. 16 (3), 224–231.
Nordio, M., Basciani, S., Camajani, E., 2019. The 40:1 myo-inositol/D-chiro-inositol plasma ratio is able to restore ovulation in PCOS patients: comparison with other ratios. Eur. Rev. Med. Pharmacol. Sci. 23 (12), 5512–5521.
Norman, R.J., Morman, R., Teede, H.J., 2023. "Tis but thy name that is my enemy"-the problem with the naming of polycystic ovary syndrome. Fertil. Steril. 120 (2), 249–250.
O'Reilly, M.W., Taylor, A.E., Crabtree, N.J., Hughes, B.A., Capper, F., Crowley, R.K., Stewart, P.M., Tomlinson, J.W., Arlt, W., 2014. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. J. Clin. Endocrinol. Metab. 99 (3), 1027–1036.
Palomaki, G.E., Kalra, B., Kumar, T., Patel, A.S., Savjani, G., Torchen, L.C., Dunaif, A., Morrison, A., Lambert-Messerlian, G.M., Kumar, A., 2020. Adjusting antimüllerian hormone levels for age and body mass index improves detection of polycystic ovary syndrome. Fertil. Steril. 113 (4), 876–884.e2.
Peña, A.S., Witchel, S.F., Hoeger, K.M., Oberfield, S.E., Vogiatzi, M.G., Misso, M., Garad, R., Dabadghao, P., Teede, H., 2020. Adolescent polycystic ovary syndrome according to the international evidence-based guideline. BMC Med. 18 (1), 72.
Pkhaladze, L., Russo, M., Unfer, V., Nordio, M., Basciani, S., Khomasuridze, A., 2021. Treatment of lean PCOS teenagers: a follow-up comparison between myo-inositol and oral contraceptives. Eur. Rev. Med. Pharmacol. Sci. 25 (23), 7476–7485.
Quinn, M., Shinkai, K., Pasch, L., Kuzmich, L., Cedars, M., Huddleston, H., 2014. Prevalence of androgenic alopecia in patients with polycystic ovary syndrome and characterization of associated clinical and biochemical features. Fertil. Steril. 101 (4), 1129–1134.
Rajala, U.M., Keinänen-Kiukaanniemi, S.M., Hirsso, P.K., Jokelainen, J.J., Laakso, M.A., Hiltunen, L.A., Ruokonen, A.O., Härkönen, P.K., Timonen, M.J., 2007. Associations of total testosterone and sex hormone–binding globulin levels with insulin sensitivity in middle-aged finnish men. Diabetes Care 30 (4), e13.
Ramezani Tehrani, F., Behboudi-Gandevani, S., Bidhendi Yarandi, R., Saei Ghare Naz, M., Carmina, E., 2021. Prevalence of acne vulgaris among women with polycystic ovary syndrome: a systemic review and meta-analysis. Gynecol. Endocrinol. 37 (5), 392–405.
Rao, P., Bhide, P., 2020. Controversies in the diagnosis of polycystic ovary syndrome. Ther. Adv. Reprod. Health 14 (2633494120913032).
Rege, J., Nakamura, Y., Satoh, F., Morimoto, R., Kennedy, M.R., Layman, L.C., Honma, S., Sasano, H., Rainey, W.E., 2013. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. J. Clin. Endocrinol. Metab. 98 (3), 1182–1188.
Rosenfield, R.L., Ehrmann, D.A., 2016. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr. Rev. 37 (5), 467–520.
Rudnicka, E., Kunicki, M., Calik-Ksepka, A., Suchta, K., Duszewska, A., Smolarczyk, K., Smolarczyk, R., 2021. Anti-Müllerian hormone in pathogenesis, diagnostic and treatment of PCOS. Int. J. Mol. Sci. 22.
Saadia, Z., 2020. Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOS) - obese vs. non-obese women. Med. Arch. 74 (4), 289–293.
Shilpa, K., Earlina, A., Larysa, S., 2023. The clinical manifestations of polycystic ovary syndrome (PCOS) and the treatment options. Eur. J. Biol. Med. Sci. Res. 11 (1), 57/91.
So, A., Sakaguchi, K., Okada, Y., Morita, Y., Yamada, T., Miura, H., Otowa-Suematsu, N., Nakamura, T., Komada, H., Hirota, Y., Tamori, Y., Ogawa, W., 2020. Relation between HOMA-IR and insulin sensitivity index determined by hyperinsulinemic-euglycemic clamp analysis during treatment with a sodium-glucose cotransporter 2 inhibitor. Endocr. J. 67 (5), 501–507.
Stein, I.F., Leventhal, M.L., 1935. Amenorrhea associated with bilateral polycystic ovaries. Am. J. Obstet. Gynecol. 29 (2), 181–191.
Tabatabaie, M., Amiri, S., Golestan Jahromi, M., Sene, A.A., Zandieh, Z., Mehdizadeh, M., Amjadi, F., 2022. The effect of myo-inositol supplement on molecular regulation of folliculogenesis, steroidogenesis, and assisted reproductive technique outcomes in patients with polycystic ovarian syndrome. Mol. Biol. Rep. 49 (2), 875–884.
Teede, H.J., Misso, M.L., Costello, M.F., Dokras, A., Laven, J., Moran, L., Piltonen, T., Norman, R.J., 2018. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum. Reprod. 33 (9), 1602–1618.
Teede, H.J., Tay, C.T., Laven, J.J.E., Dokras, A., Moran, L.J., Piltonen, T.T., Costello, M. F., Boivin, J., Redman, L.M., Boyle, J.A., Norman, R.J., Mousa, A., Joham, A.E., 2023. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Eur. J. Endocrinol. 189 (2), G43–g64.
Thomas, R.M., Nechamen, C.A., Mazurkiewicz, J.E., Ulloa-Aguirre, A., Dias, J.A., 2011. The adapter protein APPL1 links FSH receptor to inositol 1,4,5-trisphosphate production and is implicated in intracellular Ca2+ mobilization. Endocrinology 152 (4), 1691–1701.
Tosi, F., Villani, M., Migazzi, M., Faccin, G., Garofalo, S., Fiers, T., Kaufman, J.M., Bonora, E., Moghetti, P., 2021. Insulin-mediated substrate use in women with different phenotypes of PCOS: the role of androgens. J. Clin. Endocrinol. Metab. 106 (9), e3414–e3425.
Unfer, V., Carlomagno, G., Papaleo, E., Vailati, S., Candiani, M., Baillargeon, J.P., 2014. Hyperinsulinemia alters myoinositol to d-chiroinositol ratio in the follicular fluid of patients with PCOS. Reprod. Sci. 21 (7), 854–858.
Unfer, V., Dinicola, S., Russo, M., 2023a. A PCOS paradox: does inositol therapy find a rationale in all the different phenotypes? Int. J. Mol. Sci. 24 (7).
Unfer, V., Russo, M., Aragona, C., Bilotta, G., Montanino Oliva, M., Bizzarri, M., 2023b. Treatment with myo-inositol does not improve the clinical features in all PCOS phenotypes. Biomedicines 11.
Unluhizarci, K., Karaca, Z., Kelestimur, F., 2021. Role of insulin and insulin resistance in androgen excess disorders. World J. Diabetes 12 (5), 616–629.
Wallace, I.R., McKinley, M.C., Bell, P.M., Hunter, S.J., 2013. Sex hormone binding globulin and insulin resistance. Clin. Endocrinol. (Oxf) 78 (3), 321–329.
Witchel, S.F., Azziz, R., 2010. Nonclassic congenital adrenal hyperplasia. Int. J. Pediatr. Endocrinol. 2010, 625105.

Xu, X.L., Deng, S.L., Lian, Z.X., Yu, K., 2021. Estrogen receptors in polycystic ovary syndrome. Cells 10 (2).
Xu, S., Liu, Y., Xue, K., Liu, X., Jia, G., Zeng, Y., Chen, Y., 2022. Diagnostic value of total testosterone and free androgen index measured by LC-MS/MS for PCOS and insulin resistance. J. Clin. Lab. Anal. 36 (11), e24739.
Yin, W., Falconer, H., Yin, L., Xu, L., Ye, W., 2019. Association between polycystic ovary syndrome and cancer risk. JAMA Oncol. 5 (1), 106–107.
Young, C.H., Snow, B., DeVore, S.B., Mohandass, A., Nemmara, V.V., Thompson, P.R., Thyagarajan, B., Navratil, A.M., Cherrington, B.D., 2021. Progesterone stimulates histone citrullination to increase IGFBP1 expression in uterine cells. Reproduction 162 (2), 117–127.
Zhao, H., Xing, C., Zhang, J., He, B., 2021. Comparative efficacy of oral insulin sensitizers metformin, thiazolidinediones, inositol, and berberine in improving endocrine and metabolic profiles in women with PCOS: a network meta-analysis. Reprod. Health 18 (1), 171.