U.PORTO

MESTRADO EM MEDICINA TRADICIONAL CHINESA

The Effect of Acupuncture on Decreasing Infertility Associated with Polycystic Ovary Syndrome Bibliographic Revision

Ana Carolina de Freitas

M

2019

U.PORTO

Ana Carolina de Freitas
The Effect of Acupuncture on Decreasing Infertility Associated with Polycystic Ovary Syndrome
Bibliographic Revision

M.ICBAS **2019**

The Effect of Acupuncture on Decreasing Infertility Associated with Polycystic Ovary Syndrome
Bibliographic Revision
Ana Carolina de Freitas

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

ANA CAROLINA RAMOS DE FREITAS

"THE EFFECT OF ACUPUNCTURE ON DECREASING INFERTILITY ASSOCIATED WITH POLYCYSTIC OVARY SYNDROME"

– BIBLIOGRAPHIC REVISION –

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

Orientador – Professor Jorge Machado

Afiliação – Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

Co-orientador – Andreia Sofia Pires Fernandes

ABSTRACT

Background: Polycystic Ovary Syndrome (PCOS) is the most prevalent endocrine disorder affecting women in reproductive-age. Being a multisystem endocrine disorder with associated reproductive, metabolic and psychological comorbidities, PCOS encompasses significant health and economic burdens. Economically speaking, the estimated annual costs associated with this syndrome in the USA in 2004 was 4 billion dollars, with 40% of this value associated exclusively to PCOS-related diabetes. As for the health related consequences, hyperandrogenism, chronic oligo-/anovulation, hirsutism, acne, oligomenorrhea, and infertility are well documented, as such increased risk of cardiometabolic disease like dyslipidemia, insulin resistance, and type 2 diabetes mellitus. Obesity is present in the majority of PCOS patients, as so is increase psychological disease and reduced health-related quality of life, among other associated co-morbidities. Despite the etiology and pathophysiology of the syndrome are far from being completely understood, the association of PCOS with anovulatory infertility is, at this days, an irrefutable evidence. There are several ways that Western medicine uses in order to increase menstrual frequency and ovulation patterns in these patients, aiming to increase their fertility, but success rates are still low, and sometimes women cannot achieve pregnancy, even after many attempts.

Acupuncture is an integral part of Traditional Chinese Medicine dating thousands of years, and has recently been gaining popularity in western countries. In China however, acupuncture has been used for centuries in gynecology health care, aiming to regulate the female reproductive system and help women with gynecological problems, including women with PCOS and anovulatory infertility.

Objectives: The main goal of this thesis is to review the available literature to assess the possible effect of acupuncture in the treatment of infertility associated with PCOS, influencing the anovulatory nature of the syndrome directly or indirectly, as the main therapy or adjuvant therapy in combination with the western protocols.

Methods: Bibliographic revision of articles published in the international databases like PubMed, Science Direct, and other databases, between year 2000 and 2019.

Conclusions: Although the role of acupuncture in the treatment of PCOS-associated infertility is not yet fully understood and no specific acupuncture treatment protocol is associated with the treatment of the disease, recent studies suggest that acupuncture may be an important and safe candidate to consider as a complementary treatment in PCOS

and infertility. As a secondary analysis, and although not being the main objective of the study, in addition to treating the syndrome-associated infertility, studies have shown a possible beneficial effect of acupuncture on the treatment and improvement of some symptoms associated with the disease, such as insulin resistance, type-2 diabetes mellitus, among others. Despite this, the already done studies are not of sufficient quality to draw conclusions. Further good quality studies, such as randomized controlled trials, are needed to confirm the role of acupuncture in treating PCOS, and the symptoms associated with the disease, including anovulatory infertility.

Keywords: PCOS, acupuncture, anovulatory infertility, menstrual cycle, ovulation induction.

RESUMO

Revisão da literatura: O Síndrome do Ovário Policístico (SOP) é o distúrbio endócrino mais prevalente que afeta as mulheres em idade reprodutiva. Sendo um distúrbio endócrino multissistémico com comorbilidades reprodutivas, metabólicas e psicológicas associadas, o SOP abrange custos de saúde e económicos significativos. Economicamente falando, os custos anuais estimados associados a este síndrome nos EUA em 2004 foram de 4 bilhões de dólares, com 40% desse valor associado exclusivamente ao diabetes relacionado à SOP. Quanto às consequências relacionadas com a saúde, o hiperandrogenismo, oligo-/anovulação crónica, hirsutismo, acne, oligomenorréia e infertilidade estão bem documentados, assim como o aumento do risco de doença cardiometabólica, como dislipidemia, resistência à insulina (RI) e diabetes mellitus tipo 2 (T2DM). A obesidade está presente na maioria das pacientes com SOP, assim como o aumento de doenças psicológicas e a redução da qualidade de vida relacionada com a saúde, entre outras comorbilidades associadas. Apesar da etiologia e fisiopatologia do síndrome estar longe de ser completamente compreendida, a associação do SOP com a infertilidade anovulatória é, hoje em dia, uma evidência irrefutável. São várias as maneiras que a medicina ocidental usa para aumentar a frequência menstrual e os padrões de ovulação nestas pacientes, com o objetivo de aumentar sua fertilidade, mas as taxas de sucesso ainda são baixas e, por vezes, as mulheres não conseguem engravidar, mesmo após muitas tentativas.

A acupuntura é parte integrante da Medicina Tradicional Chinesa (MTC), datada de milhares de anos, e recentemente tem ganho popularidade nos países ocidentais. Na China, no entanto, a acupuntura tem sido usada há séculos nos cuidados de saúde ginecológicos, com o objetivo de regular o sistema reprodutor feminino e ajudar as mulheres com problemas ginecológicos, incluindo mulheres com SOP e infertilidade anovulatória.

Objetivos: O principal objetivo desta tese é fazer uma revisão da literatura disponível para avaliar o possível efeito da acupuntura no tratamento da infertilidade associada ao SOP, influenciando direta ou indiretamente a natureza anovulatória do síndrome, como principal terapia ou terapia adjuvante em combinação com a protocolos ocidentais.

Métodos: Revisão bibliográfica de artigos publicados em bases de dados internacionais como PubMed, Science Direct e outras bases de dados, entre os anos de 2000 e 2019.

Conclusões: Embora o papel da acupuntura no tratamento da infertilidade associada ao SOP ainda não esteja totalmente esclarecido e nenhum protocolo específico de tratamento com acupuntura esteja associado ao tratamento da doença, estudos recentes sugerem que a acupuntura pode ser um candidato importante e seguro a ser considerado no tratamento complementar do SOP e infertilidade. Como análise secundária, e apesar de não ser o principal objetivo do estudo, além de tratar a infertilidade associada à síndrome, estudos têm demonstrado um possível efeito benéfico da acupuntura no tratamento e melhoria de alguns sintomas associados à doença, como resistência à insulina, diabetes mellitus tipo 2, entre outros. Apesar disso, os estudos já realizados não são de qualidade suficiente para tirar conclusões. A elaboração de mais estudos de boa qualidade, como ensaios clínicos randomizados, são necessários para confirmar o papel da acupuntura no tratamento do SOP e os sintomas associados à doença, incluindo infertilidade anovulatória.

Palavras-chave: SOP, acupuntura, infertilidade anovulatória, ciclo menstrual, indução da ovulação.

ACRONYMS

ACTH – Adrenocorticotrophic hormone

AI – Aromatase Inhibitor

AMH – Anti-Mullerian Hormone

ART – Assisted Reproductive Technology

ASRM – American Society for Reproductive Medicine

BBT – Basal Body Temperature

BC – Before Christ

BMI – Body Mass Index

CAM – Complementary and Alternative Medicine

CC – Clomiphene Citrate

CNS – Central Nervous System

CRH – Corticotrophin-Releasing Hormone

CVD – Cardiovascular Disease

DA - Dopamine

EA – Electro-Acupuncture

ESHRE – European Society for Human Reproduction and Embryology

FSH – Follicle Stimulating Hormone

GH – Growth Hormone

GHRH – Growth Hormone-Releasing Hormone

GnRH – Gonadotropin-Releasing Hormone

hCG – Human Chorionic Hormone

HPA – Hypothalamic-Pituitary-Adrenal

HPG – Hypothalamo-Pituitary-Gonadal

HPO – Hypothalamus-Pituitary-Ovarian

ICSI – Intracytoplasmic Sperm Injection

IR – Insulin Resistance

IVF – In Vitro Fertilization

IVF-ET – In Vitro Fertilization-Embryo Transfer

LH – Luteinizing Hormone

NGF – Nerve Growth Factor

NIH/NICHD – National Institute of Health/National Institute of Child Health and Human Disease

OCP – Oral Contraception Pill

PAI-1 – Plasminogen Activator inhibitor

PCO – Polycystic Ovaries

PCOS – Polycystic Ovary Syndrome

PIF – Prolactin Releasing-Inhibitor Factor

PRF – Prolactin-Releasing Factor

RCT – Randomized Controlled Trial

SS - Somatostatin

T2DM – Type 2 Diabetes Mellitus

TCM – Traditional Chinese Medicine

TRH – Thyrotropin-Releasing Hormone

TSH – Thyrotropin Stimulating Hormone

US – United States

USA – United States of America

USS – Ultrasound Sonography

VIP – Vaso-active intestinal Peptide

WHO – World Health Organization

WHR – Waist-Hip Ratio

INDEX

FIGURE INDEX

TABLE INDEX

INTRODUCTION

Infertility is a problem which prevalence has been increasing over the years. Much has been studied about the consequences of infertility and assisted reproductive technology (ART) treatments related to pregnancy and newborns health: increased risks of preterm delivery, low birthweight, and perinatal mortality are well documented (Hanson, et al., 2017). In a review made by Boivin et al. in 2007, global estimates suggest that nearly 72.4 million couples experience fertility problems, and out of the 72.4, 40.5 million are seeking infertility medical care. This results indicated an increasing and emergent need for more research on the prevalence of infertility and treatment seeking behavior worldwide, and not least important, on the factors that may impact these estimates, such as accessibility to the needed medical care (Jacky Boivin, 2007).

Data from the National Counsel of Medically Assisted Procreation reports that in 2015, 2504 babies were born in Portugal has a result of the various available techniques of medical assisted procreation, which represents 2.9% of the total number of birth occurred in Portugal that year (Conselho Nacional de Procriação Medicamente Assistida, Setembro de 2017). Historically, the first in vitro fertilization (IVF) therapeutic cycle made in Portugal dates 1985, and the first baby fertilized by IVF was born in 1986. Worldwide, it is estimated that more than 3 million children have been born as a result of their use (Conselho Nacional da Procriação Medicamente Assistida, 2019). In 2003, it was estimated that 932 000 IVF and related cycles were performed worldwide, resulting in 232 000 babies born as a result of these techniques. European countries count lower IVF success rates because they are significantly reducing the number of multiple pregnancies, moving towards the single embryo transfer. The success rate may be smaller, but the rates of health risks – for both mother and baby – associated with multiple pregnancies is approximately half of those in the USA (Eric Manheimer, 2013). In Australia, which has one of the highest levels of utilization of IVF, the average cost of a standard IVF cycle is estimated in 5,492 dollars (Caroline A Smith, 2012), which corresponds to approximately 3409,17 euros. Worldwide data from the International Committee for Monitoring Assisted Reproductive Technologies world report, accounts that during the period between 2008 and 2010, based on both reported and estimated numbers for the countries proving data for this report, a total of > 4 461 309 ART cycles were initiated: 1 364 943 in 2008; 1 452 910 in 2009; 1 643 456 in 2010. This numbers resulted in an estimated 1 144 858 babies born between this 3-year

period. The estimated overall number of initiated cycles and of babies born increased by almost 9,5% and 9,1% per annum, respectively, during this period (S. Dyer, 2016).

Polycystic ovary syndrome (PCOS) affects an average 6%–21% of women and is the primary cause of anovulatory infertility, with major health and economic costs. Being PCOS a syndrome, the clinical manifestations are represented by an extensive and heterogeneous list of signs and symptoms, more or less frequents, and more or less identifiable, forming a spectrum of a disorder, with a mild to severe presentation, which can cause different outcomes in reproductive, endocrine and metabolic function (Adam H. Balen, 2016). The American Society for Reproductive Medicine (ASRM) and European Society for Human Reproduction and Embryology (ESHRE) (2004) held a joint consensus meeting, which agreed the definition of the PCOS as the presence of two out of the following criteria: (1) hyperandrogenism (clinical evidence of hirsutism, acne, alopecia, and/or biochemical Hyperandrogenaemia); (2) oligomenorrhea or amenorrhea and/or anovulation (disturbance of menstrual cycle); and (3) polycystic ovaries, which is assessed by ultrasound sonography (USS). Significant health and economic burden are associated with this syndrome: the estimated annual costs for the condition in USA in 2004 was 4 billion dollars, with 40% attributed to PCOS-associated diabetes (Samantha Cassar, 2016). The health implications of PCOS has been thoroughly documented, including an increased risk for endometrial cancer, metabolic disorders, cardiovascular disease, obesity, and a predisposition to insulin resistance and diabetes. Since PCOS makes women 10 times more likely to develop infertility, it is crucial to understand the connections between a diagnosis of PCOS and overall health of the women (Hanson, et al., 2017).

Being now PCOS a much more commonly diagnosed cause of infertility than it was many years ago, and despite being far from completely understanding the syndrome, with more recent research and knowledge there are now more different and new ways in which both western medicine and Traditional Chinese Medicine (TCM) can approach this disease, in more orthodox or in more holistic ways.

In vitro fertilization-embryo transfer (IVF-ET) is the most successful infertility treatment and, for many people, it provides the last possibility for pregnancy; even in PCOS women, IVF is the most successful method in ART in order to achieve pregnancy. Unfortunately, the average IVF success rate is low, around 30-33% (Cui Hong Zheng, 2012; Eric Manheimer, 2013), and due to this relatively low success rate per cycle, some patients do not achieve their goal even after many attempts. Therefore, repeated cycles will place enormous stress and economic pressure on the patients and their family, because each cycle is not only expensive, but lengthy and stressful: many studies show high prevalence of anxiety and

depression symptoms associated with fertility problems (Cui Hong Zheng, 2012). Even though science is investing and searching for more therapies, the progress in developing safe and effective therapies has been limited, driving patients to consider IVF adjuvant therapies, despite the cause of their infertility (Eric Manheimer, 2013). Complementary and alternative medicine (CAM) – which is typically described as being outside the orthodox medicine – consists of a broad spectrum of interventions that aim to promote health and wellbeing and to treat illness. More than 70% of the world's population rely on some form of CAM for health treatment, mainly as a complement to standard care or as a second-line alternative after trying first-line and more established treatments (Xiaoke Wu, 2014). There have been some benefits reported for women's use of CAM for reproductive health, and recent studies have shown that several different CAM strategies could be beneficial as adjuncts to the conventional medical management of reproductive disorders in women. Traditional Chinese Medicine is one of these CAM that could be helpful in the treatment and amelioration of the symptoms and co-morbidities associated with PCOS, such as infertility. Acupuncture is, according to Smith et al. (2010), the most commonly used adjuvant CAM fertility treatment among couples seeking fertility care in US fertility clinics (Eric Manheimer, 2013).

Acupuncture is an important and essential part of TCM that dates back to approximately 3000 years, and has been gaining increase popularity in western countries due to its convenience, lack of side effects, and unique therapeutic effects (Cui Hong Zheng, 2012). It has been used in China for centuries to regulate the female reproductive system. The basic foundation of treatment of infertility with TCM remained stable over the years, and it requires a deep understanding of menstrual and reproductive physiology. TCM still has its roots deeply settled in tradition and beliefs of thousand years ago, and it is being practiced not only in China but also in the West, mainly as a complementary approach to the well-established health medical system. Despite remaining stable in terms of diagnosis and treatment, the amount of medical research in human biochemistry and physiology came to improve the knowledge of TCM doctors and therapists, increasing their results and treatment outcomes (Lyttleton, 2013). Medical science has contributed hugely to the knowledge about the body anatomy, biochemistry and physiology, and scientists were able to develop extraordinary technological systems in the area of fertility treatments, but both medical approaches (western vs TCM) have strengths and weaknesses, and can use different methods to treat the same diseases (Lyttleton, 2013).

The aim of this review is to summarize the main findings about this syndrome and its implication in infertility, and to address in what way research in the area of acupuncture and

PCOS treatment is being conducted. Given the apparent relative lack of information on this subject in Portugal, the necessity of some research seemed even more urgent. So the goal is to review the new views on the etiology and pathophysiology of the syndrome, and to see in what way TCM and Western medicine are working together with the same goal: improve the fertility outcomes in PCOS patients and ameliorating the anovulatory nature if this syndrome. It is important to understand what type of research has been made in the last few years about the possible use of acupuncture in the treatment of infertility associated with PCOS, and the quality of the studies. The number of study protocols that are being accepted and put in practice is also important, being a nice marker on how science is investing in this area of interest.

METHODOLOGY

This master thesis is a bibliographic review about the effect of acupuncture on decreasing the infertility associated with polycystic ovary syndrome. The bibliographic search was performed mainly in the Research Gate, Pubmed and Science Direct, among others databases. Only articles published between the year 2000 and 2019 were included.

Given the fact that it is a subject of relatively recent research, there are still not enough high quality studies. Therefore, it was a priority to research for systematic reviews, meta-analyzes and some randomized controlled trials, which are included in the review. Due to the lack of sufficient number of these high quality studies, also some non-randomized controlled studies, prospective studies, secondary RCT analyzes, and a small number of feasibility, experimental and experimental animal studies were also included. Also included are three randomized controlled trial protocols, already approved by the scientific committees, which are in the process of being carried out and will bring some conclusions in the future.

THE FEMALE REPRODUCTIVE SYSTEM

In order to achieve the main goal of this review, the entire female reproductive system must be carefully but briefly explained, in anatomical, physiological and regulatory/endocrine terms.

In this way, anatomically speaking, the female reproductive system consists of the gonads, called the ovaries, and the female reproductive tract, which includes the fallopian tubes, the uterus, cervix, vagina, and external genitalia.

In the matter of the female tract regulation, and physiologically speaking, the endocrine system, specifically the hypothalamic-pituitary axis, is one of the main regulators of the reproductive system. The neurons of the hypothalamus secret specific hypothalamic releasing hormones (XRHs) which in its turn will stimulate the secretion of pituitary trophic hormones (XTHs), also specific. This pituitary hormones act over specific peripheral target endocrine glands, and stimulate the glands to release peripheral hormones (X) (Koeppen & Stanton, 2009).

The hypophysis is responsible for secreting releasing-hormones such as Thyrotropin-releasing hormone (TSH), Gonadotropin-releasing hormone (GnRH), Corticotrophin-releasing hormone (CRH), Growth hormone-releasing hormone (GHRH), Somatostatin (SS), dopamine (DA), Prolactin-releasing factor (PRF) and Prolactin releasing-inhibiting factor (PIF). This hormones will act over the adenohypophysis, releasing the trophic hormones: growth hormone (GH), thyrotropin stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH), gonadotrophins such as Follicle Stimulating hormone (FSH) and Luteinizing hormone (LH), Prolactin and Melanin stimulating hormone (Koeppen & Stanton, 2009).

Reproduction is, in this way, controlled by the neuroendocrine axis, the hypothalamo-pituitary-gonadal (HPG) axis, which drives the growth, development and maturation of the germ cells, and the synthesis of the gonadal steroids in the gonads. Likewise, also the gonadal hormones play a key role in the establishment, activation, and regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Rivier & Rivest, in 1991, said that elevated stress hormones can negatively affect the reproductive neuroendocrine axis and consequently, disturb the levels of circulating gonadal hormones (Oyola & Handa, 2017 September).

So the gonadotrophs, which are located at the lateral portion of the pituitary gland (Palermo, 2007), constitute 10 to 15% of the adenohypophysis, and secret FSH and LH, and regulate

the function of the gonads in both sexes. They have a pulsatile, periodic and cyclic secretion, and have significant differences between male and female system (Koeppen & Stanton, 2009). This pulsatile rhythmic activity is an inherent property of GnRH neurons (Palermo, 2007), and this pulsatile fashion of liberation is critical in puberty and reproduction. The activity of GnRH is regulated by neurotransmitters, steroid hormones and growth factors, and the pulses of liberation are required for reproductive functions in both sexes, which importance has been described both in primates and humans. Oyola et al. and many other authors have analyzed several studies that have demonstrated that the slow release of GnRH can selectively drive the synthesis of FSH-β, while fast pulses selectively drive the synthesis of LH-β (Palermo, 2007). This stimulation of the anterior pituitary gonadotrophs in this receptors will eventually lead to elevations in intracellular calcium, driving to the release of LH and FSH by exocytosis into the general circulation until it reaches the gonads (Oyola & Handa, 2017 September).

The GnRH is commonly accepted as the main hypothalamic hormone responsible for controlling reproduction, being responsible for the stimulation of the secretion of the anterior pituitary hormones, LH and FSH, that will themselves finally control the production of gonadal steroids and the gametogenesis (Oyola & Handa, 2017 September; Palermo, 2007). GnRH, with a regular pulsatile production and liberation, has important effects over the gonadotrophs: constant infusion promotes a down-regulation of GnRH receptors, diminishing FSH and LH secretion, not desensitizing the gonadotrophs and the secretion remains normal. The gonadotrophins LH and FSH are responsible for regulating the development, growth, pubertal maturation, reproductive processes and secretion of sex steroids in the gonads. They are packaged in separate secretory granules, which allows them to be independently secreted by gonadotrophs (Koeppen & Stanton, 2009; Oyola & Handa, 2017 September). Both hormones contain two subunits, α and β: the structure of all subunits α of all pituitary glycoproteins is similar; while the β chains are unique and when connected to the α chain, determine the specific hormone function (Palermo, 2007).

The FSH β gene is regulated by two proteins, inhibin and activin, and by GnRH. Inhibin is considered to be a selective suppressor of FSH synthesis and secretion, whereas activin increases the synthesis of FSH β chains and, consequently, the secretion of FSH itself. LH synthesis and secretion are mainly controlled by the GnRH secreted by hypothalamus (Palermo, 2007). Overall, FSH is responsible for mediating and controlling follicular growth via granulosa cell proliferation, while LH controls ovulation (Cox & Takov, 2019).

In the ovary, the main functions of LH hormone is to increase the synthesis and secretion of sex steroids in the female gonads by acting upon theca cells – which are the endocrine

cells associated with ovarian follicles – to increase synthesis of enzymes involved in the production of the androgen, androsterione. These theca cells have an essential role in infertility, because they are responsible for producing the androgen substrate required for further ovarian estrogen biosynthesis (Magoffin, 2005): androsterione is release from theca cells and taken up by adjacent granulosa cells in the primordial follicle (Drummond, 2006), where it is converted to estrone and then to estradiol. In the granulosa cells, these enzymes are mainly regulated by FSH binding to the FSH receptor. FSH, on the other hand, is also responsible and important for the growth and maturation of the ovarian follicles (Oyola & Handa, 2017 September; Koeppen & Stanton, 2009), has it will be explained in more detail along this review.

In woman, the whole secretion of gonadotrophins from the pituitary is under ovarian control through negative and positive feedback mechanisms, and menstrual cyclicity is significantly dependent on these feedback mechanism. For example, before puberty, only negative feedback is active, while after menopause, both mechanisms are eradicated (Messinis I. E., 2006). These mechanisms of action will be explained along the review, in every step of the folliculogenesis and the menstrual cycle different phases.

THE OVARY AND THE MENSTRUAL CYCLE

The ovary is located within a fold of the peritoneum denominated the broad ligament, adjacent to the lateral wall of the pelvic cavity. It has a flattened almond shape of 4 centimeters wide by 2 centimeters wide and 1 centimeter thick in an adult woman (Loza, 2012) and has two fundamental functions: first, it is responsible for producing and secreting hormones that control and drive the female reproductive system; second, to control the development, selection and, finally, the release of a mature oocyte for fertilization (Cox & Takov, 2019; Palermo, 2007). This released ovules remain briefly within the peritoneal cavity, before they are taken by the fallopian tube. The ovary is divided in an external cortex, composed by a dense cellular stroma, where the ovarian follicles development happens, and an internal medullar. The ovarian follicles are the reproductive unit of the ovary, and have both gametogenic and endocrine functions (Koeppen & Stanton, 2009).

The development of the dominant follicle and the consequent production of good quality oocytes, are under control of a complex and interactive extra and intra-ovarian control systems. This systems can be influenced by genetic and/or external environmental factors, such as nutrition, for example, which has been shown to have an impact throughout follicle

development, including on oocyte quality (R. Webb, 2016). This process of growth and differentiation of follicles from the primordial pool is called folliculogenesis (Drummond, 2006).

Ovarian follicles are the functional unit of the female gonads, and their maturation involves several stages: initiation, growth, selection, ovulation and luteinization. Although many factors are involved in follicle development and maturation, the two pituitary glycoproteins FSH and LH have a central and more pronounced role in this delicate and complex process (Palermo, 2007). Also, the use of technology of ultrasound scanning throughout the cycle in monovular species, was able to clarify the different stages of follicular development: first, early gonadotropin-independent (primordial to early pre-antral); second, gonadotropin-responsive (pre-antral to small antral); third: gonadotropin-dependent (antral to large antral) stages (R. Webb, 2016). The classification of the ovarian follicles was proposed firstly by Gougeon, in 1996. He proposed that ovarian follicles were classified according to the development stage of the follicle, which could be assessed by observing the morphological criteria (Palermo, 2007).

The ovarian follicular development begins in-utero (Drummond, 2006). During the 5th week of gestation, the female fetus's ovary contains around 500-1300 primordial germ cells which, by the 20th week will have increased to approximately 6-7 million germ cells. Of this initial number, many of them are lost during the process of folliculogenesis, and the female is born with 1-2 million primordial follicles. By the time puberty arrives, approximately 400.000 to 500.000 primordial follicles remain (Cox & Takov, 2019). Out of all the follicles existing in birth, only approximately 400 will ultimately develop into an ovulating dominant follicle, meaning that nearly 99,9% of the original follicle reserve will never complete their development, and will suffer atresia. In human, only a single follicle is selected to gain dominance and ovulate every cycle (Palermo, 2007). Although follicle development is a continuous process, in 2001, Hillier divided it into 3 successive phases: initial follicle development, FSH-dependent progression, and LH-responsive maturation (Hillier, 2001).

As for the menstrual cycle, the first half is named the follicular phase of the ovary and is characterized by the recruitment and growth of 15 to 20 big antral follicles. This process is then followed by the selection of one of these follicles as a dominant one, and his continuous growth until ovulation occurs. The second half of the menstrual cycle is called the luteal phase and is controlled by the hormonal secretions of the corpus luteum. This refers to the gonadotropin-independent growth and gonadotropin-dependent growth, also known as pre-antral and antral growth, respectively. Summing up, gonadotropin-independent phase growth depends on local growth factors, beginning with the maturation of the primordial

follicle, and ending before the follicle develops a fluid-filled space (Cox & Takov, 2019; Koeppen & Stanton, 2009).

FOLLICULOGENESIS

Beginning with the most simple and premature structure of the ovary – the primordial follicle – which represents the reserve follicle of the ovary, it is defined as a primary oocyte surrounded by flattened granulosa cells. The moment a few of these flattened cells become cuboidal, the follicle is then classified as transitory or intermediary, but they're still considered to belong to the quiescent pool (Palermo, 2007). As it's been said before, this reserve is reduced from an initial incredibly high number to a much more small number, around 300 thousand follicle at sexual maturity. Due to the fact that the ovarian follicular reserve is a fixed and finite number, the rate at which the quiescent primordial follicle die or begin to develop (or both) will determine a woman's reproductive life expectancy.

The transition from primordial into primary follicle begins with the enlargement of the oocyte and the proliferation of the granulosa cell layer, which later will start to express FSH receptors. This primary follicle stroma develops a blood supply and differentiates into the theca interna – which develops LH receptors – and theca externa (Cox & Takov, 2019). A primary follicle is characterized by a full cuboidal granulosa cell layer surrounded by a basement membrane (Palermo, 2007). As the primary follicles continues proliferation, the simple cuboidal epithelium transforms into stratified columnar epithelium, becoming a secondary follicle, also known as preantral follicles. The FSH and LH receptors allow the follicles to respond to their respective gonadotropins (Cox & Takov, 2019; Koeppen & Stanton, 2009).

Continuous maturation of secondary follicles and the selection of a dominant follicle for ovulation are gonadotropin-dependent events (Cox & Takov, 2019), and secondary or preantral follicles growth can be divided into two phases: a vascular and as avascular phase. Theca cells are recruited from interstitial stroma cells. As soon as the follicle reaches the secondary stage, the formation of a distinct theca cell layer occurs in all follicles (Palermo, 2007). At this stage, FSH binds to the FSH-receptors in the granulosa cell layer of the preantral follicle. As a result of this stimulation, follicles continue to growth and eventually develop fluid-filled spaces inside the granulosa cell layer, and so the follicles are then called early antral. Only when a fluid-fill space have merged into a large crescent-shaped cavity, which is referred to as an antrum, are the follicles called antral. At the same time, LH binds to the LH-receptors of the theca interna, and starts producing androgens, which, as ovarian follicles mature, the larger follicles use the enzyme aromatase to convert

these androgens into estrogen (Koeppen & Stanton, 2009; Palermo, 2007). Granulosa cell will then differentiate into mural and cumulus cells (Palermo, 2007). The increasing estrogen levels will act by negative feedback on the HPA axis, decreasing the circulating levels of FSH. Only the larger follicles remain sensitive to this reduced FSH levels, and the rest of the follicles in fast grow will suffer atresia, until only one – the largest and the one with most FSH receptors – follicle is left. This one, becomes the dominant follicle (Koeppen & Stanton, 2009; Cox & Takov, 2019). This selection occurs in the initial follicular phase. This follicle continues to mature through a rapid proliferation of the granulosa and theca cells. The antrum enlarges rapidly. At the mid of the cycle, the dominant follicle turns into a big preovulatory follicle, which has 20mm diameter and contains about 50 million granulose cells (Koeppen & Stanton, 2009). At this stage, the granulosa cells of the preovulatory follicle achieve high concentrations of LH receptors and, consequently, become very responsive to the LH levels. The midcycle LH surge is caused by further increases in circulating estrogen made by the preovulatory follicle and leads eventually to ovulation (Cox & Takov, 2019). The preovulatory follicle is characterized by high intrafollicular levels of estradiol, due to the size of its granulosa cell population and its capacity of aromatization (Drummond, 2006).

Simultaneously and being overlapped by the ovulation process, a shift in the steroidogenic function of the theca and mural granulose cells occurs. This is called luteinization process, which culminates with the formation of the corpus luteum. The corpus luteum is capable of producing large amounts of progesterone, alongside with estrogen, few days after ovulation. As so, the LH surge induces the start of a set of complex processes during the periovulatory period (Koeppen & Stanton, 2009). At last, the final stage of follicle development is the Graafian follicle, which can reach a diameter from 15 to 25 mm. At the time of ovulation, the mature oocyte is released from the Graafian follicle for fertilization, and the granulosa and theca cells will differentiate into luteinized cells under the influence of the LH surge (Palermo, 2007; Drummond, 2006).

The corpus luteum secrets large amounts of progesterone and estrogen, supporting implantation and early pregnancy (Cox & Takov, 2019). The corpus luteum lives for about 14 days, unless he is recruited by the human chorionic gonadotrophin (hCG), which is a similar hormone to the LH, but originates from the implanted embryo. Progesterone production is highly correlated with a pulsatile liberation of LH in women, and both LH and FSH are reduced to basal levels during the luteal phase, under the action of the negative feedback of progesterone and estrogen. Usually, the life of the luteum corpus is very regular, and a short luteal phase leads generally to infertility. Several factors that disturb the

hypothalamic and pituitary secretion during the follicular phase, including heavy exercise, fasting, high levels of prolactin and abnormal thyroid function, can lead to a luteal phase deficiency and infertility (Koeppen & Stanton, 2009). When implantation fails or does not happen, the corpus luteum degenerates into a connective tissue structure – the corpus albicans. Follicular atresia refers to the death of an ovarian follicle, and it happens through apoptosis of the granulose cells and oocytes. This process can happen at any time of the cycle (Cox & Takov, 2019; Koeppen & Stanton, 2009).

THE MENSTRUAL CYCLE

As previously mentioned, the two main steroids secreted by the ovaries are estradiol, in the follicular phase, and progesterone, in the luteal phase. The role of this two hormones is more pronounced in each phase, which does not mean that their action is exclusive of that phase of the menstrual cycle. They are important for the overall control and regulation of the whole cycle (K.Dafopoulos, 2004; Messinis, Messini, & Dafopoulos, 2014). In several studies, where women were subjected to ovariectomy or other surgery, has been showed that progesterone is also produced during the follicular phase, even though in small amounts. It is probable, therefore, that during this phase of the cycle it is not only estradiol that plays an important role in the control of LH secretion (Messinis, Messini, & Dafopoulos, 2014).

Besides this, non-steroidal substances, such as inhibin, activin, follistatin, and anti-Mullerian hormone (AMH) are also produced by the ovaries (Messinis I. E., 2006). Inhibin is an ovarian substance that suppresses the basal secretion of FSH from the pituitary. It is now established that inhibin B is mainly secreted by the growing antral follicles during the early follicular phase of the cycle, while inhibin A is secreted especially during the luteal phase, by the corpus luteum. This can be confirmed by measurements of these substances in blood along the whole menstrual cycle: Inhibin B levels increase in the early follicular phase, decline continuously until midcycle, where a peak is noted, and the concentration is low in the luteal phase; on the contrary, inhibin A shows low levels at the follicular phase, levels which increase markedly during the luteal phase of the cycle (Messinis, Messini, & Dafopoulos, 2014). Although in vitro data have shown that LH secretion may also be affected, this hormone is much less sensitive to the action of inhibin than FSH (Messinis I. E., 2006). The AMH, produced by the granulosa cells of primordial and small antral follicles, remains stable during the whole menstrual cycle. AMH concentration reflects the ovarian reserves more accurately than any other substance, because lower AMH are more usual in older women, when compared to values found in younger women (Messinis, Messini, & Dafopoulos, 2014). The role of this hormone during the menstrual cycle is less clear. It

appears that serum levels of AMH on the 3rd day of the cycle decline with increasing age, and showed a negative correlation with FSH (Messinis I. E., 2006).

A very dynamic relation exists between the ovary, the pituitary gland and the hypothalamus, in which the menstrual cycle events are organized. The menstrual cycle events initiate under action of the ovary at the final of the luteum phase of a previous infertile cycle. So first, in the absence of fertilization and implantation on the previous menstrual cycle, the corpus luteum degenerates and die, which is called luteolysis. This, at the 24th day of menstrual cycle, promotes a drastic fall in the levels of progesterone, estrogen and inhibin A. at this point, under the negative feedback mechanism effect, the gonadotroph realizes the final of the luteal phase (Koeppen & Stanton, 2009). It is known that estradiol is the main secretory product of the follicle during the follicular phase, and this induces the negative feedback regulation in the hypothalamic-pituitary system (Messinis, Messini, & Dafopoulos, 2014). This leads to an elevation of FSH levels, around two days before the beginning of the menstruation, which will consequently recruit a group of big antral follicles to initiate a fast and gonadotrophin dependent growth. This is followed by a decrease in the progesterone and estrogen levels which, by negative feedback, increases the frequency of pulsatile production of GnRH, increasing this way, selectively, the synthesis and secretion of LH by the gonadotroph. As so, the LH/FSH relation increases slowly during the follicular phase (Koeppen & Stanton, 2009). As a response to this declining levels of FSH, the follicular atresia of all the recruited follicles begins, and only the dominant one remains. The follicles extreme dependence of FSH during the continuously diminishing levels of this hormone, is the fundamental aspect to find the dominant follicle of each cycle: only the largest follicle, with the higher number of FSH receptors and the best sanguine supply will survive. This dominant follicle will then produce high levels of estrogen. Once the values are up to 200pg/ml for around 50 hours, the estrogen acts through positive feedback at the gonadotroph, inducing the midcycle LH outbreak – the LH surge (Koeppen & Stanton, 2009). Estradiol sensitizes the pituitary to GnRH via an increase in the GnRH receptors on the pituitary gonadotrophs (Messinis, Messini, & Dafopoulos, 2014). This positive feedback is even more emphasized by the small amount of progesterone that's segregated at this part of the cycle (Koeppen & Stanton, 2009), in which elevated LH levels not only are responsible for ovulation, but also drive the luteinization of the follicle and the development of the corpus luteum (Oyola & Handa, 2017 September).

This LH outbreak induces meiotic maturation, ovulation and luteinization, increasing consequently the levels of progesterone which, alongside with estrogen and inhibin will negatively feedback the pituitary gonadotrophs, lowering the FSH and LH to basal levels.

At this point, the luteal phase begins, with a significant reduction in estradiol and progesterone concentrations (Messinis, Messini, & Dafopoulos, 2014; Koeppen & Stanton, 2009). The corpus luteum, being absolutely dependent of basal levels of LH, will gradually become insensible to the basal levels of the LH hormone and eventually degenerates. In a non-fertile cycle, the menstruating corpus luteum will die in about 14 days, and the progesterone and estrogen levels will decline in 10 days, leading to the beginning of the cycle once again (Koeppen & Stanton, 2009).

This typical change in hormonal secretion during this transition from luteal to follicular phase is also known as the intercycle rise of FSH. It starts around 2 to 3 days before the beginning of the menstrual period and ends in the midfollicular phase of the next cycle. This change in FSH concentration is the already known “FSH window”, the physiological phenomenon responsible for the selection of the dominant follicle of each cycle. Messinis, Messini & Dafopoulos concluded, based on the available data, that inhibin A seems to be important in the opening process of the FSH window, while inhibin B seems more responsible for the closing of the FSH window (Messinis I. E., 2006).

Having an overview of the whole cycle it is fair to admit that, undoubtedly, the ovary is one of the major regulators of the menstrual cycle. The timing in which the two main events of pituitary origin occur are both determined by two ovarian events: the FSH transitory elevation (which recruits the big antral follicles) and the LH outbreak (which induces ovulation) (Koeppen & Stanton, 2009). It is evident that in the follicular phase of the cycle the two most important mediators of the ovarian negative feedback effect are estradiol and inhibin B, while during the rest of the follicular phase progesterone activity is added to the estrogenic effect. During the luteal phase, it is clear that is the combined action of estradiol, progesterone and inhibin A that mediates the ovarian negative effect on gonadotrophin secretion (Messinis, Messini, & Dafopoulos, 2014).

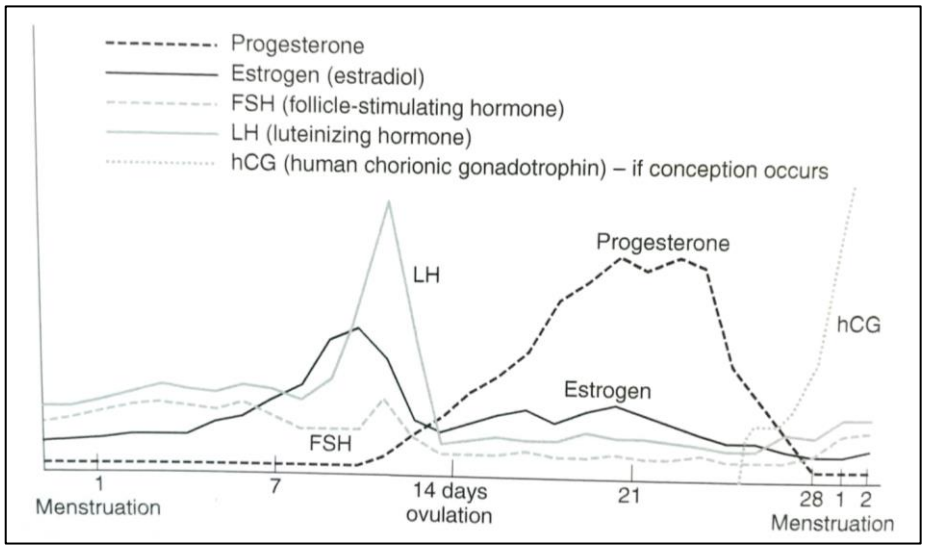

Figure 1 – The hormonal production during the menstrual cycle (Lyttleton, 2013)

THE FALLOPIAN TUBE

After the formation of the cumulus-oocyte complex during the ovulation process, the uterine tube is responsible for the capture of this complex and to transfer it to the medial portion (the ampulla-isthmus junction), where fertilization can happen (Koeppen & Stanton, 2009). The fallopian tubes are muscular structures of 10-12 cm, which distal terminations are close to each ovary, and which proximal terminations go through the uterus wall. They are divided into four segments: the infundibulum, also known as the open termination of the uterine tube, with digitiform projections called fimbriae; the ampulla, the major portion of the uterine tube, which has many folds in the mucosa; the isthmus, which has a narrow and less folded mucosa; and then the intramural or interstitial segment, which goes through the uterine wall. The uterine tube wall is composed of a mucosa, the endosalpinge; a double muscular layer, the miosalpinge; and an external layer of connective tissue, the perisalpinge (Koeppen & Stanton, 2009; Loza, 2012).

The estrogen produced during the follicular phase stimulates the growth of the endosalpinge epithelial cells, which grow in size and weight. This will increase the blood flow, the production of glycoproteins and the ciliogenesis of the uterine tube wall. The estrogen is also responsible for the increase secretion of the thick mucus on the isthmus, maintaining the cumulus-oocyte complex in the ampulla-isthmus junction long enough for the fertilization to happen. During the initial luteal phase and until the mid-luteal phase, the levels of progesterone increase, alongside with estrogen, reducing the size and function of the epithelial cells. Contrary to the estrogen, the progesterone alone diminishes the secretion

of the thick mucus and relaxes the isthmus tonus (Koeppen & Stanton, 2009). As the spermatozoid remains viable by adhering to the epithelial cells which cover the isthmus wall, this structure of the female reproductive system provides a place of storage for the sperm, so that women who ovulate until five days after a sex relation can get pregnant. The uterine tube secretions also induce the hyperactivity and capacitation of the spermatozoid and segregate fluids that provide nutritional support for the embryo before the implantation.

The movement of the embryo into the uterus is critical. The uterus has an implantation timing of 3 days, so the uterine tube needs to retain the recently formed embryo until he reaches the blastocyst stage (five days after fertilization), and then allow the blastocyst in to the uterine cavity (Koeppen & Stanton, 2009).

THE UTERUS AND THE VAGINA

The non-pregnant uterus of a nulliparous woman measures 7-8 cm in length, 4-5 cm width and 3-4 cm in the anteroposterior sense and weighs about 70 grams (Loza, 2012). The uterus locates at the medial line of the pelvic cavity, between the bladder and the rectum, and is divided into the fundus, which is the uppermost portion located at the end of the uterine tubes; the corpus, which forms almost the entire part of the uterus; the isthmus, a small and straight portion at the inferior final part of the corpus which lengthens until the cervical canal; and the cervix, which extends until the interior of the vagina. The vagina is one of the copulatory structures of women and acts like a birth canal. It's covered by a squamous stratified non-keratinized epithelium and there are no glands in the vagina, so the lubrication during the sexual act is provided by the cervical mucus, from the sanguine vases and from the vestibular glands (Koeppen & Stanton, 2009).

The mucosa of the uterus – the endometrium – is smooth, rich in vessels and simple tubular glands; the thick muscular layer, which is divided in three parts, is called the myometrium, and is in charge of the expulsion of the fetus and suffers hypertrophy during the pregnancy; and the external connective tissue and serosa are called perimetrium (Loza, 2012).

The functions of the uterus are mainly related to the fertilization and gestation. The uterus helps the movement of the spermatozoid from the vagina to the uterine tube and also provides an adequate place for the adhesion and implantation of the blastocyst. The uterus is responsible to limit the invasiveness of the embryo in implantation, so that it remains in the endometrium and does not reach the myometrium. It provides mature placenta structure, which includes the basal plaque, to which the fetus adheres, and big spaces full of maternal blood after the first trimester. One of the main functions of the uterus is to grow

and expand alongside with the fetus in growth, in a way that the fetus develops in an aqueous and non-adhesive environment. At the end of the gestation, the uterus produces muscular contractions to expel the fetus and the placenta (Koeppen & Stanton, 2009).

INFERTILITY

Fecundity is a woman's biological capability to reproduce, based on the monthly probability of conception (G.F. Homan, 2007), while fertility is the capacity to establish a clinical pregnancy. The failure to achieve a clinical pregnancy after a year of regular and unprotected sexual intercourse, or due to an impairment of function to reproduce, either alone or with his/her partner, is called infertility. According to the latest definition by the World Health Organization, infertility is a disease that causes disability as an impairment of function (Mélodie Vander Borght, 2018), and has already been recognized as a public health issue worldwide by WHO (J. Boivin, 2017).

Subfertility is a term that can be used mutually with infertility, but is not the exact same problem. Gnoth et al. described subfertility as any form of reduced fertility with prolonged time of non-conception, while trying to conceive (C.Gnoth, 2005). Although this two definitions of infertility (infertility and subfertility) always refer to a limited time period or possibly treatable type of infertility, sterility refers to a permanent state of infertility (Mélodie Vander Borght, 2018). Primary infertility was defined as infertile couples without previous pregnancy, whereas secondary infertility refers to couples that cannot become pregnant after a previous pregnancy achieved without medical help (Z Zhou, 2018).

A study made by Boivin et al. estimated the international prevalence of infertility with values taken from 25 population surveys, assessing 172 413 women. The prevalence rate varied from 3.5% to 16.7% in the more developed countries, and from 6.9% to 9.3% in the less-developed nations. The overall median prevalence was 9% (Jacky Boivin, 2007). According to another article published on the Journal of Clinical Biochemistry, the prevalence of infertility worldwide was estimated to be present in more than 186 million people, the majority belonging to developing countries (Mélodie Vander Borght, 2018). Secondary infertility is the most common form of female infertility around the globe (Mélodie Vander Borght, 2018). In Portugal, according to the last study made in infertility prevalence in 2009, with 864 women in reproductive age (25 to 44 years), the prevalence of infertility was estimated in 7,9% (Silva-Carvalho J, 2009). World regions differ widely in their demographic tendencies, with high fertility rates and population growth at the poorest countries, while population decline and lower rates of fertility are seen in many developed countries (Mélodie Vander Borght, 2018).

Although some work has been done in this regard, determining the prevalence and epidemiology of infertility may be difficult to establish, due to differences in defining the disease, lack of worldwide research and data, and poor seek for medical help. A previous systematic review conducted by Boivin et al. in 2007 reported that only around half the people who experienced infertility problems decided to seek fertility medical care, both in developing countries and in the more developed countries. In numbers, this translates to: out of 72.4 million women who are currently infertile, 40.5 million are currently seeking infertility medical care (Jacky Boivin, 2007). On one study conducted by Zhou et al., about 85% of the couples admitted they did not seek medical help. Different reasons lead the couples to make this decision: most of them still wanted to conceive naturally or though there was no fertility problem with them; only a small portion did not seek medical help for economic problems and busy schedules. Infertility is, undeniably, a heavy burden, with important consequences for both individuals and public health, including other associated problems as psychological stress and anxiety, social stigmatization, economic limitations and later-onset disease (Z Zhou, 2018).

INFERTILITY – A MULTIFACTORIAL CONDITION

The 3 main factors known to affect fertility are the time of unwanted non-conception, the age of the female partner and infertility related with diseases (Mélodie Vander Borght, 2018), but many wide ranging causes are associated with infertility, like ovulatory disorders, tubal disease, chromosomal abnormalities, sperm factors and unexplained infertility (G.F. Homan, 2007).

So the main factor affecting the female fertility is the time of unwanted non-conception, which refers to how long it takes couples to reach their goal – achieve pregnancy. Fortunately, and despite being the main factor affecting fertility, about 80% of women will conceive and become pregnant in the first 6 cycles of regular intercourse in the fertile period. Timing the fertile period in order to have intercourse during that time of the menstrual cycle showed to increase the chance of spontaneous pregnancy. From the rest of the 20% that do not get pregnant at the first 6 months, approximately half will conceive spontaneously in the next 6 cycles. As for the rest of the 10%, only after 12 unsuccessful cycles are the couples considered to be infertile. Regardless of this diagnosis, out of this 10% approximately 55% of them will be able to conceive without help. After 48 months, only 5% of them are definitively infertile and have nearly zero chance of becoming pregnant in a spontaneous way without any fertility medical help (Mélodie Vander Borght, 2018; C.Gnoth, 2005).

The second main cause of infertility is the age of the female partner. This days, in most European countries, most women have the first child at the mean maternal age of 30 years old. Many women even deliver their first child at age 35 or older. The problem with this is that female fertility begins to decline around 25-30 years of age, and delaying a pregnancy can become problematic and can possibly increase the infertility rate (Mélodie Vander Borght, 2018). A population-based study made in China found a clear rise of infertility with increasing age (Z Zhou, 2018). From 25 until 38 years old, the rates of fertility seem to decrease more slowly and gradually, while after 38 the rate of fertility loss increases rapidly. According to a study of Eijkmans et al., loss of fertility associated with age slowly rises from 4.5% at age of 25 years, 7% at 30 years, 12% at 35 and 20% at 38 years old. After that, rates rapidly rise about from 50 to 90% between the ages of 41 and 45 years. At 50 years old, conception is nearly impossible to achieve (Eijkmans J.C. Marinus, 2014).

This theory of fertility decline assumes that the age-dependent loss of fertility is mainly determined by the unceasing degeneration of oocytes stored in both ovaries, leading first to a decreased fertility and then to its expiration at the onset of menopause. It's no doubt and well established that oocyte quality declines with advancing age, increasing ovulatory disorders, reducing ovary frequency and impairing luteal phase, which all combined (or individually) can eventually lead to a decrease in the conception rates (Mélodie Vander Borght, 2018). However, during ART treatments, the response and the success rates were highly variable among women, within groups with similar or different ages. This can be suggestive that age alone could not be a reflex of the reproductive potential of a woman or of hers ovarian reserve. The variation between women in ovarian reserve is, in the first place, determined by the size of the primordial follicular pool at birth and after, by the rate of its deterioration during reproductive life, two factors genetically determined (Kannamannadiar Jayaprakasan, 2010). This genetic predisposition is not an excuse for women delaying their motherhood experiences. In fact, several studies suggest that many women do not recognize the risk of infertility in delaying pregnancy and, most of the times, the ovarian reserve is only a matter of analysis when fertility problems arrive. In the end, being an age-related problem or genetic predisposition, the problem remains. Some women even think that IVF may be able to resolve their infertility associated with advancing age: most of the time this is, unfortunately, a misconception (Mélodie Vander Borght, 2018).

The third more frequent cause of infertility is the disease-related infertility, which may affect both genders or be specific to women or man (Mélodie Vander Borght, 2018). As this revision focus on the female infertility, only some examples of diseases related to women will be approached. The most known diseases that can lead to female infertility are the

premature ovarian insufficiency (which is a rare disease that affect about 1% of women), the polycystic ovarian syndrome, endometriosis, uterine fibroids and endometrial polyps. PCOS is a heterogeneous condition, and is the most prevalent endocrine disorder in women (Mélodie Vander Borght, 2018). As this is the matter of study for this review, this theme will be explained in more detail.

Other lifestyle factors can influence the fertility rates, such as dietary restriction and over-exercise, stress, obesity, cigarette smoking, marijuana consumption and alcohol intake (Mélodie Vander Borght, 2018). The impact of lifestyle on reproductive performance may vary depending on individual etiology and circumstances, but lifestyle factors can have a high impact on general health and ability to conceive. An evidence-based review made by Homan et al. in 2007 about the lifestyle factors that can influence fertility, found strong evidence in female age, smoking and weight. Some inconclusive evidence was found for other factors like psychological stress, caffeine and alcohol intake and environmental pollutants: they may impact on reproductive health and adversely affect fertility but the evidence is limited and not consistent across studies. About diet and exercise some studies have been made but the evidence is not strong enough to make assumptions just yet. Good diet and exercise seem to improve reproductive ability by improvement of the whole body health (G.F. Homan, 2007).

There's strong evidence about cigarette smoking and infertility issues, both in general population and in couples doing fertility treatments. In women, menopause has been reported to occur in 1-4 years earlier for women who smoke, compared to non-smokers. In female, smoking may affect the follicular microenvironment and negatively affect hormone levels in the luteal phase. In addition, during fertility treatments, smoking has been shown to adversely affect the chance of pregnancy, with studies suggesting that smokers may need nearly twice the IVF cycles to conceive compared to non-smokers. In man, smoking has been associated with decreased sperm production, abnormal motility and morphology and is associated with an increased risk of DNA damage.

Weight problems are also strongly associated with fertility issues: both obesity and low body weight can impact on reproductive function by causing hormonal imbalances and ovulation dysfunction. Obesity has been shown to decrease the probability of pregnancy for women undergoing fertility treatments. In addition, weight problems are usually present in PCOS. Despite this, obesity or overweight problems are related to infertility in both PCOS and no polycystic ovary women (G.F. Homan, 2007).

Apart from this factors, cases of unexplained infertility are also reported. Some authors say that unexplained infertility is best characterized as subfertility, because some of this couples will conceive without intervention (Deidre D. Gunn and Wright Bates, 2016). Unexplained infertility is a term usually used to define a diagnosis, or a lack of diagnosis, made in couples in whom no problem associated with infertility could be found. The proportion of couples suffering from unexplained infertility is estimated in 16%, but the rates vary from 0-37%, so the mean number found in unexplained infertility is about 30% of the couples (Deidre D. Gunn and Wright Bates, 2016; Ray A., 2012).

When tubal patency, normal ovulatory function (basal body temperature, cervical mucus changes, serum LH surge or mid-luteal progesterone) and normal semen analysis are established, the couple is considered to be “unexplainably infertile”. For this couples, the diagnosis is highly frustrating, and treatment has generally been indicated if duration is more than 2 years or if the female is > 35 years. The potential causes for unexplained infertility have been described as disturbances in endocrinological balance, immunology, genetic and reproductive physiology, but the current knowledge of the reproductive system assessment is far from complete (Ray A., 2012).

FERTILITY AWARENESS METHODS

Several studies have demonstrated the positive success rates of pregnancy for women using the timed intercourse method, as they increase the probability of conception having intercourse in their fertile period. This was highlighted also by Stanford et al., in 2002. Some different methods can be used to observe the fertile window, but vulvar mucus observations seems to be an effective tool in self-assessment of peak fertility in the menstrual cycles, and is considered to be a good sign to mark the several stages of menstrual cycle. This sign seems to be superior to the relative timing of intercourse to ovulation. In cases of infertility or subfertility with a good prediction, and especially in unexplained infertility where no problem can be found, couples should be encouraged to self-monitor the menstrual cycle to identify their peak of fertility, in order to increase the probability of pregnancy in a natural way. In many cases this seems to be all they need to achieve pregnancy (C.Gnoth, 2005).

For most women, there are only around 30 days in a whole year when conceiving is more probable to occur naturally. As so, being able to correctly identify these relative few days seems, undeniably, a very powerful – and better, independent, as it is in a self-assessment way – tool to increase considerably the success rates of conception (Lyttleton, 2013). Fertility self-assessment methods is a term that includes all family planning methods that are based on the identification of the fertile window. So these methods consist on the

observation of physiological signs and symptoms of the fertile and infertile phases of the menstrual cycle. Several studies have been made to identify the best way to assess the optimum fertile window (Pedro-Antonio Regidor, 2018), and Frank Herrmann et al. described a cohort of couples who used a method that consisted of recording the cervical secretions pattern, changes in basal body temperature and the application of a calculation rule. The window of fertility was defined by Rene Ecochard et al. as the period of the cycle during which sexual intercourse may result in conception. Wilcox et al. estimated that the fertile window, in which probability of conception is higher, begins 5 days before ovulation and ends on the day of ovulation (Rene Ecochard, 2015), which corroborates with the study made by Antonio-Pedro Regidor et al. where he says the exact same thing about the fertile window. Although traditionally the menstrual cycle has been divided into two phases, the preovulatory or follicular phase, and the postovulatory or luteal phase, when recognizing the importance of the fertile window, it may be more appropriate to divide the follicular phase of the menstrual cycle into two sub-phases: the latency phase and the fertile window; the postovulatory or phase would be the third phase of the cycle (Rene Ecochard, 2015). Very recently Pedro-Antonio Regidor et al. (2018) made a study about this new device to assess the fertile window, where the device measured the body core temperature of women each 5 min, and women observed the consistency of the cervical mucus: the rise of the temperature occurs not only after the surge of progesterone and the lowest core temperature is associated with the peak of estrone-3-glucuronide, a urinary metabolite of estradiol. It was concluded that this measure is not always associated with an exact prediction of the ovulation day, so the fertile window is not always easy to address (Pedro-Antonio Regidor, 2018). More or less consistent findings are available showing higher metabolic heat production in the luteal than in the follicular phase, and that this differences in diurnal rhythmicity of thermoregulatory processes between the menstrual cycles are crucial to identify the peak of fertility, but the studies are rarefied and can be misleading (Kurt Krauchi, 2014). Both female and male gametes are subjected to temperatures cooler than those in neighboring tissues in the hours shortly before the onset of fertilization, and the temperature seems to be cooler in the follicular phase (Ronald H.F. Hunter, 2018), but most studies are still made in animals and the authors admit that more research in needed to achieve more reliable conclusions. Despite this, the basal body temperature chart (BBT) is a useful and powerful tool in women trying to conceive. It is called basal because the temperature is measured at a time when the body is at rest, and so the body temperature is at baseline. A woman's basal body temperature begins to rise after she has ovulated and progesterone increases (Lyttleton, 2013).

The studies about cervical mucus are more conclusive and the literature more revised (Emily Evans-Hoeker, 2013; Jamie L. Bigelow, 2004; Vivek Dubey, 2016). Bigelow et al. in 2004 showed a clear evidence of increased chance of pregnancy with increases in the cervical mucus: the start of the fertile interval generally correlates to a significant rise in estrogen levels, which results in the secretion of estrogenic cervical mucus and characteristic changes in vaginal discharge (Jamie L. Bigelow, 2004). It is well documented already that the cervix acts as a valve, assisting perm transportation up the cervix until the uterus when the cervical mucus is more fluid, and inhibiting sperm transport when the cervical mucus is stickier. The main observable sing aiding in the clinical fertile window identification is the presence of the cervical mucus felt or seen by women at the vulva. The peak of ovulation occurs is the last day on which the mucus is clear, slippery and stretchy.

According to the same author, this clinical fertile window ends on the third day of high temperature established using the basal body temperature (Rene Ecochard, 2015). Mistiming this period of high fertility, has been shown to have significant impact on conception rates. This appears to be connected with the recognized role of estrogenic mucus in enhancing progressive sperm motility, and allowing for penetration, storage and transport of normal spermatozoa (Jamie L. Bigelow, 2004). Over the cycle, and alongside with the hormonal and anatomic changes through the 28-day cycle, the cervical mucus changes too. First, the cervical mucus produced at infertile times is associated with a dry feeling at the vulva: it is called the G-type mucus and it is thick, pasty and impenetrable. As estrogen levels begin to rise, the cervix starts producing a more liquid type of mucus, the L-type, which causes a more wet or sticky sensation. As ovulation approaches, the mucus appears more stretchy and slippery: the S-type. This is usually referred to as egg-white-like type of mucus. At the time of ovulation, the mucus loses its stretch as the cervix starts producing P-type mucus, a more rich in potassium mucus which aids in the boost or activation of sperms as they climb through the cervix. The last day of P mucus production is the peak day of fertility, the day before the egg is released or the day it is released (Lyttleton, 2013).

POLYCYSTIC OVARY SYNDROME

PCOS is recognized as the most common endocrine disorder of reproductive-aged women around the world (Neil F. Goodman, 2015). With the evolution of science, there was an alteration in recognition of PCOS, which formerly recognized the syndrome as a gynecological and dermatological condition, to an international position of statements and guidelines now presenting PCOS as a multisystem disorder with associated reproductive, metabolic and psychological comorbidities (Anuja Dokras, 2018).

PCOS – PREVALENCE RATES AND DIAGNOSTIC CRITERIA

The prevalence rates of PCOS vary considerably according to the diagnostic criteria used, ranging from 9% in women in reproductive age worldwide, according to NIH criteria, and up to 18% with the Rotterdam criteria (Spritzer, 2014). This widely variety in the estimated prevalence of PCOS is mainly a consequence of differences in population studied, diagnostic criteria used, among other confounder factors, and is very hard to point one fixed prevalence rate, with values raring from 4-8% of women of reproductive age (Sehar Toosy, 2018), to 6-25% of reproductive-aged women (Setji, 2014), 6-10% of women of childbearing age according to Weiss in 2019 (Weiss NS, 2019), 6-21% according to Kite el al. also in 2019 (Chris Kite, 2019).

Possibly, one of the reasons leading to this difficult assessment of prevalence numbers, is that the diagnostic criteria of PCOS is not easy to make, and it has been suggested by 3 different groups: the Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproduction Medicine (ESHRE/ASRM); the National Institute of Health/National Institute of Child Health and Human Disease (NIH/NICHD); and the Androgen Excess and PCOS Society. All of these groups have made and proposed several diagnostic criteria for PCOS, and there is unanimous agreement that PCOS is a diagnosis of exclusion: meaning that the diagnosis of this syndrome must be made once conditions such as Cushing's syndrome, thyroid disorders, idiopathic hirsutism and hyperprolactinemia have been ruled out (Sehar Toosy, 2018; Spritzer, 2014). So, the NIH Consensus defined, in 1990, PCOS as the presence of clinical and/or biochemical hyperandrogenism and oligomenorrhea/anovulation. Later in 2003, the Rotterdam Consensus introduced the polycystic ovary appearance on ultrasound as a new criteria to be added to the two previous criteria. Then, the Androgen Excess and PCOS Society considered that androgen excess is a central event in the pathogenesis and development of PCOS and established that this criteria should be present and accompanied by one of the others: oligomenorrhea and/or polycystic ovary (Spritzer, 2014). In the end, the

Rotterdam criteria has considered PCOS a diagnosis of exclusion, and the diagnosis requires two of three criteria: hyperandrogenism, polycystic ovaries and/or anovulation/oligo-ovulation; the NIH/ NICHD also considers PCOS a diagnosis of exclusion, and requires hyperandrogenism and menstrual disturbance as diagnostic criteria; the Androgen Excess Society has also considered PCOS a diagnostic of exclusion, establishing that hyperandrogenism and polycystic ovaries and/or ovarian dysfunction must be present to make a PCOS diagnose (Sehar Toosy, 2018). Recently, an Expert Panel from a NIH Evidence-based Methodology supported the use of the wider Rotterdam criteria to diagnose the syndrome (Spritzer, 2014) and all international guidelines now recommend the use of this criteria in the diagnosis of PCOS (Samantha Cassar, 2016; Neil F. Goodman, 2015). Correct diagnosis of PCOS is important. Early and correct diagnosis may be sufficient to diminish the incidence of some comorbidities associated with the disease, like associated metabolic and cardiovascular risks. Furthermore, correct and early diagnosis leads to appropriate intervention, depending upon the woman's age, reproductive status and her own concerns. The management of these women should include reproductive function, as well as care for hirsutism, alopecia and acne (Neil F. Goodman, 2015).

PCOS – PATHOPHYSIOLOGY

The pathophysiology of this condition is still enigmatic, despite years of research: it is complex and is thought to be an interaction between genetics, epigenetics, ovarian dysfunction, endocrine, neuroendocrine and metabolic alterations, amongst other changes (Sehar Toosy, 2018). Even though the exact origin of each comorbidity associated with the syndrome is unclear, several observations show that the interaction of genetic and environmental factors are necessary for PCOS development, and the hypothalamus-pituitary-ovarian (HPO) axis seems to be involved. The increased frequency and amplitude of the release of GnRH and, consequently, the elevation of LH levels seems to be the most important pathophysiological feature of PCOS. One of the hypothesis for hyperandrogenism is the disruption of hypothalamic-pituitary axis and increase in LH secretion. Consequently, LH affects ovarian theca cells and increases the synthesis of androgens (Zahra Shaaban, 2019). Ovarian pathology is a key element of PCOS, but the whole condition has several more aspects and dysfunctions than the ovarian one. The polycystic appearance of the ovaries, which is characteristic in PCOS, is the result of continuous growth of high numbers of primordial follicles and their subsequent growth arrest (Sehar Toosy, 2018). Although the etiology of this aspect is still poorly understood, there is some evidence for a primary abnormality in ovarian folliculogenesis and androgen production which is manifested at puberty, but may have its origins in childhood or even during fetal development (Stephen

Franks, 2006). As an example of this, obesity (which is an important factor in PCOS symptomatology and development), is particularly important in children. PCOS typically appears or develops during adolescence and high incidence of childhood obesity has an important impact on the presentation of PCOS: recently, it has been shown in a large cohort study made in Finland with Finnish women, that the prevalence of symptoms of PCOS in adulthood was more severe in women who were obese as adults, but had also been obese during their early teenage years (Stephen Franks, 2006). So, has it's been said before, modifications of ovarian follicle morphology and function are well documented in women with the syndrome, including increased numbers of growing preantral follicles, failures of follicular growth beyond the mid-antral stage, evidence of granulosa cell degeneration and theca cell hyperplasia. Evidence suggest that androgen excess favors the typical abnormal follicle formation in PCOS and, perhaps, follicle viability (R. Jeffrey Chang, 2013). The premature development of follicles in PCOS ovaries is thought to be caused by the typical elevated levels of androgen. This androgen excess seems to be related to the abnormal steroidogenic function of the ovaries, impacting the level and distribution of adiposity in this patients, predisposing them to insulin resistance and anovulation. So in this syndrome, follicular development is either blocked, as it is seen in anovulatory PCOS, where the follicles remain healthy and steroidogenic but don't develop past mid-antral stage; or tending towards atresia as in ovulatory PCOS. Elevated levels of androgens are thought to activate cell death pathways in preantral granulosa cells (Drummond, 2006). It is known that women with PCOS show normal levels of serum FSH, while LH is either normal or elevated. The inability of follicles to mature to the preovulatory stage in these patients may be due to (at least in part) the lack of an intercycle rise of FSH (Messinis I. E., 2006). Histologically speaking, growing pre-antral follicles in women with and without PCOS seems similar. Early antral formation also seems equal (R. Jeffrey Chang, 2013). But development beyond this stage is impaired, and when compared to females without PCOS, ovaries of patients with PCOS have at least twice the number of growing pre-antral and antral follicles, which do not develop properly (Cox & Takov, 2019), and further development is not observed. As a consequence, the follicle begins to exhibit evidence of arrested growth and degenerative change, with progressive accumulation of follicular fluid and expansion of the antrum, leading to an eventual loss of granulosa cells in the follicle wall and subsequent appearance of a cyst (R. Jeffrey Chang, 2013). This cystic changes can be a cause for anovulatory infertility in some women (Cox & Takov, 2019).

Although this dysregulation, or dysfunction, of the HPO axis followed by elevated serum LH levels, hyperandrogenism, and metabolic alterations contribute to the complex and poorly understood etiology of the syndrome, some of these phenotypes of the human disease

replicate in hormone-induced PCOS models; thus, evidence from animal studies can be a helpful tool to clarify and elucidate about the pathophysiology of PCOS (Satoko Osuka, 2019).

PCOS – COMORBIDITIES, SYMPTOMS AND CLINICAL FEATURES

The consequences, clinical features and comorbidities associated with PCOS are broad-spectrum and encompass various organs and body systems: reproductive, metabolic and psychological (Adam H. Balen, 2016). PCOS is characterized by hyperandrogenism and/or chronic oligo-/anovulation which can manifest with a range of symptoms (hirsutism, acne, oligomenorrhea, and infertility); is associated with increased risk of cardiometabolic disease, including hypertension, dyslipidemia, insulin resistance (IR), and type 2 diabetes mellitus (T2DM) and sleep apnea (sleep apnea being associated with IR and T2DM, as well as obesity) (Setji, 2014); and is also linked with increase psychological disease (including increased risk of stress, depression, low self-esteem, poor body image, eating disorders and reduced health-related quality of life and there are also increasing evidence that women with this syndrome have an increased risk of mood and anxiety disorders (Laura G. Cooney, 2017)) (Chris Kite, 2019; Weiss NS, 2019; Adam H. Balen, 2016). This symptoms and comorbidities will be explained in more detail along the review.

Obesity

Most cases of women with PCOS are obese or overweight, with a prevalence of about 80% of individuals, although a small but significant proportion of patients present with normal body mass index (BMI), being normal BMI considered has ≤ 25 kg/m^2. This cases of patients with PCOS without obesity or overweight are termed as lean PCOS, and this makes diagnostic work up and therapeutic approach more difficult. The symptoms and clinical signs between women with high BMI and low/normal BMI are comparable, but obese individuals with PCOS suffer from more severe hormonal and metabolic derangements compared to the lean PCOS individuals (Sehar Toosy, 2018). A study made in India with Indian women with PCOS, compared the prevalence of some of the most common comorbities in PCOS syndrome in overweight and lean individuals. Hypertension showed no significant differences between overweight and lean patients. Significantly higher differences has been showed in hyperandrogenism (with 74,2% prevalence in overweight women compared to 50,6% in lean women) and in menstrual dysfunction (with 79,2% in overweight patients, compared to 44% in the lean ones). T2DM was higher in overweight women, as so impaired glucose tolerance (Sehar Toosy, 2018). Undeniably, almost 90% of women with PCOS are overweight or obese and even moderate weight losses of around

5% may result in significant clinical improvements in hyperandrogenism and menstrual regularity (Chris Kite, 2019).

So obesity is a prevalent characteristic in PCOS, and the presence of obesity alone, or the combination of obesity with disordered eating habits further exacerbate reproductive, metabolic and psychological disease (Adam H. Balen, 2016) which may, by its turn, aggravate insulin resistance, dyslipidemia, and metabolic-syndrome (Spritzer, 2014). Indeed, around 33-50% of US women with PCOS have metabolic syndrome. In Italy, however, only 8,2% of women with PCOS met criteria for this diagnosis (Setji, 2014). A meta-analysis has shown that women with PCOS have higher levels of triglycerides, LDL-cholesterol and total cholesterol, and lower LDL-cholesterol levels compared with non-PCOS patients, independently of BMI (Spritzer, 2014). This weight gain associated with PCOS women can aggravate the anovulatory nature of the syndrome and increase hirsutism, and weight loss in overweight and obese women with PCOS as shown to improve ovulatory frequency (Setji, 2014), with significant benefits demonstrated with weight losses of 5-10% in overweight women with PCOS (Adam H. Balen, 2016), and higher rates of spontaneous ovulation and pregnancies, as well as diminished rates of miscarriage, are associated with weight loss (Erin K. Barthelmess, 2015).

A meta-analysis made by Zhu et al., showed that even without obesity, women with PCOS still showed higher prevalence of glucose metabolic disturbance including hyperinsulinemia, IR, impaired glucose tolerance, and T2DM when compared with BMI-matched controls. In addition, the risk of high triglycerides, low-HDL, and metabolic syndrome was higher in non-obese women with PCOS, which indicates an increased risk of cardiovascular disease in these patients (Shinqin Zhu, 2019).

Impaired Glucose Tolerance and Insulin Resistance

Indeed, up to 40% of obese young women with PCOS have impaired glucose tolerance and are estimated to be 3 to 7 times more likely to develop non-insulin-dependent diabetes in later life, when compared to young obese women without PCOS (Stephen Franks, 2006). In another study of Setji et al. the estimated rate of obese women with PCOS with impaired glucose tolerance was of 30%, and the rate of obese women with T2DM by the age of 40 was of 10%. In thin women, the estimated rates are much smaller, being estimated that only 10% have impaired glucose tolerance and 1,5% have T2DM (Setji, 2014). In other recent review made by Toosy et al., rates between 44-70% have been reported. Nonetheless, IR has proven to be a difficult aspect to assess, due to limitations of methodology use (Sehar Toosy, 2018).

As so, IR and associated hyperinsulinemia have an important role in the reproductive and endocrine features of PCOS, as they can aggravate other related symptoms with the disease: they contribute to hyperandrogenism and disruption of gonadotropin secretion, as they may directly stimulate the production of androgens via ovarian tissue steroidogenesis, independently of changes in gonadotropin concentrations (Samantha Cassar, 2016). Insulin resistance can be defined as a diminished availability of insulin to mediate metabolism in skeletal muscles, adipocytes and liver: which includes glucose uptake, glycogen synthesis and lipolysis inhibition, leading to compensatory hyperinsulinemia to achieve glucose homeostasis. The main function of insulin is to regulate glucose homeostasis by stimulating glucose uptake in target tissues including skeletal muscle and adipocytes and by suppressing hepatic glucose production (Samantha Cassar, 2016). IR, even though not being part of the criteria to diagnose PCOS, it's definitely central in the pathogenesis of the syndrome in most women. In fact, PCOS was listed by the International Diabetes Federation, as a non-modifiable risk factor for T2DM (Adam H. Balen, 2016). Reduction of IR has been suggested as the principal goal of PCOS treatment (Kleopatra Papavasilou, 2017).

Polycystic Ovaries

So, some of the main characteristic features of the syndrome related to the reproductive system are the polycystic ovaries, menstrual disturbance, and hyperandrogenism (around 70% of women with hyperandrogenism have hirsutism, and acne is also a marker for hyperandrogenism) (Sehar Toosy, 2018). The Rotterdam consensus proposed the definition of polycystic ovaries as the presence of more than 12 follicles of 2-9 mm diameter, as a mean on both ovaries, and/or increased ovarian volume (> 10 cm^3). This recommendation was based mainly on a paper, which studied 214 women with PCOS and 112 with normal ovaries to determine the significance of follicle number per ovary (Adam H. Balen, 2016), but there are some other authors corroborating with this definition (Erin K. Barthelmess, 2015; R. Jeffrey Chang, 2013). Not all women with polycystic ovaries have PCOS, as this typical polycystic morphology is present in about 20% of female without the syndrome, many of whom don't even have hirsutism, are regularly menstruated and have normal serum concentrations of testosterone and gonadotropins (Stephen Franks, 2006). So has it's been said before, the abnormal steroidogenesis by the ovary is responsible for the androgen excess (hyperandrogenemia), which is thought to impact on the level and distribution of adiposity in PCOS patients and predispose them to insulin resistance and anovulation (Drummond, 2006). Hyperandrogenemia is clinically characterized by the presence of elevated free testosterone, reduced sex hormone binding globulin and elevated

free testosterone index, dehydroepiandrostene sulphate (which is an endogenous androstane steroid that is produced by the adrenal cortex) and usually manifests clinically by hirsutism (Sehar Toosy, 2018). This important marker of PCOS associated with menstrual irregularity, hirsutism and acne, has been suggested to increase risk for metabolic disorders in women (S. West, 2014). Despite being challenging to diagnose, hyperandrogenism has both clinical and biochemical manifestations and is detected in around 60-80% of cases with PCOS (Adam H. Balen, 2016). Long term hormonal contraception can prevent hyperandrogenism, and symptoms may only develop when oral contraceptives are stopped (Setji, 2014).

Ovulatory Dysfunctions

Another characteristic related to the reproductive system is the menstrual cycle irregularity, oligo-/anovulation and infertility. Irregular cycles (defined by cycles of more than 35 days or less than 21 days) with a duration of 2 years, beginning in the menarche, are likely to reflect oligo-ovulation or anovulation (Adam H. Balen, 2016). Whilst cycle length > 35 days suggests chronic anovulation, cycles slightly longer than normal or slightly irregular needs assessment for ovulatory dysfunction (Neil F. Goodman, 2015). Ovulation is evaluated by measuring serum progesterone during the mid-luteal phase (Adam H. Balen, 2016), and measure between days 21 and 22 of the cycle is the best way of assessing ovulation (Neil F. Goodman, 2015). The peak value for progesterone only remains for a short time (Adam H. Balen, 2016). Whereas progesterone levels > 2.5 ng/ml may indicate ovulation, values of at least 7 ng/ml or more are generally needed for regular luteal function (Neil F. Goodman, 2015). Ovulatory dysfunction is associated with increased prevalence of endometrial hyperplasia and endometrial cancer, in addition to infertility (Neil F. Goodman, 2015), and there has been some studies suggesting an increased rate of endometrial and breast cancer among women with PCOS. The anovulatory features of PCOS have been shown to increase proliferation of the tissue in the endometrium, leading to higher risk of carcinoma. Endometrial carcinoma has, by its turn, additional risk factors including obesity, IR, and T2DM, which can all be associated with PCOS too (Erin K. Barthelmess, 2015). Overall, infertility related to PCOS seems to show a strong relationship with subsequent endometrial cancer risk, but whether the primary cause of endometrial cancer in these women stems from PCOS, obesity, their infertility diagnosis, or a combination of all, remains unclear (Hanson, et al., 2017).

Indeed, this syndrome is the most known common cause of anovulatory infertility, and around 90-95% of women with anovulatory infertility attending to infertility clinics are diagnosed with PCOS (Sehar Toosy, 2018). The prevalence is variable, but even smaller

numbers reported by Balen et al. are considered to be high numbers. In their study, they talk about an 80% prevalence of PCOS in women with anovulatory infertility (Adam H. Balen, 2016). It is known that in PCOS, various factors influence ovarian function and so, consequently, fertility is adversely affected by obesity, hyperandrogenism and elevated serum concentrations of LH (Adam H. Balen, 2016). The low pregnancy rates are suggested to be related to the elevated secretion of LH or premature LH surge. In women with PCOS, approximately 20-26% of the controlled ovarian stimulation cycles have been reported to suffer from premature LH surge. It has been demonstrated that elevated LH levels in the follicular phase affect oocyte quality by the early resumption of meiosis and premature oocyte maturation and ovulation, causing either lower implantation or increased miscarriage rates (Runa Ozelci, 2019). Subfertility occurs approximatively in 1 in 10 couples world-wide, and in about 1/3 of couples, this number is associated with PCOS (Weiss NS, 2019). Infertility caused by chronic anovulation is the most common reason for women with PCOS to seek counselling or treatment. The first line pharmaceutical treatment for these women is ovulation induction with clomiphene citrate, with or without metformin. A recent review showed that letrozole is an effective alternative to clomiphene citrate too (Weiss NS, 2019). Although the frequency of ovulation has not been well studied, in a placebo arm of a relatively large randomized clinical trial of women with PCOS, spontaneous ovulation occurred in about 32% of the cycles, and other study reported this value too (Setji, 2014; Erin K. Barthelmess, 2015).

So as it's been said before, in a normal fertile women, a single follicle matures and goes on until ovulation out of a pool of primordial follicles present in the ovaries since birth. The rate at which primordial follicles are selected for growth is strictly controlled, in order to maintain ovarian reserve and guarantee fertility is intact. In PCOS, an imbalance between androgens, anti-Mullerian hormone (which, as it's been said before, is produced by the ovarian granulosa cells and is important in preventing primordial follicles from transitioning into primary follicles) and FSH, cause a break on follicular growth (Sehar Toosy, 2018). AMH production (synthesized predominantly by growing pre-antral and small antral follicles ranging from 2-9mm dimeter (R. Jeffrey Chang, 2013)) begins during the menstrual cycle with the recruitment of primordial follicles for development into small antral follicles. So naturally, once the dominant follicle is selected, production decreases. Serum AMH levels seems to be useful in the diagnosis of PCOS as levels are 2-4 times greater in women with the condition compared with controls (Sehar Toosy, 2018; Spritzer, 2014; Adam H. Balen, 2016). As high levels of LH are required for androgen synthesis by ovarian theca cells, high levels of LH combined with low FSH and decreased estradiol synthesis through the

conversion of androgens results in anovulation due to the absence of a dominant follicle (Sehar Toosy, 2018).

Still among the reproductive system derangements and pregnancy problems related to PCOs, it is known that women with PCOS are thought to have higher rates of first trimester pregnancy loss, reaching 30-50% rates, a 3-fold increased risk in comparison to women without the syndrome (Joham A.E., 2014).

Psychological Health

As it's been said before, among the PCOS associated comorbidities is the psychologic health. Women with PCOS seem to have higher risks of developing moderate and severe depressive and anxiety symptoms, independent of obesity (Anuja Dokras, 2018), and, according to a recent meta-analysis made by Cooney et al., this symptoms are weakly associated with age, BMI, elevated testosterone, hirsutism and IR (Laura G. Cooney, 2017). In addition, some recently published studies indicate an augmented prevalence of eating disorders and disordered eating in women with PCOS, but the degree of risk is still doubtful and there is no sufficient data about this subject (Anuja Dokras, 2018). Previous studies already reported that women with PCOS have an increased prevalence of mild depression and anxiety symptoms, or an increase in mean depression and anxiety scores, however, the underlying factors that may predispose women with PCOS to an increased risk of this symptoms are not understood (Laura G. Cooney, 2017). Several meta-analysis demonstrated not only this higher risk of anxiety and depressive symptoms, but also reduced health-related quality of life in women with PCOS (Anuja Dokras, 2018). This meta-analysis made by Cooney et al. included 18 studies from different regions of the world and showed that women with PCOS are over 3 times more likely to develop depression symptoms and have 5 times the risk of developing anxiety symptoms when compared to controls. This meta-analysis have caught the attention for the importance of the appropriate screening and follow-up of this population. This increased prevalence of anxiety and depression symptoms seems to be not exclusively connected to infertility issues, as this factor was examined as a potential confounder, however obesity was associated with increased risk of depression (Laura G. Cooney, 2017). Health-related quality of life is a significant health outcome measure in chronic disease, and relates to the physical, social and emotional effects of a disease and/or of its associated treatments. In PCOS, health-related quality of life is considerably reduced (Anuja Dokras, 2018; Erin K. Barthelmess, 2015).

In the face of this facts, the Australian and Endocrine Society practice guidelines recommend routine assessment of mental health in women with PCOS, evaluating symptoms of depression and/or anxiety. Despite these recommendations, the vast majority of women assessed in North America, Europe, and Australia reported not feeling satisfied with the attention given to this symptoms, and were not properly followed up regarding their mental health – data estimate that less than 10% of women were satisfied with the information provided regarding long-term complications, less than 5% were satisfied with emotional support and counseling they received, and more than 50% assumed that they did not receive any information on this matter at all (Anuja Dokras, 2018).

PCOS – TREATMENT OPTIONS

The management options to improve health in women with PCOS and to ameliorate the symptoms associated with the syndrome are variable and have different goals. The main interventions to minimize cardiovascular and metabolic risks in PCOS are lifestyle changes, pharmacological therapy and bariatric surgery, among others (Spritzer, 2014).

Indeed, diet and lifestyle changes is one of the first line therapies in this syndrome, combining several behavioral changes: stress, diet and exercise management.

As so, weight loss is considered to be the first-line treatment in overweight women with PCOS: diet modifications and regular physical activity have demonstrated to considerably improve insulin resistance and ameliorate hyperandrogenism, amongst other beneficial effects on PCOS symptoms (Sehar Toosy, 2018). As, at the moment, no curative treatment for PCOS is established, management of overweight/obese women with PCOS focuses mainly on weight loss through regular exercise and diet prescription, aiming to improve the clinical manifestations and lower the risks of associated comorbidities to the syndrome (Chris Kite, 2019). A systematic review made by Kite et al. showed recent and valid evidence supporting the combination of exercise interventions in the management of PCOS, in order to improve overall health of this patients (Chris Kite, 2019). Lifestyle changes (diet plus physical activity) in combination with weight loss in the order of 5 to 10%, are recommended as first-line approach for improvement of insulin sensitivity, ovulatory function and diminishing circulating values of free testosterone in women with PCOS (Kleopatra Papavasilou, 2017).

In patients with anovulatory infertility which desire to achieve pregnancy, the principals of therapy are first to optimize overall health, before initiating treatment. In those who are obese, therapists should recommend weight loss, as it should improve not only the endocrine profile, but the probability of ovulation, both naturally and in response to ovulation

induction therapy, and also prospects of having a more healthy pregnancy when it is achieved (Adam H. Balen, 2016). The main goal is then to induce regular unifollicular ovulation, whilst minimizing the risks of ovarian hyperstimulation syndrome and multiple pregnancies (Adam H. Balen, 2016). Only after this intervention, should pharmaceutical intervention begin – here, the first-line drug therapy to induce ovulation is the oral anti-estrogen clomiphene citrate (CC) or the aromatase inhibitor letrozole (AI), with second-line therapy being parental gonadotropin therapy or laparoscopic ovarian diathermy. In some cases also metformin can be used, an insulin sensitizer, alone or in combination with one of the options above. IVF may be required for women with anovulatory PCOS who do not respond to the ovulation induction therapies mentioned (Adam H. Balen, 2016). The options of treatments and ovulation induction will be assessed one by one in this review.

So, as it's been said before, restoring ovulation is naturally one of the most important goals in the polycystic ovary syndrome, and anovulation is more frequent – and probable – in obese rather than in thin women with PCOS. Some authors believe that overweight women have higher chance of being less responsive to pharmacological procedures to induce ovulation. There is still no adequate data to justify this (Sehar Toosy, 2018), but this may suggest that we should first adequate the state of whole body health, before the start of pharmacological therapy.

Clomiphene Citrate, is an anti-estrogen therapy that has been traditionally used as first-line drug therapy for anovulation in PCOS. The anti-estrogen action of this pharmaceutical blocks estradiol receptors in the hypothalamus, inducing a change in the pulsatile action of GnRH and consequent release of FSH from the anterior pituitary, which will, eventually, change follicular development. A meta-analysis made by Brown et al. in 2009 has confirmed that CC as a first-line therapy is effective in increasing pregnancy rates when compared with placebo (Adam H. Balen, 2016), and a summary of outcomes from CC therapy has shown a global ovulation rate of about 73%, a pregnancy rate of 36% and live birth rate of 29%, over 6 months of treatment. Despite the widespread use of this pharmaceutical, the use of CC is associated with negative side effects. CC is associated with 11% risk of multiple pregnancy and so careful monitoring with ultrasound is recommended. Additionally, side effects like visual disturbances (which in case of happening treatment must be stopped immediately), hot flushes, breast tenderness, dizziness and nausea can happen (Adam H. Balen, 2016).

Patients who do not respond to CC are more likely to be obese or overweight, insulin resistant and androgenic, when compared with those who do respond to the therapy. The non-responsiveness to treatment can be "defined" in two ways – "clomiphene-resistance"

indicates the failure to ovulate; while “clomiphene-failure” refers to the failure of become pregnant despite the achievement of ovulation. In fact, approximately 75% of pregnancies that occur with CC happen in the first 3 cycles of treatment, and a few occur in the following 6 months. Although ovulation is restored in about 80% of patients, pregnancy is only achieve by 35-40% of women. In this context, CC should be 1st line pharmacological therapy to improve fertility outcomes in women with this syndrome, considering that in the face of CC resistance or failure, other options should be explored, for example aromatase inhibitors (AI), gonadotropin therapy or laparoscopic ovarian surgery are some of them (Adam H. Balen, 2016).

Regarding aromatase inhibitors, the active action is to prevent estrogen biosynthesis from androgens and through hypothalamic/pituitary feedback, increasing FSH secretion. These agents supposedly avoid the adverse effects of CC, but other side effects are associated, such as gastrointestinal disturbances, asthenia, hot flushes, headache and back pain. Also, by preserving ovarian/pituitary feedback, there is reduced risk of multiple follicle development when compared to CC, because they don’t affect estrogen receptors centrally or within the endometrium (Adam H. Balen, 2016). In a large, multicenter double-blind randomized control trial, made by Legro et al. in 2014, 750 women with anovulatory PCOS and a mean BMI of 35 kg/m^2, were randomly assigned to receive CC or letrozole. As a result, letrozole improved pregnancy outcomes when compared to CC (Adam H. Balen, 2016).

Frequently, however, it will be necessary to add an insulin-sensitizing drug: metformin and thiazolidinedione are the main used (Spritzer, 2014). Metformin is a synthetically derived biguanide and is the preferred and most cost-effective oral therapy chosen to treat T2DM (Adam H. Balen, 2016). It is an insulin sensitizing agent which works reducing serum glucose levels by improving glucose uptake and its use in the periphery, and reducing hepatic glucose output (Sehar Toosy, 2018). It acts first in the liver where it reduces hepatic glucose production, stimulates insulin-mediated glucose uptake by the liver and skeletal muscle and reduces subtract availability for gluconeogenesis by lowering serum lipid levels (Adam H. Balen, 2016), but with no significant direct effect on pancreatic insulin production (Baillargeon JP, 2003). As it has been said before, early studies of the use of metformin in the management of PCOS suggested an improvement in reproductive function and the possibility of benefits in long-term health (Adam H. Balen, 2016), but it was shown to be more successful in restoring menstruation and ovulation in lean women compared with overweight patients with the syndrome (Sehar Toosy, 2018). In fact, a well conducted and high quality large prospective randomized double-blind, placebo controlled, multicenter

study made by Tang et al. in 2006, aimed to assess the combined effects of lifestyle modification and metformin in obese anovulatory women with PCOS. All subjects were evaluated by a clinic dietitian, who prescribed dietary intervention, aiming for an average reduction of 500kcal/day. The results were coherent with what has been said before in this review: those who lost weight, experienced an increase in menstrual cyclicity. As there were no statistically significant differences between the 2 arms of the study (those with lifestyle modifications and those with lifestyle modification plus metformin), this came to reinforce the notion of weight reduction holding the key to improve reproductive function (Adam H. Balen, 2016). On the other hand, there is fair evidence form one large, well designed Randomized Controlled Trial (RCT) that metformin alone is less effective than CC alone for the achievement of ovulation induction, clinical pregnancy and live birth in women with PCOS. Despite this findings, CC combined with metformin seems to improve ovulation and pregnancy rates compared with CC alone in CC-resistant patients (Practice Committe of the American Society for Reproductive Medicine, 2017).

When this two previously explained approaches fail, the 2nd-line pharmacologic therapy in women with PCOS is gonadotropin therapy. The downside of this treatment, is that it can be very challenging to stimulate the development of a single dominant follicle, so the risk of multiple pregnancy is not low, and it is difficult to establish the starting dose of gonadotropin. Being the risks higher, this option must be chosen only in CC resistance and/or failure, AI non-responsive, anovulatory and infertile, with no other associated infertility factors (Adam H. Balen, 2016). An ovulation induction strategy using CC as 1st-line drug treatment and gonadotropin therapy as 2nd-line drug treatment presents live birth rates of around 75-80%. If pregnancy does not occur after 6 ovulatory cycles in a women younger than 25 or after 12 ovulatory cycles in women older than 25, then anovulation is unlikely the cause for the couples infertility, and other factors associated with infertility should be investigated (Adam H. Balen, 2016).

As last options, laparoscopic ovarian surgery and IVF treatments may be explored (Adam H. Balen, 2016; Erin K. Barthelmess, 2015). Assisted reproductive technologies is one of the treatment options for women with PCOS that show impaired fertility. Lean women with PCOS have significantly higher rates of fertilization when compared to the overweight patients. BMI appears to be a fundamental factor in the outcomes of IVF treatments. Significantly higher fertilization rates are seen among lean women compared to overweight women with the syndrome. Surgical techniques such as unilateral or bilateral laparoscopic ovarian drilling was associated with comparable rates in successful pregnancies in a recently made meta-analysis (Sehar Toosy, 2018).

In women not trying to get pregnant, the oral contraception pill (OCP) is one of the key medical therapies used in PCOS to regulate menstrual cycles and can be used has the 1st-line treatment (Erin K. Barthelmess, 2015), provide uterine endometrial protection and treat symptoms of hyperandrogenism including hirsutism. Possible adverse effects of OCP are mood disturbance, possible weight gain and venous thromboembolism risk, and there are some reports that this drugs can worsen insulin resistance, a key factor in women with PCOS (Joham A.E., 2014), but it is known to reduce the risk of endometrial cancer which, in this patients, is an important aspect (Erin K. Barthelmess, 2015).

Given the success rates of the previously discussed treatments and the negative side effects that are associated with them, other options should be taken into account when it comes to improve menstrual irregularity, anovulation and the other symptoms associated with the syndrome, in women trying to conceive, and in women not trying to achieve pregnancy. As so, other treatment options should be considered, both from patients and medical care professionals. As it was said before, Traditional Chinese Medicine – and probably acupuncture specifically – may represent a good treatment alternative, as a main or as an adjuvant therapy in women with PCOS and anovulatory infertility. It is the main goal of this master thesis to assess in what way acupuncture might be helpful for these patients, and assess the type of investigation that has being done in this matter. Furthermore, in order to understand the possible implication of acupuncture in PCOS treatment, a brief explanation of the basic foundation of TCM must be made.

TRADITIONAL CHINESE MEDICINE

Men has always tried to fight pain and disease, reaching his full state of health and wellbeing, through different methods and means. One of this methods is the use of Traditional Chinese Medicine (TCM), born in China for over 5000 thousand years ago (Greten H. , 2007).

The first reliable documents informing us on acupuncture and moxibustion use in China go back to the First Han-Period, i.e. the second century before our era. The oldest findings known, where references to the most primary forms of care are shown, are the *I Ging* (The book of changes) and the *Huangdi Neijing* (Principles of Interne Medicine of the Yellow Emperor). The *I Ging* is considered to be oldest book of mankind. This book is believed to date back at least 3000 years, and it describes the course of life, its changes and modalities and it would offer advice and guidance to the emotional and personal life-style of men (Greten H. , 2007). The first mention of acupuncture and moxibustion in a medical text is dated at the beginning of the 2nd century Before Christ (B.C.), in a book called *Huangdi Neijing* (Principles of Interne Medicine of the Yellow Emperor), and it dates back to 2300 years. Already at that time the course of the major conduits was described and the location of the sensitive points was already indicated. This book also mentions the different needles that were used, some of the application techniques and also the use of some acupuncture points. It is fair to say that the Yellow Emperor's Classic is the beginning of the Chinese Medicine (Hempen, 2006).

In the following timetable, there is a time-line from year 2000 to 500 years BC. The right side is relative to Chinese Medicine, while the left side refers to significant events in Wester history.

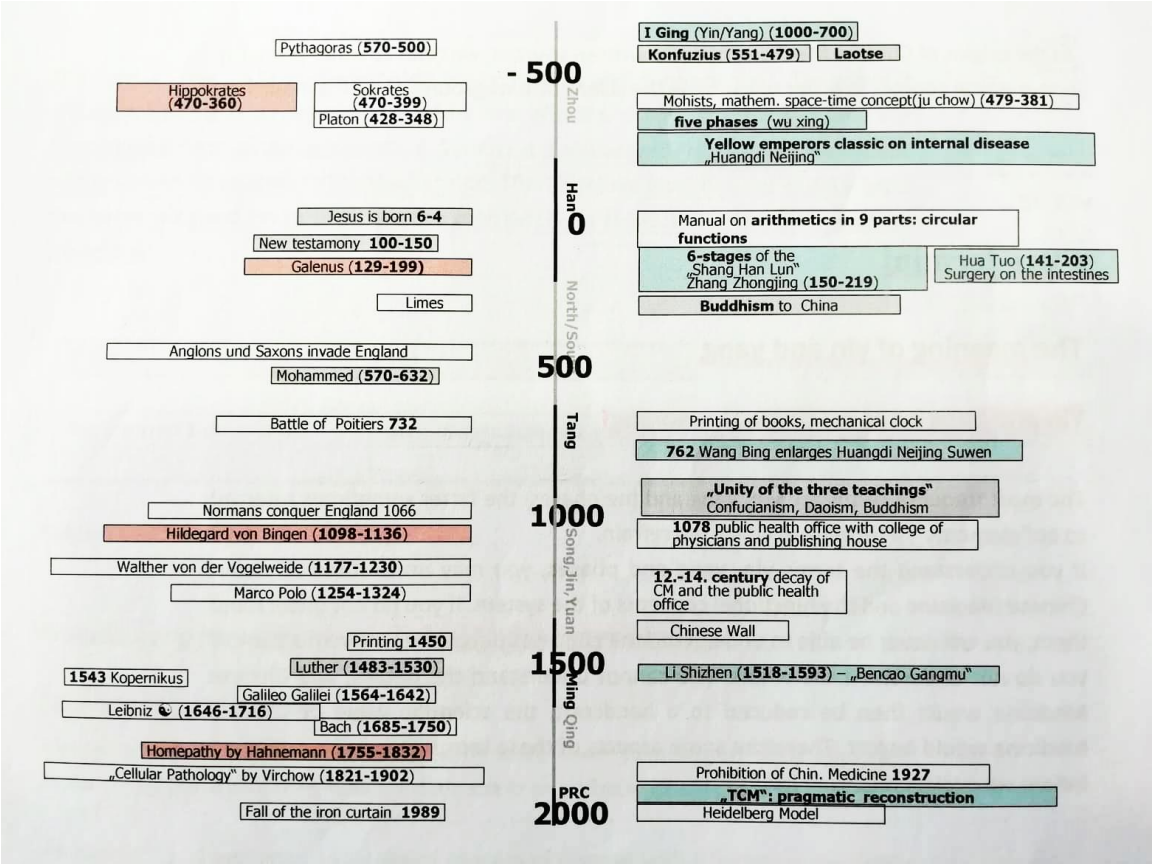

Figure 2 – Time-line: Chinese Medicine development in correlation with Western major events (Greten H. , 2007).

Historically speaking, Chinese medicine was practiced with very few understanding of the biochemical or internal physiological processes of the body. All treatments and diagnosis were made using careful and detailed observation of external signs. This observations are, until today, one of the very great strengths of TCM, but, despite this, with the continuous evolution of science and knowledge that we have available today, given by recent scientific research in reproductive medicine and acupuncture, the process of diagnosis and treatment is not the same anymore. Currently, TCM doctors will be more skilled, and will still apply the well-tested theoretical framework and treatments but with another level of sophistication and knowledge to their clinical approach (Lyttleton, 2013).

With the continuous development of TCM, the search for this type of therapy increased over the years. The World Health Organization (WHO) met the needs of promoting safety, quality and effectiveness of traditional medicines, and published the “WHO Traditional Medicine strategy: 2002-2005”, but since much has changed until recently, the same institution found the need to update the objectives and the methodology and so published the “WHO – Traditional Medicine Strategy: 2014-2023”. Traditional Medicines have a long history of use

in health maintenance and in disease prevention and treatment, particularly in chronic patients. Given the increased search for this therapies, many countries have recognized the need to develop a cohesive and integrative approach to health care, with the goal of allowing governments, health care practitioners and, most importantly, patients who use health care services, to access this therapies in a safe, respectful, cost-efficient and effective manner (World Health Organization, 2013). For WHO, the importance of safe and effective practice in Traditional Medicines depends on an adequate education and training of the therapists, but not least importantly, is also dependent on some sort of regulation of practices. Currently, there is a form of modern understanding of TCM considered crucial for their integration into the Western health care system and in research. The Heidelberg model fits in this context.

In agreement, Hempen reported that, in order to TCM be considered scientific, it must respect and fulfil some scientific criteria. Therefore, it should be based on extensive clinical experience, use a specific language and terminology in order to express their views, and it must be integrated into a more rational and comprehensive systematization of concepts. The aim is to simplify the language of TCM in order to improve the communication between Western health care professionals and TCM professionals (Hempen, 2006). Also Lyttleton (2013) reported that the improved knowledge of internal reproductive physiology and biochemistry will help the communication between the TCM therapists with their patients and their gynecologists, in more understandable language. This communication bridge is, ultimately, beneficial to everyone. This applies to all the specialties of medicine and care (Lyttleton, 2013).

The entire language of this master's thesis will be adapted to the Heidelberg model of TCM, in order to maintain the guiding thread throughout the theoretical description of the work and to improve and facilitate the understanding of terms and theories.

In this context, Traditional Chinese Medicine is defined, according to the Heidelberg model, as a system of findings and sensations designed to establish the functional vegetative state of the body. This state can be treated by Chinese pharmacotherapy through the administration of plants, minerals and other products; by activation and reflex action at central and vegetative nervous system through acupuncture and Chinese manual therapy (Tuina); by the practice of Qi gong exercises, which is a group of movements that activate the vegetative system of biofeedback; or dietetics, through the food itself (Greten H. , 2007). It consists on a rational science with medical diagnosis, based on symptoms and feelings that constitute the reality of the currant evidence of disease and pathological changes in the patient's body. So, this signs and symptoms described by the patient are a result of

dysfunctions in the body, particularly in the neurovegetative system, and the collection of this data indicates possible diseases, disharmonies or dysfunctions of the functional systems (Greten H. , 2007).

In order to understand the basis, mechanisms of action and pathological processes in TCM, some basic concepts must be explained and understood.

To begin with, understanding the terms of Yin and Yang is fundamental in order to comprehend the basis of Traditional Chinese Medicine, and to understand, further on, gynecology in TCM. Yin means structure, and the yang comes from the yin, because we need structure in order to have function. Yin is considered to be the structural basis for the development of yang, as yin produces the yang. So yang refers to function, to something more vivid, more replete, and warmer. It refers to the outside, to the exterior of the body. Yang refers to the induction, the movement, the dynamisation and to something in expansion. Yang is associated with more Qi. In contrast, yin refers to something less replete (or more depleted), to the inside, something more constricted and colder. Something at rest, static and more stable. Yin is considered to have less Qi. Yin and Yang are always comparative terms and measures, used relatively to something, which means that they are not fixed terms, they are mutable. So yin is not cold and yang is not warm. Yin is colder than yang, and yang is warmer than yin. For example, women are considered to be more yin than men, but both men and women have yin and yang functions in them. A patient which can be considered to be more yang would be a more tense and dynamic person, while a more yin patient would appear to be less tense, less dynamic, less energetic. Apart of this, there are more 3 types of Yin separately from Yin itself, or at least we may say that there are 3 things considered to be a part of Yin: Xue, body fluids and Jing (Greten H. , 2007). These terms will all be explained, since all of them are important in MTC and in gynecology.

Body fluids is a term that describes the general fluids balance of the body, and this includes all the homeostatic environment of the individual cell population. So this means that the functions of sweating and also the function of humidity of different tissues are included. This is, therefore, comparable to tissue hydration with its minerals, ions and various factors. Lack of body fluids can be seen, in this form, in dry skin, dry mouth and mucous membranes, bad lubrication in loins among other symptoms (Greten H. , 2007).

Jing refers, in the western medicine, to the nuclear functions and cell nucleus. It is as our DNA, through the western point of view. Congenital abnormalities, sperm problems or deficiencies, and reduced response to functional stimuli of the respective tissue are some examples of Jing damage in TCM. It is believed that jing finds its highest concentration

within the sperm and the vaginal fluid, and also that the jing of the mother and the jing of the father unite in order to create a baby. This is why it is fair to say that, in terms of gynecology, infertility and embryology, understanding the jing function is fundamental (Greten H. , 2007).

Xue is a term that can be compared with blood, in western medicine, since the clinical effects of Xue can be compared to the western concept of the effects of microcirculation, including the functions of microcirculation, blood cells, plasma factors, endothelium and parenchyma (Greten H. , 2007). As Porkert defines, it is a moved structivity (Porkert, 1995). This definition actually is relatable with Xue being a part of yin, since Yin means structure. According to the Heidelberg Model definition, Xue is a form of functional capacity bound to body fluids with functions such as warming, moisturizing, creating Qi and nutrifying a tissue. So one of the functions of Xue is to lead Qi and move it through the body (Greten H. , 2007).

As for Qi, it is frequently translated as “energy” or “vital force”. According to Porkert, Qi is immaterial energy with a qualification and direction (Porkert, 1995). According to the Heidelberg Model, Qi is defined as vegetative capacity of an organ or a tissue to function, which can possibly produce sensations of pressure, tearing or flow. Qi runs the body through the body conduits (Greten H. , 2007).

The body conduits are considered to be the connection of a group of points, with effects on a particular set of clinical signs from a specific organ, and which are believed to be responsible for the free movement of Qi through the body. In order to understand the way in which conduits and acupuncture points are connected with body organs, some terms will be explained. The term “phase”, in the Heidelberg model, refers to a vegetative tendency, i.e. the more likely occurrence of signs and symptoms, like a pattern of signs. The clinical manifestation of this signs and symptoms is called an “orb”, and are named after a body region. “Orb” refers to a group of relevant signs which indicate the functional state of a body region. So the conduits, being a connection of points with effects on a particular set of clinical signs manifested by specific organs, are also related to the “phases” and “orbs” (Greten H. , 2007).

There are 12 main conduits traversing the body, each one related to an organ system: the Hepatic (Liver) and Felleal (Gallbladder) conduits, belonging to the phase Wood; the Cardial (Heart), Tenuintestinal (Small Intestine), Pericardial (Pericardium) and Tricaloric (Triple Heater) conduits, belonging to the phase Fire; the Pulmonal (Lung) and Crassintestinal (Large Intestine) conduits, belonging to the phase Metal; the Renal (Kidney) and Vesical

(Bladder) conduits, belonging to the phase Water; and finally the Stomach (Stomach) and Lienal (Spleen) conduits, belonging to the phase Earth (Greten H. , 2007).

According to the Heidelberg model of TCM, there are four major mechanisms of pathogenesis that can cause disease/dysregulation. One of them is the transition problem from one “phase” to the other, which can cause blockages; second, the excess of an agent (a pathogenic factor that illicit a specific group of signs and symptoms); the imbalance between antagonists (refers to a coordinated system failure of the “phases”); and finally, yin deficiency. Yin deficiency refers to a structural deficiency condition, which causes an instable regulation in the patient (Greten H. , 2007).

This model of Heidelberg provides an overview of some words and concepts by translating them as states of vegetative functions, which proved to be essential to the integration of the TCM in the western health system. Greten was able to make a western physiological description of the neurovegetative function and patterns previously addressed. In the following picture it is possible to see this western approach to the TCM “phases”.

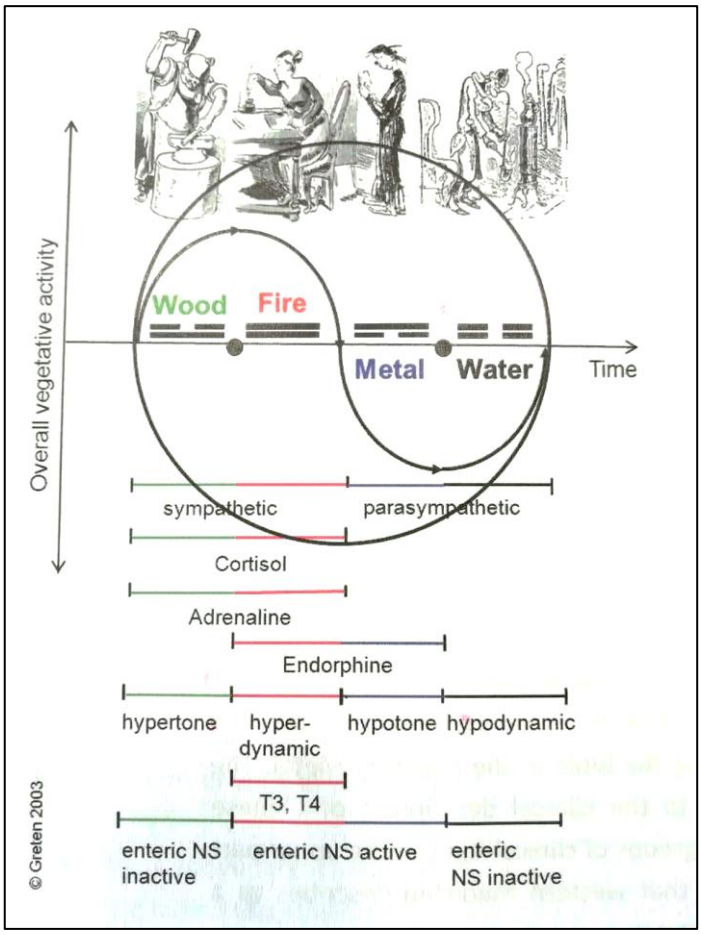

Figure 3 – Western physiological description of a vegetative sinus wave. The upper part of the picture refers to the symptoms in the language of Chinese Medicine, while the lower part shows the same symptoms in the language of Western Medicine (Greten H. , 2007).

The sinus wave represents a sinus curve of regulation, a circular movement, as so the classic circle of Yin/Yang does. This descries the regulatory model of the phases (Wood, Fire, Metal and Water), and the "western view" of their vegetative functions (Greten H. , 2007).

In the sinus wave it's possible to see that, in the yang phases, the sympathetic functions dominate more than in yin phases. In the yin phases, however, parasympathetic (vagal) activity is relatively more present. Of course, the vegetative system consists in more than parasympathetic and sympathetic activity. The first major description of the vegetative system, categorizing vegetative functions and its nervous system into sympathetic, parasympathetic and intestinal nerve fibers, actually lead to a Nobel Prize in 1927 (Greten H. , 2007).

As an example, according to Greten (2007) and the Heidelberg model, stress is believed to be accompanied by a strong increase of the sympathetic action, which is equivalent to

phase Wood. According to western medicine, at the same time vagal impulses act to counter-balance this sympathetic action and effect. In Chinese Medicine, this is a case of imbalance of antagonists, one of the four major ways of becoming ill, as previously been said. The functional tendency, or phase Wood, is more or less comparable to stress and sympathetic action, with adrenaline increase, high muscle tension, higher blood pressure, and others. This is most important in the diagnosis method of this Heidelberg model of TCM (Greten H. , 2007).

Another example is the case of asthma which, in Chinese Medicine, is a case of Wood-Metal imbalance. Wood is associated with contraction, bloated chest, tension and high blood pressure. Metal on the other hand, not only represents much less action and tension, but also stands for localizing the symptoms in the respiratory tract, and a lack of clearing mucus. Asthma consists of cramps in the bronchi, bloated chest by air trapping and incapability of breathing out. Mucus is normally present, too. It can be, and normally is, associated with stress or anger moments, like exercise-induced or psychosomatic-induced asthma. This is another good example to understand the imbalance of antagonists previously discussed (Greten H. , 2007).

As for the diagnosis in the Heidelberg model, it has four important components: the constitution, the agent, the orb and the guiding criteria (Figure 4). The constitution refers to the patient's inner nature and the overall of his individual functional properties. The agent refers to the functional power that may change the patient's individual functional properties: in other words, is the pathogenic factor that affects the balance and homeostasis of the individual. So these pathogenic factors produce specific signs and symptoms that may promote specific orb patterns, which is another of the components of diagnostic tools (Greten H. , 2007). The orb is a group of diagnostically relevant signs present at the moment of evaluation which helps to identify the dysregulation of the patient. Once all of this is established, in a second step, the symptoms are interpreted on the background of overall body regulation by the means of the guiding criteria. The guiding criteria is an assessment of the clinical signs according to an underlying regulatory model of physiology, where some of previous described factors are evaluated: the repletion/depletion (refers to Qi); the calor/algor (which refers to the state of microcirculation and so, Xue functions); the extima/intima (which refers to symptoms manifesting outside or inside the body); and finally yin/yang, which refers to the current regulatory capacity of the individual (Greten H. , 2007).

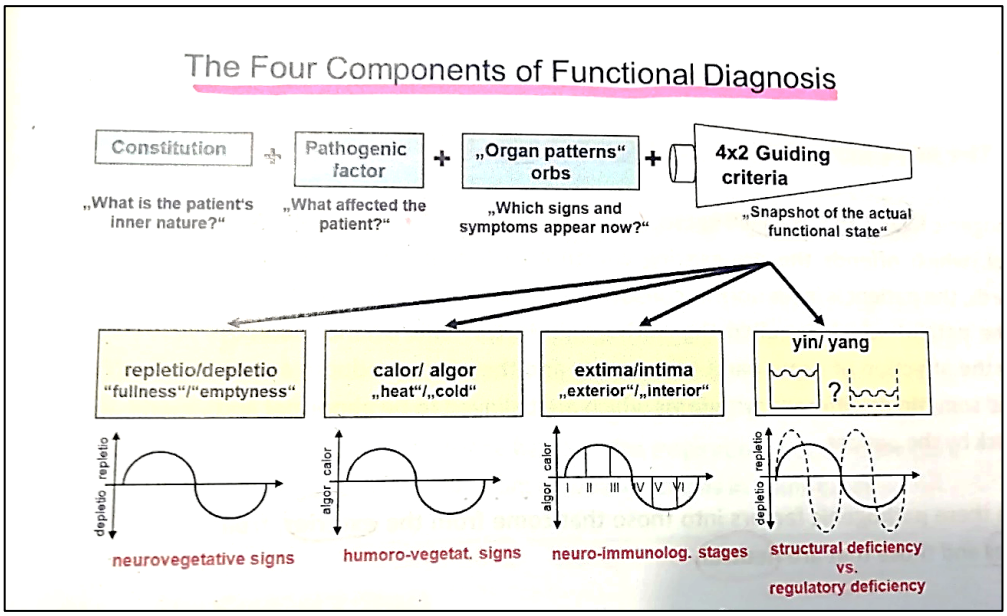

Figure 4 – Four components of the functional diagnosis in TCM (Greten H. , 2007).

GYNECOLOGY IN TRADITIONAL CHINESE MEDICINE

When studying Gynecology through the TCM point of view it becomes clear that, the first important thing to say, is that women are a rhythmical being, as both physiological and biological functions are cyclic and can be allocated into the sinus wave explained before (Greten H. , 2007).

According to Chinese Medicine, life is a cyclic process of 10 x 7 years. This cycle can be allocated into the sinus wave, where the life phases can also be related to the signs and symptoms associated with the previous presented sinus curve. In the following figure this can be seen in more detail (Greten, 2007).

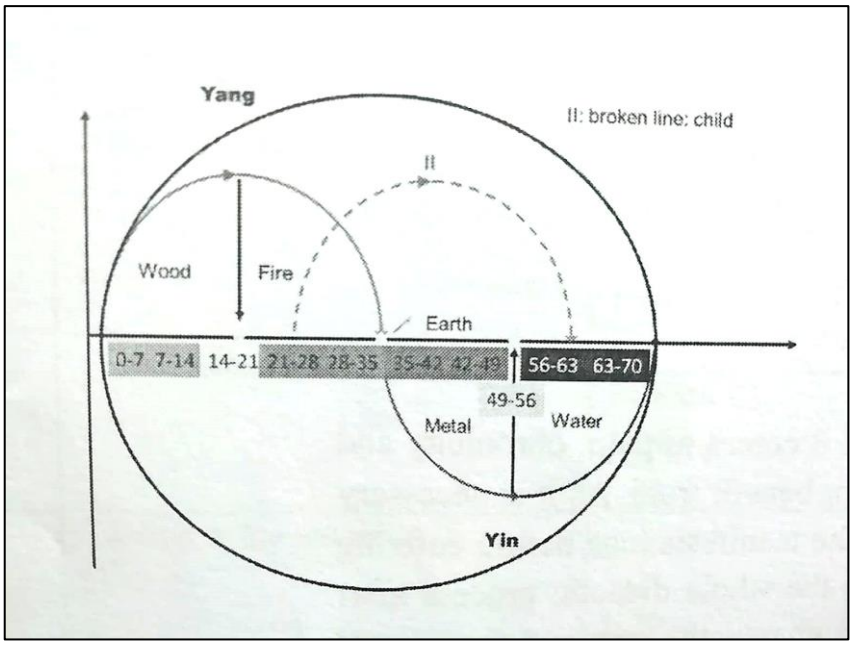

Figure 5 – Life as a cyclic process of 10 x 7 years (Greten H. , 2007)

So life as a whole can be regarded as a cyclic process. According to this, the yang phases of life normally last until the 35th year, and the yin phases last until the 70th year. It is quite interesting to see that, according to western medicine, the fertile window of a women coincidently starts to decline around the age of thirty, which, by looking at this sinus curve, is (coincidently or not) more or less the time in which the yang function of female starts to decline. So regarding the menstrual cycle, this means that energies are higher and in their best shape in the beginning of the Fire phase, between 21 and 28 years old, and then are reduced in the late fire phase between 28 to 35 years old. This is still a good moment of becoming pregnant, although according to Chinese Medicine and also westers statistics, pregnancy is less probable after the age of 35. Until this point, both western data and statistics and TCM theory are in harmony (Greten, 2007).

Treatment of infertility in TCM clinics often sees women older than 35 with significant lack of qi and xue. This will be easier to understand once the menstrual cycle is later explained through the TCM point of view. The truth is, from a western point of view, the cycle can be still intact and regular, the uterine tubes, hormonal situation, receptivity of the uterus and all of the factors can seem normal and, still, some women don't achieve pregnancy. This would be a case of western diagnosis of unexplained infertility, and the couple is considered to be infertile without a palpable reason. Regarding the Chinese diagnosis, most of the times a lack of qi and xue can be diagnosed, meaning that there are vegetative changes, which prevent the women of getting pregnant. These vegetative changes are referred to a lack of

qi (neuro-vegetative activation patterns), and lack of xue, which refers to specific properties of the body fluids and microcirculation functions (Greten H. , 2007).

In order to explain infertility and PCOS through the TCM gynecology perspective, it is important to explain and understand the menstrual cycle and female reproductive system in the Chinese Medicine perspective.

So TCM describes the aspects of female reproduction (the organs, the glands and their secretions, and the psyche) in terms of Renal orb function (Kidney function), Cardiac orb function (Heart function) and the Uterus/paraorbis Uteri – "The Uterus, the Heart and Kidney form the core of reproductive activity", some TCM texts say (Lyttleton, 2013). It is important to underline that these are not the only organs involved in the whole process. They are described has the main controllers of the reproductive function and process, but TCM is always a process of the whole body, the whole system, so they are not the only organs or systems necessary for the effective functioning of the menstrual cycle.

As it's been said before, when TCM refers to Renal/Kidney Jing, is what modern Western medicine refers to as the gametes or eggs and sperm themselves. Renal/Kidney functions include the influence of the hormones which regulate the different parts of the cycle, and also influence libido and sexual function. The Cardiac orb (Heart) includes the mind and the activity of the hypothalamus and pituitary, which controls the whole cycle. The mind and spirit – "Shen" in TCM – exerts a delicate but powerful influence over many aspects of the menstrual cycle (Lyttleton, 2013). According to the Heidelberg model, "Shen" is defined as a "functional capacity to put order into mental associativity and emotions, thus creating "mental presence"". Both in Chinese and West fertility clinics, the role of the mind or Shen is recognized not only in physical well-being but also in many of the physiological processes leading to successful conception, and the main emotional disturbances responsible for menstruation problems are a lack of inner tranquility or stress (Greten, 2007). Emotional stress, causing a dysregulation of the Cardiac orb, can affect the function of the hypothalamus, causing pituitary gland dysfunction, and ovulation may be delayed or completely absent. As for the Uterus, it describes the area where all of this happens. The use of the term Uterus in Chinese medicine refers to all the reproductive organs: uterus, ovaries, fallopian tubes and cervix. Old Chinese medicine texts describe the Cardiac orb (Heart) as the master controller of the other organs. In the same way, Western medicine often refers to the hypothalamus and pituitary as the master controllers of the other glands in the body (Lyttleton, 2013).

The Lienal/Spleen and Hepatic/Liver orbs also contribute, although in a less direct way, to aspects of reproduction and fertility. The Lienal produces the Xue (from the nutrients it can extract from food) and the Hepatic stores and moves the xue – so, both can have a positive or negative effect on the nourishment of the Uterus. The Xue and, therefore the Lienal and Hepatic orbs, are important in fertility by nourishing the endometrium, making it a moist, juicy and nutritious place for an embryo to settle in. Also, as the hepatic orb is responsible for the smooth movement of Qi, it plays a critical role during events surrounding ovulation and menstruation, two pivotal moments of the menstrual cycle (Lyttleton, 2013). Qi is easily blocked by emotional stress: the obstruction of Qi in these conduits (Hepatic and Lienal) can affect the release of the egg from the ovaries and the flexible movement of the fallopian tubes (Lyttleton, 2013). Xue is stored by the Hepatic orb, especially when the body is at rest. Some of this must be passed to the Uterus before preparing a pregnancy or menstruation can occur. If the Hepatic blood is deficient, menstruation may be scanty, infrequent or absent (Greten, 2007).

As it's been said before, there are 12 main conduits in TCM that were described previously. In addition, TCM texts describe a number of "extra" conduits, some of which play an important role in gynecology and in the function of the menstrual cycle. Speaking about the conduits in gynecology, the main conduits are, of course, those related to the respective orbs described has being the more important ones: the cardiac conduit (Heart Meridian), the hepatic conduit (Liver Meridian), the lineal conduit (Spleen Meridian) and the renal conduit (Kidney Meridian). As for the extra conduits, the most important ones for gynecology are the Bao Mai and Bao Luo, which are the ones responsible for the communication between the Cardiac orb, the Renal orb and the paraorbis Uterui (Lyttleton, 2013); the Impedimental Sinartery/Chong Vessel, and the Respondens Sinartery/Ren Vessel are of primary importance in controlling the menstrual cycle and play an important role in conception and pregnancy; the Regens Sinartery/Dumo vessel and the Zonal Sinartery/Daimo Vessel (also known as Zonalis), are also important in gynecology, although their application in the treatment of functional infertility is not central. They are, however, important in the treatment of PCOS and infertility associated with this syndrome (Lyttleton, 2013; Greten, 2007).

THE MENSTRUAL CYCLE THROUGH THE TRADITIONAL CHINESE MEDICINE PERSPECTIVE

In the context of the menstrual cycle, Renal Yin relates to the hormonal triggers which stimulate follicles to develop, as well as to the factors which support the follicle's growth and maturation – so it embraces aspects of pituitary function as well as ovary structure and function (Lyttleton, 2013). The function of the glands in the cervix gives us a particularly useful indication of Yin function, as they produce the fertile mucus, the easiest observation of yin functions. In contrast with the Yin's still, cool, moist and nourishing nature, is the dynamic, active and warming nature of Yang. The effect of Yang begins to be seen in the ovulation part of the menstrual cycle, as for in the moment of ovulation, there is much dynamic activity: the egg is launched out of its follicle and the fimbrial fingers embrace and guide the egg into the fallopian tube. This journey down the tube is also dynamic, with both the egg and the tube needing to be able to move smoothly and flexibly, which is also a function of the hepatic orb. The moment of fertilization also relies of the power of yang to succeed. Yang is the motivating force for all transformations in the body (Lyttleton, 2013). As it's been said by Greten (2007) and can be seen in Figure 2, it is during the yang phase that occurs the providing of energetic potential (hepatic orb), and the transformation of potential into function (cardiac orb) (Greten H. , 2007). The moment a sperm's head breaks through the egg, and the DNA starts developing, the greatest transformation of all occurs – the beginning of a potential human life. Renal yang also performs a very important role after ovulation. For many years the Chinese have described the inability of a woman to achieve pregnancy as a "Cold womb", or, in others terms, lack of Renal yang energy. Is it now known that "Cold womb" refers to lack of progesterone, which means that implantation and early development of the embryo will not be supported. When there is insufficient progesterone produced, the body temperature is considerably lower, about 0.4ºC colder than when progesterone levels are high (Lyttleton, 2013).

So when the subject of study is the menstrual cycle, TCM therapists break it up into four phases: the period, post-period, ovulation and the post-ovulation and pre-period, which, when considering infertility, have no separation and are treated the same way. The period, post-period and ovulation phases all fall into the follicular part of the cycle, and so are more yin phases. The post-ovulation and pre-period are more yang phases, has they belong to the luteal or secretory phase (Lyttleton, 2013).

As it's been said before, the follicular phase is a more proliferative or estrogenic phase of the cycle. It is called follicular phase because it is in this part of the cycle that the follicles in the ovary grow, in order for ovulation to occur. It is also called the proliferative and

estrogenic phase because more estrogen is produced rather than progesterone, and because the lining of the uterus is enlarging from almost nothing to a thick and receptive padding to receive a newly fertilized egg. So, the first day of the cycle is usually defined as the first day of menstrual bleeding (Lyttleton, 2013). In TCM terms, this phase associated with the stimulation of the pituitary hormones, corresponds to the Yin aspect of the ovarian activity. Day one of menstruation is the day in which the Impedimentalis Sinartery begins to "empty", the day where the Qi of the Respondens Sinartery drives the flow of xue downwards to the cervix (Lyttleton, 2013). The amount of shedding of the endometrium varies from woman to woman, but from the TCM point of view, the variance of quantity and quality of blood flow during the period places great importance: the nature of the menstrual flow and symptoms accompanying it are all significant in order to understand the Qi and Xue movement (Greten, 2007; Lyttleton, 2013). Any obstructions to the menstrual flow can be valuable to do a good diagnosis and subsequently apply a correct treatment of infertility. For example: the presence of clots alerts the TCM therapist that the flow of Qi and Xue is not smooth and is blocked – known as Qi and Xue stagnation – which requires treatment (Lyttleton, 2013; Greten, 2007).

Over the next 2-3 days, the Impedimental conduit empties, and the building of yin and blood begins again, until the conduit is completely refilled. So although menstrual bleeding may continue for more days, endometrial regeneration usually begins 2 days after the onset of menstruation and, by day 5, the remodeling of the endometrium is complete. At this stage, the levels of estrogen begin to rise, and more tissue develops, until the proliferation phase is completed and ovulation is imminent, when the endometrium achieves at least 8mm thick (Lyttleton, 2013). This proliferation of the endometrium, blood vessels and glandular cells reflects the filling of the Impedimental Sinartery. When concentrations of estrogen secreted by the dominant follicle reach a certain level, the surge of FSH and LH from the pituitary is initiated, which will trigger ovulation over the next 36-40 hours and increases the production of progesterone from the follicle. This events coincide with the beginning of the Yang part of the cycle (Lyttleton, 2013).

So, when ovulation is coming, the Yin is at the peak of the cycle, the Impedimental conduit is full of Xue and the Yang is in the beginning of its action. When ovulation occurs and the events of the next few hours occur, such as the rupture of the follicle and the movement of the fallopian fimbriae, the Yang part of the cycle begins. In the TCM perspective, the Cardiac orb controls the collaterals of the uterus, which means that if the Cardiac Qi is obstructed due to emotional stress, for example, the function of the uterus will be affected and the process of ovulation can be disturbed. It is an action of the Cardiac orb to maintain the

paraorbis Uteri “open”, in order to allow the egg to be released and the sperm can be granted passage into the uterus. The cervix also responds to the increased levels of estrogen by opening and producing the fertile mucus which, as it’s been said before, helps the survival of the sperm and their movement through the cervix, providing a more hospitable pH and containing nutrients that activate the sperm and make them swim more vigorously. This is still a reflection of the Yin part of the cycle.

At the midcycle, the elevated estrogen levels before and during ovulation drop sharply after the ovulation. Obviously, the cervix corresponds equally quickly: the cervical mucus dries up to be replaced by a thick, dry or pasty discharge. This change from wet to dry represents perfectly the switch from Yin (moisturizing nature) to Yang (warming nature) (Lyttleton, 2013).

In this Yang part of the cycle, not only the egg and follicle have active rolls, but also the fallopian tubes fimbriae start aiding and moving, catching the sticky mass and helping it to move into the ampulla of the tube. According to TCM, the release of the egg and its smooth passage through the fallopian tube requires free and unobstructed movement of the Qi: this is an action of the hepatic orb, mainly. At this point, the cycle is at the Yang part or, in Westerns terms, in the luteal phase. This luteal phase – the period between ovulation and the next menstruation – is the most constant and predictable part of the cycle (Lyttleton, 2013). In this phase, the progesterone is the most important hormone, causing the endometrium to secret nutrients in case of a conception and switching off the hormones who would keep ripening up more eggs. Progesterone also acts on the temperature-regulating centers in the hypothalamus. TCM doctors described the importance of a “warm womb” hundreds of years ago and, despite physiology was not able to ensure why it is so beneficial to the embryo or fetus to be in a slightly warmer environment, TCM describes the “cold womb” as a common cause of infertility for hundreds of years. In TCM, warm womb environment is not only refereeing to the temperature, but also to the metabolic activity of the uterus, to its secretion of nutrients and nurturing home for a fetus (Lyttleton, 2013).

About a week after ovulation, and under the influence of the LH surge, the corpus luteum peaks in size and is responsible for maintaining the endometrium. Without LH, the corpus luteum starts to degenerate and about day 26 of a 28-day cycle, the secretory function finishes and the endometrium starts to disintegrate again. So, when conception occurs, the corpus luteum produces enough progesterone to ensure implantation and survival of the embryo. When this does not happen, and an inadequate luteal phase is diagnosed, means that there might not be enough Yang to sustain the luteal phase and give time to the embryo

to implant successfully, which can be a cause of infertility. Fortunately, with TCM, the treatment of Renal Yang deficiency can be achieved (Lyttleton, 2013).

The stage of development of the endometrium must be synchronized with the development of the embryo in order to implantation be successful. The previously discussed “Window of Implantation” or Window of Fertility occupies 4-5 days of interval in the whole 28-day cycle (it’s around day 19 or 20), when progesterone reaches its higher levels. This means that if the Renal Yang is not enough, this Window of Implantation can be shortened of deficient, and the embryo will not survive (Lyttleton, 2013).

The most crucial part of the Yang phase, if conception has occurred, is the implantation of the embryo. In the most successful cases, implantation occurs in the next 8-10 days after ovulation. The majority of cases that implant 11 days after ovulation will not last, and the majority of embryos that implant over the next 9 days after ovulation are the ones more expectable to develop into healthy fetuses, and pregnancy is more secure. Embryos with strong and abundant Renal Jing are the ones with more expectancy to develop quickly. This process of implantation is also a function of the Yang phase of the cycle. If the implantation does not occur, it could be a case of a slippery or damp uterus for the fetus to attach, and this may be a case of humor presence. Humor is a TCM pathological agent that represents the accumulation of liquid and mucus, analogous to oedema (Lyttleton, 2013).

The two tables below show the summary of the parallels between TCM and Western medical terms. Table 1 shows the parallel between Western physiology and TCM; and table 2 shows the comparison of the action of estrogen and progesterone in western and in TCM terms.

Western physiology	**TCM**
The gametes	Jing
Influence of sex hormones	Renal Yin and Yang
Influence of hypothalamus and pituitary	Cardiac orb
Ovaries, tubes, endometrium and cervix	Paraorbis uteri

Table 1 – Parallel between TCM and Western medical terms, adapted from Treatment of Infertility with Chinese Medicine, from Jane Lyttleton (Lyttleton, 2013).

Western physiology	TCM
Estrogen promotes the growth of the follicle and egg	Yin supports action of Jing
Estrogen stimulates cervical glands to produce fertile mucus	Yin creates fluids
Progesterone lifts the body temperature	Yang warming nature
Progesterone stops the production of fertile mucus from the cervix	Yang dries fluids

Table 2 – Comparison of the action of estrogen and progesterone in TCM and Western terms, adapted from Treatment of Infertility with Chinese Medicine, from Jane Lyttleton (Lyttleton, 2013).

Last, but not least important, according to the Heidelberg model of TCM, in order to conceive a child, there are 7 laws of menstruation that should be intact, and the TCM therapist should address this before continuing infertility treatment with the women.

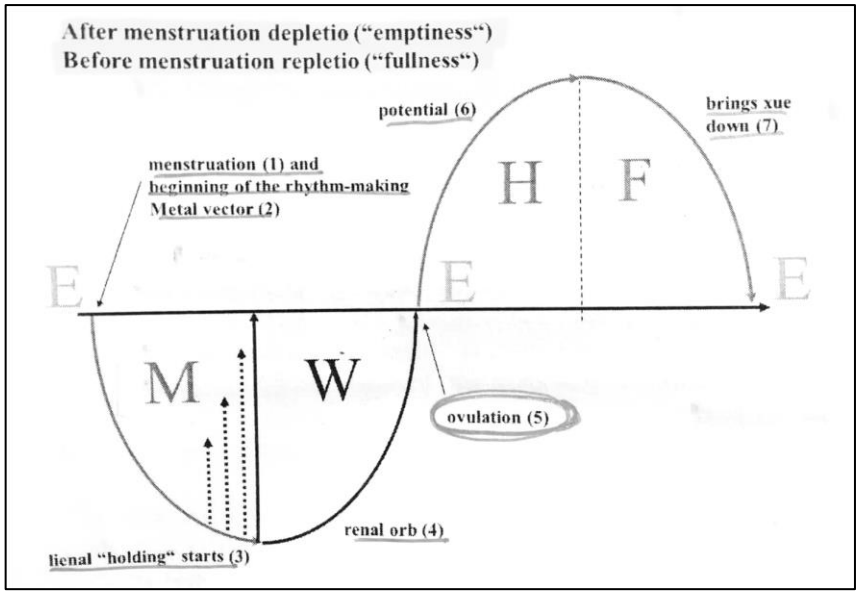

Figure 6 – Physiology of menstruation in the Heidelberg model. The representation of the menstrual events according to the phases previously mentioned (Greten, 2007).

This figure 6 shows that Greten and Lyttletlon are in agreement with the allocation of menstrual cycle events in the yin and yang phases. Before ovulation is a more yin phase and after ovulation a more yang and potential phase.

The first law of menstruation explains that the menstrual cycle has the purpose of preparing the women to conception, pregnancy and delivery and, in order to do so, the body must have enough Qi and Xue. Conceiving a child and maintaining a pregnancy is a process that requires a lot of energy and blood, and Chinese medicine believes that there should be a supply of this two factors before the body starts using them for nine months. If the body of the female is not prepared for pregnancy and not nourished enough, even if conception is achieved, the risk of miscarriage is higher (Greten, 2007).

Second, the orbs and conduits responsible for bringing the energy downwards to the uterus must be stronger than the conduits which lead the energy upward, in order to lead xue and qi downward to the main organs of reproduction. This means that the cardiac orb, the stomach orb and the pulmonary orb must be stronger than the rest of them at this time of the cycle, underlining again that the cardiac orb is the main source of this descending power (Greten, 2007).

Law number three says that, even having enough qi and xue, and the force of the downward conduits is enough, the pathways downwards may be free and the movement shoud be smooth. The downward conduits (the main and the extra) must be open and unblocked, so the Impedimental Sinartery, the Respondens Sinartery, the cardiac conduit, the stomach conduit and pulmonal conduit must be open and free, underlining the most important one – the Impedimental Sinartery. The Zonal Sinartery is also very important in fertility and pregnancy, because of the anatomical location: it comes from the uterus, feeds the ovaries and leads the energy through the tubes back to the uterus (Greten, 2007).

Fourthly, the uterus must be intact – if the uterus is at any pathological condition, this may result in in menstrual disturbance or difficulty at conception (Greten, 2007).

Law number five is about the hepatic orb function and the need for it to be in balance, as it is the hepatic orb responsibility to initiate the blood flow at menstruation. In cases of hepatic yang deficiency, amenorrhea can happen due to the lack of hepatic power. So menstrual cyclicity and health is also dependent on the healthy state of the hepatic orb, and TCM therapists should always be aware of this importance (Greten, 2007).

The sixth rule of menstruation is about the need for a stopping mechanism of the bleeding process, and this is done by the stomachal orb. So in cases where the stomachal orb is not

strong enough, there could be menstrual disturbances like metrorrhagia, intermenstrual bleeding and menorrhaghia, for example (Greten, 2007).

Lastly, but not least importantly, the seventh law says that, in case of hepatic blockage, lumps in the menstrual blood can appear, coming by xue stasis. This must be treated in order to improve reproductive health, even though the women is not trying to conceive. Nonetheless, if it is a case of infertility problems, xue stasis must be addressed and treated before starting the actual infertility treatment (Greten, 2007).

POLYCYSTIC OVARY SYNDROME THROUGH THE TRADITIONAL CHINESE MEDICINE PERSPECTIVE

Understanding the etiology of PCOS is difficult both in Western medicine and in TCM. Chinese medical doctors believe that the congenital Jing and the acquired Jing can be the root of this ovarian dysfunction, even before the beginning of puberty around the 2nd 7-year cycle, when the Impedimental and the Respondens Sinartery become active (Lyttleton, 2013). PCOS, being a complex and difficult disorder, have numerous interactions of factors that can contribute to its development, but one of the factors that's more or less established, is that this acquired Jing disturbance is the realm of influence of the Lienal and Stomachal orbs. Unfortunately, the diagnosis of this syndrome is hard to do before the beginning of puberty, sometimes even after many years, which will increase the disease and delay the start of treatment (Lyttleton, 2013).

As it has been said before, TCM sets the base of diagnosis and treatment in the interpretation and analysis of clinical symptoms and signs given by the body. One of the first and most consistent signs is the irregular or infrequent nature of their ovulation, which could be mild or more severe. Either way, one should consider that the function of the Impedimental Conduit and the Respondens Conduit is not optimal. If amenorrhea starts at puberty, then a Renal Jing deficiency must be consider; if there are more regular but still long or irregular cycles, then the Hepatic Qi and the Impedimental and Respondens Sinartery must be addressed (Lyttleton, 2013).

Another common symptom is the weight gain or the high waist-to-hip ratio, and there is a clear case of humor accumulation (Phlegm) in this PCOS patients. Patients who present obesity or even slight weight gain (5kg is enough) usually present some disturbances in the menstrual cycle. From the TCM point of view, in PCOS this could be a Zonal Sinartery dysfunction, which may allow the build-up of humor in this area. Loss of weight has shown

to improve ovulation frequency, appearing to mobilize humor, and unblocking and improving the function of both Impedimental and Respondens Sinartery (Lyttleton, 2013). Bad food choices and junk-food consumption and lack of exercise are associated with increased humor accumulation and, obviously, with blocked movement of Qi, especially of the Lienal orb. Most of the typical androgenic signs and symptoms of PCOS are also associated with this humor and blood stagnation, as so some of the cardiovascular diseases associated with the syndrome (Lyttleton, 2013).

So in the end, irregular or absent periods is the main feature that infertile women with PCOS present, and the main diagnosis is Impedimental and Respondens Sinartery disturbance. As known, the foundation of this conduits is the Renal orb and both require nourishment of Qi and Xue in order to function optimally, and both can be obstructed by humor accumulation or Xue and Qi stagnation. The Zonal Sinartery is also very important in this syndrome, because it's allocated in the precise area of the disturbance: is where the waist expands, the fat accumulation occurs and the place of the typical swollen ovaries of this PCOS patients (Lyttleton, 2013).

Research in this area of interest has been growing and growing. In various parts of the world, researchers have demonstrated that acupuncture can improve ovarian function and increase ovulation frequency. Some of the studies have demonstrated that LH, testosterone and insulin levels improved and were beneficially reduced by acupuncture treatment, and some studies even studied the effect of this treatment in insulin resistance (Lyttleton, 2013). As this is the main matter of study of this review, this studies will be analyzed in more detail in the next chapter.

ACUPUNCTURE AND POLYCYSTIC OVARY SYNDROME

So as it's been said before, due to the uncertainty of etiology of the syndrome as well as the heterogeneity of the symptoms, no cure can currently be offered to women with PCOS. There is no consensus on long-term management, mainly to women not trying to conceive. Women with this syndrome are, instead, treated in a symptom-guided way, usually for long duration, but this treatments are associated with metabolic and gastrointestinal adverse effects most of the time (Elisabet Stener-Victorin , 2013; Elizabeth Jedel, 2011; Julia Johansson and Elisabet Stener-Victorin, 2013). The pharmacological treatments and lifestyle modifications aim to reduce clinical and biochemical hyperandrogenism, to restore menstrual cycles and ovulation, and to improve reproductive function and aid in achieving pregnancy. Given the fact that most women with this syndrome require long-term treatment, it is important to investigate if any treatments or new strategies with fewer side effects are available, such as acupuncture (Elisabet Stener-Victorin , 2013), which is now much more accepted in the western world as the main treatment or adjuvant treatment for more and more conditions, being a relatively safe therapy, with few side effects (Julia Johansson and Elisabet Stener-Victorin, 2013).

ACUPUNCTURE IN IVF-ET TREATMENTS

The first report suggesting that acupuncture can increase the clinical pregnancy rate of IVF was made by Stener-Victorin et al. in 1999. Since then, the application of this therapy to assist in ART treatments has attracted great interest from the international community. Despite this, and even though more than 40 clinical trials have been made in recent years, the efficacy of acupuncture in improving the reproductive function and pregnancy rate is still a matter of debate: some studies have suggested a positive impact from adding acupuncture to IVF, but some other studies do not confirm this effect (Cui Hong Zheng, 2012). The first meta-analysis performed by Manheimer et al. in 2008, which included 7 trials with 1366 participants, reported that acupuncture given around embryo-transfer (ET) day could improve the rates of clinical pregnancy, ongoing pregnancy, and live-birth in women undergoing IVF treatment (Eric Manheimer et al., 2008). Another analysis conducted by Ng et al. which included 10 trials with 2003 women, published also in 2008, demonstrated that the IVF rate significantly increased, especially if acupuncture was performed in the day of the ET. Another analysis, conducted by Cheong et al., published by the Cochrane Collaboration in the same year, concluded that acupuncture treatment in the day of ET increases live birth but does not increase pregnancy rate, and concluded that

there are no beneficial effects on pregnancy outcomes when acupuncture is performed around the time of oocyte removal. Other 4 meta-analysis conducted by different authors could not confirm the beneficial effect from using acupuncture during the IVF treatment (Cui Hong Zheng, 2012). The meta-analysis conducted by Cui Hong Zheng et al. (2012), concluded that acupuncture, especially around the time of controlled ovarian hyperstimulation, improves pregnancy outcomes in women undergoing IVF. On another hand, one other systematic review made by Eric Manheimer et al. in 2013, that included 16 RCT (4021 participants) found no benefit of adjuvant acupuncture for IVF treatment, when assessing the clinical pregnancy rate among women undergoing IVF treatment (Eric Manheimer, 2013).

One prospective randomized trial made in 2006 evaluated the effect of acupuncture on reproductive outcome in patients undergoing IVF or intracytoplasmatic sperm injection (ICSI). In this study, a total of 273 patients were randomly assigned into 3 different groups: 87 patients were allocated in the no acupuncture group (control group); 95 patients received acupuncture on the day of embryo transfer; and the last 91 received acupuncture on the day of embryo transfer and again 2 days after embryo transfer. Apart from the acupuncture treatment, all patients were treated with the established western fertility treatment protocol of the clinic. No demographic characteristics were significantly different between groups: not in age, BMI, duration of fertility problems, proportion of primary infertility and previous attempts in IVF/ICSI, or even the main cause of infertility. As results of this intervention, Lars G. Westergaard et al. published that clinical and ongoing pregnancy rates were significantly higher in the group that received acupuncture on the day of embryo transfer, when compared with the control group. The acupuncture treatment on the day of embryo transfer plus 2 days after showed no additional benefits and, in addition, despite having higher rates of pregnancy and ongoing pregnancy rates when compared to the control group, it did not reach statistical difference. This results showed that acupuncture may be effective as an adjunct treatment alongside with the established treatments of western medicine in FIV/ICSI, and could be taken in consideration during ART treatments (Lars G. Westergaard, 2006).

One other study was made with the goal of assessing the outcomes of acupuncture intervention in IVF/ICSI treatments in infertile women. This randomized, prospective, controlled clinical study conducted by Stefan Dieterle et al. in 2006, aimed to evaluate the effect of luteal-phase acupuncture in clinical and ongoing pregnancy rates in 225 women with infertility undergoing IVF/ICSI treatments, and all women had at least one embryo transferred. All patients were, at the same time, under the established Western treatment

implemented in the University IVF center. Patients were randomly assigned into 2 groups: group 1 with 116 patients, which received luteal-phase acupuncture according to the principals of TCM; group 2 with 109 patients receiving placebo acupuncture, where acupuncture treatment was designed not to influence fertility outcomes. As results, the implantation rate was significantly higher in group 1, with a 14,2% rate of implantation, when compared to a 5,9% rate of implantation on group 2. In addition, clinical pregnancy and ongoing pregnancy rates per transfer were also significantly higher in group 1, with rates of 33,6% and 28,4%, respectively, than in group 2, with 15,6% and 13,8% rates. It is of importance to note that according to the German IVF/ICSI register of 2003, the average clinical pregnancy rates for this age are 24,6% for IVF and 22,6% for ICSI, being lower than the results achieved in the luteal-phase acupuncture group. This study showed that acupuncture might be a good complementary option for improving the outcomes of this patients undergoing IVF/ICSI treatments (Stefan Dieterle, 2006). On another hand, there was another prospective, randomized, controlled, single blind trial which results differed from some previous research, and found no significant differences in pregnancy rates between acupuncture group and control group; however, the patients who went under acupuncture treatment reported feeling significantly less anxious after embryo transfer, as well as being more relaxed and able to enjoy their sessions more, when compared to the control group patients (Alice D. Domar, 2009).

Author	Year	Study	Results
Lars G. Westergaard	2006	Prospective Randomized Trial	Clinical and ongoing pregnancy rates improved
Manheimer et. al	2008	Meta-analysis	Improved the rates of clinical pregnancy, ongoing pregnancy and live-birth in women undergoing IVF-ET
Ng et. al	2008	Meta-analysis	IVF rate increased significantly
Cheong et. al	2008	Meta-analysis	Increased rate of live-birth
Cui Hong Zheng et. al	2012	Meta-analysis	Increased pregnancy outcomes in IVF
Eric Manheimer	2013	Systematic review	No benefit of adjuvant acupuncture for IVF

Stefan Dieterle	2006	Prospective randomized controlled clinical study	Increased clinical and ongoing pregnancy rates
Alice D. Domar	2009	Prospective randomized controlled single blind trial	No significant differences in pregnancy rates Significantly less anxiety during treatments

Table 3 – Results of the evaluated studies on the effect of acupuncture on IVF-ET outcomes and parameters.

Despite this discordance between studies and analysis made in order to evaluate the effectiveness of acupuncture as an adjunct therapy in ART treatments, some evidence says that acupuncture treatment may be able to improve reproductive and metabolic disturbances with no adverse effects in women with PCOS. Although their efficacy has not yet been established, studies have shown that in women with PCOS or undefined ovulatory dysfunction, repeated acupuncture treatments decrease total testosterone and other sex steroids levels, reduce the LH/FSH ratio, and improve menstrual frequency without adverse effects. Some case-control studies suggest that acupuncture has stronger effects than physical exercise on these same variables (Elizabeth Jedel, 2011) but the etiology of PCOS is, as described before, poorly understood, and this can be an aspect which easily potentiates the miss information and the lack of good quality evidence in this matter of study.

ACUPUNCTURE IN PCOS – MECHANISM OF ACTION

High activity of the sympathetic nervous system is associated with factors related to PCOS, such as disturbed central and peripheral β-endorphin release, hyperandrogenemia, hyperinsulinemia and insulin-resistance, as well as abdominal obesity and cardiovascular disease (Elisabet Stener-Victorin , 2013). Neuroendocrine defects contribute to a persistently rapid LH pulsatility and increased amplitude, which will further augment ovarian androgen production. The role of β-endorphin as an inhibitor of the GnRH pulse generator and pituitary LH release suggests that PCOS results, at least in part, from insufficient central inhibition of GnRH by β-endorphin. One of the most prominent and consistent feature in PCOS is, as described earlier, the ovarian hyperandrogenemia, which is likely to play a key role in the pathogenesis of the syndrome, although hyperinsulinemia, insulin-resistance and abdominal obesity are thought to be important in the nature of the syndrome too (Elisabet Stener-Victorin , 2013). The way in which acupuncture influences the sympathetic nervous system and, consequently, controls some features associated with PCOS is still highly

hypothetical, but some researchers have already made some conclusions about this correlation.

The treatment of any disease with Traditional acupuncture involves inserting disposable sterilized needles into the skin at acupoints along the conduits. The needles can then be stimulated by hand or by a small electric current in the case of electroacupuncture (EA), or, a new and more modern form of this treatment, with laser acupuncture, where modern science and technology combines with traditional methods by using a low-energy laser beam directly to the acupoints (Cui Hong Zheng, 2012). The mechanism of action of this therapy in the reproductive system is still not completely elucidated, but seems to be set in three potential base mechanisms: first, acupuncture may mediate the release of neurotransmitters that may stimulate the secretion of GnRH, thereby influencing the menstrual cycle, ovulation and fertility; secondly, acupuncture seems to stimulate blood flow to the uterus by inhibiting uterine central sympathetic nerve activity; thirdly, acupuncture may stimulate the secretion of endogenous opioids, which may inhibit the central nervous system (CNS) outflow and the biological stress response (Eric Manheimer et al., 2008; Hongying Kuang, 2013). According to Stener-Victorin, who described this hypothetical mechanism of action more thoroughly, the insertion of acupuncture needles intramuscularly appears to initiate a specific pattern of afferent activity in sensory nerve fibers. These needles are then stimulated by manual manipulation and/or by electrical stimulation, the so called EA, for approximately 20-40 minutes. When talking about EA, the needles are then connected to electrodes for passing electric current. It is suggested by numerous authors that low-frequency EA, this is 1-15/20 Hz, is the most suitable frequency for the treatment of infertility and reproductive related disorders (Elisabet Stener-Victorin , 2013; Elizabeth Jedel, 2011; Julia Johansson and Elisabet Stener-Victorin, 2013; Fan Qu et al., 2017). So low-frequency EA with repetitive muscle contraction seems to activate physiological processes similar to those induced by physical activity, which in turn modulate the activity in the sympathetic nervous system which is affected in women with PCOS (Elisabet Stener-Victorin , 2013; Hongying Kuang, 2013).

Starting at a peripheral level, both manual and electrical stimulation of acupuncture points in muscle tissue leads to the release of a number of neuropeptides, such as substance P, calcitonin gene-related peptide, vaso-active intestinal polypeptide (VIP) and nerve growth factor (NGF), from peripheral nerve terminals into the surrounding area, resulting in increased microcirculation. Additionally, electrical stimulation increase skeletal muscle glucose update (Elisabet Stener-Victorin , 2013; Julia Johansson and Elisabet Stener-Victorin, 2013). So the stimulation with acupuncture needles into skeletal muscle excites

ergoreceptors that activate afferent sensory fibers. These signals are then transmitted to the spinal cord where they through sympathetic reflexes, may modulate organs, for example ovaries, located in the same innervation area as the stimulated acupuncture points. Stener-Victorin demonstrated in two different studies that low-frequency EA increases ovarian blood flow when needles are placed in the abdominal and posterior limb muscles, which have the same somatic innervation as the ovaries and uterus. The response was mediated by ovarian sympathetic nerves as a reflex response controlled by supraspinal pathways. This also demonstrates that the nervous system transfers signals to the brain which, by response, may affect the organ. The total effect of acupuncture treatment in the female reproductive system is, most likely, dependent on both mechanisms (Elisabet Stener-Victorin , 2013).

Signals also reach the CNS via supraspinal pathways where they can exert central effects. It has been demonstrated that low-frequency EA causes the release of a number of neuropeptides like serotonin, dopamine, endogenous opioids, and oxytocin in the CNS, of which all are essential for inducing functional changes in different organ systems. Modulation of hypothalamic β-endorphin secretion results in a general decrease of sympathetic tone, shown by decrease in blood pressure and muscle sympathetic nerve activity. So, as hypothalamic β-endorphin also interacts with the hypothalamic-pituitary-ovarian axis, acupuncture may hypothetically modulate the GnRH pulse generator and the release of pituitary LH (Elisabet Stener-Victorin , 2013; Elizabeth Jedel, 2011; Julia Johansson and Elisabet Stener-Victorin, 2013). Interestingly, repeated low-frequency EA in combination with manual stimulation acupuncture have been shown to induce more regular cycles, to decrease circulating LH/FSH ratio and high circulating testosterone. Additionally β-endorphin in plasma is thought to be related to the hyperinsulinemic response. Because low-frequency EA in combination with manual stimulation has been demonstrated to lower high circulating β-endorphin concentrations in women with PCOS, it may hypothetically decrease hyperinsulinemia and/or increase insulin clearance, and improve insulin sensitivity as demonstrated in rodent studies (Elisabet Stener-Victorin , 2013).

Women with PCOS have higher levels of circulating β-endorphin, which may contribute to the pathogenesis of the syndrome, possibly by insufficient inhibition of GnRH by β-endorphins in the CNS. So the relation between the β-endorphin levels and sympathetic activity seems to be sustained also in PCOS, where acupuncture treatment has been shown to reduce both high plasma β-endorphin and sympathetic nerve activity and increase low hand-skin temperature (Julia Johansson and Elisabet Stener-Victorin, 2013). Because this is, still, hypothetical, the research made in the area of acupuncture being used in relieving

PCOS symptoms or improving infertility problems associated with the syndrome, is very controversial and different outcomes are seen in some studies designed to evaluate the same variables. Nonetheless, there are already some good quality studies made in this area of interest, and many study protocols have already been accepted for some nice randomized controlled trials aiming to study the effect of acupuncture in PCOS related symptoms.

ACUPUNCTURE IN PCOS SYMPTOMS AND COMORBIDITIES

In 2000, Stener-Victorin et al. conducted a non-randomized, longitudinal, prospective study, aiming to elucidate the effect of EA in oligo/anovulatory women with PCOS, and related endocrine and neuroendocrine markers associated with PCOS. This study included 24 women with a mean age of 32 (ranging from 24 to 40 years), with amenorrhea or oligomenorrhea with no more than 4 spontaneous bleedings per year, and with a typical untrasonographic presentation of PCOS. Out of this total 24 women, 19 were clomiphene resistant, which means that no ovulation occurred after 150mg of clomiphene citrate for 5 days. The treatment period consisted in 3 months before the start of EA treatment, 3 months of EA treatment and 3 months after EA treatment was over, so 8-9 months of study, with 10 to 14 treatments in total. The main outcome measures in this study were basal body temperature (BBT) and menstrual pattern, and secondary outcomes measures were BMI and WHR. The BBT was measured with a digital infrared thermometer over each EA session: first measurement (considered the basal) after 10 minutes rest and just before EA started; several measures made every 7 minutes during EA; and one measure immediately after EA. BMI and WHR were measured before EA treatments and after the study period. In order to evaluate ovulation rates, menstrual and ovulation patterns were measured by recording self-reported vaginal bleedings and BBT daily. Ovulation was recorded and assessed during the 3 months prior EA, during the 3 months of EA treatment period and during the 3 months following EA treatment. A woman was defined as having good outcome if the BBT disclosed repeated ovulation (or pregnancy) during the 3 months period of EA treatment and in the following 3 months. As a result of the study, out of the 24 women, 9 had experienced good outcome (38%), and 15 no effect (62%). From this 9 women which had good outcome after 3 months of EA, they counted a total of 4 ovulations in the period of 3 months prior to the start of the EA treatments; this number increased to 31 ovulations in the period during and after EA treatment (6 months). This means that from a 0,15 ovulations/woman monthly prior treatment, they increased to 0,66 ovulations/woman monthly during and after EA treatments. Also importantly, out of the 19 women who were considered clomiphene resistant, 7 had experienced good outcome and ovulated (37%),

and 12 had no effect (63%). In the group of 9 women with good outcomes, BMI was significantly lower when compared with the group with no effect, but BMI and WHR did not change during the study period (Stener-Victorin, 2000). This study is in line with what has been described before: overweight and obesity do decrease the ovulation rates and increase the menstrual disturbances in this patients with PCOS.

A prospective RCT comparing low-frequency EA and physical exercise, conducted by Elizabeth Jedel et al. in 2011, showed that a total of 14 treatments of low-frequency EA over 16 weeks was more effective than a 16 week program of regular exercise at reducing serum testosterone, hyperandrogenism markers (such as sex steroids precursors, estrogens, androgens, and glucuronidate androgen metaboliotes) and acne and at increasing menstrual frequency in women with PCOS. In this study, 84 women were randomly allocated in 3 different groups: 33 women in low-frequency EA group during 16 weeks; 34 women in exercise group, for at least 3 times a week, during 16 weeks; and no intervention group with 17 women. The main goal of this study was to assess whether low-frequency EA could decrease hyperandrogenism and improve oligo/amenorrhea in women with PCOS. So the main measure was the concentration of total testosterone, followed by the secondary outcome measures that included changes in menstrual frequency, concentration of sex steroids, acne and hirsutism. The outcomes were measured at baseline, after 16 weeks of treatment and again after 16 weeks, so a 32 week follow-up. Both low-frequency EA and physical exercise were more effective than no intervention at improving menstrual frequency and several of the measured sex steroids, showing that despite both treatments may be helpful in interrupting the vicious cycle of ovarian dysfunction and androgen excess typical of this syndrome, acupuncture apparently is more effective. After the first 16 weeks, serum testosterone decreased in the low-frequency EA group and was lower than the value in exercise group. Menstrual frequency improved from 0.28 to 0.69/month in the low-frequency EA, which was significantly higher than in the exercise group. Between baseline and 32 weeks, at follow-up, acne score decreased in low-frequency EA group and menstrual frequency also remained improved when compared with the exercise group. The insulin sensitivity was not affected, which suggested that the intensity, frequency and duration of low-frequency EA in this treatment was too low and insufficient to affect insulin sensitivity. This study is important to understand that, despite not having all the answers or not being able to fully understand the underlying mechanisms involved, acupuncture may in fact be helpful in the control or even amelioration of PCOS syndrome. Menstrual dysfunction and hyperandrogenism are typically treated with oral contraceptives, which have been associated with side effects, contrarily to acupuncture (Elizabeth Jedel, 2011).

Also, clomiphene citrate, already previously described as an exogenous gonadotropin therapy used to induce ovulation in PCOS patients, and laparoscopic ovarian drilling are commonly used to induce ovulation in women with PCOS, but given the often reported negative side effects, the use of acupuncture can be beneficial. Besides, although being considered the first-line treatment to induce ovulation in women with PCOS, a compilation of published results from 5268 patients revealed an ovulation rate of 73% per cycle per patient, but a small rate of 36% of pregnancy and an even smaller rate of live birth of 29% per patient, during 6 months of treatment (Hongying Kuang, 2013). An RCT conducted by Julia Johansson et al., in 2013, with two treatments/week of 30 minutes of acupuncture during 10-13 weeks, aimed to assess if frequent acupuncture treatment could improve ovulation frequency, LH secretion and pulsatility, and sex steroids secretion in lean/overweight PCOS patients. Repeated acupuncture treatments with both manual and electrical stimulation resulted in a higher ovulation frequency during the treatment period than in the control group, but it is not associated with a decrease in the LH pulsatility pattern. Instead, this effect on ovulation seems to be more related with the decrease in circulating sex steroids, their precursors and metabolites, and also a decrease in inhibin B. There was no difference between groups in terms of menstrual cycle pattern prior to the start of the study, which clearly indicates that the higher ovulation frequency in acupuncture group is attributed to the treatment effect (Johansson J, 2013). In PCOS women with ovulatory dysfunction, several nonrandomized trials revealed that repeated acupuncture treatments decreased total testosterone and other sex steroids levels, reduced LH/FSH ratio, and improved menstrual frequency without side effects (Hongying Kuang, 2013).

A quasi-randomized study made in 2010 by Lai MH et al., aim to assess the effect of abdominal acupuncture on the endocrine and metabolic level in obesity-type PCOS patients, when compared to metformin treatment. Abdominal acupuncture performed once daily during 6 months improved menstrual frequency and decreased circulating testosterone more effectively than metformin during the same period. Also BMI, waist-to-hip ratio, fasting blood glucose and ovarian volume were reduced significantly in both groups, and menstrual frequency increased evidently in abdominal acupuncture and in metformin groups. The effect of abdominal acupuncture was significantly superior to the effect of metformin group in down-regulating BMI, and also superior in up-regulating menstrual frequency. Both groups had decreased levels of testosterone, with abdominal acupuncture being more effective when compared to metformin during the same time of treatment (Lai MH, 2010). Another Chinese study, made in 2018 by Xu J. and Zuo Y, aim to assess the efficacy of acupuncture as adjunctive treatment on infertility patients with PCOS, comparing the different outcomes of acupuncture combined with medication and

medication alone. The patients were divided into two groups: in the control group patients were medicated with Diane-35 (an oral contraceptive used mainly in the treatment of androgen-dependent diseases in women), and an observation group, where patients were given the same dose of Diane-35 plus acupuncture during the non-menstruation period, once every two days, 3 treatments per week. Treatment in both groups had the duration on 2 menstrual cycles. After the treatment, BMI, LH and testosterone were lower in the observation group, while only LH reduced in control group. Ovulation rate was 93,3% in observation group, which was higher than 80% in control group. As for clinical pregnancy, after treatment, the rate of clinical pregnancy was 43,3% in observation group, which was higher than 33,3% of control group (Xu J, 2018).

As it's been said before, women with PCOS have a three to sevenfold increased risk of developing type 2 diabetes, which represents a major health burden. Independently of total body weight, insulin sensitivity is approximately 40% lower in women with this syndrome. The potential role of acupuncture in the treatment of women with PCOS and IR has not yet been determined with conclusive data, and this is an important area of investigation, since most women with PCOS require long term treatment for this symptom (Zheng Y, et al., 2015). A review found that acupuncture can correct various metabolic disorders contributing to the development of IR, such as hyperglycemia, overweight, hyperphagia, hyperlipidemia, inflammation, altered activity of the sympathetic nervous system and insulin signal defect. Thus, acupuncture may have the potential to improve insulin sensitivity. This publication provided limited, but clinical evidence, in support of the effectiveness of acupuncture in IR (Liang F., 2010). Obviously, more well-designed, evidence-based clinical RCT are needed to confirm the effects of acupuncture in IR, but numerous studies have demonstrated that acupuncture could correct some metabolic disorders associated with IR and also has the potential to improve insulin sensitivity. This study made by Liang F. in 2010, however, was not performed in IR PCOS patients, so one cannot conclude that the same improvement would happen in PCOS women with IR (Liang F., 2010). Some other findings suggest that EA or manual stimulation acupuncture may affect metabolism in different ways: while EA stimulation affects more functional signaling pathways related to insulin sensitivity, manual stimulation of acupuncture needles has a greater effect on glucose tolerance in PCOS model in rats (Li et al. Trials, 2017). The mechanisms underlying these results remains to be elucidated. This study protocol for a RCT aiming to evaluate the effect of acupuncture on the insulin sensitivity in PCOS combined with IR, emerged from a pilot study of acupuncture affecting the insulin sensitivity in 81 cases of women with PCOS with IR, showing that acupuncture may have a significant effect on improving the insulin sensitivity in these patients. Although this study has confirmed that the combination of manual and low

frequency EA can improve insulin sensitivity, hormone levels and anxiety situation, it had the limitation of being a single center study without comparison groups (Li et al. Trials, 2017).

One experimental and feasibility study made by Stener-Victorin et al. in 2016, tested the hypothesis of low-frequency EA treatment for 5 months, 3 times/week, in improving glucose regulation and androgen levels in 17 obese women with PCOS. After 5 weeks of treatment, no changes in whole glucose homeostasis was observed, but HbA_{1c} decreased by 9,5%. As for circulating testosterone, a decreased in 22% was seen and dihydrotestosterone decreased by 12%. Curiously, this changes seen in this study occurred with no changes in whole body weight and waist circumference (E. Stener-Victorin, 2016). Considering the small sample size and the design of the study, and knowing that definite conclusions cannot be made or taken from studies of this dimension, the results of this study show that EA may be important in the control of insulin resistance in women with PCOS, and could be a good tool in preventing or, at least delay, the beginning of a type 2 diabetes mellitus. In the very least, it shows the necessity and the reason why some RCTs should be made in this area of interest.

In women, as it was explained before, AMH is an important regulator of folliculogenesis in the ovaries. It is secreted by granulosa cells of the ovarian follicles and its levels are elevated 2 to 3 times in women with PCOS, when compared with normo-ovulatory women (Deepika Garg, 2016). When the antral follicles become larger and the follicles begin their FSH-dependent cyclic recruitment process, AMH expression decreases. This balance between AMH and FSH is essential in human ovarian folliculogenesis (Yin Shi, 2019). Specifically, some animal and clinical studies have shown that EA promotes follicle development and corrects reproductive endocrine dysfunction in ovaries by regulating the functions of the hypothalamus, pituitary and ovaries, but the exact mechanisms implicated in this influence have yet not been clarify (Yin Shi, 2019). This study of 2019 aiming to demonstrate whether EA improves hyperandrogenism, follicular development dysfunction, and ovulation by regulating the imbalance between FSH and AMH in PCOS rats, showed that EA increases the expression of the FSH receptor and decreases the expression of AMH and AMH receptor II, to ameliorate androgen excess and anovulation in PCOS. Some other studies showed that rats exposed to letrozole develop metabolic and reproductive alterations that resemble the features observed in women with PCOS. So low-frequency EA stimulation can potentially improve reproductive endocrine disorders and follicle arrest in PCOS, by decreasing the excessive expression of AMH to regulate FSH and AMH imbalance (Yin Shi, 2019). This plays an important role in fertility problems associated with

this syndrome, which has to be seen has a whole, and not as independent symptoms that coincidently occur. This means, that in order to achieve fertility, the more symptoms are controlled, the better.

Altogether, there are few quality studies studying the effect of acupuncture in the AMH levels. Yet another study, conducted by Leonhard et al. (2015) aimed to investigate whether EA or physical exercise could influence serum AMH levels, antral follicle count or ovarian volume in women with PCOS. This study was a secondary analysis of a prospective, randomized controlled trial made by Jedel et al., already described and analyzed above. In this study, AMH was measured by recollecting a blood sample, in the morning after fasting overnight at baseline (independently of cycle day, given majority of women had irregular cycles), after 16 weeks of treatments and at follow up after 32 weeks. Antral follicle count and ovarian volume were assessed by magnetic resonance image at baseline and after 32 weeks at follow up. Results showed that between baseline and after 16 weeks – which can be indicative of a short-term effect – serum AMH reduced significantly in the EA group by 17,5%, and differed from the change in the exercise group. Revealing also a long-term effect, this AMH levels remained significantly decreased by 15% at follow-up of 32 weeks, but did not differ from the exercise or the no intervention group. As for ovarian volume assessment, there was a 8,5% decreased between baseline and follow-up in the EA group. The exercise group proved to be effective in decreasing antral follicle count, given an 11,7% reduction in the follow-up assessment (Henrik Leohnardt, 2015). Since there are few studies on lifestyle interventions and ovarian morphology in humans, this results can be important in this area of research, indicating that more research can be made. This study, was the first to show that low-frequency EA can reduce AMH levels and ovarian volume in women with PCOS.

On another hand, another study (Jason Franasiak, 2012) that was a secondary analysis from another randomized, double-blind, sham-controlled clinical trial (Lisa M. Pastore, 2011), studied the impact of EA on AMH concentrations in women with PCOS. The original study was conducted by Pastore et al. in 2011, were a total of 84 women were randomly assigned into true acupuncture group (40 women) and sham acupuncture group (44 women), in a 5 months protocol, aiming to assess whether true, sham or both acupuncture protocols could normalize pituitary gonadotropin hormones and increases ovulatory frequency in women with PCOS. Besides the improvement on LH/FSH ratio, the results were similar in the sham and true acupuncture interventions. Also, ovulation rates were similar in both groups. So they were unable to discern a difference between the true and sham acupuncture protocols for these women with PCOS in this specific study. As said

above, the secondary analysis of this original article was conducted by Jason Franasiak et al. in 2012, aiming to examine the impact of EA on AMH concentration in women with this syndrome. As a result, they found no significant changes in AMH levels after the use of active or sham acupuncture (Jason Franasiak, 2012).

A RCT made by Zheng et al. in 2013 had the goal of assessing the effectiveness of abdominal acupuncture at the endocrine and metabolic level in patients with obesity-type PCOS (Zheng YH, 2013). In order to assess the metabolic and endocrine function in overweight and obese women, 86 women with a diagnoses of PCOS and with a BMI ≥ 25 kg/m^2 were randomly distributed between receiving 6 months of abdominal acupuncture (once a day); or oral metformin daily, also for 6 months. The following markers were measured at baseline and after 6 months of both treatments: BMI, Waist-Hip-Ratio (WHR), ovarian volume, menstrual frequency, LH, testosterone, FSH, fasting blood glucose, 2-hour postprandial blood glucose and insulin, fasting insulin, total cholesterol and both low-density lipoprotein (LDL) and high-density lipoprotein (HDL). After 6 months, BMI, WHR, ovarian volume, LH/FSH ratio, testosterone, LDL, total cholesterol, triglycerides, fasting blood glucose, 2-hour postprandial blood glucose and insulin and fasting insulin were significantly reduced in both groups. Menstrual frequency and HDL had significantly increase in also both groups. FSH increased in both groups, but not significantly. The only measures in which abdominal acupuncture showed to be considerably advantageous over the metformin group were the reduction of BMI and WHR and in improving menstrual frequency. As showed, both approaches improved endocrine and metabolic function of patients with the obesity-type PCOS (Zheng YH, 2013). One thing that is important to remember, is that acupuncture shows fewer side effects when compared to pharmacological approaches, so one should consider if this could be a good alternative treatment in controlling not all, but at least some symptoms associated with the syndrome.

A study conducted by Stener-Victorin et al., in 2004, evaluated the effect of EA stimulation of different frequencies and intensities on ovarian blood flow in rats with steroid induced polycystic ovaries (PCO). The ovary, being a highly vascularized organ, benefits from a well and good maintenance of high blood flow in order to the ovulatory process occur smoothly. The sympathetic nerves appear to be distinctly involved in the control of ovarian secretory activity and are important for the overall regulation of ovarian function. This study concluded that not only low-frequency EA at strong intensity can increase ovarian blood flow in rats with steroid induced PCO, it seems to be more effective than high frequency EA stimulation, which goes in harmony with what other authors have been concluding about this matter. Of

course, one cannot translate this findings into human PCOS model, but this may encourage more research in this area of interest (Elisabet Stener-Victorin R. K., 2004).

As it was described before in the etiology and symptoms associated with PCOS, this women may be at increased risk of pro-thrombotic state. This can also be reflected by increased activity of circulating plasminogen activator inhibitor (PAI-1) and fibrinogen, independently of BMI, because both are markers that can be used to evaluate CDV health or, in this case, disease. This factors can contribute to increased risk of CVD, and this circulating homeostatic factors are influenced by insulin resistance, which has been already well described in this syndrome. Also, the association of PCOS with high sympathetic nerve activity is another factor that can contribute to the risk of CVD and to the etiology of the syndrome. Having this in mind, Elisabet Stener-Victorin et al. made, in 2012, a secondary analysis of the prospective, randomized controlled trial conducted by Jedel et al. in 2011, already described in this review. As already been described before, in this original study 84 women were randomized into 3 different groups: 33 women received 16 weeks of low-frequency EA group (14 treatments in total), 34 women were allocated in exercise group (exercise for 16 weeks at least 3 times/week) and 17 women allocated in no intervention group (control). This secondary conducted by analysis aimed to evaluate the possible effect of low-frequency EA and physical exercise on markers of coagulation and fibrinolysis, insulin sensitivity, and adipose tissue characteristics in women with PCOS, in order to investigate if acupuncture could decreased the risk of CVD in this patients. Between baseline and week 16, serum PAI-1 decreased significantly in the low-frequency EA group and differed significantly from the levels of the exercise group and the no intervention group. At the 32 week follow-up, serum PAI-1 remained lower in the EA group when compared to the other groups (Elisabet Stener-Victorin F. B.-H., 2012). This study showed that EA at low-frequency may be able to decrease the possible pro-thrombotic state of this patients with PCOS, but more research is needed before taking definite conclusions.

So, adding to the overall use of acupuncture in PCOS, one of the goals is directed towards improving the health-related quality of life by alleviating co-morbid psychiatric symptoms and preventing long-term physical and psychiatric complications. Lifestyle modifications, diet alone and diet with exercise programs showed to be effective in decreasing depression and improved menstrual problems, body weight problems and emotional problems (Elisabet Stener-Victorin G. H., 2013). Acupuncture with manual stimulation and low-frequency EA has been showed to be effective in the treatment of major depression disorders in women without PCOS, in women with depression during pregnancy and in post-partum. Because PCOS is a chronic disease usually accompanied by symptoms of anxiety and depression,

which impairs health related quality of life, the need to evaluate rather acupuncture could improve this symptoms emerged. A secondary analysis of a prospective RCT in women with PCOS showed that health-related quality of life was greater in the acupuncture group, improving social functioning, energy/vitality, and general health perception and mental component. The emotional component in PCOS patients also improved significantly after the 16 week acupuncture intervention, which, most importantly, persisted at 32 weeks. The results in this study are in line with previous reports indicating that acupuncture may indeed reduce symptoms of depression, anxiety and improve health related quality of life in other conditions than PCOS (Elisabet Stener-Victorin G. H., 2013). The effect of acupuncture was more pronounced on anxiety symptoms and there was a clear tendency of decrease within the acupuncture group immediately after the treatment period but it did not reach statistical significance.

Based on results presented by Wu et al. in 2016, in a review of a total 31 RCTs that included 2371 women with PCOS, out of the 31 RCTs , only one trial fail to find significant differences in the outcomes between true and sham acupuncture – this is the Franasiak et al. trial made in 2012 that was described above; in the remaining 30 RCTs, there was an improvement in at least one of the indicators of PCOS after acupuncture treatment, when compared with control groups. Menstrual frequency, hormones (FSH, LH, FSH/LH ratio, and testosterone), anthropometrics, insulin-sensitivity, blood lipids, and fertility were the main measurements used to assess the effects of acupuncture in the PCOS patients (Yan Wu, 2016).

Author	Year	Study	Results
Stener-Victorin et. al	2000	Non-randomized, longitudinal, prospective study	EA increased ovulation rates
Elizabeth Jedel et. al	2011	Prospective randomized controlled trial	EA decreases serum testosterone, hyperandrogenism markers and acne. EA increase menstrual frequency
Julia Johanson et al.	2013	Randomized controlled trial	EA increased ovulation frequency EA decreased circulating sex steroids
Lai MH et. al	2010	Quasi-randomized study	EA increased menstrual frequency

			EA decreased circulating testosterone, BMI and WHR, fasting blood glucose and ovarian volume
Xu J. and Zuo Y	2018	Clinical study	EA decreased BMI, LH, testosterone EA increased ovulation rate and clinical pregnancy rate
Liang F.	2010	Review article	EA may improve hyperglycemia, overweight, hyperphagia, hyperlipidemia and inflammation
Stener-Victorin et. al	2016	Experimental and feasibility study	EA decreased HbA_1C and sexual steroids
Yin Shi	2019	Experimental animal study	EA increased the expression of FSH receptor and decreased the expression of AMH and AMH receptor II EA improved anovulation and androgen excess
Leonhard et. al	2015	Secondary analysis of a prospective, randomized controlled trial	EA decreased significantly serum AMH and decreased ovarian volume
Jason Franasiak	2012	Secondary analysis of a randomized, double-blind, sham-controlled clinical trial	EA improved LH/FSH ratio No significant changes in AMH levels
Zheng et. al	2013	Randomized controlled trial	EA significantly decreased BMI, WHR, ovarian volume, LH/FSH ratio, testosterone, LDL, total cholesterol, triglycerides, blood glucose, isulin

			EA increased significantly menstrual frequency and HDL, and increased FSH
Stener-Victorin et. al	2004	Animal experimental study	EA increased ovarian blood flow
Elisabet Stener-Victorin et. al	2012	Secondary analysis of a prospective randomized controlled trial	EA decreased significantly serum PAI-1
Elisabet Stener-Victorin	2013	Secondary analysis of a prospective randomized controlled trial	EA improved health-related quality of life, social functioning, energy and vitality, general health perception and mental component

Table 4 – Results of the evaluated studies on the effect of acupuncture on PCOS symptoms and anovulation.

A major weakness of research in acupuncture and of acupuncture studies is the variability of sham methods (placebo needles, superficial needling, e.g.), the location and depth of needling and the number and duration of treatments. Increasing the treatment frequency and the number of treatments leads to higher ovulation frequency, indicating the importance of correct treatment dose (Zheng Y, et al., 2015). Also, the double randomization in acupuncture studies is very difficult to achieve, since acupuncture in which both participant and practitioner are unaware of the treatment are practically impossible to conduct (Elizabeth Jedel, 2011).

DISCUSSION

Having in mind that the main goal of this review was to assess the studies that have been made in the area of acupuncture and the treatment of infertility associated with PCOS, several studies described the also beneficial effect of acupuncture in the management of other symptoms and co-morbidities associated with the syndrome. While writing the thesis, the more I read about the syndrome, the more I realized that it seemed important to make a summary of the effect of acupuncture in some of the other aspects of this syndrome. Learning about fertility or, in this case, infertility clarify the idea that this disease can be caused by a single problem of the female or male reproductive tract, but it also can be a dysregulation of the whole body, where diet, exercise and changes in lifestyle might be mandatory in the treatment. Saying this, while treating infertility associated with the PCOS, therapists should have in mind the treatment of the whole, of each symptom, and not focus exclusively on the reproductive issue. As it was said in the beginning, weight losses around 5% showed to increase fertility outcomes in this patients, which is a good example that small improvements in whole health can improve the fertility problem associated with the syndrome.

Also, health care providers who treat and care for women with infertility or PCOS should be aware that their patients may face challenges beyond symptoms of the reproductive system. Symptoms related to anxiety, depression and possible substance abuse should be assessed in consultation, and assessing the psychiatric needs of infertility patients is crucial to provide comprehensive care to this unique patient population. Appropriate patient counseling should also include healthy lifestyle modifications in women with PCOS in order to improve their overall health, and alleviate some of the health risks associated with the syndrome (Hanson, et al., 2017).

The etiology, definition and diagnosis of this syndrome is still, until today, a matter of debate, although PCOS has been recognized for more than 70 years (Julia Johansson and Elisabet Stener-Victorin, 2013). Despite this, it became clear that the vicious circle of PCOS features and symptoms aggravating each other is mediated mainly by androgens, insulin, insulin resistance, weight gain and obesity, and some other factors. In order to improve health and quality of life in women with PCOS, this vicious circle must be broken. Although pharmacological treatment may be effective, they are often associated with negative side-effects that can diminish the health-related quality of life in this patients. Several clinical and animal experimental studies that have already been made indicate that acupuncture is beneficial for ovulatory dysfunction in PCOS, and some studies showed improvements in some other PCOS-related symptoms (Julia Johansson and Elisabet Stener-Victorin, 2013).

After analyzing this studies, one can conclude that acupuncture may be considered a good tool in improving PCOS and PCOS-related symptoms as an adjuvant therapy, but for some symptoms, acupuncture may be used as the main therapy. Despite this, the mechanisms of action of acupuncture in the treatment of anovulatory infertility and other symptoms associated with the syndrome are still poorly understood, and this naturally represents a problem for the scientific community. More research is needed in order to affirm the role of acupuncture and TCM in the treatment of the PCOS, and to be possible the implementation of acupuncture in the conventional treatment strategy, it is also mandatory to compare it with the first-line pharmaceutical options and first-line lifestyle modifications (Julia Johansson and Elisabet Stener-Victorin, 2013). In this way, some good quality study protocols have proposed this, and some have already been accepted by the responsible scientific committees.

A previously mentioned pilot study of a 5 week acupuncture treatment with combined manual and low-frequency EA stimulation, showed improvements in whole-body glucose homeostasis in insulin-resistant women with PCOS (E. Stener-Victorin, 2016), but this pilot study was an uncontrolled trial, which underlined the urgent necessity for more controlled and well-designed studies. A new study protocol of a combined multinational cross-sectional case-control study and randomized controlled trial has already been approved (Elisabet Stener-Victorin et al., 2019). This study protocol aims to evaluate if acupuncture treatment with lifestyle modification, metformin treatment with lifestyle modification or lifestyle modification alone have different outcomes in insulin sensitivity and related symptoms in overweight/obese women with PCOS. This will be assessed in the end by the main outcome: measurement in HbA_{1c} concentrations. In this way, patients will be randomized into 3 groups for 4 months: group 1 with EA 2-3 times a week plus lifestyle management; group 2 with metformin therapy daily plus lifestyle modification and; group 3 with lifestyle alterations alone (Elisabet Stener-Victorin et al., 2019).

Investigation is increasing and more studies are on the way. Another study protocol for a RCT aims to compare if a pretreatment of acupuncture followed by letrozole compared with letrozole alone produce different outcomes on the live birth rate in women with PCOS. The protocol study, conducted by Juan Li et al. in 2016, has already been approved by the ethics committees. A systematic-review made by Lim CE, Ng RW, Xu k, et al., published in the Cochrane Database of Systematic Reviews in 2016, concluded that there were no studies designed to investigate live birth in PCOS patients. Live birth is, for obvious reasons, one of the most important clinical outcomes is this patients (Juan Li et al., 2016). One other study protocol was proposed by Li et al. in 2017, and was already accepted by scientific

committee. This is a protocol for a multi-center, controlled, double-blind, and randomized clinical trial with the aim to evaluate the effect of acupuncture on the insulin sensitivity in women with PCOS and associated IR. This will be analyzed by randomizing 342 patients in 3 groups: true acupuncture plus placebo metformin; sham/placebo acupuncture plus metformin; placebo acupuncture plus placebo metformin (Li et al. Trials, 2017).

This serves to show the need for more investigation in this area, given the numerous amount of factors, outcomes and parameters that can be analyzed in this area of interest.

The use of acupuncture is increasing in the West, and so well-designed, good quality research should be conducted these days: this is highly necessary, as Hopton et al. reported in 2012 that “fertility problems” are the second main reason for which people seek acupuncture treatment in the UK (Hopton AK, 2012).

Despite the lack of sufficient data to conclude that acupuncture is well established in the treatment of infertility or in the infertility associated with PCOS, the truth is that acupuncture has been, until now, one of the very few interventions that in meta-analysis or in some RCT, showed to improve the outcomes of some ART treatments (Andersen D, 2010). Having in mind that not all the studies addressed in this review have the best quality in order to provide good and high quality evidence in science and health, the fact that women are using acupuncture should not be ignored (Elisabet Stener-Victorin , 2013), and the existence of so many studies and so many success cases is, at the very least, a valid reason to motivate the investment of money and time on some researchers in this area of interest (E. Stener-Victorin, 2008). Knowing that there are no relevant randomized controlled trials on the use of acupuncture to treat metabolic abnormalities in women with PCOS, a number of experimental studies indicate that acupuncture may improve metabolic dysfunction, such as hyperglycemia, overweight, hyperphagia, hyperlipidemia, inflammation, and insulin signaling defects, all of these contribute to the development of insulin resistance. Hopefully in the near future, new well-designed randomized controlled studies investigating the efficacy of acupuncture on menstrual and ovulatory dysfunction will be made, both in women who want and don’t want to conceive (Elisabet Stener-Victorin , 2013).

The reason why some meta-analysis addressing the same question produce such different results is an often asked question, and one of the main problems in area of acupuncture research. Some of the most common reasons for these discrepancies in all areas of scientific research are differences in inclusion criteria, methods of searching the literature, data extraction and data analysis (Cui Hong Zheng, 2012). In acupuncture, this can be worsen by the fact that different acupuncture schemes may result in different clinical effects,

even slightly different changes may lead to different results. Also, most of the acupuncture treatments protocols seemed too short to completely correct infertility problems caused by long-term insufficiencies or imbalances. Another problem in acupuncture research is the fact that, in most cases, acupuncture trials lack in syndrome differentiation and individual diagnosis, which is the main diagnosis method used in TCM. TCM diagnosis is, as it's been said before, based on signs and symptoms shown at the moment, along with the different pathogenic factors, constitution and emotional status of the patient, which means that individualized diagnosis can change the treatment selection completely. Furthermore, two women with PCOS despite having the same disease, can have different diagnosis based on their constitution and, for example, their emotional status, leading to two different acupoints selection. Some experts predict that better therapeutic outcomes would be achieved if individualized diagnosis and consequently, individualized acupuncture treatments, were tested (Cui Hong Zheng, 2012). In fact, a study design for a multicenter, randomized, double-blinded, controlled trial for women with PCOS in China, already aims to make a TCM diagnosis before the start of the treatment, allocating the patients into four different categories, with the goal of a more individualized diagnosis and, consequently, a more individualized treatment. This is expected to improve the outcomes of this RCT. This clinical trial as the main goal of testing the efficacy of acupuncture with or without clomiphene citrate on live birth in PCOS women suffering from ovulatory dysfunction (Hongying Kuang, 2013).

In addition, the duration of the whole treatment and the interval between treatments is also a variable that needs consideration, because it can produce different outcomes in acupuncture intervention. As an example, while acute main require one or two sessions of treatment, chronic diseases (including disorders of the endocrine system) may need more sessions to achieve good outcomes, for weeks or even months (Fan Qu et al., 2017). As some diseases may need pharmacological treatment in long-term, the same can happen with acupuncture in order to maintain the good quality of life related to chronic diseases.

It is fair to say that PCOS is one of the most complex and challenging endocrine and reproductive disorders. Having in mind the inherited factor in many cases, in others the etiology is not clear, but it may be related to the way women are behaving with their bodies: anorexia, excessive weigh and obesity, poor diet, stress, lack of physical exercise or excessive exercise, and other adverse influences to the human body. Chinese medicine is well established to find ways of treating and ameliorating the comorbidities the body shows with this syndrome, including infertility. By gathering all the signs and symptoms given by each body, TCM could be a good, effective and holistic help to treat these patients,

addressing the disharmony of each women in a more personal and customized way (Lyttleton, 2013).

Acupuncture carries many potential advantages when compared with the conventional treatments, like low-cost, few complications and, most importantly, the possibility of a more personalized and individualized treatment (Liang F., 2010). The most common adverse effects seen in acupuncture treatment are minor, like needle pain, tiredness and little bleeding, but the manifestation of such effects is relatively low (Ernest Hung Yu Ng, 2008). Nevertheless, acupuncture is a safe intervention in the hands of competent practitioners. Yamashita et al. prospectively evaluated 55,291 acupuncture treatments administered by acupuncturists with medical training and documented only 64 adverse events, which represents a really low rate of 0.12%. All of these adverse events were minor, the most common being bruising, dizziness, perspiration, discomfort, and dermatitis. This adverse effects of acupuncture may be avoided by using the procedure properly. In addition to proper knowledge of the anatomy, appropriate antiseptic practice by practitioners is important (Liang F., 2010). Therefore, and with this in mind, acupuncture can indeed be considered a good alternative for PCOS patients. Knowing that no approach can promise results, the use of acupuncture is associated with very few side effects, and is relatively well accepted by the patients most of the time. Thus, patients with PCOS should seek the help of acupuncture as, regardless of the results, this therapy is not associated with serious negative effects, and any improvement that it provides should be well received by patients, improving their health-related quality of life.

CONCLUSION

As a nutritionist and a TCM therapist, the area of gynecology seemed a good area of health care to specialize, in which the two professions could come together to achieve the same goal: improve quality of life and aid to treat or alleviate the symptoms of women with gynecological problems. After writing this theses this became even clearer: the area of gynecology is definitely an area of personal interest, and it's my personal belief that nutrition advice, diet and lifestyle modifications and TCM together can have a great impact on these patients. In alignment with this, and knowing that it is not the goal of this master thesis, after studying the subject of PCOS and infertility and the role that TCM could play in this disease, the idea for a future investigation in this area of interest emerged, combining nutrition, TCM and PCOS patients. As it was described before, PCOS patients have increased risk of overweight or obesity. This predisposition to obesity also increases, consequently, the risk for some other PCOS related co-morbidities, such as IR, T2DM, glucose intolerance, disturbed menstrual cyclicity and anovulation, among others. It is now well established that even small weight losses can improve the overall health and, in particular, the menstrual cycle and ovulation patterns in these women. As a continuum for this master's thesis, it would be of great interest to investigate, in a PhD final thesis, in what way TCM and nutrition could improve the menstrual cyclicity and ovulation patterns in these patients, both alone or combined. Independently of this being possible or not, the process of doing this master's thesis was enough to increase my interest in this area of health care, particularly the idea of being able to treat and improve the symptoms associated with gynecological problems, thus improving the quality of life of all women who struggle with gynecological diseases daily.

BIBLIOGRAPHY

Adam H. Balen, L. C.-V. (2016). The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidelines. *Human Reproductive Update*, Vol. 22, No. 6, pp 687-708.

Alice D. Domar, I. M. (2009, March). The impact of acupuncture on in vitro fertilization outcome. *Fertility and Sterility, 91*(3).

Andersen D, L. K. (2010). Acupuncture on the day of embryo transfer: a randomized controlled trial of 635 patients. *Reproductive BioMedicine Online, 21*, 366-372.

Anuja Dokras, E. S.-V. (2018). Androgen Excess - Polycystic Ovary Syndrome Society: position statement on depression, anxiety, quality of life, and eating disorders in polycystic ovary syndrome. *Fertility and Sterility*, Vol. 109, No. 5.

Baillargeon JP, I. M. (2003). Insulin sensitizers for polycystic ovary syndrome. *Clinical Obstetrics and Gynecology* , 46(2):325-340.

C.Gnoth, E. P.-H. (2005). Definition and prevalence of subfertility and infertility. *Human Reproduction* , Vol.20, No.5, pp.1144-1147.

Caroline A Smith, S. G. (2012). Using a Delphi consensus process to develop an acupuncture treatment protocol by consensus for women undergoing Assisted Reproductive Technology (ART) treatment. *BMC Complementary & Alternative Medicine, 12*(88).

Chris Kite, I. M. (2019). Exercise, or exercise and diet for the management of polycystic ovary syndrome: a systematic review and meta-analysis. *Kite et al. Systematic Reviews*, 8:51.

Conselho Nacional da Procriação Medicamente Assistida. (2019, Agosto 30). *Conselho Nacional de Procriação Medicamente Assistida*. Retrieved from cnpma web site: http://www.cnpma.org.pt

Conselho Nacional de Procriação Medicamente Assistida. (Setembro de 2017). *Relatório de actividade desenvolvido pelos centros de PMA em 2015.*

Cox, E., & Takov, V. (2019, February 8). Embryology, Ovarian Follicle Development.

Cui Hong Zheng, M. M. (2012). The Role of Acupuncture in Assisted Reproductive Technology. *Evidence-Based Complementary and Alternative Medicine*.

Deepika Garg, R. T. (2016). The role of AMH in the pathophysiology of polycystic ovarian syndrome. *Reproductive BioMedicine Online, 33*, 15-28.

Deidre D. Gunn and Wright Bates, M. (2016). Evidence-based approach to unexplainde infertility: a systematic review. *Fertility and Sterility, Vol. 105, No. 6*.

Drummond, A. E. (2006). The role of steroids in follicular growth . *Reproductive Biology and Endocrinology*.

E. Stener-Victorin, E. J. (2008). Acupuncture in Polycystic Ovary Syndrome: Current Experimental and Clinical Evidence. *Journal of Neuroendocrinology, 20*, 290-298.

E. Stener-Victorin, M. M. (2016). Changes in HbA1c and circulating and adipose tissue androgen levels in overweight-obese women with polycystic ovary syndrome in response to electroacupuncture. *Obesity Science and Practice*. doi:10.1002/osp4.78

Eijkmans J.C. Marinus, F. V. (2014). Too old to have have children? Lessons from natural fertility populations. *Human Reproduction, vol. 29, No. 6*, 1304-1312.

Elisabet Stener-Victorin . (2013). Hypothetical physiological and molecular basis for the effect of acupuncture in the treatment of polycystic ovary syndrome. *Molecular and Cellular Endrocrinology, 373*, 83-90.

Elisabet Stener-Victorin et al. (2019). Acupuncture or metformin to improve insulin resistance in women with polycystic ovary syndrome: study protocol of a combined multinational cross sectional case-control study and a randomised controlled trial. *BJM Open*. doi:10.1136

Elisabet Stener-Victorin, F. B.-H. (2012, February). Effects of acupuncture and exercise on insulin sensitivity, adipose tissue characteristics, and markers of coagulation and fibrinolysis in women with polycystic ovary syndrome: secondary analyses of a randomized controlled trial. *Fertility and Sterility, 97*(2).

Elisabet Stener-Victorin, G. H. (2013). Acupuncture and physical exercise for affective symptoms and health-related quality of life in polycystic ovary syncrome: secondary analysis from a randomized controlled trial. *BMC Complementary and Alternative Medicine, 13*(131).

Elisabet Stener-Victorin, R. K. (2004). Effect of electro acupuncture stimulation of different frequencies and intensities on ovarian blood flow in anaesthetized rats with steroid-induced polycystic ovaries. *Reproductive Biology and Endocrinology, 2*(16).

Elizabeth Jedel, F. L.-K.-V. (2011). Impact of electro-acunpuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. *American Journal of Physiology-Endocrinology and Metabolism, 300*, 37-45.

Emily Evans-Hoeker, D. A. (2013). Cervical Mucus monitoring prevalence and associated fecundability in women trying to conceive. *Fertility Sterility* , 100(4): 1033-1038.

Eric Manheimer et al. (2008). Effects of acupuncture on rates of pregnancy and live birth among women undergoing in vitro fertilisation: a systematic review and meta-analysis. *BMJ*.

Eric Manheimer, D. v. (2013). The effects of acupuncture on rates of clinical pregnancy among women undergoing in vintro fertilization: a systematic review and meta-analysis. *Human Reproduction Update, Vol. 19*(No. 6), pp. 696-713.

Erin K. Barthelmess, a. R. (2015). Polycystic ovary syndrome: currant stratus and future prespective. *Front Biosci (Elite Ed)*, 6: 104-119.

Ernest Hung Yu Ng, W. S. (2008). The role of acupuncture in the management of subfertility. *Fertility and Sterility, 90*(1).

Fan Qu et al. (2017). Use of electroacupuncture and transcutaneous electrical acupoint stimulation in reproductive medicine: a group consensus. *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology), 18*(3), 186-193.

G.F. Homan, M. D. (2007). The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Human Reproduction Update*, Vol. 13, No. 13, pp. 209-223.

Greten. (2007). *Clinical Subjects - Gynaecology* (5th ed. 2011 ed.). Unrevised course version.

Greten, H. (2007). *Understanding TCM - The Fundamentals of Chinese Medicine Part I* (6th rev. ed. 2013 ed.). Heidelberg School Editions.

Hanson, B., Johnstone, E., Dorais, J., Silver, B., Peterson, C. M., & Hotaling, J. (2017). Female Infertility, infertility-associated diagnoses, and comorbidities: a review. *J Assist Reprod Genet* , 167-177.

Hempen, C. H. (2006). *Pocket Atlas of acupuncture.* New York: Thieme.

Henrik Leohnardt, M. H.-K.-V. (2015). Serum anti-M€ullerian hormone and ovarian morphology assessed by magnetic resonance imaging in response to acupuncture and exercise in women with polycystic ovary syndrome: secondary analyses of a randomized controlled trial. *Acta Obstetricia et Gynecologica Scandinavica, 94*, 279–287.

Hillier, S. (2001). Gonadotropic control of ovarian follicular growth and development. *Molecular and Cellular Endocrinology*, vol. 179, pp. 39-46.

Hongying Kuang, Y. L.-V. (2013). Acupuncture and Clomiphene Citrate for Live Birth in Polycystic Ovary Syndrome: Study Design of a Randomized Controlled Trial. *Evidence-Based Complementary and Alternative Medicine*.

Hopton AK, C. S. (2012). Acupuncture in practice: mapping the providers, the patients and the settings in a national cross-sectional survey. *BMJ Open*.

J. Boivin, L. B. (2017). International estimates of infertility prevalence and treatment-seeking: potentital need and demand for infertility medical care. *Human Reproduction*, Vol. 22, No. 6, pp. 1506-1512.

Jacky Boivin, L. B. (2007). International estimates of infertility prevalence and demand for infertility medical care. *Human Reproduction*, 22: 1506-1512.

Jamie L. Bigelow, D. B. (2004). Mucus observations in the fertile window: a better predictor of conception than timing intercoure. *Human Reproduction* , Vol. 19, No. 4, pp. 889-892.

Jason Franasiak, S. L. (2012). Longitudinal Anti-Mullerian Hormone inWomen with Polycystic Ovary Syndrome: An Acupuncture Randomized Clinical Trial. *Evidence-Based Complementary and Alternative Medicine*. doi:10.1155

Joham A.E., B. J. (2014). Contraception use and pregnancy outcomes in women with polycystic ovary syndrome: data from the Australian Longitudinal Study on Woman's Health. *Human Reproduction*, Vol. 29, No. 4, pp 802-808.

Johansson J, R. L. (2013). Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial. *American Journal of Physiology-Endocrinology and Metabolism, 304*, 934-943.

Juan Li et al. (2016). Comparison of acupuncture pretreatment followed by letrozole versus letrozole alone on live birth in anovulatory infertile women with polycystic ovary syndrome: a study protocol for a randomised controlled trial. *BMJ Open, 6*. doi:10.1136

Julia Johansson and Elisabet Stener-Victorin. (2013). Polycystic Ovary Syndrome: Effect and Mechanisms of Acupuncture for Ovulation Induction. *Evidence-Based Complementary and Alternative Medicine*.

K.Dafopoulos, C. S. (2004). Changes in pituitary sensitivity to GnRH in estrogen-treated post-menopausal women: evidence that gonadotropin surge attenuating factor plays a physiological role. *Human Reproduction*, vol. 19, No. 9 pp. 1985-1992.

Kannamannadiar Jayaprakasan, M. B.-F. (2010). A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. *Fertility and Sterility*, Vol. 93, No. 3, pp. 855-864.

Kleopatra Papavasilou, E. P. (2017). Nutritional support and dietary interventions for women with polycystic ovary syndrome. *Nutrition and Dietary Supplements*, 9: 63-85.

Koeppen, B. M., & Stanton, B. A. (2009). *Berne & Levy Fisiology.* Elsevier Editor Ltda.

Kurt Krauchi, K. K.-W. (2014). Diurnal and menstrual cycles in body temperature are regulated differently: a 28-day ambulatory study in healthy women with thermal discomfort of cold extremeties and controls. *Chronobiology International*, 31(1): 102-113.

Lai MH, M. H. (2010). Effect of abdominal acupuncture therapy on the endocrine and metabolism in obesity-type polycystic ovarian syndrome patients. *Zhen Ci Yan Jiu.*, 35(4):298-302.

Lars G. Westergaard, Q. M. (2006, May). Acupuncture on the day of embryo transfer significantly improves the reproductive outcome in infertile women: a prospective, randomized trial. *Fertility and Sterility, 85*(5).

Laura G. Cooney, I. L. (2017). High prevelance of moderate and severe depressive and anxiety symptoms in polycystic ovary syndrome: a systematic-review and meta-analysis. *Human Reproduction*, Vol. 32, No. 5, pp 1075-1091.

Li et al. Trials. (2017). *Acupuncture treatment for insulin sensitivity of women with polycystic ovary syndrome and insulin resistance: a study protocol for a randomized controlled trial.* doi:10.1186

Liang F., K. D. (2010). Acupuncture: is it effective for treatment of insulin resistance? *Diabetes, Obesity and Metabolism, 12*(7), 555-569.

Lisa M. Pastore, C. D. (2011). True and Sham Acupuncture Produced Similar Frequency of Ovulation and Improved LH to FSH Ratios in Women with Polycystic Ovary Syndrome. *Journal of Clinical Endocrinology and Metabolism, 96*(10), 3143–3150.

Loza, E. M. (2012). *Tratamento Osteopático de la Mujer: Infertilidad funcional, embarazo y postparto.* Escuela de Osteopatía de Madrid: Editorial MEDOS.

Lyttleton, J. (2013). *Treatment of Infertility with Chinese Medicine* (2nd edition ed.). Churchhill Livingdtone Elsevier.

Magoffin, D. A. (2005). Ovarian theca cell. *The International Journal of Biochemistry & Cell Biology*, Vol. 37, Issue 7, pp 1344-1349.

Mélodie Vander Borght, C. W. (2018). Fertility and infertility: Definitons and epidemiology. *Clinical Biochemistry*.

Messinis, I. E. (2006). Ovarian feedback, mechanism of action and possible clinical implications. *Human Reproductiob Update*, Vol. 12, No. 5 pp. 557-571.

Messinis, I. E., Messini, C. I., & Dafopoulos, K. (2014). Novel aspects of the endocrinology of the menstrual cycle. *Reproductive BioMedicine Online*, 28, 714-722.

Neil F. Goodman, R. H. (2015). AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS, AMERICAN COLLEGE OF ENDOCRINOLOGY, AND ANDROGEN EXCESS AND PCOS SOCIETY DISEASE START CLINICAL REVIEW: GUIDE TO THE BEST PRACTICES IN THE EVALUATION AND TREATMENT OF POLYCYSTIC OVARY SYNDROME - PART 1. *Endocrine Practice*, Vol. 21, No. 11.

Oyola, M. G., & Handa, R. J. (2017 September). Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: sex differences in regulation of stress responsivity. *Stress*, 20(5):476-494.

Palermo, R. (2007). Differential actions of FSH and LH during folliculogenesis. *Reproductive BioMedicine Online*, Vol. 15. No. 13. 326-337.

Pedro-Antonio Regidor, M. K.-F. (2018). Identification and prediction of the fertile window with a new web-based medical device using a vaginal biosensor for measuring the circadian and circamensual core body temperature. *Gynecological Endocrinology*, Vol. 34, No.3, pp.256-260.

Porkert, M. (1995). *Classical Acupuncture - the stardard textbook .* Dinkelscherben: Phainon Editions & Media GmbH.

Practice Committe of the American Society for Reproductive Medicine. (2017). Role of metformin for ovulation induction in infertile patients with polycystic ovary syndrome (PCOS): a guideline. *Fertility and Sterility*, Vol. 108, No. 3.

R. Jeffrey Chang, H. C.-A. (2013). Disordered follicle development. *Molecular and Cellular Endocrinology*, 5; 373(0): 51-60.

R. Webb, J. B.-M. (2016). Follicle development and selection: past, present and future. *Anim. Reprod.*, v. 13, n. 3, p. 234-249.

Ray A., S. A. (2012). Unexplained infertility: and update and revie of practice. *Reproductive BioMedicine Online*, 24, 591-602.

Rene Ecochard, M. O. (2015). Self-identification of the clinical fertile window and the ovulation period. *Fertility and Sterility*, Vol. 103, No. 5.

Ronald H.F. Hunter, F. L.-G. (2018). Whither human IVF? Fertilisable oocytes selected on the basis of follicular temperature. *Journal of Assisted Reproduction and Genetics*, 35: 643-644.

Rotterdam ESHRE/ASRM, S. P. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertility and Sterility*, 81: 19-25.

Runa Ozelci, S. D. (2019). Gonadotropin releasing hormone antagonist use in controlled ovarian stimulation and intreuterine insemination cycles in women with policystic ovary syndrome. *Taiwanese Journal of Obstetric & Gynecology* , 58: 234-238.

S. Dyer, G. C.-H. (2016, May 20). International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. *Human Reproduction, 31*(7), 1588–1609.

S. West, H. L.-R.-P. (2014). Irregular menstruation and hyperandrogenaemia in adoloescence are associated with polycystic ovary syndrome and infertility: Northern Finland Birth Cohort 1986 study. *Human Reproduction*, Vol. 29, No. 10, pp 2339-2351.

Samantha Cassar, M. L. (2016). Insulin Resistance in polycystic ovary syndrome: a systematic review and a meta-analysis of an englycaemic-hyperinsulinaemic clamp studies. *Human Reproduction*, Vol. 13, No. 11, pp 2619-2631.

Satoko Osuka, N. N. (2019). Animal models of polycystic ovary syndrome: A review of hormone-induced rodent models focused on hypothalamus-pituitary-ovary axis and neuropeptides. *Reproductive Medicine and Biology*, 18: 151-160.

Sehar Toosy, R. S. (2018). Lean polycystic ovary syndrome (PCOS): an evidence-based pratical approach. *Journal of Diabetes and Metabolic Disorders*, 12: 277-285.

Setji, L. T. (2014). Polycystic ovary syndrome: Update on diagnosis and treatment. *The American Journal of Medicine*, Vol. 127, No. 10, pp 912-919.

Shinqin Zhu, B. Z.-J. (2019). Metabolic disturbances in non-obese women with polycystic ovary syndrome: a systematic review and meta-analysis. *Fertility and Sterility*, Vol. 111, No. 1.

Silva-Carvalho J, S. A. (2009). Caracterização de fertilidade em Portugal, Estudo na Comunidade. *Estudo AFRODITE*, 9-73.

Spritzer, P. M. (2014). Polycystic Ovary Syndrome: reviewing diagnosis and management of metabolic disturbances. *Arq Bras Endocrinil Metab*, 58/2.

Stefan Dieterle, G. Y. (2006, May). Effect of acupuncture on the outcome of in vitro fertilization and intracytoplasmic sperm injection: a randomized, prospective, controlled clinical study. *Fertility and Sterility, 85*(5).

Stener-Victorin, W. U. (2000). Effects of electro-acupuncture on anovulation in women with polycystic ovary syndrome. *Acta Obstetricia et Gynecologica Scandinavica, 79*, 180-188.

Stephen Franks, M. I. (2006). Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *International Journal of Andrology*, Vol. 29, pp 278-285.

Takov, E. C. (2019). Embryology, Ovarian Follicle Development.

Vivek Dubey, S. M. (2016). Cervical mucus helps in the fertilization in women. *World Journal of Pharmacy and Pharmaceutical Sciencies* , Vol. 5, Issue 10.

Weiss NS, K. E. (2019). Gonadotrophins for ovulation induction in women with polycystic ovary syndrome. *Cochrane Database of Systematic Reviews*, Issue 1, No. CD010290.

World Health Organization. (2013). *WHO - Traditional Medicine Strategy: 2014-2023.*

Xiaoke Wu, E. H.-V. (2014). Effects and Mechanisms of Complementary and Alternative Medicine during the Reproductive Process. *Evidence-Based Complementary and Alternative Medicine*.

Xu J, Z. Y. (2018). Efficacy of acupuncture as adjunctive treatment on infertility patients with polycystic ovary syndrome. *Zhongguo Zhen Jiu.*, 38(4):358-61.

Yan Wu, N. R. (2016). Acupuncture for treating polycystic ovary syndrome: guidance for future randomized controlled trials. *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology), 17*(3), 169-180.

Yin Shi, L. L. (2019). Efficacy of electroacupuncture in regulating the imbalance of AMH and FSH to improve follicle development and hyperandrogenism in PCOS rats. *Biomedicine & Pharmacotherapy, 113*.

Z Zhou, D. Z.-J. (2018). Epidemiology of infertility in China: a population-based study. *BJOG* , 125:432-441.

Zahra Shaaban, A. K. (2019). Pathophysiological mechanisms of gonadotropins- and steroid hormones-related genes in etiology of polycystic ovary syndrome. *Iranian Journal of Basic Medical Sciences* , Vol. 22, No. 1.

Zheng Y, et al. (2015). How does acupuncture affect insulin sensitivity in women with polycystic ovary syndrome and insulin resistance? Study protocol of a prospective pilot study. *BMJ Open, 5*. doi:10.1136

Zheng YH, W. X. (2013, September). Effectiveness of abdominal acupuncture for patients with obesity-type polycystic ovary syndrome: a randomized controlled trial. *Journal of Alternative and Complementary Medicine, 19*(9), 740-745.