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The efficiency of a complementary approach to Stein-Leventhal Syndrome (Polycystic Ovary Syndrome) - a comprehensive review

Natália Maria Gomes de Oliveira

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The Efficiency of a Complementary Approach To Stein-Leventhal Syndrome (Polycysitc Ovary Syndrome)

- A Comprehensive Review

Natáliia Maria Gomes De Oliveira

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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“The efficiency of a complementary approach to Stein-Leventhal Syndrome (Polycystic Ovary Syndrome) – a comprehensive review "

Dissertação de Candidatura ao Grau de Mestre em Medicina Tradicional Chinesa submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto

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To my parents who taught me to persevere with passion through thick and thin, to love with discipline and to believe that making it right is always better than making it easy.

To all my valuable friends for the love, kinship, understanding and for helping me to keep focus on life core values when withstanding agitated waters.

Do the difficult things while they are easy and do the great things while they are small.
A journey of a thousand miles must begin with a single step.
Lao Tzu

Saber estar e romper a tempo, correr os riscos da adesão e da renúncia, pôr a sinceridade das posições acima dos jogos pessoais, isso é política que vale a pena.

Francisco Sá Carneiro

13-06-1974, *(Capítulo I - Entrevista ao Diário de Notícias).*

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Resumo

Introdução: O síndrome de Stein-Leventhal ou Síndrome de Ovário Poliquístico (SOP) – é hoje conhecido como a patofisiologia endócrina mais entre mulheres de idade fértil, com uma prevalência, que varia entre 5-15%, consoante as orientações de diagnóstico seguidas. Apesar da ubiquidade de estudos endócrinos, reprodutivos e epigenéticos realizados até agora, a origem deste quadro clínico mantém-se desconhecida e o seu diagnóstico é sempre feito por exclusão de patologias que espelhem fenótipos idênticos. O diagnóstico de SOP segue atualmente uma de três orientações - NICHD (1990), Roterdão (2003) ou AE-PCOS Society (2009). O denominador comum é a associação de pelo menos dois de três critérios clínicos - anovulação/disfunção do ovário, excesso de androgénio clínico/bioquímico e/ou morfologia poliquística na avaliação de ultrassom. As implicações endócrinas e metabólicas refletem uma alta co-morbilidade: infertilidade, cancro do endométrio, depressão, obesidade apneia obstrutiva do sono (AOS), síndrome cardiometabólico, doença cerebrovascular, diabetes tipo II (DM II), dislipidemia, fígado gordo não alcoólico (FGNA). As associações de especialistas aconselham orientações na vigilância da saúde e qualidade de vida da mulher com este síndrome sendo frequente necessidade de tratamento numa parte ou ao longo da vida. Inúmeros tratamentos farmacêuticos e/ou cirúrgicos são usados no controlo de sintomas clínicos de SOP e suas co-morbilidades, contudo, os efeitos secundários de uso prolongado e/ou a sua baixa taxa de sucesso tornam os tratamentos complementares como a Medicina Tradicional Chinesa (MTC) numa figura de relevo. Este estudo tem como objetivo rever a eficiência do papel dos tratamentos de MTC usados no síndrome de Stein-Leventhal, em todo o mundo.

Objetivo: O presente trabalho tem em vista rever e avaliar o papel dos tratamentos com técnicas e/ou princípios de MTC – farmacoterapia chinesa, acupunctura, Tuina, Qi gong - usados na gestão do síndrome de Stein-Leventhal ou PCOS, como complemento à intervenção alopática.

Métodos: Para revisar o trabalho científico produzido e publicado no período mais recente dentro dos objetivos definidos, foi efetuada uma pesquisa seguindo o protocolo PRISMA e respetivo fluxograma aplicada ao motor de pesquisa EBSCO disponibilizado pelos recursos online da biblioteca do ICBAS.

As palavras-chave foram aplicadas aos campos de pesquisa com uma semântica que permita um cruzamento otimizado:

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Phytotherapy OR herbal medicine

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Acupuncture OR acupuncture therapy OR acupuncture treatment

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Tuina OR massage

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Qi Gong OR Chi Kung

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) TCM OR Traditional Chinese Medicine

Critérios de inclusão: publicações em inglês, com texto completo disponível e compreendidos no período entre setembro de 2015 e setembro de 2020. Foram apenas incluídos estudo efetuados em mulheres. Critérios de exclusão: trabalhos de meta-análise e revisão, artigos em língua não inglesa, indisponibilidade de texto completo, trabalho de campo com amostras não significativas, e outros objetos de estudo que não humano.

Resultados: Foram encontrados 8 estudos dentro do objetivo, entre RCT e co-horts. Desses estudos 4 relativos a técnicas de acupunctura (acupunctura manual, a laser, electro acupunctura e com sutura embebida em farmacoterapia chinesa) e 4 relativos a tratamento com farmacoterapia chinesa.

Conclusão: Apesar da maioria dos resultados serem positivos há necessidade de enriquecer o panorama científico com mais estudos de controle randomizados, com estandardização de dialética e diagnóstico aplicado na avaliação do quadro do doente com síndrome de Stein-Levanthal.

Palavras chave: Síndrome de Stein-Leventhal, SOP, ovários poliquísticos, excesso de androgénio, hirsutismo, disfunção metabólica, resistência à insulina, disfunção ovariana, anovulação, infertilidade, amenorreia, MTC, Fitoterapia, Acupunctura, Tui-Na, Qi gong

Abstract:

Introduction: The Stein-Leventhal syndrome – as of today commonly referred to as Polycystic Ovary/Ovarian Syndrome (PCOS) - is accepted as the most frequent endocrine pathophysiology among women of fertile age displaying a prevalence between 5-15%, consigning the criteria covered by clinical diagnostic. Despite ubiquitous endocrine, reproductive and epigenetic studies carried hitherto, the underlying cause of this disorder is still unidentified, and its diagnostic is always made by exclusion of conditions which mirror identical phenotypes. Current diagnoses for PCOS follow one of three guidelines: NICHD (1990), Rotterdam (2003) or AE-PCOS Society (2009). Common hallmark to these guidelines is the association of at least two out of three criteria – anovulation/ovarian dysfunction and/or, clinical/biochemical androgen excess and/or polycystic ovarian morphology in ultrasound assessment. Both endocrine and metabolic implications of PCOS reflect a high comorbidity: infertility, endometrial cancer, depression, obesity, obstructive sleep apnoea (OSA), cardiometabolic syndrome, cerebrovascular disease, type II diabetes (DM II), dyslipidaemia, non-alcoholic fatty liver. The associations of specialists advise standard procedures on surveillance of the health and quality of life of women with this syndrome, being frequent the urge for treatment in part or throughout their life. Notwithstanding the numerous pharmaceutical and/or surgical treatments addressing both PCOS clinical features and its comorbidities, their long-term usage side effects and low efficacy turn complementary medicine treatments a relevant asset. This study aims to review the efficiency of TCM treatments used for Stein-Leventhal syndrome worldwide.

Objective: The present work aims to review and assess the role and efficiency of techniques and/or principles of TCM – Chinese pharmacotherapy, acupuncture, Tuina, Qi gong – applied to the treatment and management of the Stein-Leventhal syndrome as a potential prolific complement to allopathic intervention.

Methods: To review the scientific work produced and published in the most recent period within the defined objectives, a research was carried out following the PRISMA protocol and its flowchart applied to EBSCO research motor within the period comprehended between September 2015 and September of 2020. Keywords applied to search fields with semantics that allow optimized cross-referencing:

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Phytotherapy OR herbal medicine

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Acupuncture OR acupuncture therapy OR acupuncture treatment

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Tuina OR massage

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Qi Gong OR Chi Kung

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) TCM OR Traditional Chinese Medicine

Inclusion criteria: English publications with full text available, included in the period 2015(September) to 2020 (September), studies conducted on human females. Exclusion criteria: Meta-analysis and review articles, non-English publications, unavailable full text, field research with non-representative samples.

Results: Eight studies were found within the objective, between RCT and co-horts. Of these studies 4 related to acupuncture techniques (manual acupuncture, laser, electro acupuncture and suture in Chinese pharmacotherapy) and another four related to Chinese pharmacotherapy.

Conclusion: Although most of the results are positive, there is a need to enrich the scientific panorama with more randomized control studies, with dialectic standardization and diagnosis applied in the evaluation of the patient with Stein-Levanthal syndrome.

Keywords: Stein-Leventhal syndrome, PCOS, polycystic ovaries, androgen excess, hirsutism, metabolic dysfunction, insulin resistance, obesity cardiovascular disease, ovarian dysfunction, anovulation, infertility, amenorrhea, mental health, MTC, phytotherapy, Acupuncture, Tui-Na, Qi gong

Abbreviations

AE-PCOS - Androgen Excess – PCOS Society

AFC - Antral Follicle Count

ALT - Algor Leadens Theory

ANS – Autonomous Nervous System

AR – Androgen Receptor

ASRM - American Society for Reproductive Medicine

BMI – Body Mass Index

CC - Clomiphene Citrate

CHM - Chinese Herbal Medicine

CNS – Central nervous system

DA - Dopamine

DHEA – Dehydroxyprogesterone

DHEAS - Dehydroxyprogesterone sulphate

EA – Electroacupuncture

ESHRE - European Society for Human Reproduction and Embryology

FAH – Functional adrenal hyperandrogenism

FOH – Functional ovarian hyperandrogenism

FSH – Follicle-stimulating hormone

GABA – Gamma-aminobutyric acid

GC – Granulosa Cells

GnRH – Gonadotropin-releasing hormone

GVA – General Visceral Afferent

GVE - General Visceral Efferent

GWAS - Genome-wide Association Screening

hCG – human Chorionic Gonadotropin

hGH - human Growth Hormone

HI – Hormonal Injections

HM – Heidelberg Model

HPG – Hypothalamus-Pituitary- Gonadal axis

IR – Insulin Resistance

IVF – *in vitro* fertilization

LDL – Low Density Lipoprotein

LH – Luteinising Hormone

NE - Norepinephrine

OHSS – Ovarian Hyperstimulation Syndrome

OSA – Obstructive Sleep Apnoea

OWR – Ovary Wedge Resection

PCOS – Polycystic Ovary Syndrome

PGC – Primordial Germ Cells

PNS – Peripheric Nervous System

RCT – Random Control Trial

5-HT - Serotonin

T2DM – Diabetes Mellitus Type II

TCM – Traditional Chinese Medicine

WM – Western Medicine

1. Introduction

The Stein-Leventhal syndrome – today commonly designed as Polycystic Ovary/Ovarian Syndrome (PCOS) - is accepted as the most frequent endocrine pathophysiology among women of fertile age displaying a prevalence between 5-15%, consigning the criteria covered by clinical diagnostic (Coutinho et al., 2019, Rasquin Leon et al., 2020). Despite ubiquitous endocrine, reproductive and epigenetic studies carried hitherto, the underlying cause of this disorder is still unidentified, and its diagnostic is always made by exclusion of conditions which mirror the phenotypes (Azziz, 2004). The current guidelines for PCOS diagnosis follow the Rotterdam criteria with phenotype detailing (A to D). (Eshre, 2018, Teede et al., 2018c)

Endocrine and metabolic implications reflect a high comorbidity (Ho et al., 2020): infertility (Bertoldo et al., 2019), endometrial cancer (Meczekalski et al., 2020), depression (Deeks et al., 2010, Singh et al., 2020) and mental illnesses (Glowinska et al., 2020, Harnod et al., 2020), obesity (Lombard et al., 2010, Moran et al., 2010a), obstructive sleep apnoea (OSA) (Sam et al., 2019, Kahal et al., 2020), cardiometabolic syndrome (Kakoly et al., 2019, Gunning et al., 2020, Livadas et al., 2020), cerebrovascular disease (Zhu et al., 2019), type II diabetes (T2DM) (Kazemi et al., 2019), dyslipidaemia (Lewandowski et al., 2019), non-alcoholic fatty liver (Azziz et al., 2009, Sarkar et al., 2020).

All the above plus other reproductive, metabolic, and mental disturbances related to Stein-Leventhal syndrome knuckle it down as a public health concern(Mohammadi, 2019). Therefore, associations of specialists advise guidance on the care and surveillance of the health and quality of reproductive life of women with this syndrome being frequent need for treatment in part or throughout life (Bellver et al., 2018, Jin et al., 2018) Notwithstanding the numerous pharmaceutical and/or surgical treatments addressing both PCOS clinical features and its comorbidities (Palomba et al., 2009, Carmina et al., 2019, Witchel et al., 2019), their long-term usage side effects and, low efficacy turn complementary medicine treatments a relevant asset (Wiweko et al., 2017, Facchinetti et al., 2019, Ramanan et al., 2020) . The aim of this work is to be to review the efficiency of TCM treatments conducted worldwide on Stein-Leventhal syndrome based on literature produced during the last five-year period.

1.1. Historical background on Stein-Leventhal Syndrome

Through mankind history the survival of the fittest has been a definite concern and so has human health. In early written records from Antiquity, the theme of Medicine is recurrent. Therefore, different cases on health conditions, even if not yet named or recognized as a factual illness were described from ancient times.

Feminine issues mainly if fertility was involved got the attention of the medical practitioners once that continuity of the species was and, still is the basilar stone in society – polycystic ovaries syndrome is a notable condition that made itself known in such registers once the external characteristics of the phenotypes can be, *per se*, quite peculiar.

Hippocrates of Kos (460-377 B.C), a distinctive physician in the Age of Pericles or Classical Greece acknowledged the existence of women of robust constitution, masculinized looks, infertile with scanty or short-lived menses (Hanson, 1975).

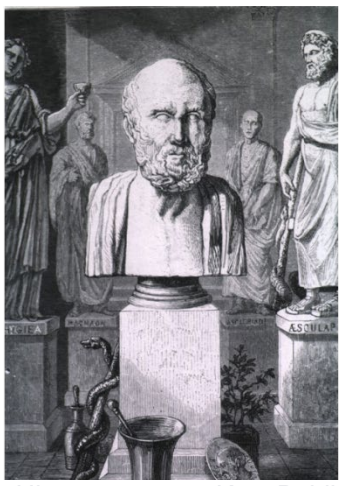


Figure 1. Hippocrates von Kos, Public Domain

Soranus of Ephesus (98-198 C.E.), a Greek physician specialized in gynaecology cit. in Lorieux, 2016 addressed *the association of amenorrhea with women who were masculine in appearance, and clitoral enlargement in women who had the characteristics of what the authors now recognize as congenital adrenal hyperplasia* (Loriaux, 2016) and also addressed the correlation between robust and man like women and sterility (Temkin et al., 1991).

Rabbi Moses Ben Maimon also known as Maimonides - a *Sephardi* physician and philosopher born in 1135´s Cordoba (Frank, 1981), considered one of the foremost intellectual figures philosopher of the Medieval Judaism – likewise pushed a pencil on women with heavy menses who displayed a manly nature and coarse skin (Rosner, 1971).

Figure 2. Maimonides (Public Domain, Rambam Institute)

Ambroise Paré (1510-1590 C.E.), preeminent obstetrician of the Renaissance Era (Shah, 1992) who in 1552 was assigned as Royal First Surgeon in the service of Henry II's and, later kept in service of his three sons - Francis II, Charles IX and Henry III – left 25 papers on his extended work on the practical side of Medicine (Popa et al., 2018). In his 24th book of the generation of Man, Paré pens about *Viragines* or Viragos – women featuring secondary amenorrhea, virile bulky structure, facial hair and a manly voice (Paré, 1634).

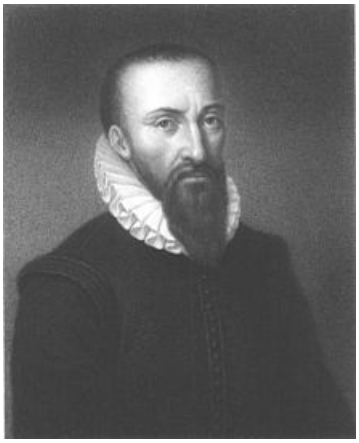


Figure 3. Ambroise Paré - Fantasy portrait by William Holl (Public Domain).

A naturalist of the 18th century-Italy and a crucial pivot at a top-notch European network of scientific correspondence of above 800 elements (Prete et al., 2009, Prete, 2012) – Antonio Vallisneri (1661-1730 C.E.) - includes in his vast work, a first remarkable description about a lady with classical clinical and morphological features of what would be associated today to PCOS (Speca et al., 2007). Cit. Vallisneri in Insler, 1990, "*Giovane rustica maritata, moderatamente pingue ed infeconda, con due ovaie piu grande del normale, come un uovo di colomba, bernoccolute, lucente e biancastre."* (Insler et al., 1990)

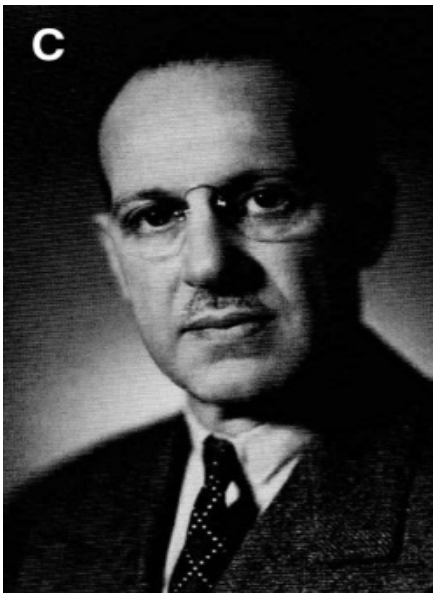
In 1844 Chereau and Rokitanski add another portrayal of a modification of the human ovary shape consisting mainly in a fibrosclerocystic structure (Dumachita-Gargu et al., 2010) and Gustav Bulius and Kreshmar registered the phenomenon of hyperthecosis for the first time by 1897 (Bulius et al., 1897). Decades downstream up to the twentieth century after this same histomorphology variation was reviewed by von Khalden in 1902 (Von Kahlden, 1902), it was proven to be correlated to absence of catamenia and infertility (Stein et al., 1935).

On this wise a new syndrome a posteriori designated as Stein-Leventhal Syndrome (Human PCOS), was then identified and consistently reported in September 1935 by two surgeons and medical researchers from Illinois, USA (Ellett et al., 1957) - Irving Freiler Stein and Michael Leventhal - who dedicated themselves to the exploratory study of the human ovaries on patients which chief complaints were secondary amenorrhea and sterility associated to bilateral polycystic ovaries through pneumoroentgenography ensued by wedge-resection of the cystic cortex of the ovaries as treatment to restore the ovaries´ functional normality (Stein et al., 1939).

Irving Freiler Stein M.D., is a natural from Chicago born on 17 September 1887 who attended the University of Michigan and then graduated in 1912 from Rush Medical College - also an affiliation of the University of Chicago from 1898 to 1942. From there he passed his residency at the Michael Reese Hospital following to each he served as an assistant in Surgery to Drs Carl Beck and D. K. Eisendrath at the department of Obstetrics and Gynaecology of the same hospital. Later he became senior staff gynaecologist at that institution and before he died in October 1976, he rose to president of the American Society for the study of Fertility in 1953. Over and above Stein served as president at the International Fertility Association (Gardner, 1953).

Michael Leo Leventhal was born in 1901, also an attendee of the University of Michigan and a graduate from the Rush Medical School – 1924. In 1925 Michael Leventhal did his internship at the Michael Reese Hospital where he met Dr Stein and to whom he

Figure 4.B- Michael Leo Leventhal M.D.



C – Irving Freiler Stein, M.D.

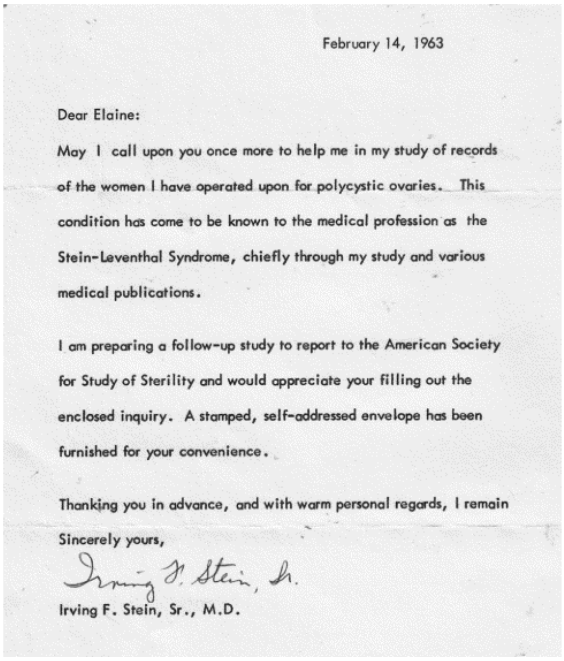
Licensed and authorized by (Azziz et al., 2016)

served as a clinical assistant from 1926 to 1935. Later Dr Leventhal became an instructor at the Northwestern University Medical School (Azziz and Adashi, 2016)

"*Amenorrhea associated with polycystic ovaries*" is a relevant by-product from an observational study initiated in 1929, when Irving Stein accomplished his first wedge resection of bilateral polycystic ovaries as a treatment for the condition of amenorrhea. Until then leading authoritative works on gynaecology associated bilateral polycystic ovaries more with uterine bleeding or metrorrhagia (Rochet et al., 1964) – fact explained by an increased number of follicles lined by granulosa cells secreting excess estrogenic hormone, a possible inducer to the confirmed pathological findings of endometrial hyperplasia (Strauss et al., 2014).

A preliminary communication of this decisive pilot study, ensued with seven amenorrhoeic women through a maximum period of five years, was made at the Central Association of Obstetricians and Gynaecologists in New Orleans in November 1934.

The seven patients who participated in this research work showed an additional cluster of identical clinical symptoms to menstrual irregularity featuring secondary amenorrhea and a history of infertility. This conjunct of findings, years later to be considered among the classic signs and symptoms of PCOS, were bilateral polycystic ovaries, a masculine type of hirsutism and to less extent breast hypoplasia and obesity.



February 14, 1963

Dear Elaine:

May I call upon you once more to help me in my study of records of the women I have operated upon for polycystic ovaries. This condition has come to be known to the medical profession as the Stein-Leventhal Syndrome, chiefly through my study and various medical publications.

I am preparing a follow-up study to report to the American Society for Study of Sterility and would appreciate your filling out the enclosed inquiry. A stamped, self-addressed envelope has been furnished for your convenience.

Thanking you in advance, and with warm personal regards, I remain

Sincerely yours,

Irving F. Stein, Sr.

Irving F. Stein, Sr., M.D.

Figure 5.Letter that Dr Stein sent to patients in whom he had performed bilateral ovarian wedge resection for the Stein-Leventhal syndrome seeking follow-up, dated Feb. 14, 1963. Licensed and authorized by: (Azziz et al., 2016)

A remark regarding the first choice of the hormonal treatment with endocrine preparations as a conservative treatment to regulate the menstrual cycle and promote ovulation: during the 30's, experimental assays with anterior pituitary-like substances as antuitrin-S (Geist et al., 1935) supported an endocrine imbalance as the origin of bilateral polycystic ovaries (Mandelstamm et al., 1932). In this case hormonal therapy positively promoted menstruation in a few of the seven women, yet it failed to foster pregnancy. As no childbearing resulted from this first strategy it was proceeded to an invasive technique of partial resection of the ovary - already implemented during the XIX century, in 1891 by Marin, in 1894 by Matthaei, in 1895 by Waldo R and even by Gustav Bulius in1897 (Goldzieher, 1982).

Given the histology studies described above the authors considered the principle of hypersecretion of anterior pituitary hormone as insufficient – endocrine imbalance of the anterior pituitary had already been previously associated to both metrorrhagia and amenorrhea (Zondek, 1932) – hence they appointed a mechanical factor as a further reason for the amenorrhea and sterility.

A test to this theory was ensued through the subsequent decade, where in a study, 46 women presenting the identical signs and symptoms were thoroughly examined. According to the collected data, it was suggested that the ovarian cortex packed with ovarian theca or granulosa-lined microcysts altogether with a thickened tunica albuginea

act as an impediment for ovulation once that the immature follicles to ripen and get to the surface (Stein et al., 1939, Stein, 1945). Such hypothesis was corroborated by the fact that after the wedge resection, the physical obstruction to normal ovulation was eliminated thus ovarian function was overall restored with ovulated menstrual cycles allowing sterility to be overcome.

From there, Stein and his fellows´ trained eyes were able to consistently report a collective of findings coinciding with the signs and symptoms described in the – by 1958 more than 114 cases have been reported by Stein and Leventhal (Barry, 1961). These would lead to the pronouncement of a definite syndrome under their name – cit. White, Barry (2008) *if we accept the definition of syndrome as being a complexus of symptoms and signs* (Duncan, 2014) . The pathogenesis behind the disorder had yet not been buttoned down by 1958, still Irving Stein and Michael Leventhal had already outlined the keystones for an early definition.

Cit. Stein in Roberts et al, the syndrome is finally defined as: "*sterility accompanied by secondary amenorrhea, hirsuties in 50-75%, and consistently enlarged symmetrical ovaries with a definite pathological pattern*". Cit Leventhal (Leventhal, 1958): " *Persistent anovulation characterizes all patterns of bleeding. The basal body temperatures show a continually monophasic curve, the vaginal smears demonstrate an estrogenic effect, and the endometrial biopsies reveal a low proliferative endometrium. (…) The demonstration of bilateral polycystic ovaries is the keystone in the diagnosis. (…) The sudden appearance of male type hirsutism is significant (…) the ovaries are enlarged grossly to a size approaching and at times exceeding the size of the uterine corpus. (…) The tunica is thick, tough, and pearly white*. (…) *One of the most significant and consistent findings is a hyperplasia of the theca interna cells, which most frequently show evidence of luteinization. (…) The hyperplasia is marked by numerous mitotic figures and hyperchromatism and is very richy vascularized. (…) the paradox of a wide range of endometrial reactions with ovarian hyperthecal activity may be explained by changes in the estrogen-androgen balance.* (Roberts et al., 1960)

So far, in review of the last pages, it is safe to say that according to modern history the true birth of the diagnosis of PCOS, started with an *in-situ* observation of abnormally enlarged ovaries by pelvic examination, combined with a history of amenorrhea and hirsutism. The morphological alterations were then confirmed by microscopic analysis of histologic cross sections of the affected ovaries and at the time, these reproductive female organs with associated endocrine role were considered the main guilty for this.

Goldzieher and Green, 1962 reported that in general, morphological polycystic ovaries was a feature that could be or could be not associated with the rest of the symptoms and signs of the syndrome. (Goldzieher et al., 1962) The hypothetical neuroendocrinal disturbance was first put on the table after a sum of studies between the late 50's and 1970 - in 1958 a study correlated high levels of urinary LH with diagnosed PCOS females and, in 1970 it was recorded that the ratio LH/FSH was also high in women diagnosed women with Stein-Leventhal's syndrome. (Mcarthur et al., 1958, Yen, 1970).

However, major breakthroughs on biochemical assays during the 70s and 80s, broadened the insight of the implications and possible origins. Rosenfield et al, 1972 identified that hyperandrogenaemia could be also related to the ovaries besides adrenal production (Rosenfield et al., 1972), and later in 1986 the study of Futterweit et al. confirmed that the administration of exogenous testosterone in female-to-male transexuals animal models led to de development of polycystic ovaries, underlining the major factor of biochemical hyperandrogenism on PCOS (Futterweit et al., 1986).

By 1983 Barbieri et al had confirmed the relation between hyperandrogenism, acanthosis nigricans and insulin resistance (Barbieri et al., 1983) and meanwhile before 1990 several authors had noticed that the profile of hyperinsulinemia or of insulin decreased sensitivity to insulin in PCOS females was not necessarily related to an obesity pattern (Chang et al., 1983, Dunaif et al., 1989a, Dunaif et al., 1989b) and that elevated levels of insulin in synergy with LH stimulated the production of androgens by the interstitial thecal cells in the ovaries - in overall corroborating a theory that hyperinsulinemia contributed to hyperandrogenism in women with PCOS. (Cara et al., 1988, Hernandez et al., 1988)

Due to a lack of diagnostical consensus regarding this matter, in 1990, the National Institute of Child and Human Development (NIH – NICHD) definitely defined the conceptualization of PCOS as an endocrine disorder and summarized for the first time the general diagnostic criteria to be: clinical or biochemical hyperandrogenism/ hyperandrogenaemia, oligo-ovulation with an exclusion of other potential causes that mirror similar symptoms like congenital adrenal hyperplasia, Cushing's syndrome and the existence of androgen-secreting tumours. (Dunaif et al., 1992)

By 1989 there a study had meanwhile revealed another phenotype of PCOS later termed as functional ovarian hyperandrogenism which implies a general ovarian steroidogenesis hyper response - showing a dysregulation of 17-hydroxylase and 17, 20 -lyase activities by enzyme cytochrome P450c17 encoded by the gene *CYP17A1* – inducing elevated 17-hydroxyprogesterone and androstenedione. (Barnes et al., 1989, Rosenfield et al., 1990) This scenario was proved to be independent of LH surge and morphological

polycystic ovaries however sensitive to GnRH agonists. (Ehrmann et al., 1992, Barnes et al., 1993)

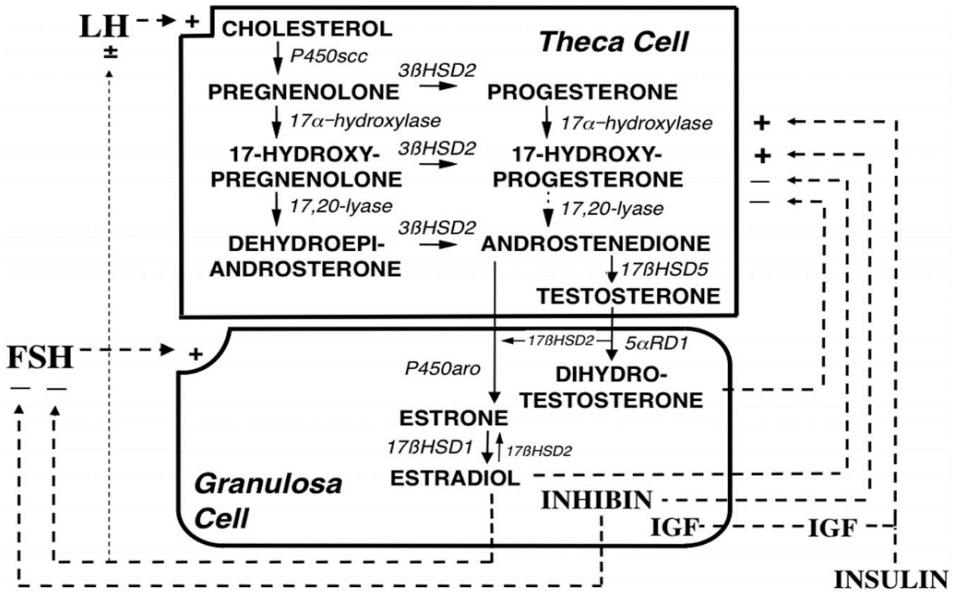


Figure 6. Depiction of the organization and regulation of the major steroid biosynthetic pathways in the small antral follicle of the ovary according to the 2-gonadotropin, 2-cell model of ovarian steroidogenesis. Licensed and authorized by (Rosenfield et al., 2016)

The perspective over PCOS origin was changing:

- On one hand the ovary was regarded as the origin of the syndrome and not as the target of a neuroendocrine imbalance.
- On the other hand, steroidogenic irregularity was attributed to an enzyme dysfunction and not to an enzymatic deficiency.

Further research work supported this hypothesis and added the information that although it could be related with insulin resistance and/or obesity it was not derived from them. (Ehrmann et al., 1995b)

By 2003 the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) reformulated the criteria to meet the diagnosis of PCOS. Two out of three criteria should be present to confirm the syndrome: oligo/anovulation, androgen excess and the confirmation of polycystic ovarian morphology by imaging (gold standard measure: presence of 12 or more follicles in each ovary measuring 2±9 mm in diameter, and/or increased ovarian volume (>10 ml)' - (Group,

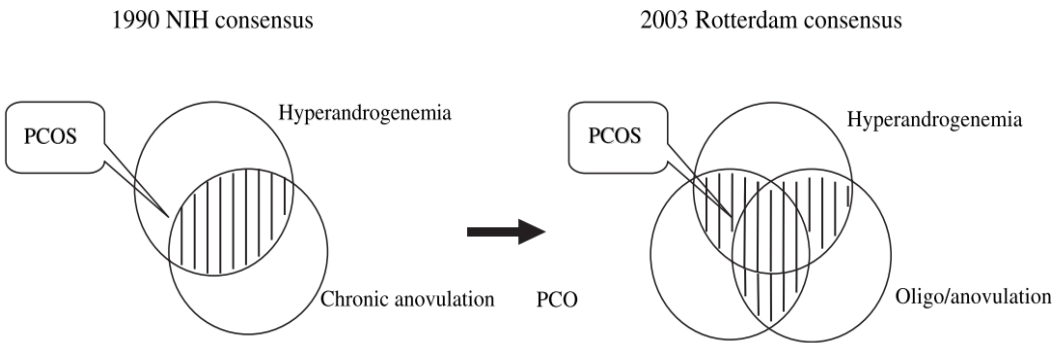


Figure 7. Diagnosis of PCOS, according to the 1990 NIH and the 2003 Rotterdam consensus criteria. Licensed and authorized by (Broekmans et al., 2006)

2004)). These are designed as Rotterdam criteria and from them it is possible to identify four distinct phenotypes of PCOS. (Broekmans et al., 2006, Franks, 2006)

The cutoff values for each criterion were also determined and put forward according to a consense and are dysplayed in the table below:

Factor	Definition and cutoff	NIH-PCOS (Rott-PCOS, no PCO)	Rott-PCOS no hyperandrogenism	Rott-PCOS normal cycle	Rott-PCOS full
Oligo/anovulation	WHO-II classification: Oligomenorrhoea (35–182 days) or amenorrhoea (>182 days) and FSH 1–10 U/l, with normal E2	+	+		+
Hyperandrogenism	Clinical: hirsutism (Ferriman–Gallwey score ≥ 9) and/or biochemical: FAI* > 4.5	+		+	+
PCO on transvaginal sonography	Volume: one or two ovaries > 10 cm^3 and/or follicle count (2–9 mm); one or two ovaries ≥ 12 follicle		+	+	+

*FAI = (Total testosterone × 100)/SHBG.

Table 1.Criteria for PCOS diagnosis according to NIH and Rotterdam consensus. Authorized and licensed (Broekmans et al., 2006)

Between September 30 and October 1, 2005, in Ravello, a second Special Scientific Meeting of the Androgen Excess Society was held by international differentiated specialists. Within the objectives of this meeting was featured: clinical features and diagnosis in PCOS. Both obstetric gynaecologists and endocrinologists, debated diagnosis and treatment of PCOS. (Francesco Orio et al., 2006)

Androgen Excess – PCOS Society (AE-PCOS) defined the diagnostic with the incorporation of two out of three criteria where it was possible to take in hyperandrogenism and oligo-anovulation or hyperandrogenism and polycystic ovaries. This new definition

made it possible to identify “ovulatory PCOS” i.e. include the estimated 10% of the women with PCOS that had hyperandrogenism with ovulatory cycles. (Wild et al., 2010)

NIH 1990	Rotterdam 2003	AE-PCOS Society 2006
• Chronic anovulation • Clinical and/or biochemical signs of hyperandrogenism (with exclusion of other etiologies, e.g., congenital adrenal hyperplasia) *(Both criteria needed)*	• Oligo- and/or anovulation • Clinical and/or biochemical signs of hyperandrogenism • Polycystic ovaries *(Two of three criteria needed)*	• Clinical and/or biochemical signs of hyperandrogenism • Ovarian dysfunction (Oligo-anovulation and/or polycystic ovarian morphology) *(Both criteria needed)*

Table 2.Diagnostic criteria for PCOS used until 2012. National Institutes of Health Evidence-based Methodology Workshop on Polycystic Ovary Syndrome, Final Report (Public Domain)

By then diagnoses for PCOS follow one of three guidelines: NICHD (1990), Rotterdam (2003) or AE-PCOS Society (2006). (Daniilidis et al., 2009). Not only the use of multiple classification systems became confusing and hampered the clinicians from a clear communication with the patients about their health issues management but it also promoted the overlapping of phenotypes as it is possible to analysed on the following table:

		Potential PCOS Phenotypes									
		A	B	C	D	E	F	G	H	I	J
Panel Terminology	**Diagnostic Criteria**	**NIH**						**AE-PCOS/ Rotterdam 1**			**Rotterdam 2**
Androgen Excess	Hyperandrogenemia	+	–	+	+	–	+	+	–	+	–
	Hyperandrogenism*	+	+	–	+	+	–	+	+	–	–
Ovulatory Dysfunction	Oligo-anovulation	+	+	+	+	+	+	–	–	–	+
Polycystic Ovarian Morphology	Polycystic Ovaries	+	+	+	–	–	–	+	+	+	+
	NIH 1990 Criteria	x	x	x	x	x	x				
	Rotterdam 2003 Criteria	x	x	x	x	x	x	x	x	x	x
	AE-PCOS 2006 Criteria	x	x	x	x	x	x	x	x	x	

Table 3.Potential Phenotypes of PCOS by NIH 1990, Rotterdam 2003, and AE-PCOS. National Institutes of Health Evidence-based Methodology Workshop on Polycystic Ovary Syndrome, Final Report (Public Domain)

Later in 3-5 December 2012, under the scope of the international workshop series "Pathways to Prevention (P2P)" promoted by National Institutes of Health, an independent panel came to consensus of adopting the Rotterdam criteria with the discrimination of one of the possible 4 phenotypes as a new standard for researchers and clinicians (table 4).

Adult Diagnostic Criteria (Rotterdam)

Otherwise unexplained alternative phenotypes:

1. Phenotype 1 (classic PCOS)[a]
   a. Clinical and/or biochemical evidence of hyperandrogenism
   b. Evidence of oligo-anovulation
   c. Ultrasonographic evidence of a polycystic ovary
2. Phenotype 2 (Essential NIH Criteria)[a]
   a. Clinical and/or biochemical evidence of hyperandrogenism
   b. Evidence of oligo-anovulation
3. Phenotype 3 (ovulatory PCOS)[a]
   a. Clinical and/or biochemical evidence of hyperandrogenism
   b. Ultrasonographic evidence of a polycystic ovary
4. Phenotype 4 (nonhyperandrogenic PCOS)
   a. Evidence of oligo-anovulation
   b. Ultrasonographic evidence of a polycystic ovary

Table 4.Diagnostic criteria for PCOS by NIH 2012 – a) AE-PCOS Society only recognizes hyperandrogenic phenotypes. Licensed and authorized by (Rosenfield et al., 2016)

The hyperandrogenism and ovulatory disfunction turns less severe from phenotype 1-3 (or A-C). Phenotype 4 (or D) does not consider hyperandrogenism – only anovulatory cycles and polycystic morphology of the ovaries, reflecting a possible functional hypothalamic amenorrhea not considered as a constitute of PCOS by AE-PCOS Society. (Lauritsen et al., 2015) Furthermore, phenotype 3 (or C) makes it possible to diagnose PCOS in mildly hirsute women with normal androgens serum level who would be considered to have idiopathic hirsutism according to the Endocrine Society Clinical Practice Guidelines. (Martin et al., 2008) These are two more contested examples however more weaknesses are found within the current guideline:

- Biochemical assessment of hyperandrogenemia is difficult: testosterone serum levels go through diurnal episodic and cyclic alterations (Rosner et al., 2007), commercial issues with testosterone assays added to the fact that many steroid assays are not accurated and even the best showed slight differencees between them.(Rosner et al., 2007, Rosner et al., 2010, Auchus, 2014)
- Clinical assessment of hyperandregenemia is then based on hirstutism, though as aforementioned the diagnosis can crossline witht the idiopathic hirsutism diagnosis on other guidelines. (Martin et al., 2008)

- Criteria defined for the imaging diagnosis of polycystic morphology of the ovaries can tend for an overdiagnosis in women if the count of antral follicles is based on cut edge high-definition techniques (Dewailly et al., 2014)

Facing these difficulties, it has been proposed to use a substituting criterion to the count of antral follicles and ovarian volume: assessment of the anti-Mullerian hormone (AMH). AMH proved to be related to androgen status hinting a main and direct part of androgens in the reproductive dysfunction in PCOS - therefore and considering AMH reflecting PCOS status, it may also be useful in PCOS diagnosis. (Cassar et al., 2014)

A study with 262 women divided according to phenotypes derived from Rotterdam criteria for PCOS compared the ratio of serum AMH to the total antral follicle count (AFC) – AMH/AFC ratio was used as a tool for assessing AMH production per follicle. The results demonstrated a higher ratio in ovulatory dysfunction phenotypes, endorsing the hypothesis of a key role for AMH in the mechanism of anovulation in PCOS. (Bhide et al., 2017)

Features	Phenotypes			
	A	B	C	D
Ovulatory dysfunction	X	X	X	
Hirsutism and/or hyperandrogenemia	X	X		X
Polycystic ovaries	X		X	X
AMH/AFC Ratio	1.5	1.6	1.2	1.1

Table 5.Phenotypes of PCOS according to the NIH 1990 and Rotterdam 2003 criteria. Licensed and authorized by (Azziz, 2006) – adapted

Despite the utility, sensitivity, and reliability of AMH levels as a marker for ovarian response - hyperandrogenism and anovulation in PCOS - further studies question the current available assays for AMH assessment in the market. These assays use different reference values and face issues of sample stability, turning the interpretation of the AMH values and their clinical implications into a dichotomy and possibly misleading decisions in clinical context as IVF treatments of PCOS patients. (Liss et al., 2017, Magnusson et al., 2017)

At present, and within the scope of the latest review regarding the subject of use of AMH as a substitute tool for diagnosis of PCOS and polycystic ovary morphology, it is not recommended the use of AMH assays until some gaps are filled in. In the future, with an improvement of the assay quality and respective standardization, AMH levels can feature as a steady replacement to ultrasound techniques which are pricey and less reachable. (Teede et al., 2019a)

Meanwhile, to address the antral follicle count dilemma, the new international guidelines redefined the ultrasound check and polycystic ovary morphology parameter as: a follicle number per ovary of ≥20 and/or an ovarian volume ≥10 ml on either ovary, ensuring no corpora lutea, cysts or dominant follicles are present when using endovaginal ultrasound transducers with a frequency bandwidth that includes 8 MHz. If using an older imaging technique, it is suggested to consider as threshold a volume on each ovary ≥10 ml. Moreover, the high prevalence of multifollicular ovaries during the first 8 years after the menarche led to the update on international inputs disregard the use of ultrasound on those cases. (Teede et al., 2018b)

1.2. Prevalence and impact on society

Nowadays, it is estimated that more than one out of ten women worldwide is diagnosed with PCOS (Barsky et al., 2020) - evaluated as a prevalent and leading condition of both female reproductive and endocrine impairment, with a series of heterogeneous implications on the metabolic and psychological status of the individual, i.e., it has marked repercussion on their quality of life. (Rao and Bhide, 2020)

A syndrome, according to Azziz, should be defined accordingly to its long-term impact or morbidity, more than the phenotypical constellations. (Azziz, 2006) Within the symptoms that can appear during the lifespan of a women carrying PCOS we can find hyperandrogenic skin conditions like acne and hirsutism during younger years plus infertility problems and metabolic disorder such as abdominal obesity, cardiovascular disease, type 2 diabetes (T2DM) and mental health related issues as anxiety and depression, among others. (Teede et al., 2010a, Teede et al., 2010b)

Figure 8 shows common clinical concerns during the lifespan of a woman with PCOS – information gathered from the 2012 workshop sponsored by NIH. The identification and management of such a cluster of symptoms brings a burden over the national health systems and even over the family's finances considering that not only the proper diagnosis precludes other conditions hence costly blood work and ultrasound testing.

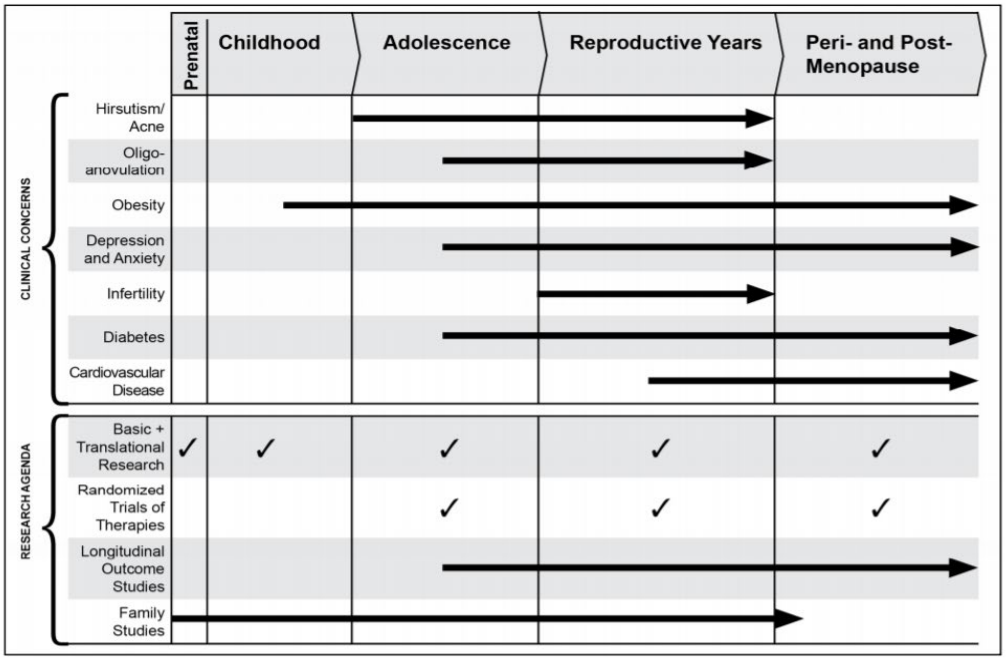


Figure 8. Common Clinical Manifestations Associated with the Syndrome Across the Life. National Institutes of Health. (Public Domain)

PCOS is estimated to be the guilty of 72–84% of adult hyperandrogenism among other conditions that should be excluded during a pertinent diagnostic. (Witchel et al., 2015) The table below synthetizes the differential endocrine conditions to be excluded from the diagnosis:

A. Physiologic adolescent anovulation
B. Functional gonadal hyperandrogenism
1. PCOS:Primary FOH (common form of PCOS)
2. Secondary FOH
a. Virilizing congenital adrenal hyperplasia
b. Adrenal rests of the ovary
c. Ovarian steroidogenic blocks
d. Insulin resistance syndromes
e. Acromegaly
f. Epilepsy ± valproic acid therapy
3. Disorders of sex development
4. Pregnancy-related hyperandrogenism
C. FAH
1. PCOS:primary FAH (uncommon form of PCOS)
2. Virilizing congenital adrenal hyperplasia
3. Other glucocorticoid-suppressible FAH
a. Hyperprolactinemia
b. Cortisone RD deficiency (and apparent RD deficiency)
c. Apparent DHEA sulfotransferase deficiency
4. Glucocorticoid-nonsuppressible FAH
a. Cushing's syndrome
b. Glucocorticoid resistance
D. Peripheral androgen metabolic disorders
1. Obesity
2. Idiopathic hyperandrogenism
3. Portohepatic shunting
E. Virilizing tumors
F. Androgenic drugs

Table 6. Differential diagnosis of hyperandrogenaemia. FAH – Functional Adrenal Hyperandrogenism Authorized and licensed by (Rosenfield et al., 2016).

Such a position determines that clinically reliable test procedures should be taken into action for a safe differentiation of the source of androgen excess on the women once that androgens are secreted by both the ovaries and adrenal glands in response LH and ACTH respectively. Hereby it is possible to follow the standard tests to determine the androgenic origin:

Test	Rationale	Method	Outcome Measures	Interpretation[a]
GnRHag	Endogenous LH and FSH release stimulates coordinated function of ovarian follicles	Leuprolide acetate 10 μg/kg sc (for maximum stimulation)	Ovarian steroid secretion peaks at 20–24 h	17OHP >152 ng/dL without steroidogenic block indicates typical FOH (PCOS-T)
hCG	Exogenous administration of LH analog stimulates theca-interstitial cells	hCG 3000 IU/m^2 (for maximum stimulation)	Ovarian steroid secretion peaks at 24 h	17OHP >152 ng/dL without steroidogenic block indicates typical FOH (PCOS-T)
LDAST	Long DAST: dexamethasone profoundly suppresses adrenal androgens over several days	Dexamethasone 0.5 mg QID per os × 4–5 d	Free testosterone, DHEAS, cortisol: sample early morning d 5	Free testosterone ≥8 pg/mL with DHEAS <70 and cortisol <1 μg/dL characteristic of FOH
SDAST	Short DAST: dexamethasone rapidly suppresses adrenal testosterone and cortisol	Dexamethasone 0.25 mg/m^2 per os at 12 noon	Total testosterone, cortisol: sample 4 PM (4 h)	Total testosterone >26 ng/mL, cortisol <5 μg/dL suggests FOH
ACTH	Exogenous ACTH stimulates adrenal steroidogenesis	Cosyntropin ≥10 μg/m^2 (for maximum stimulation)	DHEA,17OHP, steroid intermediates, cortisol peak at 30–60 min	DHEA 1500–3000 μg/dL without steroidogenic block indicates FAH

Table 7. Test procedures to determine source of female androgen excess. Licensed and authorized by (Rosenfield et al., 2016)

A broad study of 2005 tried to estimate the potential costs that women with PCOS have on the US national health plan. First it was calculated the total of woman within fertile age, 14-49: the study identified 4 million of american women with PCOS. It was assumed that at least a woman would have been evaluated at least once in their lifetime. Costs estimated for their first screening:

Test	PCOS requiring test (%)	Cost/unit of service[a]	Total cost ($)
Testosterone panel[b]	25	71.65	71.65
DHEAS	25	31.07	31.07
TSH	100	23.47	23.47
Prolactin	100	27.08	27.08
Basal 17-HP	100	37.95	113.85
ACTH test	6	121.46	121.46
TV-U/S	100	126.28	126.28
Endometrial biopsy	30	123.87	123.87
oGTT	100	60.91	60.91
Lipid profile	100	37.90	37.90
Total cost/initial evaluation		661.64	737.54

Table 8. Cost of the initial evaluation of patients with PCOS in 2004 dollars. Licensed and authorized by (Azziz et al., 2005)

To the estipend obtained from the diagnostic tests authors summed up the calculus of the treatments and follow-up of the most prevalent comorbidities linked to women with PCOS – diagnostic following the classic phenotype (A) following NIH-1990 guidelines.

Morbidity	Prevalence (%)
Menstrual dysfunction/AUB	75.0
Infertility/year	50.0
Type 2 DM	7.2
Hirsutism	70.0

Table 9. Estimates of the prevalence of morbidities associated with PCOS in the United States used for calculation of economic burden. Licensed and authorized by (Azziz et al., 2005)

Authors excluded from comorbidites : potential increases in the risks for endometrial hyperplasia and carcinoma, early pregnancy loss and obstetrical complications, cardiovascular, including cerebrovascular disease, hypertension, and dyslipidemia, and dermatological complaints such as acne and androgenic alopecia. (Azziz et al., 2005, Joham et al., 2015b)

	Annual costs in millions of U.S. dollars (% of total costs)
Initial evaluation	99 (2.3)
Treatment	
Menstrual dysfunction/AUB	1350 (30.9)
Infertility	533 (12.2)
Type 2 DM	1766 (40.4)
Hirsutism	622 (14.2)
Total cost	4370 (100.0)

Table 10. The overall health care-related economic burden of PCOS patients during their reproductive years in 2004. Licensed and authorized by (Azziz et al., 2005)

Overall, by 2004, the study estimated annual cost of about 4.37 billion of dollars spent in identification and management of the PCOS condition in USA – only during the reproductive gape. However the nowaday costs would have skyrocketed - taking into account economical inflation over the last 15 years, the present worlwide number of women affected with this syndrome (which only in US is projected to be 5 million by 2012 (NIH report, 2012)), and the fact that expenses are extended to an entire woman's lifespan due to the type of secondary health issues.

Comparing data from table 3 and table 4, T2DM is the comorbidity with heavier costs (40% of prevalence on the total burden) tought estimated to be the less present of the four paramters considered by the reviewers. UK researchers took this data and developed another broad study on a co-hort of T2DM women within the PCOS population in UK aged 14-44. The model attempted to measure healthcare costs and quality of life for a period between 2014-2019 and results are shown below:

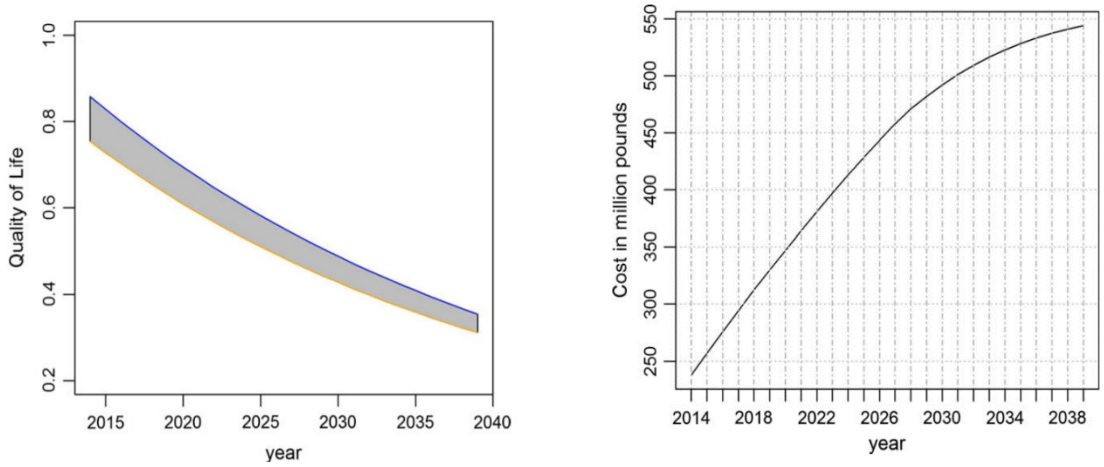


Figure 9.Left - Quality of life measured by EQ-5D simulated for the PCOS cohort (yellow line) and a healthy cohort (blue line) in the UK over the follow-up period (2014–2039).

Right- Economic burden of PCOS in the UK over the follow-up period (2014–2039).

Authorized and licensed by (Ding et al., 2018)

Authors predict that 26% of PCOS population may thrive with diabetes by the end of follow-up and quality of life of PCOS patients was lower than women without PCOS throughout the follow-up period. NHS annual expenses were projected to least £237 million. (Ding et al., 2018)

By 2002 a study showed that there is a tendency for a familial clustering with genetic predisposition maybe involved in early onset of PCOS and that environmental factors may lead to the expression of the clinical phenotype. (Battaglia et al., 2002) A cross-sectional socioeconomic study by Bharathi et. al, 2017 among 502 random young women in Chennai, India, demonstrates that PCOS as a lifestyle disorder highly prevalent within wealthy and middle income urban population as compared to rural population which might infer the association between POCS and quality of food intake. (Bharathi et al., 2017)

It is important underline that the prevalence depends on the accurate diagnostic or undiagnostic of this female condition since the disease is considered multifactorial and as

a complex syndromic disorder it is often difficult to diagnose due to overlapping symptoms. Multiple etiological factors have been implicated in PCOS. Authors estimate this condition to be undiagnosed in up to 70% of affected women and key features such as a psychological burden and metabolic risks are under-recognised. (Boyle and Teede, 2012)

The broadest study about satisfaction with the experience regarding PCOS diagnosis, drawed information at diagnosis and present worries among done among revealed belated diagnosis and defective information leading to the conclusion that a promt diagnosis, support and coaching would positively impact the patient's experience. (Gibson-Helm et al., 2017)

An early accurate diagnostic leads to an adequate treatment from an early age protecting the woman to develop more serious comorbities at middle age and to lessen the needs to go for severe and costly IVF treatments in case of infertility associated to PCOS. (Thong et al., 2020)

An assessment of the clinicians preparation is demanded and recent literature indicates a need of more education and awareness on information based on Rotterdam diagnostic criteria, management of psychosocial comorbidities and PCOS treatments among physicians (Piltonen et al., 2019)

This is evidence that current medical services lack the ability to proper educate and support women with their diverse reproductive, metabolic, and psychological care needs inside a fragmented service across health providers. Although national evidence-based guideline advocate for interdisciplinary services, a poor translation led onto wide practice variation and no optimal models of care. It is strongly advised to codevelop multidisciplinary and holistic care by consumers and health professionals. (Tay et al., 2018)

For all socio-economic reasons and high impact on quality of life as proved so far, most women try or thought about trying complementary treatments including TCM or Ayurvedic Medicine. (Sim et al., 2010, Liao et al., 2018)

1.3. Stein-Leventhal Syndrome and WM

For a better understanding of such a complex health condition it is very important to grasp a solid knowledge of the anatomy and physiology of the human female reproductive system involved in the pathology as well of the neuro-endocrine system responsible for controlling the homeostasis of a healthy and functional reproduction.

1.3.1. Uterus and Ovary

Often considered separately, the abdomen and pelvis shape the largest continuous visceral cavity of the human body. Abdomen and pelvis join forces to provide several vital functions as per example:

- support and protection of the digestive, urinary tracts, internal reproductive organs, and their respective neurovascular supplies.
- deliver the neurovascular transmission to and from the thorax and the lower limbs.
- to offer support and attachment of the internal reproductive and urinary organs to the external genitalia and access.
- To accessorize functional muscles to facilitate physiological actions such as respiration, defecation, and micturition.
- stabilize the spinal column so it can support weight and execute proper movement.

Viscera are the organs found inside or intimately related to the pleural and peritoneal cavities – these may present a solid or a hollow/tubular constitution. All of them are suspended inside of the celomic cavities by laminae of connective tissue and covered by a serous tunica through which vases, nerves and ducts take access to viscera. The walls of the peritoneal or abdominopelvic cavity are lined by a one serous membrane like aforementioned – it is denominated as peritoneum. (Tortora, 2013)

Hollow viscera are internally coated by a mucous tunica and may also exhibit an external fibromuscular coating where there is a prevalence of single-unit smooth muscle tissue, governed autonomous nervous system (ANS). The extensions of visceral epithelial and connective coating may form distinct types of glands: exocrine or endocrine. Endocrine glands may be originated from hollow viscera during embryogenesis or independently originated from the neural plate (supra-renal medulla and neurohypophysis) or may be even be originated from mesoderm (supra-renal cortex). (Standring, 2015)

The low pelvic viscera comprise part of the gastrointestinal system, the urinary system, and the reproductive system – in both, male and female. Female reproductive system can be anatomically divided in external and internal genitals. (Hoare Bs, 2020) For this research two main organs are considered of major importance: uterus and ovary, once they are the ones involved in the reproductive cycles affected by the neuro-endocrinal disruption in the Stein-Leventhal syndrome.

Internal Female Genitalia

Inside the true pelvis we can find the viscera that belong to the female internal genitalia: vagina, cervix, uterus, fallopian tubes, and ovaries.

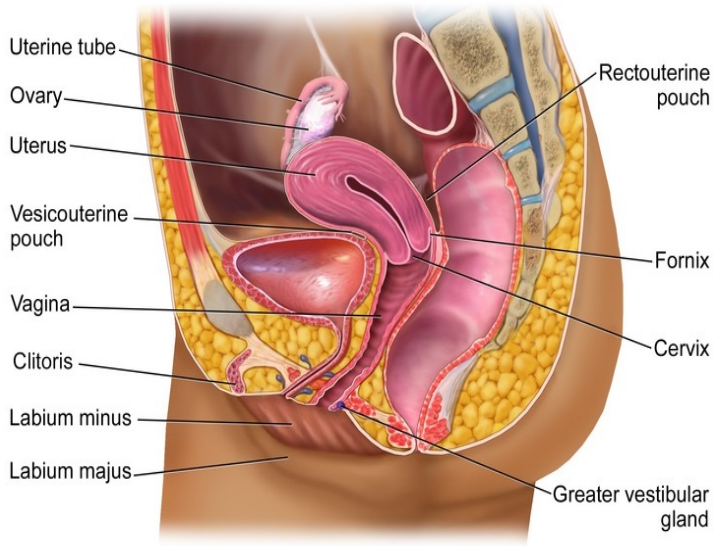


Figure 10. Female reproductive anatomy. Under the terms of the Creative Commons Attribution 4.0 International License. Source:(Hoare BS, 2020).

Uterus

The uterus is the largest anatomic structure of the female reproductive system serving the purpose of blastocyst implantation, embryo development, carrying and protecting the future child. Therefore, to successfully adapt to the foetus development this hollow organ goes under hypertrophy and hyperplasia during pregnancy - reaching to the epigastric region in the later stages of the process – fact that underlines the thick, elastic and muscular nature of the uterus. It is normally situated in the lower pelvis posteriorly to the urinary bladder and anteriorly to the rectum.

The uterus is anatomically divided into four main regions: the fundus, the body, the isthmus, and the cervix. The upper two thirds constitute the fundus and the body of the uterus (corpus uteri) and the lower third forms the isthmus and cervix (cervix uteri). The body of the uterus presents an inverted pyriform shape and contains a lumen that is flat antero-posteriorly. The lower part offering passage to the cervix is narrower and is cylindrical in shape and it continues downwardly to the vagina. The adult nulliparous woman normally accommodates a uterus that is about 7.5 cm long x 5.0 cm in broad x 2.5 cm thick, and weighs between 30 and 40 gr. In females who have recently been pregnant the

structure presents wider dimensions, contrary to menopause when sex hormones are low, and the uterus becomes smaller (atrophied). At the top of the body of the uterus we find the uterine tubes which are two symmetrical structures laterally attached to it from both sides, with their ostia opening into the lumen. (Standring, 2008, Hoare Bs, 2020)

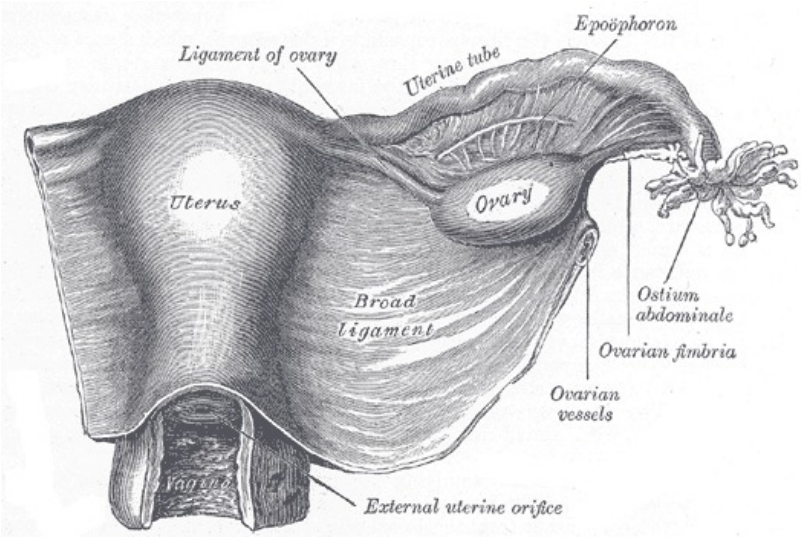


Figure 11. The female genital organs, uterus, and right broad ligament - under the terms of the Creative Commons Attribution 4.0 International License. Source: (Hoare BS, 2020)

In a healthy nulliparous state, the uterus normally tilts forwardly along its axis – when the bladder is empty the whole uterus leans forwards at an angle to the vagina, and in this position is described as anteverted. However, in some cases - about 10% to 15% of women - the whole uterus leans backwards, and it is considered a retroflexed uterus. (Drake et al., 2009)

In respect to its histology it is possible to find three distinct functional layers in this organ:

- An outer layer or serosa
- A medium layer rich in smooth muscle – myometrium
- An internal mucosa: endometrium

The internal layer or endometrium plays a fundamental role in the preparation for implantation, and in nurturing the pregnancy if the latest does take place or to promote menstruation if fecundation is absent during that reproductive cycle. This layer is a pluricellular complex made of steroid-target tissue – particularly sensitive to progesterone - which goes through hypertrophy during the follicular phase of the cycle involving an intricate conjunct of events that involve resolution of inflammation, angiogenesis, tissue remodulation and formation - this repair after previous lining shedding occurs without

residual scarring or even functional loss). In the absence of an ovum to implant at the proliferated endometrium it enters a breakdown stage with its release through menses. This interplay between proliferation and desquamation of the endometrial layer of the uterus leads to a uterine cycle commonly designated as menstrual cycle and it is part of the female reproductive cycle along with the ovarian changes – both uterine and ovarian cycles are driven by coordinate positive and negative hormonal feedbacks involving the hypothalamus-pituitary-ovarian axis (HPG axis) that is later reviewed in the section of the physiology of the reproductive system. (Critchley et al., 2020)

Uterine Vascular supply

The vascular net of the uterus consists of two different vessels, distinguished by the type of blood they carry respectively: arterial or venous blood. Hence, we can find an arterial supply and a venous drainage accordingly. The uterine artery nurtures the uterine body, the uterine cervix, tubes, and the upper part of the vagina. Authors consider this blood vessel as the most important in the pelvic region due to its stated hyper development during a woman's pregnancy. This artery derivates from the anterior division of the internal iliac artery and crosses anteriorly to the ureter at the broad ligament, subdividing in two canals that pass superiorly and inferiorly along the lateral surface of the uterus. One major subdivision meanders up the uterus within the broad ligament until it reaches the region of the ovarian hilum where it anastomoses with derivates of the ovarian artery. Another branch descends to provide the cervix and blends with branches of the vaginal artery to originate the azygos arteries of the vagina, which in turn descend anterior and posterior to the vagina.

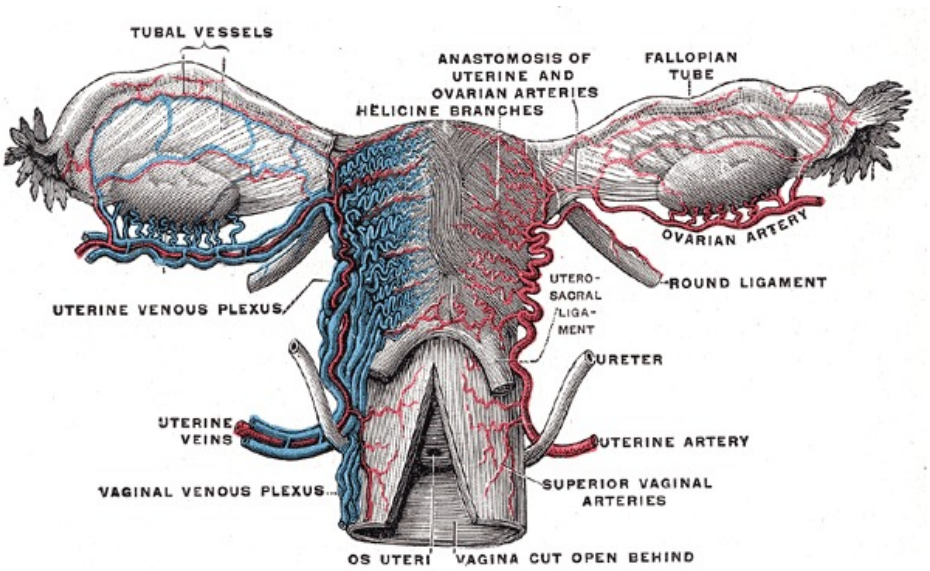


Figure 12. Female reproductive blood supply. under the terms of the Creative Commons Attribution 4.0 International License. Source: (Hoare BS, 2020)

Each uterine artery gives off numerous branches which enter the uterine wall, divide, and run circumferentially as groups of anterior and posterior arcuate arteries. Arcuate arteries progressively narrow as they multiplicate and approach the midline turning into helicine arterioles that follow a centripetal direction through the myometrium towards the deepest tissue of the body of the uterus: the endometrium layer. Those dense capillary plexuses within the uterus wall undergo distinct morphology through the different phases of the menstrual cycle. In the proliferative phase helical arterioles are less prominent, whereas they grow in length and calibre, becoming even more tortuous in the secretory phase. The venous drainage of the uterus pursues the route of the uterine venous plexus that anastomose with the vaginal and ovarian venous plexuses, drawing out laterally in the broad ligaments and join the internal iliac veins. (Standring, 2008)

Ovary

The ovaries are the main feature of the female reproductive system for the study of the condition we are considering within this thesis. The ovaries are found in pairs, within the pelvis, laterally to the uterus close to the pelvic wall in both sides, suspended by a double fold peritoneum - mesovarium - which is attached to the upper limit of the posterior aspect of the broad uterine ligament. The ovaries connect the uterus through fallopian tubes, which are responsible to carry the zygote to the uterine cavity for implantation. (Oktem et al., 2008, Mihu et al., 2011)

The human ovary carries a gametogenic and an endocrine function. The first of which determines the liberation of a mature ova ready to be fecundated by sperm and to ensue embryonic development. The second designated function aides the accessory reproductive organs to potentiate and support pregnancy and birth by secreting steroid hormones into the blood circulation. (Leung et al., 2018)

This pair of organs rests within the ovarian fossa. Posteriorly to the ovarian fossa we find extraperitoneal structures such as the ureter, internal iliac vessels, obturator vessels and nerve, and the derivation of the uterine artery. The medial surface faces the uterus and uterine vessels in the broad ligament, forming a peritoneal recess coined as the ovarian bursa. Above the superior extremity are located the fimbria and the most distal section of the uterine tube that connects the ovary to the uterus. The inferior extremity points downwards towards the pelvic floor and the anterior border faces the posterior sheet of the broad ligament and encloses the mesovarium. The posterior border is free and faces the peritoneum, which covers the upper part of the internal iliac artery and vein, and the ureter.

Superior and laterally to the right ovary are situated the ileocecal junction, the caecum and appendix. Passing over the superior pole of the left ovary we find the sigmoid colon and joins the rectum, which lies between the medial surfaces of both ovaries.

These gonadal structures are oval shaped with normal dimensions of approximately 4 cm long × 2 cm wide in reproductively mature women and they are homologous with the testis in the male gender. In the female neonate their dimensions are about 1.3 cm long × 0.6 cm wide. Before the menarche, the ovaries present a size of about one third of the normal reproductive age and increase progressively along the body development. After the menopause, the average size of the ovary reduces to 2.0 cm long × 1.5 cm wide and further to 1.5 cm long × 0.75 wide in late menopause. Former to puberty, these almond like structures present a dull white colour with a smooth outer surface, but in healthy adult women and adolescent females they tend to appear greyish with a creased surface due to the periodic scarring process of ovulation to which follows the degeneration of successive corpora lutea. (Standring, 2008)

The female gonads are supplied by ovarian arteries which are vessels that derivate of the abdominal aorta and originate below the renal arteries. Each of the ovarian artery descends behind the peritoneum, and at the brim of the pelvis crosses the external iliac artery and vein to enter the true pelvic cavity. Here the artery turns medially at the ovarian suspensory ligament dividing into a branch to the mesovarium that supplies the ovary, and into another branch that follows into the uterine broad ligament, below the uterine tube, to provide blood nourishment the tube. On each side, a branch passes lateral to the uterus to unite with the uterine artery. Other branches accompany the round ligaments through the inguinal canal to the skin of the labium major and the inguinal region. The blood drainage happens through the ovarian veins which emerge from the ovary as the pampiniform plexus in the mesovarium and suspensory ligament. Two veins come off this plexus ascending with the ovarian artery usually merging into a single vessel before entering either the inferior vena cava on the right side, or the renal vein on the left side. (Paulsen et al., 2018)

Anatomically if we observe the mid-section of a fully matured ovary, we can distinguish an outer area and an inner area: designated as cortex and medulla, respectively. From a histological point of view the ovary can be sectioned into a tunica albuginea, a germinal epithelium (germen sprout or bud), the ovarian cortex and medulla. The tunica albuginea consists of a whitish capsule made of dense irregular connective tissue and is the outer layer of the ovary – before puberty it shows as a smooth surface, but it turns into a cicatrized tissue as ovulations start to take place. The germinal epithelium or surface epithelium ensues the tunica albuginea and covers the ovarian cortex – it consists of a

specialized mesothelium and it is made of low cuboidal or squamous cells. The ovarian cortex comprises the area between the surface epithelium and the ovarian medulla - it consists ovarian functional units called follicles surrounded by condensed irregular connective tissue rich in collagen fibres and fibroblast-like cells (stroma). Stromal cells originate the thecal layers of maturing ovarian follicles and the theca interna turns into steroid hormone-secreting (oestrogen and progesterone) in the corpus luteum. Ensuing the cortex settles the ovarian medulla which is the deepest layer, and it is formed by loosely arranged connective tissue and it is well packed with blood vessels, lymphatic vessels, and nerves. Resting follicles will lie within the less vascularized layer of the cortex contrary to the maturing, atretic follicles and embryological remnants (rete ovarii) that are located in the cortical medullary border which is eminently vascularized. (Tortora, 2013, Leung et al., 2018)

Before the female enters the puberty, the ovarian cortex occupies 35%, the medulla 20%, and interstitial cells up to 45%, of the volume of the ovary. Post-puberty the cortex constitutes the vast part of the ovary and encloses the medulla except at the hilum – place where the vessels and nerves enter the ovary. It encloses the ovarian follicles in different developmental stages, and corpora lutea and their degenerative remnants, depending on age or phase of the menstrual cycle. (Standring, 2008)

Pelvic innervation of the female internal genitalia

As mentioned above the reproductive female system is located mainly on the abdominopelvic cavity. This region is innervated by an important neuronal network. For understating this net, we should first grasp a notion of how the nervous system is composed.

The anatomy of the nervous system is classically divided into central nervous system (CNS) and peripheric nervous system (PNS). The CNS is formed by the brain and spinal cord, both of which develop from the neural tube in the embryo. The PNS is composed of all nervous structures outside the CNS and are responsible for connecting the CNS to the body: spinal and cranial nerves, visceral nerves and plexuses, and the enteric system. These elements develop from neural crest cells and as outgrowths of the CNS. (Squire et al., 2012)

Functionally speaking the PNS can be subdivided into a somatic and an autonomic nervous system (ANS). The ANS derives from the splanchnic nerves and cranial nerve X or Vagus nerve and it is the component responsible for the control of involuntary events such as respiration, digestion, sexual arousal and reproduction, digestion, blood pressure and heart rate. To carry these functions, the ANS integrates, besides the enteric system, two important parts: the sympathetic part and parasympathetic part. To the sympathetic

nervous system (SNS) belong the abdominopelvic splanchnic nerves, prevertebral sympathetic ganglia, and abdominal aortic plexus. While, to the parasympathetic nervous system (PSN) belong the anterior and posterior vagal trunks, abdominal autonomic nerves plexuses, pelvic splanchnic nerves, and intrinsic parasympathetic ganglia. (Sharabi et al., 2020)

According to Sanvictores et al., 2020 the ANS is also constituted by a component extra to SNS and PNS, made essentially of general visceral efferent (GVE) fibres which create a motor response to a general visceral afferent (GVA) fibre stimulation. GVA fibres carry sensory impulses – such as hunger, blood pressure, organ distention, and visceral inflammation - from internal organs to the CNS. These visceral sensory nerves often colocalize within sympathetic and parasympathetic nerves (Mazzone et al., 2016) and overall they allow the body to act upon a homeostasis vigilia. (Sanvictores et al., 2020)

Both sympathetic and parasympathetic fibres are controlled by upper motor neurons derived from the hypothalamus. Both SNS and the PNS contain afferent and efferent fibres that providing sensory input and motor output to the central nervous system (CNS). The motor pathway of both SNS and PNS contain a two-neuron series: a preganglionic neuron with a cell body in the CNS and a postganglionic neuron with a cell body in the periphery that innervates target tissues. (Shoja et al., 2013)

The stimulation of the SNS conducts a "fight or flight" response with an overall increase of activity and attention – processes like the elevation of the blood pressure and heart rate, glycogenolysis, cease of gastrointestinal peristalsis. The PNS promotes the "rest and digest" processes being responsible for heart rate and blood pressure decrease and activation of gastrointestinal peristalsis/digestion. (Koopman et al., 2011)

The neurochemical language of the presynaptic neurons of both the SNS and PNS and of the postsynaptic parasympathetic neurons uses acetylcholine as their messenger while the postsynaptic sympathetic neurons generally produce norepinephrine. (Waxenbaum et al., 2020)

The ovarian innervation is made of autonomic plexuses where the upper part of the ovarian plexus is formed from branches of the renal and aortic plexuses while the lower part is reinforced from the superior and inferior hypogastric plexuses. These plexuses consist of postganglionic sympathetic, parasympathetic, and visceral afferent fibres. The ovarian vasoconstriction is leaded by efferent sympathetic fibres derived from T10 and T11 segments and ovarian vasodilatation is promoted through the parasympathetic fibres derived from the inferior hypogastric plexuses. (Drake et al., 2009)

The uterine tube is innervated by autonomic fibres that travel along the ovarian and uterine arteries and it has both sympathetic and parasympathetic supply. The vagus nerve delivers

preganglionic parasympathetic fibres to the lateral or distal half of the tube while pelvic splanchnic nerves provide for the medial or proximal half of the tube. Spinal segments from T10 to L2 supply sympathetic innervation. Visceral afferent fibres together sympathetic nerves go into the cord through corresponding dorsal roots. The ampullary submucosa contains modified Pacinian corpuscles sensitive to pressure and vibration. (Standring, 2008)

As described before, the uterus sits beneath the broad ligament in the female pelvis and through this ligament travel along with uterine arteries it is possible to observe the ascendance of neuronal fibres from the autonomic inferior hypogastric plexus to supply the uterine body. Later these fibres connect with the tubal nerves also derived from the inferior hypogastric plexus and with the ovarian plexus. The uterine nerves end at in the myometrium and endometrium along their vessels. A few fibres connect to the cervix originating a plexus that contains small paracervical ganglia. Efferent preganglionic sympathetic fibres are born from L12-L1 segments and preganglionic parasympathetic fibres arise from S2-S4 spinal segments and relay in the paracervical ganglia. Sympathetic is responsible for uterine contraction and vasoconstriction while parasympathetic activity leans forward uterine inhibition and vasodilatation – hormonal signalling from ovarian and uterine cycles interferes with this autonomic control. (Morizaki et al., 1989)

The lower vagina is supplied by the pudendal nerve (S2, S3 and S4). The upper vagina is supplied by the splanchnic nerves (S2, S3 and sometimes S4). (Standring, 2008)

1.3.2. Physiology of the Human Female Reproductive System

The reproductive system of the human female is governed the hypothalamic - pituitary – gonadal (HPG) hormonal axis. This can be triggered by either, endogenous factors like emotions or by external factors such as sexual visual stimuli, that will arouse the hypothalamus' release of short chain polypeptides named gonadotropin releasing hormone (GnRH) via the activation of limbic region of the brain. These circulating GnRH control the pulsated secretion of luteinizing hormone (LH) and follicle stimulating hormone (FHS) by gonadotrope cells of the anterior pituitary gland - FSH initiates follicular growth, while LH stimulates further development of the ovarian follicles. (Vogazianou, 2019) In turn gonadotropins induce the secretion oestrogen and progesterone by the ovaries (Coutinho et al., 2019) turning them into an endocrine structure responsible for the direct regulation of the menstrual cycle and ovulation. (Coutinho and Kauffman, 2019)

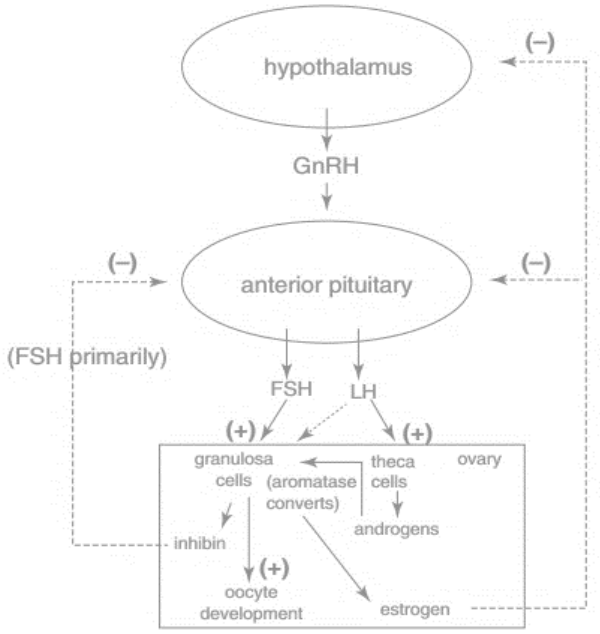


Figure 13. The interplay between the hypothalamic, anterior pituitary, and ovarian hormones. Authorized and licensed by Vogazianou, 2019.

So far, six different oestrogens have been isolated from the plasma of human females, but only three are present in significant quantities: β-oestradiol, estrone, and estriol. In a nonpregnant woman, the most abundant oestrogen is oestradiol synthesized from cholesterol in the ovaries. (Mccartney et al., 2004). According to human physiology literature the oestrogens secreted by ovarian follicles are involved in the development and maintenance of female reproductive structures and the evolving of secondary sex characteristics that include: distribution of adipose tissue in the breasts, abdomen, mons pubis, and hips, voice pitch; a broad pelvis; a pattern of hair growth on the head and body; increase protein anabolism; including the building of strong bones; lower blood cholesterol level, and moderate levels in the blood inhibit both the release of GnRH by the hypothalamus and secretion of LH and FSH by the anterior pituitary. (Tortora, 2013)

The progesterone, secreted mainly by cells of the corpus luteum, synergizes with oestrogens to prepare, and maintain the endometrium for the implantation of a fertilized ovum and to prepare the mammary glands for milk secretion. High levels of progesterone act by a negative loop upon GnRH and LH.

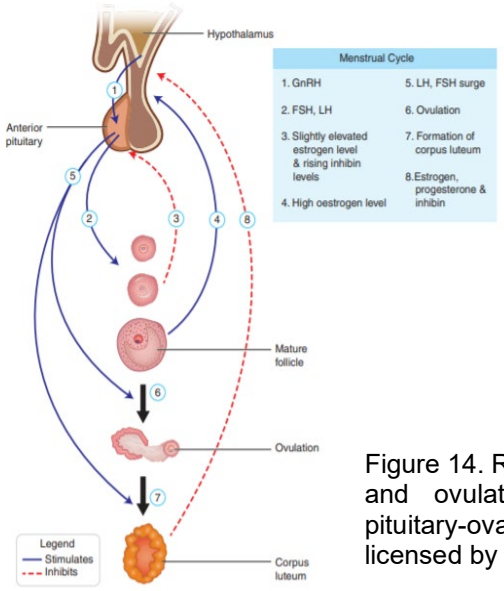


Figure 14. Regulation of menstrual cycle and ovulation in the hypothalamic-pituitary-ovarian axis. Authorized and licensed by (Vogazianou, 2019)

During their fertile years, nonpregnant females normally exhibit cyclical changes in the ovaries and uterus. Each cycle takes about a month and involves both oogenesis/folliculogenesis and preparation of the uterus to receive a fertilized ovum. These cycles are driven by hormones secreted by the hypothalamus, anterior pituitary, and ovaries. (Walters et al., 2018)

The ovarian cycle is a series of events in the ovaries that occur during and after the maturation of an oocyte while the uterine (menstrual) cycle is a concurrent series of changes of the endometrium, preparing this for the arrival of a fertilized ovum and respective implantation. If fertilization does not occur at the fallopian tubes, ovarian hormones wane, leading to the desquamation of the endometrium's *stratum functionalis*. Apart from the ovarian and uterine cycle, the female reproductive cycle also comprises the hormonal changes implicated in their regulation and the cyclical changes in the breasts and cervix associated with the events. (Leung et al., 2018)

Days of the female Reproductive Cycle	1-5	6-7	8	9-14	15-28
Ovarian Cycle	Follicular phase		Ovulation	Luteal phase	
Uterine Cycle	Menstruation	Early proliferative phase		Secretory phase	

Table 11. Ovarian and Uterine Cycle comparison within the period of an estimated 28-day female reproductive cycle.

The ovarian cycle

The ovarian cycle corresponds essentially to cyclic changes within a fertile woman's oocytes and ovarian follicles though the early stages of oogenesis have their spring in utero. During a woman's reproductive years, it is a roughly 28-day cycle that can be correlated with, but is not the same as, the menstrual cycle that will also be discussed below in this work. The cycle can be subdivided in three phases:

- Follicular or Pre-ovulatory phase
- Ovulation
- Luteal or Post-ovulatory phase

This cycle comprises the events of oogenesis (the production of female gametes), and folliculogenesis (the growth and development of ovarian follicles). At birth, the ovary contains approximately 400,000 primordial follicles and each one encloses a primary oocyte. Following sexual maturity at the completion of puberty, the production of FSH and LH by the anterior pituitary gland induces the growth of these primordial follicles to develop through a repetitive process of three steps happening every ovarian cycle: recruitment, selection and dominance. In each ovarian cycle, FSH induces follicular growth in the ovaries and about 20 primordial follicles are activated to begin maturation and recruited for accelerated growth – recruitment step. Only one of those 20 primordial follicles will then be selected to follow fully mature and achieve ovulation – selection step - this mature follicle will become the dominant follicle. While dominant follicle frees its oocyte by the phenomenon of ovulation the remainder recruited follicles enter atresia. Over the span of the female reproductive life, only *circa* 300 to 400 eggs will be released, through ovulation. (Vogazianou, 2019)

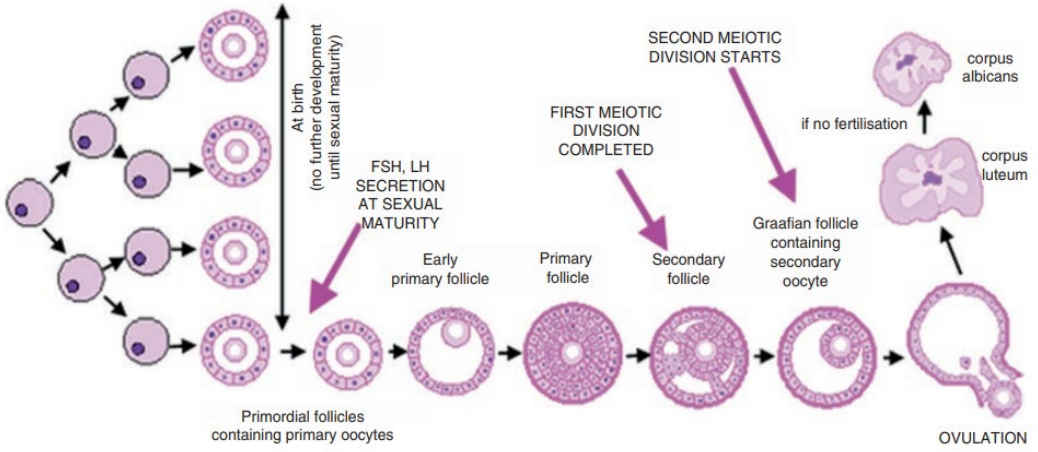


Figure 15. Ovarian follicular development. Authorized and licensed by (Vogazianou, 2019)

The pre-ovulatory or follicular phase is considered the time between the end of menstruation and ovulation and it is variable period in comparison to the post-ovulatory or luteal phase lasting in average 6 to 13 days in a 28-day cycle. During this stage, some of the secondary or antral follicles in the ovaries begin to secrete oestrogens and inhibin. By about day 6, like mentioned above, a single secondary follicle is selected to become the dominant follicle. Oestrogens and inhibin produced by this dominant follicle lead to a negative feedback on the anterior pituitary which reduces the secretion of FSH. (Vegetti et al., 2006) As soon as the Graafian follicle is ready for ovulation it leads to a peak secretion of oestrogens which stimulates a more frequent release of GnRH from the hypothalamus that in turn promotes the release of FSH and additional LH by the anterior pituitary. Elevated levels of LH causes rupture of the secreting mature Graafian follicle with expulsion of a secondary oocyte about 9 hours after the peak of the LH surge. (Tortora, 2013) The disrupted Graafian follicle turns into corpus luteum responsible for increasingly secrete progesterone with a peak around 6-8 days after ovulation. During this phase body basal temperature increases about 0.5°C, due the thermogenic effect of progesterone. In the luteal phase the high levels of oestradiol, progesterone and inhibin exert a negative feedback on LH and FSH levels. If eventually fecundation does not take place until 48h after ovulation the levels of oestradiol and progesterone decrease and corpus luteum turns into corpus albicans. (Vogazianou, 2019)

The uterine cycle

Early in puberty, the hypothalamus becomes less sensitive to the inhibitory actions of oestrogen and progesterone on GnRH-release, fact which induces an increase in the release of GnRH stimulating the secretion of gonadotropin (LH and FSH) secretion. Gonadotropin secretion then stimulates production of ovarian steroid hormones, primarily oestrogen, which drives the development of secondary sexual characteristics.

According to Novello and Speiser, 2018 adrenarche happens when a child's adrenal cortex beings the secretion adrenal androgen precursors and ultimately resulting in an increase of the adrenal androgens dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS). Adrenarche occurs years before the onset of puberty and is thought to originate secondary sexual features such as the growth of pubic and axillary hair growth. DHEA, a weak androgen receptor agonist, is the most abundant product of the adrenal cortex and is thought to be responsible for the clinical signs of pubarche (first appearance of pubic hair) by conversion to stronger androgens - testosterone, and dihydrotestosterone. DHEA is sulphated outside the adrenals becoming DHEAS - a stable marker for adrenal androgenic

activity. The physical manifestation of androgenic hormone production – pubarche - embodies the development of pubic and axillary hair, adult body odour, and acne. Usually there is a typical pattern in the physical changes that a girl undergoes during puberty: the first sign of puberty is the breast budding (thelarche) at an average age of approximately 11 years. Pubarche follows telarche and is ensued by the growth of axillary hair. The start of menstruation – menarche – normally comes after all the other physical changes and occurs *circa* 2.5–3 years after the first signs of the onset of puberty. (Novello et al., 2018) Menstrual cycles start off as irregular at menarche and literature indicates that it can take a gap of 2–3 years to become regular with ovulatory cycles. Several studies illustrate that this period of anovulatory cycles tends to be longer as the age at menarche increases. (Hosokawa et al., 2012)

Citing Vougazianou, 2019 menstruation can be defined as: *the cyclic discharge of blood and disintegrated endometrium, from the uterus through the cervix and vagina*.

In the absence of fertilization and pregnancy, the ovaries react with the quick drop in the production of progesterone and oestrogen leading to the flaking of the uterine endometrium. It normally is a repetitive cycle that occurs throughout a woman's reproductive life when she is not pregnant as aforementioned. When the female ceases the reproductive years and enters the climacteric period – menopause – the menstrual cycle stops to exist.

The menstrual cycle comprehends:

- Menstruation
- Early proliferative phase
- Secretory phase

The menstruation phase corresponds to the shedding of uterine lining caused by myometrial contractions and vasoconstriction in the uterus. It is commonly accepted that the menstruation happens within the first 1-4 days of the reproductive the cycle though the duration of the uterine bleeding may last to a period of 7 days with a blood loss within a range of 15–80 mL per cycle, usually with a maximal flow at the second day of the menses. This discharge occurs once that declining levels of progesterone and oestrogens lead to the release of prostaglandins which in turn make the uterine spiral arterioles constrict resulting in cell death by hypoxia. (Maybin et al., 2011, Cousins et al., 2016) The *stratum functionalis* comes off, remaining only the *stratus basalis* so that endometrium turns thin again. Due to the presence of fibrinolysin and other factors that inhibit clotting, blood clots should not be present in menstrual blood, unless menorrhagia takes place. (Dockeray et

al., 1987) Menorrhagia is a heavy blood loss which may occur due to numerous factors such as myomas and clotting disorders. (Gleeson et al., 1993)

Early proliferative phase of the menstrual cycle usually takes place between the end of the last menstruation and ovulation – between 5th and 8th days of the reproductive cycle. In early proliferative phase the oestrogens liberated into the blood stream by fast developing ovarian follicles lead to the repair of the endometrium where cells of the *stratum basalis* undergo mitosis and produce a new *stratum functionalis*. As the endometrium thickens and the remnant disrupted follicle at the ovary secretes progesterone and oestrogens to promote further growth and coiling of the endometrial glands with enriched vascularization and further thickening of the superficial endometrium to *circa* 12–18 mm. This phase of the uterine cycle, after ovulation, comprehends an intense secretory activity of glycogen by the endometrial glands, designated as secretory phase of the uterine cycle. The peak of this phase happens about 1 week after ovulation and by the time this one occurs, if fertilization is absent until 48h the levels of circulating progesterone and oestrogens decline due to degeneration of the corpus luteum of menstruation originating the beginning of a new menstrual cycle with the onset of menstruation. (Hall, 2015)

1.3.3. Etiology and pathophysiology of Stein-Leventhal Syndrome

The mechanisms behind PCOS pathophysiology are complex and to be completely determined (Sanchez-Garrido et al., 2020). So far, evidence identifies developmental, environmental, genetic, and epigenetic mechanisms related to the etiology of this disorder (Fenichel et al., 2017, Escobar-Morreale, 2018).

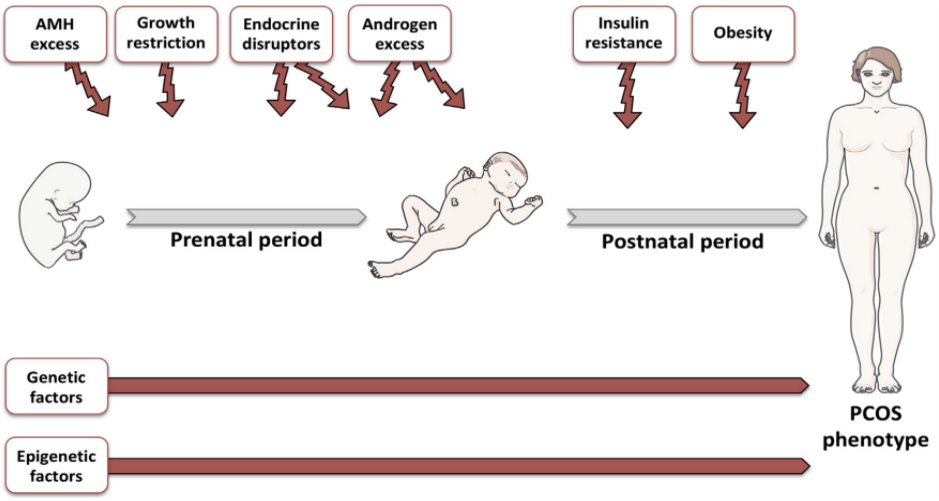


Figure 16. Potential pathogenic factors of PCOS, under the terms of the Creative Commons Attribution 4.0 International License. Source:(Sanchez-Garrido et al., 2020)

According to figure 18, a simplified current etiology of PCOS condition is attributed to a “two hit” theory. A first hit or congenital predisposition *in utero* interplayed by multiple factors - including increased AMH levels, restriction in foetal nutrition, endocrine disruptors, and androgen excess - lead to development of a PCOS-like phenotype in adulthood when a second hit happens. The second hit is the exposure to post-natal provocative factors such as obesity and insulin resistance. (Sanchez-Garrido et al., 2020)

Congenital virilization is a widespread method for PCOS experimental models as it lies as a frequent cause of secondary PCOS. However, this mechanism is downplayed within the context of primary PCOS once that foetal-maternal passage of testosterone is bottlenecked by high placental aromatase activity, and embryonic ovarian follicle development does not begin until mid-gestation. (Rosenfield et al., 2016)

Changes in foetal nutrition have been appointed as an intra-uterine etiologic co-factor to metabolic syndrome and related cardiovascular disease in adulthood. Both low birth and high birth weight have been identified as risk factors though most PCOS cases occur in girls with normal birth weight. (Paschou et al., 2015)

The role of intrauterine perturbations on development of adult disease happens via altered epigenetic programming (Heijmans et al., 2008) in PCOS granulosa cells with over 100 differentially methylated sites impacting on several functions (Makrinou et al., 2020), androgen receptor splice variants (Walters et al., 2016), ovarian aromatase defective methylation, genes involved in insulin/insulin-like growth factor signalling (Yu et al., 2015) and anomalous miRNA expression in PCOS women’s cells and adipose tissue (Rosenfield et al., 2016). Records on databases, indicate that PCOS etiology involves 241 gene variations (Joseph et al., 2016). Polymorphism or any nucleotide alteration causing a defect in the transcriptional activity of a related gene might lead to PCOS – examples are genes that encode for the androgen receptor, LH receptors, FSH receptors, Leptin receptors are responsible (Ajmal et al., 2019). Gene defect perturbs the biochemical pathway and leads to dysfunction of an ovary - polymorphisms such as StAR (steroidogenic acute regulatory protein) polymorphs, FSHR (FSH receptor) polymorphism, FTO polymorphism, VDR (vitamin D receptor) polymorphism, IR (insulin receptor) and IRS (insulin receptor substrate) polymorphism, GnRHR (GnRh receptor) polymorphism are found to be involved in the origin of PCOS (Chen et al., 2018).

Further genome-wide association screening (GWAS) allowed the identification of a regulatory protein variant which explains the typical PCOS hormonal disarray:

DENND1A.V2 (DENND isoform 1A, variant 2) (Mcallister et al., 2014, Rosenfield et al., 2016). V2 is an extrapotent splice variant of DENND1A - a guanine nucleotide exchange factor localized to cytoplasmic pits adjacent to the cell membrane - upregulated in PCOS theca and zona reticularis cells and mediates the PCOS steroidogenic abnormality in vitro. (Dapas et al., 2019) DENND1A.V2 expression is inversely correlated to miR-130b-3p, which participates in the post-translational regulation of gene expression (Mcallister et al., 2019). miR-130b-3p also interacts with five other 22 genes apparently related to PCOS: the LH receptor and RAB5B (RAS-related protein 5B). Pathway and network studies suggest that miR-130b-3p inhibits DENND1A and RAB5B up-regulation of theca cell luteinizing hormone receptor expression at the cell surface and interacts with insulin signalling through the MAP kinase pathway. Forskolin activation led DENND1A.V2 and RAB5B translocation to the nucleus, suggesting they also directly stimulate steroidogenesis (Kulkarni et al., 2019) Coding variants of AMH and AMH receptor (AMHR) with defective signalling were singled out by next-generation sequencing in 6.7% PCOS patients with increased serum AMH levels. These defective proteins compromise the inhibition of folliculogenesis and steroidogenesis by AMH, evoking that PCOS cases can also be originated by a primary folliculogenesis defect rather than a primary theca cell defect. (Gorsic et al., 2019)

S no	Gene	Cytogenic location	Anomalies	Author
1	***AR***	Xq12	X inactivation	Urbanek
2	***FSHR***	2p16.3	Gene variation	Aesha Sh
3	***FTO***	16q12.2	SNP rs9939609	Rizwan S
4	***CAPN10***	2q37.3	Polymorphism	Margrit Urbanek
5	***CYPA1A***	15q24.1	T6235C	K Arvind Babu
6	***CYP11A1***	15q24.1	SNP rs4077582	Cheng-wei zhang
7	***CYP17A1***	10q24.32	T > C	Li Li
8	***CYP1A1***	15q24.1	Ile/Val	Ibrahim Esinler
9	***CYP21A2***	6p21.33	Heterozygous mutation	Settas N
10	***CYP3A7***	7q22.1	Variant allele	Mark O goodarzi
11	***CYP19A1***	15q21.2	Arg264Cys	K Ranjith reddy

Table 12. Cytogenic location and anomality's found in genes associated with PCOS. Authorized and licensed by (Ajmal et al., 2019)

Post-natal environmental factors highlight adolescent obesity derived from IR and T2DM (Yilmaz et al., 2018) . Exposure to synthetic compounds found in plastic bottles, detergents, toys, cosmetics, or pesticides may interrupt the endocrine system. Bisphenol A (BPA) is a synthetic chemical with estrogenic activity and preclinical in vivo studies indicate that prenatal exposure to BPA may alter the HPG axis and lead to a PCOS classic phenotype 1 or A. (Fernández et al., 2010) Further studies in human females confirm a

positive correlation between the developmental exposure to BPA and increased risk of T2DM and obesity (Rezg et al., 2014), and serum BPA and PCOS condition (Hu et al., 2018). Therefore, an unhealthy lifestyle, being it diet or exposure to environment factors, predisposes to PCOS. (Goodarzi et al., 2011) IR and hyperinsulinemia disrupt the androgen secretion by the ovaries leading to anovulation. (Diamanti-Kandarakis et al., 2006) Imbalance of gonadotrophin-releasing hormone, follicular stimulating hormone (FSH), luteinizing-hormone (LH) and prolactin are also identified in PCOS (Marx et al., 2003) Evidences of Stein-Leventhal pathogenic mechanisms identify functional ovarian hyperandrogenism as a primary physiologic disorder aggravated by IR hyperinsulinism found in half of PCOS. (Ehrmann et al., 1995a)

Elevated levels of circulating testosterone and androstenedione are the most frequent biochemical perturbation in patients with PCOS (Franks, 1991). Research findings indicate the ovary as primary source of excess of androgens in women with PCOS (Wajchenberg et al., 1986, Franks et al., 1999) or FOH (functional ovarian hyperandrogenism) (Rosenfield et al., 2016), however a minority of PCOS women is found to have the adrenal gland responsible for hyperandrogenism (Moran et al., 2004, Kumar et al., 2005) or FAH (functional adrenal hyperandrogen (Rosenfield et al., 2016). Different studies demonstrate an intrinsic hyperstimulation of steroidogenic machinery in ovarian theca cells in PCOS as a result of an upregulated expression of cytochrome P450c17, P450scc (a key rate-limiting enzyme in steroid production), 3β-HSD2, and 17β-hydroxysteroid dehydrogenase type 5 (17 βHSD5), also referred to as aldo-keto reductase type 1C3 (AKR1C3) (Wickenheisser et al., 2006) with a higher response in the presence of exogenous LH in both basal conditions or suppression of endogenous LH levels by a GnRH antagonist (Gilling-Smith et al., 1997).

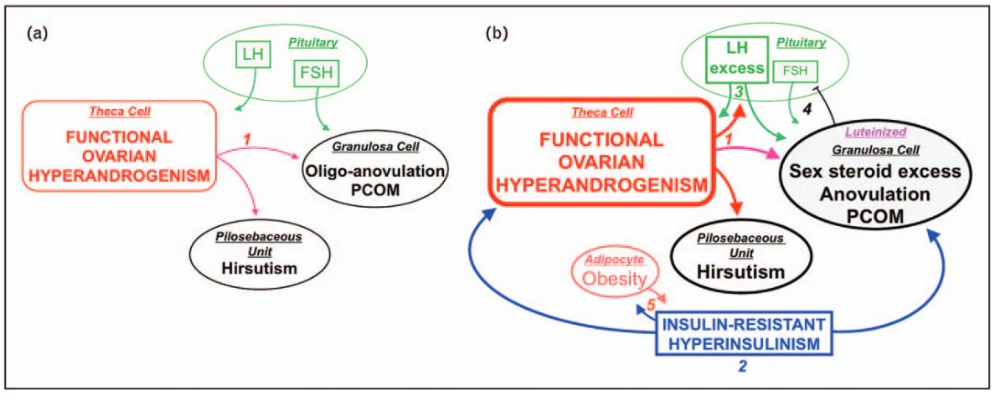


Figure 17. Unified parsimonious model of PCOS pathophysiology - Heaviness of lines and fonts connotes severity. Authorized and licensed by (Rosenfield, 2020)

The presence of an elevated amplitude and pulse of LH release with consequent increase of LH/FSH ratio in PCOS women, indicates a neuroendocrine dysregulation to play role on androgen excess. Literature reports that hyperandrogenism enhances hypothalamic gonadotropin releasing-hormone (GnRH) pulse frequency through the inhibition of the negative feedback on LH secretion by of sex steroid hormones (Lewandowski et al., 2011), resulting in increased LH and androgen levels.

Plus, as consequence of an excessive count of antral follicles in the ovaries of PCOS women, AMH levels are commonly increased in these patients (Lauritsen et al., 2015) which may act as an additional factor for excess of androgen production once that GnRH neurons express AMH type II receptor, and this hormone stimulates hypothalamic GnRH neuron activity (Cimino et al., 2016)

IR might also boost the frequency and amplitude of GnRH and LH pulse secretion by the upregulation of GnRH gene expression through activation of MAPK pathway in hypothalamic GnRH neurons reinforcing hyperandrogenaemia. Androgen excess has a deleterious impact on metabolic homeostasis in women with PCOS, acting on different metabolic tissues such as the adipose tissue, liver, muscle, and pancreas as well as on the brain. (Sanchez-Garrido et al., 2020)

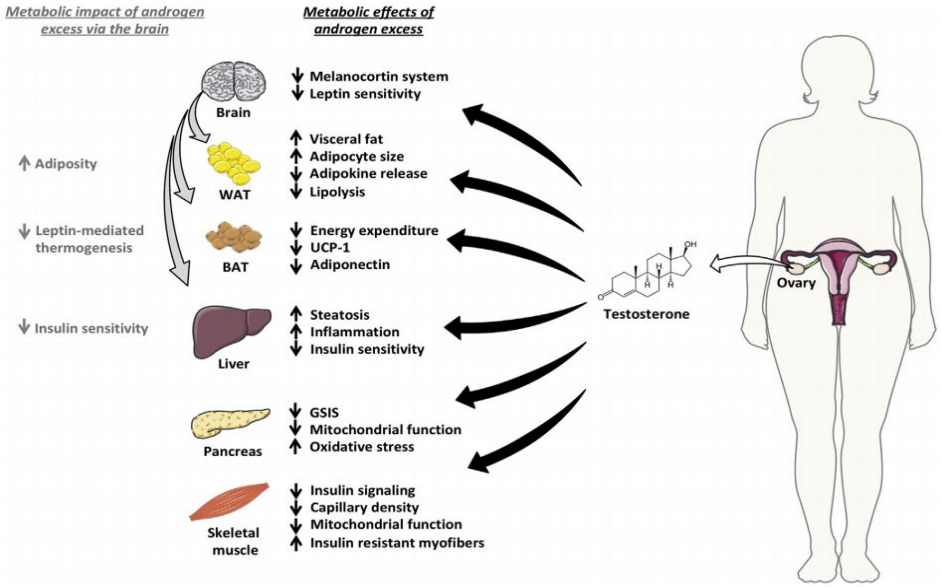


Figure 18.Metabolic impact of androgen excess in PCOS. In women with PCOS, androgen excess has a detrimental impact on different metabolic tissues, including the adipose under the terms of the Creative Commons Attribution 4.0 International License. Source:(Sanchez-Garrido et al., 2020)

Women with PCOS carry a varying constellation of symptoms according to the age at which the condition phenotype expresses. PCOS clinical comorbidities include criteria belonging to three main domains: reproductive, metabolic, and psychological (Tay et al., 2018). Due to this multiple health implications many PCOS women require long-lasting treatment which range from lifestyle interference to precise medical or surgical methods. Medical treatments might be effective despite the possibility of several complications. (Lanham et al., 2006)

1.3.4. WM management of PCOS metabolic features

PCOS has insulin resistance as a cardinal feature, affecting 75% of lean women and 95% of obese women (Stepto et al., 2013) therefore associated with a higher risk of d impaired glucose tolerance, pre-diabetes, T2DM, cardiovascular disease including dyslipidaemia and obstructive sleep apnoea (OSA).

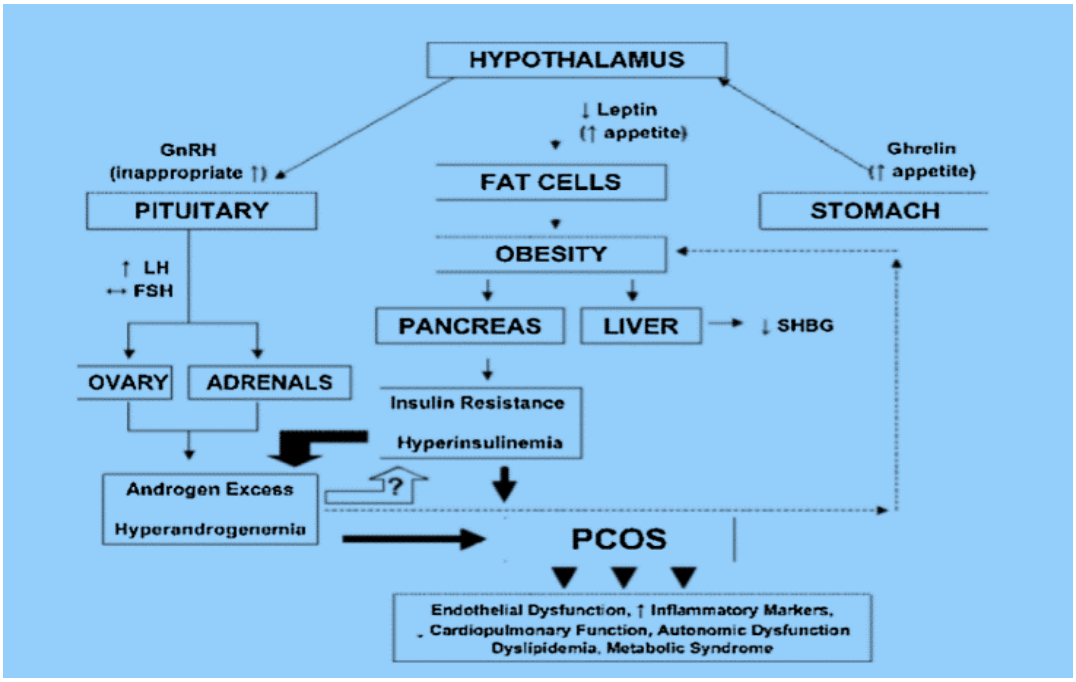


Figure 19.Flowchart that illustrate how insulin resistance leads to elevated level of androgen. It also explains decrease level of androgen that can be possible by using drugs that prevents insulin resistance. Authorized and licensed by (Ajmal et al., 2019)

Research work strongly correlates bi-directionally PCOS and obesity. Clinical history demonstrates that PCOS occurs independently of obesity but of an obesity phenotype is often found within PCOS patients (Hoeger et al., 2012). Plus, PCOS' prevalence is parallel to a BMI increase - key driver of dyslipidaemia, which is characterised by higher triglycerides and lower high-density lipoprotein (LDL) cholesterol levels (Koivuaho et al., 2019) - where

obesity aggravates metabolic, reproductive, and psychological features of PCOS (Lim et al., 2013, Teede et al., 2013).

Name	Expression cells/ tissues	Regulation by:	Target cells or tissues	Function
Adipokines				
Leptin	Adipocytes, gonadotrophs, thyreoidotrophs, somatotrophs	Circadian rhythm during menstrual cycle	Hypothalamus, pitutary, ovaries	↑GnRH, pSTAT3, LH, FSH, estradiol *Outcomes:* Sexual maturation, puberty, seasonal regulation of sexual behavior
		Energy homeostasis, adipose cell mass and number	Hypothalamus, brain stem, cortex	↓NPY, AgRP and ↑a-MSH/ POMC, CART *Outcomes:* Decreased appetite
Adiponectin	Adipocytes, pituitary, theca cells, cumulus cells, oocytes, Leydig cells, spermatozoa, epididymis	Estrous cycle, GnRH, LH, FSH	Pituitary	↑FSH, progesterone, insulin-induced LH, IGF-1-induced progesterone and E2
		Energy homeostasis, adipose cell mass and number	Immune cells	↑M2 macrophages, ↑monocyte apoptosis, ↓NFkB signaling *Outcomes:* Local and systemic anti-inflammatory effects and protection of Leydig cells
			Pancreas	↑Survival pancreatic β-cells *Outcomes:* Insulin-sensitizing effects
			Arcuate nucleus, adipose tissue	↑Fat oxidation, ↓local inflammatory response *Outcomes:* Energy balance
Visfatin	Adipocytes, human primary granulosa cells, human granulosa KGN cell line, human cumulus cells, oocytes	Obesity, type 2 diabetes, cardiovascular disease	Immune cells	↑TNFa, IL-6, IL-1β *Outcomes:* Monocyte chemotactic activity
			Ovaries	↑Leydig cell steroidogenesis *Outcomes:* Ovarian function
Resistin	Adipocytes, porcine ovaries	Gonadotrophins, gonadal steroids, IGF1	Ovaries	↓ Steroids *Outcomes:* Ovarian steroidogenesis

Table 13. Adipokines with an impact on the HPG axis – adapted. Authorized and licensed by (Tsatsanis et al., 2015)

Studies have shown that women with PCOS have a higher calorie intake and a more sedentary lifestyle than women without PCOS (Moran et al., 2013). Further research is required into the biopsychosocial drivers of obesity such as increased hunger signalling and/or the impact of emotional well-being on lifestyle habits.

Mechanistically, insulin resistance and hyperandrogenism are both exacerbated by obesity with adipose tissue producing pro-inflammatory signals mediating insulin resistance and hyperandrogenism - adipose tissue biochemical activity leads to a pleiotropic effect on the HPG axis affecting fertility at multiple levels (Tsatsanis et al., 2015).

Obesity and insulin resistance are related with decreased production of sex hormone binding globulin (SHBG) leading to an elevated level of free circulating androgens hence hyperandrogenism. Even if a higher body mass index (BMI) is a prevalent feature in women

with PCOS, lean women should be under vigilance in order not to miss an early and efficient diagnosis of PCOS. (Mario et al., 2012)

First line interventions of current international guidelines pass through weight management, diet, and lifestyle adaptation following additional pharmacotherapy if the first approach fails.(Naderpoor et al., 2015)

Medications (metformin, orlistat, incretin mimetics) and bariatric surgery improve insulin resistance, reduce hyperandrogenism and alleviate PCOS clinical severity (Vosnakis et al., 2013, Jensterle et al., 2015) In PCOS, metformin acts upon insulin resistance and inhibits ovarian androgen production (Teede et al., 2007, Palomba et al., 2010, Balen et al., 2016) via effects on steroidogenic acute regulatory protein and 17-α-hydroxylase. (Strauss et al., 2014) This drug increases insulin sensitivity by decreasing gluconeogenesis, lipogenesis and enhancing glucose uptake in the liver, skeletal muscle, adipose tissue, and ovaries.(Tenenbaum et al., 2004) thus it is used prevent weight gain and appears to assist with weight loss, to prevent and manage T2DM, gestational diabetes, fatty liver, and to reduce cardiovascular disease in T2DM. (Teede et al., 2010b, Harrison et al., 2013, Powers et al., 2020) Secondary effects of metformin identified: diarrhoea, nausea, vomiting, abdomen bloating, and flatulence. (Saluja et al., 2020)

Glucagon-like peptide-1 (GLP-1) are suggested as new anti-diabetic drugs with excellent therapeutic profiles (Tudurí et al., 2016), improving glycaemic control and insulin resistance together with weight loss.(Müller, 2014)

PCOS women screened with dyslipidaemia profile have sometime been prescribed statins. This therapy has been shown to reduce testosterone levels.(Almalki et al., 2020) However, women pursuing pregnancy are not advised to take statins for these birth defect effects.(Jiao et al., 2020, Pang et al., 2020)

Although the risks of this intervention of bariatric surgery have decreased over time due laparoscopy is more common. Bariatric surgery is primarily recommended for extremely obese patients who exhibit metabolic comorbidities and do not achieve therapeutic goals after lifestyle and pharmacological intervention. (Li et al., 2019)

1.3.5. WM management of PCOS reproductive features

Reproductive features in PCOS include hyperandrogenism, oligomenorrhoea, subfertility, and pregnancy complications. (Rosenfield, 2015, Shea et al., 2014)

Hyperandrogenism can be assessed by biochemical screening levels of free androgens or by clinical evaluation through Ferriman-Gallwey (mFG) scoring tool. (Moran et al., 2011) Less commonly acne and scalp alopecia can be used as diagnostic criteria for excess of androgen. (Wong et al., 2019, Pena et al., 2020) Women with PCOS are also at increased high risk-pregnancy with possible preeclampsia, preterm delivery, and gestational diabetes, as well as higher rates of subfertility and use of assisted fertility treatments. These may happen interdependently or alone with higher incidence in obese phenotypes (Joham et al., 2015a) Furthermore, PCOS is also associated to endometrial cancer as these women share many of the risk factors including obesity, hyperinsulinemia, T2DM, and anovulation with unopposed uterine oestrogen exposure (Lee et al., 2020). However, routine ultrasound screening for endometrial thickness is only recommended in the presence of risk factors - prolonged amenorrhoea or oligomenorrhoea. (Al Wattar et al., 2020)

Hyperandrogenaemia symptoms as hirsutism and alopecia are addressed with aesthetic measures as laser body hair removal and administration of combined contraceptive oral pill (COCP) to regulate the androgen excess as well as irregular menses. (Teede et al., 2019b) Use of COCP is associated to the increase insulin resistance, elevation of triglyceride levels and turns the individual inflammation prone: low grade generalized inflammation and IR is already a marked common background in PCOS women, especially by midlife, thus common sense would advise not to compulsively prescribe such drug to PCOS women.(Kluft et al., 2002) Metformin combined with the COCP may be useful for management of metabolic features. There is minimal evidence of benefits of adding an anti-androgen to COCP therapy. (Carmina et al., 2019, Teede et al., 2019b)

Based on current guidelines, metformin is indicated in PCOS in some scenarios to improve fertility for management of menstrual irregularity if women are unable to take COCP, and in co-existent prediabetes or T2DM, where lifestyle modification fails – it alone proved benefits for adult women for management of weight, hormonal and metabolic outcomes, especially for women with BMI ≥ 25 kg/m^2.There is inadequate evidence to suggest the optimal COCP formulation, or dosing regimen and formulation of metformin. (Costello et al., 2019)

Infertile anovulatory PCOS women who are overweight or obese are recommended a lifestyle change alone as first-line treatment though studies have come to suggest Letrozole as a valid treatment for first-line pharmacological approach in ovulation induction to improve fertility outcomes. (Costello et al. 2019) Letrozole as others aromatase inhibitors,

are suggested to be linked to sides effects such as musculoskeletal syndrome and sleep disorders. (Nabieva et al., 2018)

Clomiphene citrate (CC) alone and metformin alone could also be used as first-line pharmacological therapy, even being both less effective than letrozole and metformin being less effective than clomiphene citrate in obese women. (Abuelghar et al., 2013) Side effects of clomiphene citrate have been identified. A 2005 study with 315 women that had taken one or more cycles of CC reported high frequencies of psychological side-effects (Choi et al., 2005). Stimulation of ovulation with CC can cause side effects on endometrial receptivity for which new drugs have been tested to promote a sustained release of CC and improve the efficiency on implantation. (Ajdary et al., 2020) Other symptoms have been also referred: ovarian enlargement, abdominal discomfort, flushing, mood disturbances, visual symptom as blurred vision, headaches, nausea and/or vomiting and breast tenderness. (Nahid et al., 2012) Moreover, it was reported a differential between PCOS women and healthy women doing treatment. The differential treatment response is correlated with BMI and androgen index -designated clomiphene resistance and often calls for the addition of insulin lowering medications. (El-Gharib et al., 2015)

Gonadotrophins or laparoscopic ovarian surgery are usually second-line ovulation induction therapies. In the absence of an absolute indication for *in vitro* fertilisation (IVF) / intracytoplasmic sperm injection, women with PCOS and anovulatory infertility could be offered IVF as third-line therapy where first or second-line ovulation induction therapies have failed. (Costello et al., 2019) PCOS women respond well to these injectable fertility drug but also are more prone to the problems associated with the use of gonadotropins such as ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy. (Lindenberg et al., 2018)

PCOS ovulation issues an also be improved by destroying or removing portions of the ovaries, probably by the fact that the number of theca cells producing testosterone decreases and are reduced number of resting follicles producing AMH and inhibin. Wedge resection, ovarian drilling or cystectomy and ovarian diathermy are applied techniques for removing parts of the ovary.(Farquhar et al., 2012) The risks are those associated with any surgery and include damage to ovaries and the formation of adhesions. (Farquhar, 2004)

1.3.6. WM management of PCOS psychological features

From a WM perspective there is a bicorrelation between anatomy and function therefore it is possible that anatomical modifications imply functional or vegetative changes

and vice-versa. Psychological modifications in PCOS phenotypes have been recorded as mentioned before and it has been associated to a change in neurotransmitters' status and brain circuits.(Coutinho et al., 2019)

Not only anxiety and depression, including in women with PCOS, lead the second world cause of global disease burden (Ferrari Aj, 2013) with a 12-month prevalence of 6–18% (Andlin-Sobocki et al., 2005, Bale et al., 2015) but also other comorbid psychiatric disorders as bipolar disorder (Annagür et al., 2015, Moore et al., 2017), have a higher prevalence than in healthy age-matched controls (Moran et al., 2012).

Psychological disorders can be classified within two categories: psychosocial issues related to PCOS (body dissatisfaction with weight and overall image, intimate relational functioning, femininity, fertility concerns, coping difficulties); psychiatric disorders (bipolar disorder, mood disturbances, major depressive disorder, eating disorders, and borderline personality disorder) (Azizi et al., 2017).

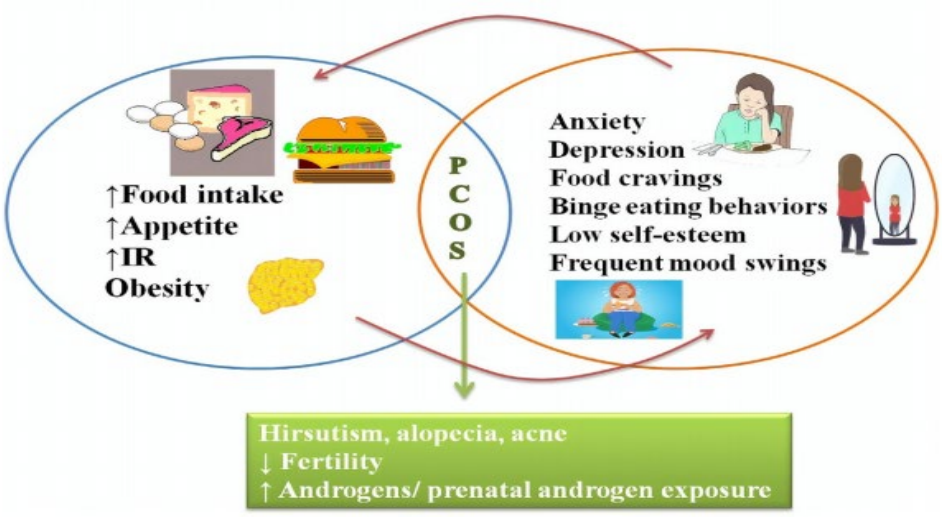


Figure 20.Psychiatric disorders, obesity and eating disorders in PCOS. Authorized and licensed by (Ilie, 2020)

Multifactorial component of Stein-Leventhal syndrome includes an overstimulated HPG axis, with increased adrenal androgen production and intensification of cortisol turnover (Vassiliadi et al., 2009) impacting the body image as a less feminine phenotype and as a source of increased anxiety – both related to self-image insecurities and hormonal disbalance related. Figure 22, below, describes the theory behind the impact on the brain structure by the hormonal alterations on PCOS women including a possible etiological exposure to excessive androgens *in utero.* Androgen receptor (AR) expression is found to be decreased in amygdala - evocating a down regulation of androgen signalling - while the serotonin2c and γ-aminobutyric acid (GABA) A receptors were increased in females of PCOS-like models. (Moore et al., 2017)

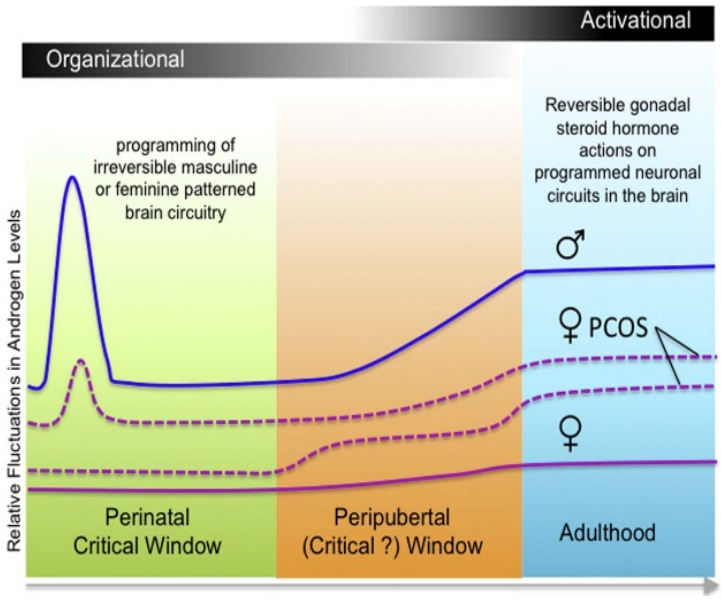


Figure 21. Postulated organisational and activational impact of androgen actions in PCOS development. Blue line: typical in the developing male (blue line). Solid pink line – healthy females. Both dashed pink lines – PCOS female increased androgen exposure *in utero*. Two dashed pink lines - PCOS phenotype. Authorized and licensed by (Moore et al., 2017)

Both solid lines - blue and pink – depict the typical development with respective normal levels of androgen production in healthy males and females, respectively. High androgen levels in male development *in utero*, masculinizes brain circuitry and coordinates brain circuits sensitive to reversible (activational) effects of gonadal steroid hormones in adulthood. On the other hand, females produce relatively very low levels of androgens in perinatal life hence a feminized brain construction and adequate steroid hormone sensitivity in the brain. PCOS phenotype derived from increased androgen exposure during perinatal and/or peripubertal windows of development (dashed pink lines) is thought to arrange modifications in brain circuitry leading to inappropriate activational responses to steroid hormones. (Moore et al., 2017)

Neurotransmitter's imbalance in the PCOS female is a factor of anxiety and depression. Central monoaminergic and noradrenergic systems are key at regulating several forebrain actions, including mood and behaviour. Anxiety and depression are linked to changes in norepinephrine (NE), dopamine (DA) and serotonin (5-HT) (Ruhé et al., 2007). These neurotransmitters interact with the neuro-steroids in the brain keeping their levels and regulating the functional status of the gonads (Do Rego et al., 2009). Literature corroborates the fact that neurotransmitters handle several body functions comprising reproduction and mood (Chaudhari et al., 2017).

The prevalence of depression in women with PCOS shown to be four times more than that of women without PCOS (Hollinrake et al., 2007). Even in absence of a diagnostic

of depression or allied mood disorders, women with PCOS are prone to anxiety and burdensome mood comparing to women without PCOS (Barnard et al., 2007, Benson et al., 2009, Jedel et al., 2010). The higher prevalence of psychiatric disorders in PCOS women, especially depression and anxiety disorders, may be due to both hyperandrogenism and the resulting somatic symptoms. These symptoms can lead to stigma in these women and lower their quality of life. (Berni et al., 2018, Brutocao et al., 2018) Antidepressants are the widest used therapy for depression and can be divided into five main categories according to their mechanism of action: monoamine oxidase inhibitors, tricyclic antidepressants, tetracyclic antidepressants, selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors. Common side effects of antidepressants include thirst, nausea, low blood pressure, drowsiness, sexual dysfunction, and weight gain, amenorrhoea. Hepatic necrosis has been reported as a serious side effect of monoamine oxidase inhibitors and has limited the use of this type of antidepressant. (Zhuang et al., 2013) Obesity or weight gain is highly related with PCOS so the effect of antidepressants therapy on weight can be a no-go in these patients. Obesity and difficulties in weight management impacts negatively on quality of life of these women (Mccook et al., 2005) and it is likely to play a part in the greater prevalence of depression in women with PCOS (Himelein et al., 2006)

2. Traditional Chinese Medicine

2.1. Brief historical context

Traditional Chinese medicine (TCM) has been integrated within the Chinese health care system for more than 2,000 years now (Liu et al., 2015a). It was originally a system of healing based upon the Chinese philosophy of the correspondence between nature and human beings. Currently the therapeutic treatments of TCM involve different approaches, such as acupuncture and moxibustion, tuina massage, phytotherapy or herbal medicine and Qi gong or Chi Kung.(Keji et al., 2003, Greten, 2017e)

It is usually said as common sense that “Studying history makes one wise” which means that understanding the origins and evolution on a determined matter allows us to a deeper insight for a wiser deductive thought on that same matter. For that, acknowledging the profound relation between TCM and Chinese culture is fundamental to perceive the relation between society and disease, disease and environment, and disease and health once that in Chinese culture the concept of health and disease is deeply associated with their concept of life.

Very briefly, the archaeological findings indicate that ancient Chinese started a sedentary lifestyle based on agriculture by the early Neolithic period with a beginning at the Yellow River, Yangtze river and Pear River Basins. From here their worldview folded into a shape of unique features where, later by the Spring and Autumn and Warring States Period (770-221 B.C.) the dialectic thinking included concepts as: yin, yang, The Bagua trigrams, Qi and a theory of five phases as their foundation and they are intimately related with their holistic point of view on universe, man and health.

The concept of Qi in ancient Chinese philosophy was initially related to air in the sky, the man's breathing and the atmosphere of the Earth and eventually this concept evolved to the foundation of all living things on Earth. Cit. Guan Zhong in *Guanzi* "*one survives when he is full of Qi, and one dies when he loses all Qi*". Therefore, Ancient Chinese view "spirit" something like Qi and essence. Citing from the Yellow Emperor's Inner Classic (Huang Di Neijing): "*The superior focuses on the spirit, while the inferior focuses on the body*". Spirit here represents mental activity, emotions, movements, and physiological functions hence the vitality of the body. Then, TCM wholistic approach is born from an attentive study of the interaction of man and nature with a focus on heaven and earth, sun, and moon. Cit. Porkert, 1982: "*Chinese medicine, like other Chinese sciences, defines data on the basis of the inductive and synthetic mode of cognition. Inductivity corresponds to a logical link between places in time*." (Porkert, 1982) This inductive thought rules the perception of the natural world by the ancient Chinese quoted in I Ching as: "*transformation is caused by interaction between two things. This is the constant pattern of life*."

The original meaning of yin-yang was an analogy to the comparing sides of a mountain facing or opposing the sun being the sunny side of yang nature and the opposed side describing the yin essence. Later these concepts evolved to a relation where yang qualifies bright, warm, relative upper position and motion and by polarity yin qualifies for dark, cold, inferior position and motionless. Energy would be yang; form would be yin and so all things and phenomena in nature would be classified into yin and yang. (Ya et al., 2016)

TCM teachings during antiquity were orally passed from master to disciple hence the first written records like I Ching or The Book of the Changes appeared many centuries after the beginnings of TCM. In I Ching the yin-yang duality is translated into a language based on a pair of symbols further arranged into 64 hexagrams. This book served as basis for the scientific binary arithmetical model of the Sinophile Leibniz, on the 17th century, where yang assumes the value of 1 and yin of 0. This Leibniz mathematical model defends a scientific

core of the natural philosophy of TCM that is based on the ancient Chinese abstract cultural concepts of Qi, yin, yang, five phases and Bagua (Wilhelm et al., 2001, Greten, 2017e)

Despite the slowdown on its development between the 17th century and the pre-communist modern era in China, from the latest mid-century on, there was a major investment in formation of skilful professionals and on medical research on traditional models the quality of TCM emerged with a great impact leading to a global expansion of the subject and cit. Hempen, 2020 *TCM is the most comprehensive and widely practiced system of medicine in the world*. (Hempen et al., 2020)

Moreover, after the NIH consensus conference in 1998, the understanding of TCM has been the subject of solid methodological studies. By the 21st century the World Health Organization (WHO), to promote a cohesive and a safe practice, it published general guidelines for TCM and the latest update being, *Traditional Medicine Strategy: 2014-2023 (WHO, 2013).*

During the 20th century, he research of several sinologists as Needham, Unschuld, Porkert, allowed to overcome linguistic barriers between TCM and WM, making it possible the design of a contemporary model based on updated terminology applied to physiological concepts, the method of diagnosis, and the classical clinical findings. (Porkert, 1982, Lu et al., 2002, Unschuld, 2010) In order to promote an integrative parallel between a western view of functional medicine and the scientific approach of TCM treatments of PCOS, this work follows the language and reasoning of the Heidelberg Model (HM) of TCM which based on Porkert's theory. And so, according to this model, TCM is defined "*as a system of findings and sensations designed to establish a functional vegetative state of the body"*. (Greten, 2017e)

2.2. Basic concepts in TCM

QI is defined by HM *as a vegetative capacity to function of a tissue or organ* (Greten, 2017e) and also considered by Porkert, as *a qualified and directed immaterial energy*. (Porkert, 1982) Within Qi we find a yin/yang duality that according to the HM is considered as a pair of terms used to describe functional relations in Chinese culture and language which within a regulatory context could mean that - a yin problem is a translation of an unbalanced regulation for lack of substrate or a that functional values are below target and, a yang problem is a functional disorder in homeostasis or that the functional values are above target. In medical circumstances yin may be related to a depletion of Qi, algor, intima or even to structure and yang may be related to repletion of Qi, calor, extima or function.

These mutable two concepts are highly interchanging and so yang functional state means an overall more presence of Qi compared to a yin state. Both should co-exist in harmony in order to maintain well-regulated state orthopathy. (Greten, 2017e) Within the structure notion of Yin, we can find: Xue, body fluids and Jin. Three concepts deeply involved in the seven laws of Menstruation of the HM and gynaecological disorders such as ovarian cysts and infertility highly associated to PCOS. Xue is defined as the effects of microcirculation and the flowing of substance needed and it can assume a relation to Shen (functional capacity to put order to mental associativity and emotions – mental presence), the constructive Qi (the tissue itself) and Jin (passive fluids moisturizing the tissue). Body fluids represent the balance of the body, functions of active fluids as sweating and the function of mucous membranes. Jing embodies the function of nuclear DNA within the physiology of the cell nucleus hence with a central role in the oogenesis of the ovarian cycle and fecundation. The conceptual triad Qi, Xue and Shen constitute the Three Treasures in TCM. (Greten, 2017c)

2.3. Yin -Yang and the Phases

The theory of Chinese medicine lays its foundation on the theories of Yin and Yang as well as that of 5 Element Cycles which are correlated with the tidy arrangement of 8 trigrams or Bagua by King Wen (1099-1050 B.C.). The yin and yang permanent dynamic leads to a mechanism of subsequent movements that manifest themselves on the control of the Qi. These movements first described as an arithmetic function through Bagua acquired the names of phases and these phases illustrate a natural cycle where a “mother” phase derives the “son” cycle. If the movements are influenced by an exterior force, they may change their course and acquire a pathological countermovement losing homeostasis. (Coutinho et al., 2015)

The Yin-Yang and a Five Element system translated to a HM language of TCM lead to a dynamic self-regulatory model of phases that regulate the content of Qi within the body, compared to a sinus wave of regulation and simultaneously perceived as a circular movement from a perspective perpendicular to the movement axis. HM uses this circular movement as an analogy to explain yin and yang bidirectional movement towards the target value – the centre of the axis corresponds to the Earth phase considered to the responsible for downregulation from Phase II and takes on upregulation from Phase III of the water basin model, much like a pivot role. The mathematical function of a sinus embedded within a circle resemble nothing more than the Taiji symbol – metaphor for balance between yin and yang in the Chinese philosophy.

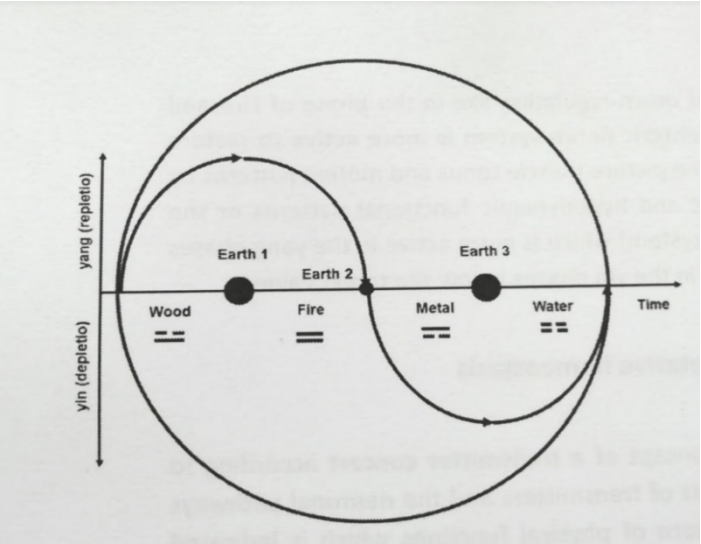


Figure 22. Taiji symbol translated as a circular function: sinus curve, representing the four phases associated with the respective yin-yang bigrams. (Greten, 2017d)

Each transitioning phase corresponds to a quartile within the circle: Wood (phase I), Fire (phase II), Metal (phase III) and Water (phase IV). From a WM point of view this pattern depicts the sinusoidal variance of the vegetative physiology of the ANS where the sympathetic activity is associated to the Yang phases movement and the parasympathetic activity is compared to the Yin movement as can be seen in the following figure 23.

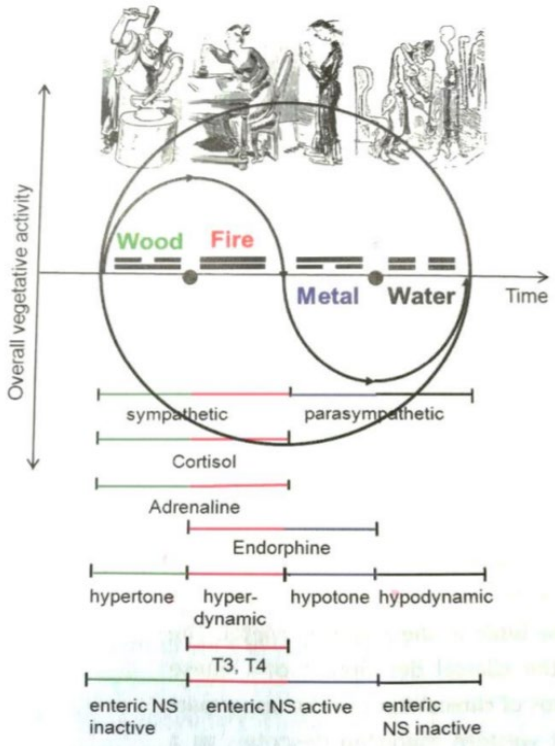


Figure 23. Comparison of human functional states in HM language of TCM and their causal. vegetative functional mechanisms. (Greten, 2017e)

In sum, according to HM phases of TCM are cybernetic terms of a cyclic process referring to vegetative functional tendencies in man and, they can be depicted through constellations of clinically relevant signs derived from vegetative functional tendencies, also known as Orbs. These clinical signs can manifest within the conduit – also defined as the conjunct of points with effect on the clinical signs of an orb, believed to serve as conduit for the flow of Qi and Xue – and can also manifest a constitution – a tendency to express the signs of one orb predominantly so that they show in the physical phenotype.

The conduits are designated as sinarteries or Chinese arteries for being pathways strongly associated to arteries in WM once they were thought to guide and carry Qi and Xue respectively. (Greten, 2017e) Conduits are distributed in internal and external orbs. The main conduits of the four internal orbs of the respective phases follow a course identical to these while the circuits paired to the external orbs, follow the opposite direction of the respective phases. This subsequent countermovement of the external conduits allows a slowdown and repletion of Qi and Xue favourable to a Qi transformation according to the body demands. (Greten, 2017e)

Phase	Catchword	External Conduit	Physiologic direction	Internal conduit	Physiologic direction
Wood	Over-potential	Felleal	↓	Hepatic	↑
Fire	Over-function	Tennuintestinal	↑	Cardiac	↓
Metal	Functional relaxation, Rithimicy	Crassintestinal	↑	Pulmonary	↓
Water	Regeneration	Vesicle	↓	Renal	↑
Earth	Phase Transformation and Assimilation	Stomach	↓	Lienal	↑

Table 14.Relation of phases physiological movement of the external and internal orb-paired conduits.

2.4. Pathogenesis in TCM

As read above in Chinese medicine the healthy state of the individual appoints towards a homeostasis of the vegetative regulation. If an imbalance of yin-yang sets in and breaks the course of the physiologic movements, then we have a compromise of health and the origin of imbalance and progression of disease according to TCM. (Pan et al., 2020)

The HM defines four mechanisms for the onset of disease (Greten, 2017e):

Mechanism	Description
Transition problem	When the natural movement inter phases is disturbed by a blockage
Excess of an agent	When a pathogenic factor that illicit a specific group of signs and symptoms
Imbalance between antagonists	Permanence of a prolonged state of opposed vegetative actions
Yin deficiency	Structural deficiency condition, which causes an instable regulation in the patient

Table 15.Four main mechanisms responsible for disease onset according to HM on TCM.

Source: (Greten, 2017e)

These mechanisms leading to imbalance are directly correlated with a WM physiological description of the neurovegetative function. For example, an excess of an agent refers to a counteracting force on the movement of Qi and Xue on a conduit leading to signs and symptoms related with the pattern of a specific orb or body island of the same conduit. The agents may be of internal origin (such as emotions), of external origin (such as climate factors that challenge the body's vegetative capacity to respond to the changes induced) and of neutral origin (associated to the individual's lifestyle). (Greten, 2017e)

Exterior agents are defined as a functional power that elicits reactions of the "as-if-type" resembling climatic excesses over the vegetative functioning to the body and each agent is named after a climatic influence: ventus, ardor, aestus, humor, ariditas and algor. According to TCM, each agent refers to a physiological movement or phase representative of the signs and symptoms elicited by that agent on the vegetative nervous system. (Greten, 2017e)

Internal agents arise from endogenously and according to the HM they are described as a vectorial inner motion from outwards of the balanced emotional Earth state or target value – ira, voluptas, pavor, maeror, cogitatio, solicitude and timor.

Finally, the deviation of the vegetative regulation from the target value or homeostatic state of the individual can be derived of neutral factors as an unhealthy lifestyle as malnutrition, over exhaustion, infections or even trauma as was mentioned above.

2.3. Concept of Diagnosis in TCM

A correct and precise diagnostic is crucial for a quick success of a pertinent treatment with the minimum costs and collateral effects. The inspection or attentive observation is the key factor for a well-defined diagnostic in TCM and HM divides this observation in 4 major components as elucidated in the following figure:

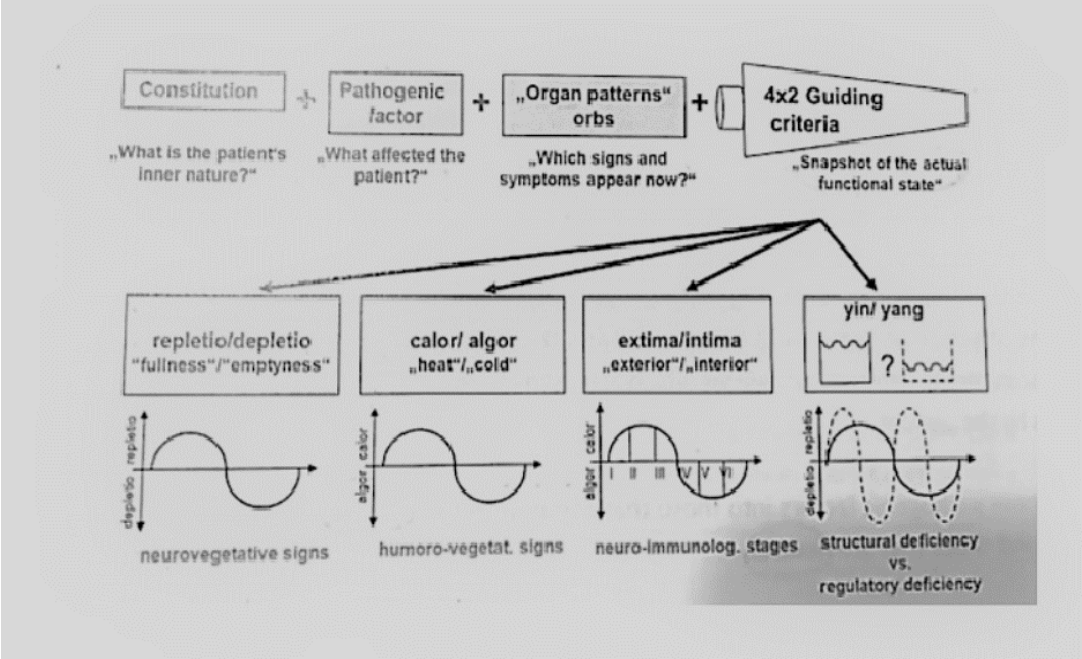


Figure 24. Diagram of main four components of a HM diagnostic in TCM. (Greten, 2007)

In sum, a rigorous diagnostic according the HM normative should start with the evaluation of the constitution of the patient that starts immediately as the therapist interacts with the individual and this step involves the assessment of the whole patients vegetative state of function through direct observation including tongue, hearing, questioning, palpation including pulse evaluation. (Anastasi et al., 2009) After determining the orb through which the vegetative system of the individual tends to run in (constitution), the therapist proceeds to identify the orb of body island that is affected by the pathological agent for then take the third step and identify the agent that is the root of the physiological imbalance. The last step is to make a reading of the symptoms by a fundamental matrix divided in guiding criteria or regulatory models as cited above. (Greten, 2017e)

The definition of each component of the diagnosis construct relative to the HM is referred in the following table.

Component of diagnostic	HM Definition according to (Greten, 2017e)
Constitution	Tendency to manifest a determined orb pattern proper of the functional phenotype of the individual
Orb	Constellation of the most significant signs manifesting a specific functional imbalance named after the activated body region or body island
Agent	Pathogenic factor which manifests of signs and symptoms resemblant of an orb pattern: • neutral • endogenous • exogenous origin
Guiding Criteria	Matrix of symptoms in TCM based on four regulatory models: • Neurovegetative signs of repletion or depletion of Qi levels • Humor-vegetative signs of the microcirculation state: Calor/Algor • Neuro-immunological stages – manifesting on Extima or Intima • Manifests structural or Regulatory deficiencies

Table 16.Definition of each diagnostic component according to HM of TCM.

2.4. TCM and PCOS diagnosis

As seen above, according to WM, the reproductive dysfunction in PCOS is endocrine related and it is characterized by hyperandrogenism with subsequent disturbance of follicular development in polycystic ovaries with oligo/anovulation – carrying metabolic and psychological comorbidities. (Teede et al., 2010a, Yin et al., 2020)

HPG axis is activated with the onset of sexual maturity usually somewhere between 10-14 years old, when menarche happens. This activation leads to the activation of the ovarian and menstrual cycles that constitute the foundation of the female reproductive physiology. As seen in the picture below LH levels suffer a cycle variation during the reproductive age a woman so it is within this age that women will more often the cardinal features associated to PCOS.

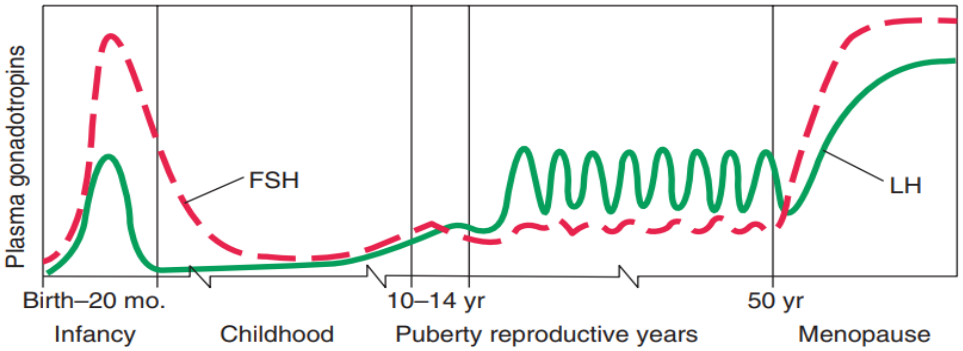


Figure 25. LH and FSH levels throughout a female´s life. Authorized and licensed by (Vogazianou, 2019)

This cyclic nature of the feminine reproductive function is highly considered by TCM and it has long been used to address gynecological disorders and respective research has been quite prolific within the past 20 years (Zhou et al., 2009).

Moreover, pointing out the relation of PCOS with the reproductive age of a woman the first step to take into consideration is the woman in perceived at the perspective from TCM. HM states that women are a rhythmical being, as both physiological and biological functions are cyclic and can be allocated into the sinus wave explained as seen in the following figure.(Greten, 2017c)

As it is depicted above and compared with the figure below, the reproductive functions of a female estimated to be between the sexual maturity at the puberty until the climacteric around the 50 years old – is coincident with the initiation of the Fire phase and extends to the initiation of water phase of the individual's cycle in HM of TCM.

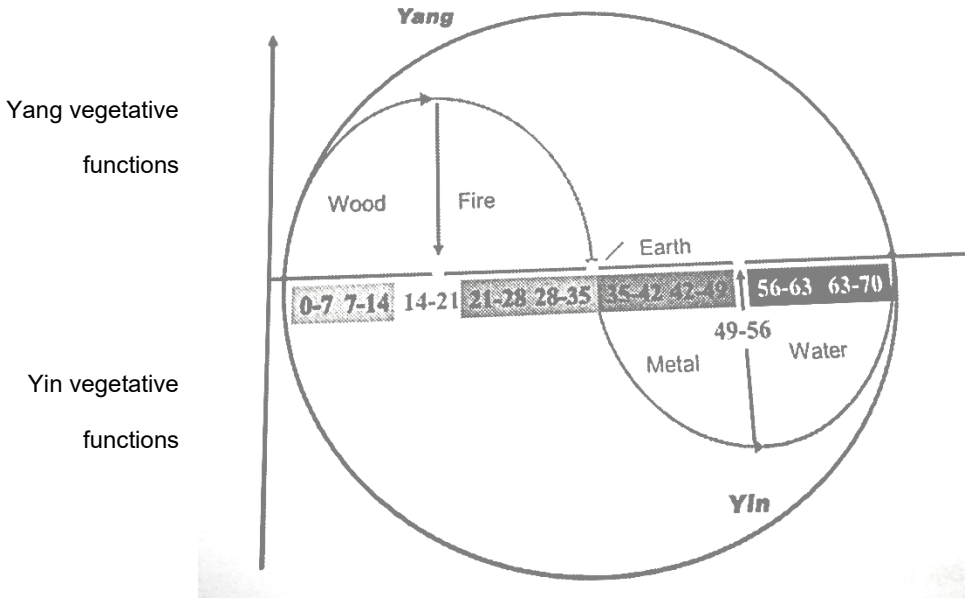


Figure 26. Life as cyclic process of 10x7 years (Greten, 2017c)

TCM regards life as a cycle, and female reproduction physiology can be regarded as a cycle within a cycle. A healthy functional reproduction shows a well-established equilibrium in both physical and emotional status of an individual with the respective manifestation through signs and symptoms associated to the Orbs related to vegetative reproduction functionality.

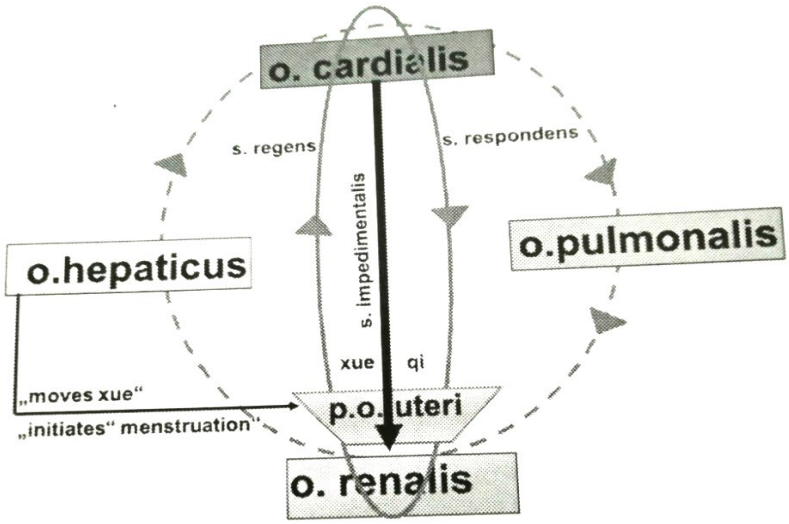


Figure 27. Conduit relations and orbs involved in the cycle formation.(Greten, 2017c)

According to scheme in figure 30 it is possible to understand the importance of xue and qi regulation between the cardiac and renal orb (cardio-renal rapport) by the hepatic and pulmonary orbs that define the direction of qi and xue flow during the cycle, according the phases that are activated at the moment – if phase Fire is more active the vegetative

movement is downwards which happens before menstruation or if phase water is predominant then the vegetative movement is upwards which happens before ovulation. (Greten, 2017c)

A brief parallel can be made between WM and TCM for a clear understanding:

- HPG axis controls the female reproductive cycles: Hypothalamus (associated to Shen – and Shen belongs to Cardiac Orb in TCM); Pituitary gland and gonads produce gonadotropins and steroid hormones respectively (requires a healthy yin-yang balance of the Renal Orb)(Greten, 2017e);
- Hypothalamus receives inputs from the limbic system (related to emotionality (Daisy, 2017)), therefore associated to emotional response and control and coherent with TCM association of Hypothalamus and Shen – Shen is considered as constellar force for mental presence, HM in TCM (Greten, 2017e);
- Female reproductive organs – associated to Paraorb uteri in TCM. Ovary is involved in gamete formation (Jin – related to the yin nature) and maturation as well with hormonal production while uterus is a highly irrigated structure (see figure 14) involved in pregnancy and menstruation (xue – also part of yin nature).(Greten, 2017c);
- Powerful influence of blood channels for feeding the reproductive system: Impendimentalis sinartery or Chong Mai (pathway close by to aorta ramification from the level of the renal arteries downwards)
  Respondens Sinartery or Ren Mai (upper pathway close by epigastric, internal thoracic, subclave arteries, until its terminus)
  Regens sinartery or Du Mai (conduit close to the pudendal artery branches)
  Zonal sinartery or Dai Mai (pathway correspondent to the complex vessel network that derives from the internal iliac artery for local uterus and ovarian perfusion (view human female reproductive system 1.3.1);
- Regular rhythmic flow of qi to guide xue (“Qi is the mother of the Xue”. A functional blood circulation and local micro perfusion requires an optimized energy status and flow (qi) thus a sympathetic/parassympathetic balance for a rhythmic timing in the cycles – hepatic orb (wood phase of creating energy potential) and pulmonary orb (metal relaxation phase) qi vectorial movements counterbalance each other acting within the cycle allowing a timed phenomenon of ovulation and menstruation (Greten, 2017c)

- Regular rhythm of the blood and perfusion in menstrual cycle, where major apport is done during the proliferative phase with an upward movement (associated to water phase and regeneration – renal orb) and cut of supply with shedding of the uterus lining that requires a downward movement by the fire phase (cardiac orb)(Greten, 2017c);
- Preeminent role of yin phenomena in the female body preparation for conception: increased glandular function and secretion during follicular and secretory phases of the ovarian and menstrual cycles respectively – yin (material and moist nature of secretions) and body fluids; with respective nourishing tissue by blood and local microcirculation – xue factor - of the endometrium (coincident with proliferative phase of menstrual cycle). Gamete and follicle formation are governed by genes and enzymatic function (Jing or essence in TCM, also considered part of the yin)
- Pivot role of Earth phase (stomach and lienal) in the xue movement and qi outflow.

For a better understanding of the cycle in TCM we can define it as a sinus curve regulated by functional vegetative patterns:

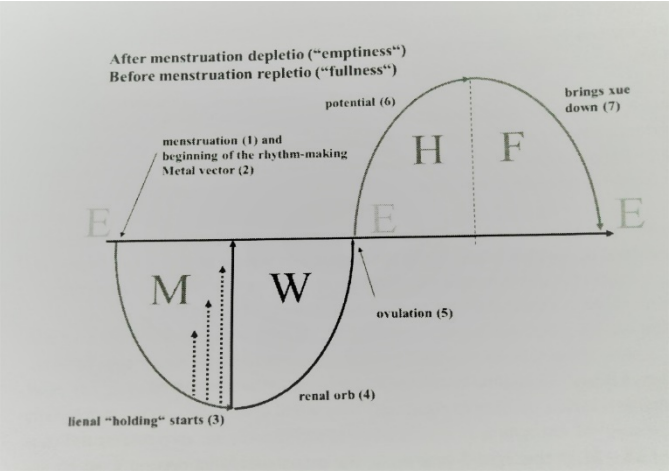


Figure 28. Menstruation cycle represented within the sinus curve as a process that can be described by the phases.(Greten, 2017c). M (metal), W (water), E(earth), H (wood), F(fire)

For balance in female functioning all should work like a chronometer. If timing fails, the cycle is disrupted, and consequent pathologies happen. Considering the previous mentioned WM pathomechanism PCOS and parallel to HM in TCM language (Greten, 2017e) :

- Compromised flow of energy and blood (hyperactivated sympathetic system leading to vasoconstriction, low apport of nutrients and dehydration) – Hepatic orb over-repletion or qi blockage in the hepatic and felleal orbs.

- Under functional gastric transformation and assimilation of food and liquids – Weak lienal/stomach orbs with accumulation of pathogenic fluids (humor). Poor nutrition and energy with less erythropoietic function: deficiency of acquired qi or xue deficiency (weak lienal orb)
- This originates blocks in the center (blocked tricaloric orb and disruption within Chong-Ren communication) with heat accumulation in the center (waist and abdominal fat deposits = pituita (accumulation of condensed humor through calor, over time)
- Swollen shiny ovaries with lack of microperfusion, lack of fluids transformation, humor accumulation with xue stasis - ovulatory dysfunction

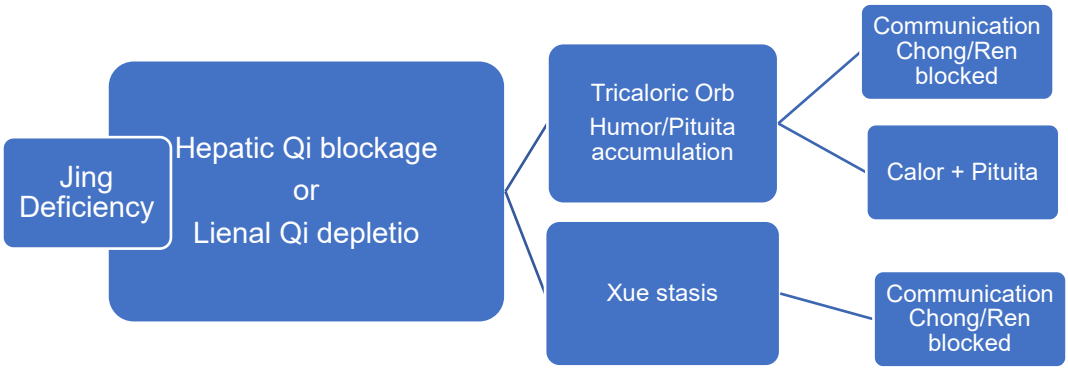


Figure 29. Pathomechanism of PCOS in TCM.

These physiological alterations reflect signs and symptoms to be observed within a diagnosis. Considering a diagnostic according to HM we should consider: the constitution of the individual, the orb associated to the chief complaints, the agents associated to the disruption of the functional vegetative balance and the status of the general guidelines implicated in the healthy homeostasis of the same vegetative function (energy status, micro perfusion status, inflammation status, and yin/yang balance).

Recalling chief complaints of WM women with PCOS: clinical or biochemical hirsutism, alopecia, ovulatory dysfunction or amenorrhea, ovarian cystic morphology, obesity with high waist-hip ratio, IR hyperinsulinemia.(Witchel et al., 2020) Translating chief complaints into TCM language in the next table refers to TCM findings that might be associated to a HM diagnostic in a woman pinpointed with PCOS in WM.(Greten, 2017c)

PCOS Diagnostic components according to HM in TCM	
Constitution	Tendency to manifest Jing deficiency (Renal orb) or exhausted Shen (Cardiac orb) or Qi deficiency (compromised Lienal or Pulmonar Orb) or Xue deficiency (Lienal and Hepatic orbs)
Orb	Renal Orb Cardiac Orb Paraorb Uteri Lienal Orb Hepatic Orb
Agent	• Neutral – stress/overwork • Emotions – Maeror/Timor/Cogitatio • Cold • Humor/Pituita
Guiding Criteria	Matrix of symptoms in TCM based on four regulatory models: • Depletive Lienal Qi or Pulmonar Qi • Xue stasis or Xue deficiency • Calor (background low body inflammation) • Yin deficiency (xue, jing and Jin-body fluids) • Renal Yang deficiency

Table 17. PCOS diagnostic according to HM in TCM.

However, PCOS women, according to the modified Rotterdam criteria guidelines in WM divided into 4 distinct phenotypes. Analogy to TCM language follows:

Phenotype A *Classical PCOS*	Hyperandrogenism + polycystic ovary morphology+ Ovulatory dysfunction
	Pituita (plegm-damp) or Xue stasis + Jin deficiency or renal orb deficient qi (yang)
Phenotype B	Hyperandrogenism + Ovulatory dysfunction
	Humor + Jin deficiency or renal orb deficient qi (yang)
Phenotype C	Ovulatory dysfunction + polycystic ovary morphology
	Jin deficiency or renal orb deficient qi (yang)+xue statis or pituita (phlegm-damp)
Phenotype D	Hyperandrogenism + polycystic ovary morphology
	xue statis or pituita (phlegm-damp)

Table 18. Rotterdam criteria derived phenotypes of WM translated to TCM language.

2.5. Treatment in TCM

As in WM, in TCM a proper diagnosis is followed by therapy. TCM therapies of TCM include acupuncture therapy, Chinese pharmacotherapy, dietetics, Qi gong and Chinese manual therapy.

2.5.1. Acupuncture Therapy

Acupuncture as a medical approach of TCM had a marked progress in the second half of the twentieth century, mainly within the period from the New China to the Great Proletarian Cultural Revolution (1966-1975) when the development science of acupuncture was widely stimulated, both in theory and practice. (Shin, 1999) Acupuncture as a Chinese medical treatment involves insertion of very fine metal needles into specific areas or acupoints of the body shown to be efficacious to regulate and restore vegetative function. (Ee et al., 2020) According to the HM, correlating to the WM point of view, acupuncture is considered a vegetative reflex therapy with the ability to revert the vegetative state from heteropathy to orthopathy. (Greten, 2017e) Two main theories are usually applied to acupuncture treatments. The first is based the Yin-Yang and a Five Element system, which involves the meridian and acupoint system, and the other is related to the Western neurophysiological model – Heidelberg´s Algor Leadens Theory (ALT) - which is based on neuroendocrine-immunologic mechanisms. (Shen, 2001)

In TCM, acupoints are defined as skin loci where it is possible to access and intervene on vital substances qi and xue. In the Jin dynasty (265 A.D. – 420 A.D.) 349 acupoints were already referred and systemized into sets of conduits or jingmai on the Systematic Classic of Acupuncture and Moxibustion after a careful observation and exploration of the body surface with. (O'connor et al., 1981) The current 361 acupoints used in clinical practice were already identified in Acupuncture and Moxibustion Feng-Yuan (Qing dynasty, 1644 A.D. – 1911 A.D.). Research so far demonstrates several characteristics associated to acupoints: correspondence to a dermatome pattern (Man et al., 1973), different types of terminal nerves (Dung, 1984), a reduced skin electric impedance, and the presence of neurovascular bundles(Lin et al., 2011). These mechanisms of action include intramuscular stimulation to address muscular pain, nerve stimulation to treat neuropathies or even the sympathetic ganglion to treat asthma through acupuncture (Cheng, 2009). Scientific definition and anatomical substrate of acupoint remains debatable (Gunn et al., 1976, Dung, 1984, Ramey, 2001) but the common denominator for the acupoints and acupunctures efficacy is the nervous system, so it is logic to consider the nerves as the material substrate of acupuncture technique (Fu, 2000).

A dermatome refers to a cutaneous area innervated by nerve root, dorsal ganglion, or spinal segment (Apok et al., 2011). The segmental innervation of acupoints has been studied and for example, it was observed that the needle stimulation of 'correct' skin loci for acupoints F34 (Yanglingquan), L9 (Yinlingquan), and S43 (Xiangu) resulted in zones of hyposensitivity confirming to a dermatome pattern of L3 and L5 (Man et al., 1973).

With major advances made in medical physics through the development radiological instruments it was possible to correlate and prove the functions of acupuncture by functional magnetic resonance imaging and positron emission tomography (Cai et al., 2018) with Cho et al to first spot a relationship between specific acupoints and visual-cortex activation (Cho et al., 1998) and lately in 2019, Yu et al. confirming the effects of acupuncture over Alzheimer's Disease also through neuroimaging studies (Yu et al., 2019). Acupuncture nowadays is performed under distinct stimulation techniques besides the usual manual acupuncture, as for example: laser acupuncture, electro-acupuncture or catgut embedding acupuncture. Laser acupuncture is non-invasive which is a plus for patients with trypanophobia and electroacupuncture (EA) allows a deeper muscular stimulation which can be a surplus in PCOS patients. Regarding the syndrome of Stein-Leventhal or PCOS, acupuncture has been tested as therapy of co-therapy to help the regulation of the comorbidity's symptoms affecting women with this condition. Namely, it is possible to find studies on the use of low-frequency EA alone (Sun et al., 2013, Li et al., 2015) or in combination with exercise to modulate the sympathetic outflow to the adipose tissue and ovaries and improve their morphology promoting healthier antral follicles with a thinner theca layer and in that way, regulate the hypersecretion of androgens improving both reproductive and endocrine metabolic functional state.(Manneras et al., 2009). Acupuncture has been also used alone or in combination with clomiphene to ameliorate the disorder of subfertility in PCOS patients. (Xu et al., 2017, Wang et al., 2019) One of the three main impacts of PCOS on women's health is on psychological balance and on that behalf acupuncture studies have been designed to relieve anxiety and depression through the regulation of circulating β-endorphin and androgens. (Zhang et al., 2020a)

2.5.2. Chinese pharmacotherapy

Phytotherapy also designed as Chinese pharmacotherapy in the HM is considered to be a therapy or treatment based on herbs, minerals and to correctly follow such an approach the therapist should thoroughly comprehend the construct of TCM and its terminology of body functions or energetic states. (Greten, 2017e)

Although currently in need of further research, meta-analyses and studies have come so far give a positive reinforcement on the efficacy of Chinese medicinal drugs on some fields and on the surge of new medicines and prescriptions for co-treating pathologies as infections, autoimmune diseases, metabolic illnesses, dermatological disorders, and gastrointestinal diseases.(Hempen et al., 2020)

The HM considers five metagroups of pharmaco-active substances distributed through nineteen main groups. Below we can find a table relating the type of pathology and each meta-group.

Groups	Disorder
I-IX	Infections
X	Psychosomatic disorders
XI	Pain and functional disorders
XIII	Chronic pituita
XIV to XVI a)	Supplete energy in chronic diseases

Table 19. Relation between pharmacotherapy Meta-Groups and type of pathologies treated according to the HM in TMC

The main groups in Chinese phytotherapy are considered as following a practical order of importance of the respective use of the drugs. The aim of TCM is to maintain the individual in a healthy regulated state of homeostasis as far as possible – the exact point in the centre of perfect regulation will rarely be attained considering the influences of the environment on the person and due to the dynamic and transitional essence of all the life-processes being it of biochemical, physiological or even of emotional and mental nature. (Greten, 2017e) Therefore, the intuit is to regulate the vegetative state avoiding entering acute and chronic dysregulated states. If well managed even if an acute disruptive condition sets in, the relative deviation from the target value will be reconditioned to the normative avoiding the progression of the disease into a chronic evolution. For that reason, the groups of plants targeting infections, psychosomatic imbalances and pain treatment which can related to immediate and temporary dysregulation of the vegetative function are considered of major importance for being more commonly found within the complaints of the patients. (Greten, 2017d)

The specific condition of PCOS, as referred above, it is proved to be a lifelong pathology of the female endocrine system which apart from mood imbalances (Teede et al., 2010a) leads to metabolic disorders as T2DM (Kakoly et al., 2019, Liao et al., 2019) that in long run generate to the production of chronic pituita (Rachon et al., 2010) – condensed humor through calor, over time (Greten, 2017e). On the other hand, PCOS patients run on

low levels of Qi energy levels energy by a depletion of the centre or yang deficiency (Wanshan et al., 2019) thus drugs pertaining to the last two meta-groups of the table will also be found in the studies of PCOS through TCM.

In a large-scale survey-study completed by the Taiwan National Health Insurance Program database to assess TCM utilization patterns among women with PCOS in Taiwan during 1997–2010 demonstrated that 89.22% women with newly diagnosed PCOS had received TCM therapy with Jia-Wei-Xiao-Yao-San and Xiang-Fu (Rhizoma Cyperi) being most commonly used formula and single herb, respectively, in the database. (Liao et al., 2018)

2.5.3. Dietetics

According to the Academy of Dietetics and Nutrition the recent definition of dietetics comprises the application of the science of nutrition to the human being in health and disease. (Judd, 2003) Thus, nutrition is pivotal in life and in medicine. Acute and chronic diseases like PCOS have impact on macro and micronutrient intake, metabolism, and catabolism, originating nutrition-related conditions associated with increased morbidity and eventually death. However, in TCM the concept of dietetics goes beyond nutrients and calorie intake. Food is regarded as having a pharmacological effect and classified according to its effect on vegetative system. (Greten, 2017e)

2.5.4. Qi Gong

HM defines Qi Gong as a traditional vegetative biofeedback exercise system used as a preventive method or a therapy. These exercises tranquilize the mind, harmonizes emotions, and helps to keep the cartilage and bone mobility and muscular fitness. Qi Gong exercises are recommended for stress management, burnout prevention or treatment of burnout syndrome. (Greten, 2017e) A recent systematic review of both RCT and non-RCT, indicated that Tai Chi and Qigong exercise appear to have similar beneficial effect as usual care involving conventional exercise on the improvement of depression and anxiety in individuals with substance abuse disorders (Liu et al., 2020) corroborating previous findings of Qi Gong being effective in improving depressive symptoms. (Liu et al., 2015b)

Qi gong activity reinforces the circuits involved in the regulation of prefrontal cortex and this repeated prefrontal cortex stimulation may mediate the “pleasure dissonance” sustainability of the automation of cognitive control of drug addicts (Priddy et al., 2018). Reward system theory claimed that the perception and sensation of happiness experience from practicing Tai Chi and Qi gong, as an alternative where recompense is gained immediately, possibly reformulates the source of rewarding resolving the “pleasure

dissonance". (Hong et al., 2014) Studies with magnetic resonance imaging evidences that mindfulness training can create new connections of brain nerve cells of prefrontal lobe, and decrease activities of the limbic system responsible for emotion response regulation (Tang et al., 2010, Tang, 2011). Summing up the findings and the fact that group practice of Qi gong supports social interaction of the individual with their peers it might facilitate the perception of a positive outcome and community support ultimately improving their mental health (Wang et al., 2013).

Mood alterations and psychologic comorbidities as anxiety, depression and even psychiatric disorders have been associated to women with PCOS (Tay et al., 2020) for which Qi Gong treatment could be a valuable tool in the management of this condition along with usual approaches.

2.5.5. Chinese manual therapy

Tuina or Chinese manual therapy is commonly considered a component of the foundational practice of TCM and has been practiced in China for over 5000 years (Goats, 1994). Tuina therapists perform non-invasive MTC treatments to relax the muscle or soft tissue by administering manual technical interventions such as effleurage (stroking), petrissage (kneading), percussion and acupressure therapy. (Greten, 2017b) Recent studies have found that Tuina could decrease the activity of the sympathetic nervous system as well as release neuropeptide Y and vascular endothelin-1, both with a significant benefactor role in the circulation of blood in the vertebral artery (Sripongngam et al., 2015, Wang et al., 2018). Functional magnetic resonance imaging (fMRI) studies found that Tuina counterpoles the causal relationship between the posterior default mode network and the sensorimotor network (Zhang et al., 2015), and fMRI to individuals after Tuina interventions demonstrated an increased activated area in the insular and somatosensory cortices. (Sparks et al., 2017) This last findings make Chinese manual therapy a valuable candidate in treatments to remove the obstructions to Qi flow and in Xue stasis (compromised microcirculation or micro perfusion) allowing a return to tissue functional homeostasis.

2.5.6. PTTCM

Given the evidences mentioned in WM management of PCOS psychological features of PCOS, patients might benefit from an early assessment of a possible psychological support or even psychiatric intervention however, the general population guidelines on screening for depression and anxiety do not include PCOS patients as an at-risk group. PCOS women tend to seek complementary therapies, including TCM to manage

their symptoms especially menstrual disturbances and infertility problems when WM therapeutics fail or has negative secondary effects. It can be an opportunity to:

- first, identify a possible psychological disorder with due intervention once that an effective TCM diagnosis would identify emotional and behavioural symptoms related to a specific Orb besides the clinical and body signs. The Shen status in the individual, assessing the condition of the Cardiac Orb. Assessment of the Centre (Stomach and Lienal Orbs) for evaluating processing and transformation. Assessment of the Renal Orb for integration, learning and self-perception.
- Recommend a coordinated WM treatment and apply early TCM approaches to manage the condition from an early and preventive stage according to the principles of functional vegetative medicine.

From this standpoint of the PTTCM can be an asset within the clinic context when treating PCOS emotional or behavioural disorders. PTTCM or Psychotherapy in Chinese Medicine, according to HM, is a TCM psychotherapy based on the four-layered ontology where the subconscious or subliminal mechanisms take place in the neurovegetative system and respective brain centres. (Greten, 2017e)

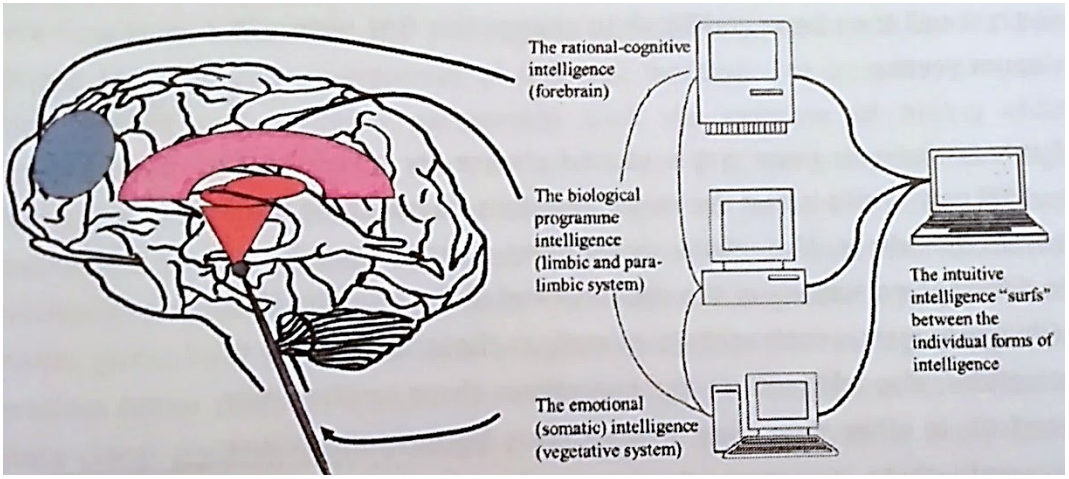


Figure 30. The four-layered ontoly – a network of thinking, acting and feeling. (Greten, 2017a)

According to HM (Greten, 2017a) the correspondent four layers of intelligence in this behavioural model are:

- Rational - cognitive intelligence or technical intelligence – frontal cortex gyri
- Biological programme intelligence – relational intelligence associated to limbic and paralimbic

- Emotional intelligence or vegetative intelligence – somatic and vegetative systems
- Intuitive intelligence - perceptual intelligence

Figure 31 elucidates how rational-cognitive, biological, and emotional components of intelligence interact with each other according to PTTCM – the emotional intelligence functions as basis for a stable harmonious triad of feeling, thinking, and acting. This fundament is coherent with WM findings show that the process of decision-making is highly dependent on the emotional state of the individual having a higher impact than the rational component of the frontal cortex mechanisms. (Angie et al., 2011)

In HM, emotions as inner agents, act upon the vegetative system leading to a dysbalanced state and manifestation of the respective orb or constellation of signs and symptoms. These can be described within the rose compass of phase as shown in the picture below.(Greten, 2017a)

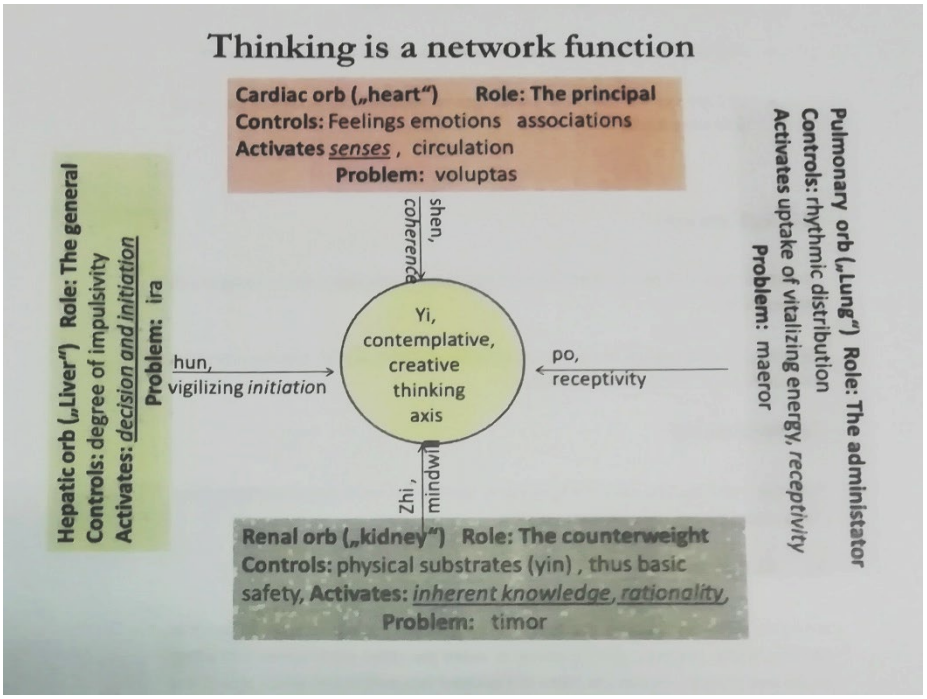


Figure 31. Emotions or inner agents associated to each constitutional type and Orb, according to HM. (Greten, 2017a)

Each constitution is associated to a tendency to act within a specific phase or range of vegetative functional patterns whether it is associated to more sympathetic or parasympathetic behaviour. Moreover, the emotional tendency of each orb has influence on the self-concept of the individual and has an impact in the relation with the social systems leading to the possibility to characterize the behavioural pattern of each constitution. A person of pulmonary constitution with a tendency to maeror or sadness is prone to get anxious, depressed, easily offended and withdraw from the social system in opposition to a

person with a hepatic constitutional type with a tendency to iratic emotions and act more decisively and pragmatic. (Greten, 2017a) TCM health concept is associated to a homeostatic functioning which is attainable through a countermovement on the vector associated to the imbalance and restore equilibrium since TCM fundaments on a dynamic and continuous balance of yin-yang. (Greten, 2017e) PTTCM aims to improve the dysfunctional behaviour of the individual. The approach follows principles that allow the person to access their self-perception and act upon self-regulation through a conscious status: the patient is the authority regarding his life (not the therapist); use of current language (not psychotherapy technical terms); the therapist is the facilitator of the process (never the “pilot”).

3. Methodology

The objective of this work is to analyse the efficiency of TCM treatments applied on women with PCOS within the last 5-year period (from September 2015 to September 2020). For assessing and reviewing the published scientific a search carried out following the PRISMA protocol and its flowchart applied to EBSCO research motor.

Keywords applied to search fields followed a conjugated semantics that allow optimized cross-referencing:

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Phytotherapy OR herbal medicine

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Acupuncture OR acupuncture therapy OR acupuncture treatment

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Tuina

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) Qigong OR Qi Gong OR Chi Kung

PCOS OR polycystic ovary syndrome OR polycystic ovarian syndrome OR Stein-Leventhal Syndrome AND (+) TCM OR Traditional Chinese Medicine

Study design and participants: randomized controlled trials (RTC) and non-RCT on strategies (chinese pharmacotherapy, acupuncture treatments, Qi gong exercises or Chinese manual therapy interventions) to improve disorders associated to women diagnosed with PCOS. The exclusion criteria were selected with the objective of finding the most recent findings and trials within TCM regarding human PCOS. For that, non-TCM complementary medicine, meta-analysis and reviews were disregarded. Human PCOS proved to have a high emotional and psychiatric component with social implications (Banting et al., 2014) for which *in vivo* and *in vitro* studies, although with high biochemical

value, have their due limitations and thus were ruled out from the study. Results comprehend 8 articles – 4 related to acupuncture studies and 4 related to Chinese pharmacotherapy. It was only found one protocol study including chinese manual techniques and none with Qigong exercises. The analysis of the selection of articles is summed in the following tables. The evaluation was segmented by type of TCM treatment and year of publishing.

A total of 161 articles were found and to which were applied the following exclusion criteria:

- Duplicates (including doubled publications) - 33
- Non-related titles and abstracts - 50
- Non-English publications - 6
- Complementary medicines not TCM -7
- Meta-analysis and review articles - 7
- Animal and *in vivo* studies - 30
- Feasibility studies – 6
- Research communications - 9
- Field research with non-representative samples - 2

PRISMA flowchart with the selection of articles displayed below

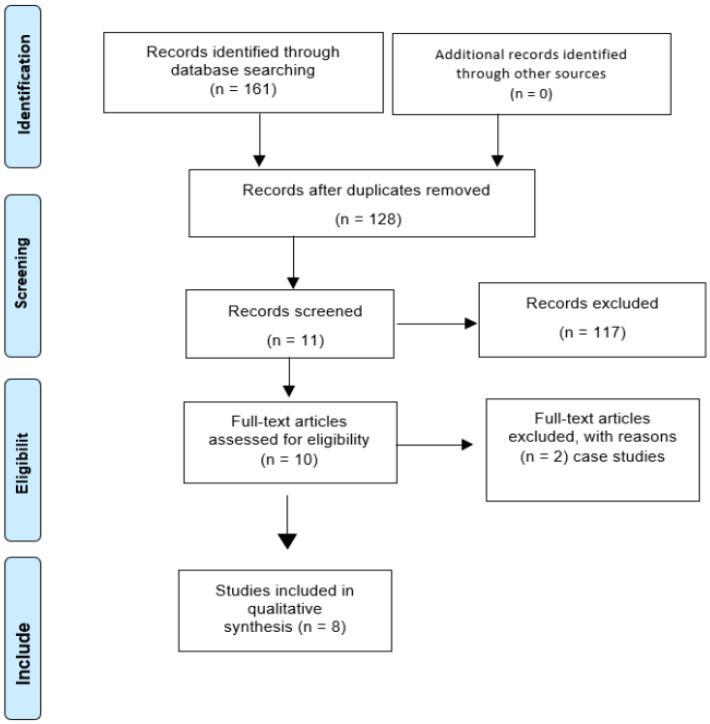


Figure 32. PRISMA flow diagram for the review study.

4. Results

4.1. Acupuncture Therapy

No.	RCT study	Type of Treatment	Objective	Duration	Outcome
1	Wenmin et. al, 2016	Catgut acupuncture + Chinese pharmacotherapy + clomiphene treatment	Verify the effect of acupoint catgut embedding therapy combined with Chinese pharmacotherapy for kidney deficiency and blood stasis on the improvement of gluco-lipid profile and pregnancy rate of infertile obese PCOS women	3 months	The therapy showed positive results regarding pregnancy rate, weight loss and lipidic profile except for LDL
2	Xiao-Ke et al, 2017	Manual and eletrocupuncture with WM therapy - clomiphene	Test the effect of acupuncture and/or clomiphene in infertile PCOS women	4 months	Acupuncture with or without clomiphene didn´t increase live births
3	El Shamy et. al, 2018	Laser acupuncture + healthy lifestyle	To test effect of laser acupuncture on the improvement of PCOS symptoms	3 months	Results suggest Laser acupuncture as a safe alternative or complement to WM in ovulatory induction
4	Wanshan et. al, 2019	Manual acupuncture	To test the effect of acupuncture on PCOS women infertility	3 months	Improved ovarian function with ovulation and pregnancy rate

Table 20. Search full text results regarding acupuncture treatment.

It was only found 4 acupuncture trials on women with PCOS within the range 09/2015-09/2020 (Wenmin et al., 2016, Xiao-Ke et al., 2017, El-Shamy et al., 2018, Wanshan et al., 2019). Apart from these studies it was possible to identify 2 reviews regarding acupuncture but not POCS specifically (Changzhen et al., 2015, Karim et al., 2019) and 7 studies with acupuncture in rats (Maliqueo et al., 2017, Cui et al., 2018, Fu et al., 2018, Ma et al., 2018, Shi et al., 2019, Peng et al., 2020, Xu et al., 2020). Below, the main outcome features of the study groups of each work:

Study	Outcomes Evaluation Criteria
1	Periodical ovulation + clinical pregnancy+ BMI+WHR + Bloodwork (TG, LDL, HDL, TC+HOMA-R)
2	Live births + BMI+WHR+ Bloodwork (FSH+LH+LH/FSH+TT+Estradiol+Progesterone+TG+TC+HDL+LDL)
3	Bloodwork (FT + TT + FSH + LH+ LH/FSH + AMH + HOMA-R)
4	Endometrial thickness +menstrual cycle+ ovulation+ pregnancy

Table 21. Outcome criteria for each study (1-4).

For a better understanding of the impact of studies towards the general objectives, the table below with summed up data of the four acupuncture studies. (*2a) -active acupuncture + clomiphene, 2b)- active acupuncture) + placebo*)

No	*Sample*	*PCOS diagnostic*	*TCM diagnostic*	*Age*	*BMI*	*Infertility course (years)*	*HOMA-IR*	*LH/FSH*	*Pregnancy rate (%)*
1B	21	Rotterdam	Kidney deficiency + blood stasis	28	27.4	3.30	3.22	-	35.29
1A	19				23.9		1.79		
2 a) B	250	Rotterdam	-	28	23.8	2.04	3.2	1.8	29.36
A	235				23.8		3.4	2.4	
2b) B	250	Rotterdam	-	28	24.2	1.98	3.3	1.8	13.9
A	223				24.0		3.5	2.3	
3.B	13	Rotterdam	-	20	27.2	-	2.9	2.4	-
3.A	13				26.6		1.9	1.3	
4.B	30	Chinese protocol	Kidney yang deficiency	28	-	3.60	-	3.22	23.33
4.A	30							2.09	

Table 22. Baseline measures of the study groups of each research article. B-before treatment. A-after treatments. (study 2 results calculated directly from the HOMA-IR algorithm online - article data were not coherent)

To facilitate the comparison between the acupuncture treatments administered in each study a list of tables for each point combination is listed on the next page and example of treatment is displayed on figure 36 – this particular example belongs to the study number one.

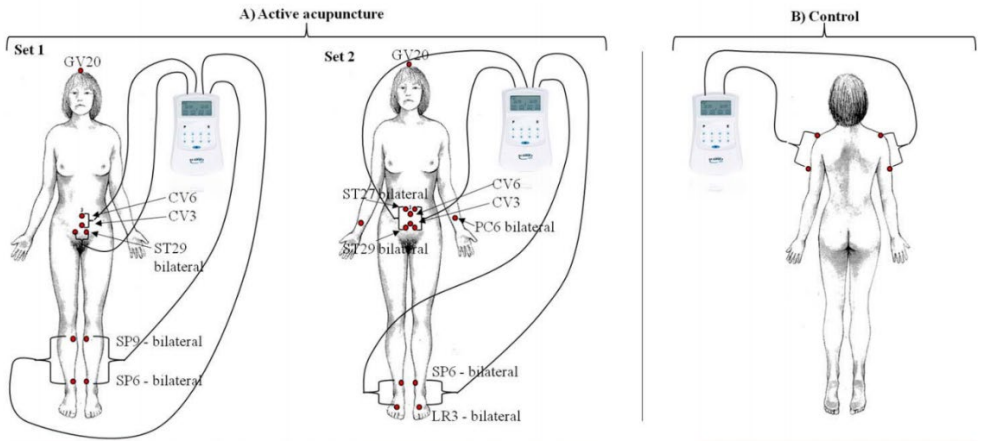


Figure 33.Acupuncture Point Localization in the Active Acupuncture and Control Acupuncture Groups in study 1. Authorized and licensed by (Xiao-Ke et al., 2017)

Study 2	
Acupoint	**Dermatode**
Active acupuncture 1	
Rs 3 (Zhongji)	L1
Rs 6 (Qihai)	Th11
S 29 (Guilai)	Th6-12
L6 (Sanyinjiao)	L4-5, S1-2
L9 (Yinlingquan)	S1-2
H4 (Hegu)	C8, Th1
Rg20 (Baihui)	C2-3, N. trigeminus
Active acupuncture 2	
Rs 3 (Zhongji)	L1
Rs 6 (Qihai)	Th11
S 29 (Guilai)	Th6-12
S25 (Tianshu)	Th6-12
L6 (Sanyinjiao)	L4-5, S1-2
H3 (Taichong)	S2-3
PC 6 (Neiguan)	C8, Th1
Rg 20 (Baihui)	C2-3, N. trigeminus
Control acupuncture	
No known point	C3-4, n. supraclavicularis
No known point	C5-6, n. cutaneous brachiilateralis

Table 23. Acupoint protocols used for each study (1-4).

Study 1	
Acupoint	**Dermatode**
Rs 12 (Zhongwan)	T7-T8
B 23 and B52 (Shenyu)	L2
Rs 3 (Zhongji)	L1
Rs4 (Guanyuan)	Anterior cutaneous nerve of the subcostal nerve – Th12
L10 (Xuehai)	L3, S1-2
L6 (Sanyinjiao)	L4-5, S1-2
S 40 (Fenlong)	L5
S 36 (Zusanli)	L5

Study 3	
Acupoint	**Dermatode**
Unilateral Rs4 (Guanyuan)	Anterior cutaneous nerve of the subcostal nerve
Unilateral Rs5	Th11
Bilateral S29	Th6-Th12
Bilateral L6	L4-L5 S1-S2

Study 4	
Acupoint	**Dermatode**
Rs3 (Zhongji)	L1
Rs4 (Guanyuan)	Anterior cutaneous nerve of the subcostal nerve – Th12
Rs6 (Qihai)	Th11
Rs12 (Zhongwan)	T7-T8
Rg2 (Yao shu)	S2
Rg3 (Yao yang guang)	L4
Rg4 (Mingmen)	Afferent ganglion of the ovary and adrenal gland.

4.2. Chinese Pharmacotherapy

No.	*Study*	*Type of Treatment*	*Objective*	*Duration*	*Outcome*
1	Lu et. al, 2017	RCT	Verify the effect of the BHF formula on PCOS women metabolism	3 months	BHF was proved effective to PCOS by reducing the inflammatory reaction and oxidative stress.
2	Liao et.al, 2018	National co-hort	Test the use of TCM for Polycystic Ovary Syndrome	-	89.22% women diagnosed with PCOS had received TCM therapy. The top five commonly prescribed single herbs and herbal formulas show promise in treating PCOS symptoms.
3	Liao et.al, 2018	National co-hort	Test the efficacy of Chinese phytotherapy in the reduction of T2DM in PCOS patients.	-	The phytotherapy showed a decreased risk of T2DM for women with PCOS
4	Zhang et. al, 2020	Co-hort	Effect of Qi Gong Wan Prescription on Patients with Phlegm-Dampness PCOS Based on Intestinal Flora	2 months	Jiawei Qi Gong Wan improves IR linked to the status of the intestinal flora in PCOS patients with phlegm-dampness syndrome.

Table 24.Search results regarding full text of Chinese pharmacotherapy.

Summing up to the acupuncture trials it was found 4 more studies with herbal therapy or Chinese pharmacotherapy within the last 5 years (Lu et al., 2016, Liao et al., 2018, Liao et al., 2019, Zhang et al., 2020b). One of these studies is a RCT and the remnant are co-horts. It was also found 4 reviews about Chinese pharmacotherapy (Ong et al., 2017, Li et al., 2018a, Moini Jazani et al., 2019, Pundir et al., 2019) and 13 studies with animals and in vitro (Ni et al., 2016, Wenmin et al., 2016, Atashpour et al., 2017, Eunkuk et al., 2017, Hengxia et al., 2017, Wang et al., 2017, Yu et al., 2017, Zhao et al., 2018, Jin et al., 2019, Liu et al., 2019, Ong et al., 2019, Darabi et al., 2020, Qiu et al., 2020, Yang et al., 2020)

5. Discussion

5.1. Acupuncture Therapy

All the studies, except for number three were done in mainland China and intended to assert the efficacy of acupuncture mostly in infertility management (study 2 and 4) - whether study 1 also considered effects on metabolic features.

All studies, except number 4, used WM diagnosis after Rotterdam criteria for diagnosing PCOS women. Study 1 used both criteria (WM and TCM diagnostic). For scientific validity and coherence, a first diagnostic step should be transversal therefore both gold standards in TCM and WM should be designed, met, and followed to reflect assertive

results. Study 4 followed WM Chinese guidelines for PCOS diagnostic which are similar to international guidelines except for the polycystic ovary morphology assessment which the latest determines that the number of follicles per ovary should be over 20 and not over 12 (which can lead to overdiagnosis of polycystic morphology within the population).

The exclusion criteria mainly included: other endocrine disorders mimicking PCOS, including hyperprolactinemia, uncorrected thyroid disease, Type I or Type II diabetes, and Cushing's syndrome along with studies 1 and 3 which is under the recommendations by the international guidelines (Teede et al., 2018d) Different acupuncture approaches were used: catgut embedding acupuncture (study one), electro-acupuncture and manual acupuncture (study 2), laser acupuncture (study 3) and manual acupuncture (study 4).

All the four studies use at least acupuncture as TCM treatment (with or without a WM therapy associated) but only studies 1 and 4 present a TCM diagnosis of PCOS. Both consider Kidney deficiency as main feature of TCM diagnosis for PCOS, which goes along with HM considerations of renal orb deficiency for this pathology – study one considers xue stasis as a secondary diagnostic criteria in opposition to study four that values humor (pathogenic fluid accumulation) as a main reason of stagnation within the pelvic region (humor is the equivalent to dampness/pituita is equivalent to phlegm or more condensed fluids status).

Authors in study 4 based their diagnosis priority on a previous study of Wang Dong Mei which investigated the statistical distribution of the syndrome where: 68% accounted for kidney syndrome (renal orb); 20% for liver syndrome (hepatic orb) and 6.1% for spleen (lienal orb). Feng, 2014 also describes PCOS as a TCM complex syndrome composed by 4 basic TCM syndromes: kidney deficiency (renal orb deficiency), phlegm stagnation syndrome (pituita accumulation), qi stagnation and blood (xue) stasis syndrome, syndrome of dampness heat of liver channel (equivalent to calor-humor in the hepatic orb) (Feng et al., 2014).

Despite these references, outcome values regarding pregnancy rate and metabolic feature improvement was higher in studies 1 and 3, where the diagnostic and treatments considered local microcirculation as a focus. Catgut implants promote local blood flow therefore prompting the follicles development and luteinization phase since the protein complex decomposition and liquification facilitates local permeability and the absorption of the drug in the area as well as microcirculation in overall. On the other hand, laser acupuncture (study 3) when acting on the identical innervation to the ovary, is known to reduce sympathetic activity, therefore, reduced androgen secretion in parallel with higher centres modulation by decreased central β-endorphins within circulation. The impact is

seen not only at a serum LH reduction but also decreasing the uterine artery resistance, improving uterine blood flow (by decreased sympathetic tonus).

A side note should be taken regarding the average age of the study group of work three that is 8 years younger than the other groups (20 years old) which might be a benefit on the outcome since metabolic features are more severe later in midlife as well the yang deficiency of the renal orb. According to HM, 20 years old belongs to the beginning of the fire phase where functional power is highlighted and 28 years old belongs to final range of the Fire phase almost entering the metal phase where the yang aspect of the renal orb is comparably reduced and yin aspects reinforced (as hormonal secretions) - *vide* figure 28.

On the other hand, in study 2, though women were advised to follow a healthy diet and practice exercise which is not mentioned on the other studies the BMI reduction was not higher. Laser acupoints in study 3 were selected according to innervations of the ovaries (Th12-L2; S2-S4) to modulate HPG and HPA axes in support of hormonal balance. Study 1 uses more Earth points (Stomach and lienal) reinforcing the upper movement needed for water phase (by the lienal orb) and downward movement of fire phase (by the stomach orb) and this way promoting a smooth qi flow between cardio-renal rapport through Respondens and Regens then showing in a better xue flow through the Chong channel or Impendimentalis Conduit to the paraorb uteri. Lienal and stomach points are used in all studies except for study four and, notably, this is the study with the second worst result regarding pregnancy rate.

Study 2 was the broadest and included initially 1000 recruited patients from 21 centres randomly allocated to one of four groups for a 2x2 trial: active acupuncture plus clomiphene, control acupuncture plus clomiphene, active acupuncture plus placebo, and control acupuncture plus placebo. The clomiphene and placebo assignments were double-blinded, unknown to patients and study investigators except the data manager. Study one and three were one blinded and in study four there was no control on placebo or control acupuncture (which is a downfall of the research work). It is urgent to design more RCT's with double blind performance or at least patient blinded and with control to compare the baseline and outcomes of study group with a similar feature (inclusion and exclusion criteria) and context.

Study 1 is much more complete compared with the others, giving a considerate amount of information – including as a supplement where it is possible to find secondary metabolic parameters. However the outcome ratios like HOMA-IR and LH/FSH had to be calculated by the reader with the given values that would be in different units and in need

of conversion which brings an added difficulty for a comparison with the other studies. To confirm the baseline and outcome HOMA-IR values, conversions were made, and automatic algorithms were used to obtain final ratio, but the values did not coincide with article. Moreover, the newly calculated ratios were more congruent with the values presented with study 1 also, conducted with Chinese women within the same average age range (hence similar western pacific body type) even if the average baseline BMI in study 1 is higher (27.4) than the average baseline BMI value for study 2 (23.8). Apart from that, both studies considered metabolic values (baseline and outcome) as well as study 3 - even if the main objective of study 1 and 2 was to evaluate the efficacy of the TCM treatments on the infertility feature of PCOS women.

Current reality of PCOS women health status calls for more complete studies regarding metabolic status within the effect of acupuncture protocols as well as regarding mental health and quality of life. The only study that applied quality of life surveys was study number 2 which shows the lack of importance that is given to this aspect despite the fact of being well documented the psychological aspects of PCOS condition. TCM includes several therapeutic techniques that could help with this aspect, but no study about Qi gong or PTTCM were found. A single protocol study about comprising Tai-chi was within the article search.(Li et al., 2018b)

5.2. Chinese pharmacotherapy

Study 2 is a co-hort study where the authors assessed the Taiwanese national health database and randomly chose 1000 000 subjects diagnosed with PCOS through gynaecological ultrasonography or testosterone/ 17-hydroxyprogesterone blood test. Files missing information on birthday or gender were dismissed as well as non-adults (less than 18 years old) and male in gender identification. Individuals with PCOS diagnosis between 1997-2010 were selected and divided in non-TCM and TCM users.

The age group with higher prevalence within TCM users is 18-29 years old and the comorbidities of higher prevalence within TCM users are infertility, amenorrhea, and anxiety. T2DM, obesity, dyslipidaemia and major depression had similar prevalence between TCM and non-TCM users. Among PCOS women recurring to TCM therapies, 50.37% were treated with Chinese pharmacotherapy, only 0.18% with acupuncture or Chinese manual therapy alone, and 49.45% with a combination treatment.

The most prescribed formulas to PCOS patients were Jia-Wei-Xiao-Yao-San (used in treatment of anxiety, irritability, stress, depression, pre-menstrual tension, climacteric syndrome and infertility); Gui-Zhi-fu-Ling-Wan (tonify xue, transform xue stasis, reduce fixed abdominal masses); Dang-Gui-Shao-Yao-San (used to treat dysmenorrhea and abdominal pain); Wen-Jing-Tang (usually used to treat dysmenorrhea, excessive bleeding, and infertility due to qi deficiency and cold in the Impendimentalis and Respondens conduits) and Ma-Zi-Ren-Wan (also known as hemp seed pill and shown to be effective in the treatment of functional constipation).

The most prescribed single herbs were Xiang-Fu (Cyperus rotundus L.) with antidepressant activity, Da-Huang (Rheum officinale Baill) – with abundant emodin proposed to be a possible treatment for T2DM and other metabolic disorders); Yi-Mu-Cao (Leonurus artemisia (Lou.) S.Y.Hu) – promotes blood flow and regulates menstruation and also shown to decrease fasting blood glucose and triacylglycerol levels, increase high-density lipoprotein and plasma insulin concentrations as well as to have anti-inflammatory); Yan-hu-suo (Corydalis yanhusuo W.T. Wang) – analgesic effect in dysmenorrhoea and Dan-Shen (Salvia miltiorrhiza Bge) – useful in the treatment of IR, obesity and T2DM for the presence of salvianolic acid B which also shown to have hepatoprotective effects on NAFLD trials (Wang et al., 2015).

Study 3 followed the same logic as study 2 but adding the criteria of being diagnosed with T2DM at the endpoint. Also, this study comprised diagnosed patients between since 1997 and follow-up terminus in December 2013.

Within this study the TCM users displayed a higher prevalence of emotional disorders, reproductive dysfunctions which may motivate the patient to look for TCM support. Parallel to a higher prevalence of these issues, it was found a higher prevalence of metformin and clomiphene use but no difference of COCP use between TCM and non-TCM users. After a follow up of *circa* 5 years the TCM users showed a reduced incidence of T2DM.

The five most efficient formulas treating T2DM in study 3 were: Jia-Wei-Xiao-Yao-Sa, Gui-Zhi-Fu-Ling-Wan, Wen-Jing-Tang, Dang-Gui-Shao-Yao-San, Shao-Fu-Zhu-Yu-Tang. The most effective single herbs at managing D2TM were Yan-Hu Suo and Xu-Duan.

Study 1 of Chinese pharmacotherapy results is related with the use of serum metabolic to assess the most common metabolites within the serum of normal-insulinemic and hyper-insulinemic PCOS patients (diagnosed by EM Rotterdam criteria).

Important values within the dyslipidaemia profile were considered before and after treatment for 3 menstrual cycles with Bushen Huatan Formula (BHF) that is known to

improve IR and ovarian dysfunction and that includes herbs: Astragali radix, poriacocos wolf, atractylodeslancea, Radix Salvia miltiorrhiza, Rizhona Coptidis and Herba Epidemium.

After intervention with BHF both normo and hyper-insulinemic PCOS women had lowered their BMI, WHR, body weight (more significant drop of values in the hyper-insulinemic group) and other signs of hyperinsulinemia as acanthosis nigricans, HOMA-IR, LDL-C, ApoB, Apob/ApoA1. SHBG increased after treatment in both groups with higher increase within the normo-insulinemic group. Furthermore, serum metabolics showed that in normoinsulin group the phospholipid metabolism improved, and, in the hyperinsulinemic group, the amino-acid metabolism had changed inverting the tendency to find decreased levels of arginine and ornithine in PCOS individuals.

Safe to say that this study shows a protective effect of this BHF against inflammation and oxidative stress.

A final fourth co-hort study analysed the effect of Qi Gong Wan in metabolic and hormonal features of PCOS women diagnosed with Phlegm-Dampness Syndrome (Pituita/Humor accumulation within the affected conduits) based on their intestinal flora.

Criteria BMI WHR, HOMA-IR fasting plasma glucose and Fasting insulins were lowered in the Phlegm-Dampness PCOS patients after formula treatment as did improve the diversity of intestinal flora with a higher presence of Firmicutes, Actinobacteria and proteobacteria after treatment and decreased values of Bacteroidetes.

Therefore, the importance of intestinal flora within a PCOS phenotype and respective management should be considered for improvement of metabolic.

6. Conclusions

From a retrospective towards the recent analysis it is safe to say that the results of the studies towards TCM interventions are quite positive – whether it is acupuncture or Chinese pharmacotherapy – however RCT within the last five years regarding PCOS treatments in TCM are extremely scarce and it is highly demanded further research. Taiwan national co-horts studies are a proof of that since that it showed clearly that half of the women that had been diagnosed with PCOS looked up for a complementary approach within TCM.

Furthermore, it was proved that most the reasons why women search for TCM treatments are endocrine, reproductive and psychological complaints what goes hand in hand with the chronic chief complaints of PCOS condition and complying with the

information that most women are not satisfied with the WM delayed diagnosis and management of their symptoms – as read above.

Most of the acupuncture studies had metabolic blood screening thought the treatment is administered mostly to address infertility. Reproductive complaints and endocrine dysfunction maybe the main reasons that take women to TCM practice though metabolic imbalance is a condition that worsens with age and gets transversal to most of lifespan, from adolescents to climacteric women. For this reason, studies exploring TCM formulas and conjugation with acupuncture should be met, preferably with blind control and study groups.

No concluded studies regarding Qi gong and Chinese manual techniques were found in this review. Only a study protocol suggesting tai-chi as treatment to address obese PCOS patients was found within the last five years. Giving the fact that psychologic an psychiatric disorders are prevalent in Stein-Leventhal syndrome, treatments like PTTCM and Qi Gong are recommended for a whole better cardio-renal balance and holistic equilibrium between Heaven-Earth – inner dialogue of the individual disrupted in serious cases of anxiety and depression (timor and maeror prevalence).

This review had several limitations that could be improved in a further step: number of used databases and a further exploration of the translation of the diagnostic and mechanism of the disease between the HM and other classical TCM approaches. This review was limited to studies published in English, thus putting the review at risk of language bias. Also, there was no contact of the study authors for missing information or data conversions.

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