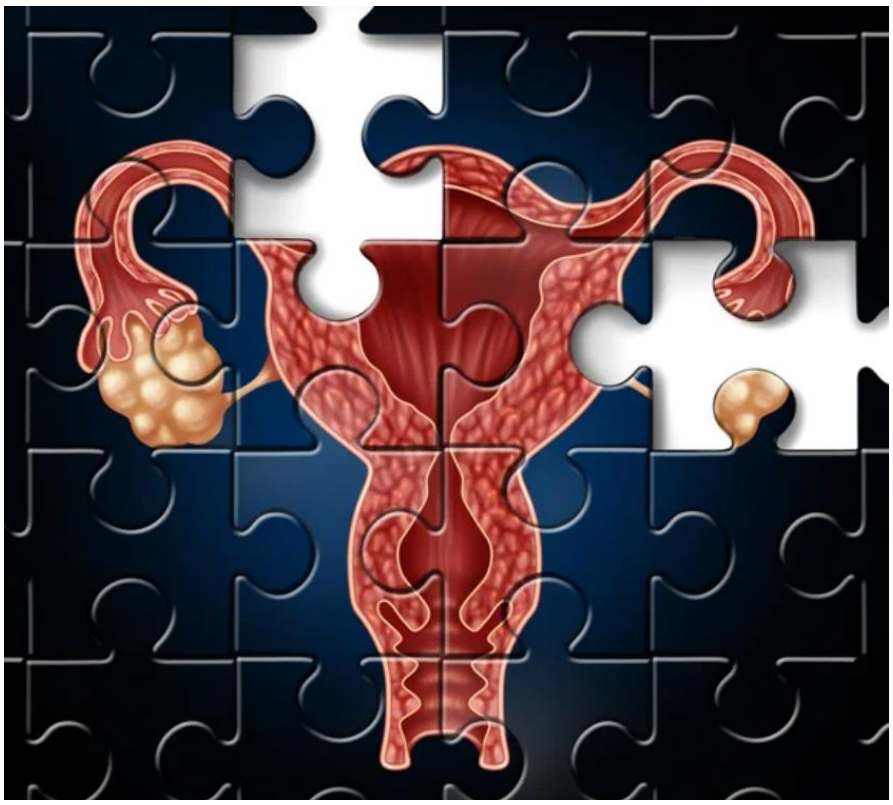
Polycystic Ovarian Syndrome and the Phenotypical Puzzle

A literary study on how the polycystic ovarian syndrome phenotypes differ in origin results in a puzzle for patients and physicians.



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BACHELOR'S THESIS

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Abstract

This bachelor thesis will shed light on the pathophysiological difference in the phenotypes of polycystic ovarian syndrome (PCOS). PCOS is defined as a syndrome with four different phenotypes, where only two diagnostic criteria are required for a diagnosis. This thesis aims to investigate the origin of these phenotypes and if all phenotypes share the same origin. In this way, patients and physicians can adjust the treatment plan to each patient's phenotype. The syndrome itself will be introduced. Then based on the necessary criteria the disorder will be unpacked. With every criterion, the underlying pathophysiology is discussed and accompanied by the necessary figures and tables. At last, a hypothetical scheme is introduced that shapes the origin of the disorder. Yet, this scheme is countered with the last peculiar phenotype. A conclusion is formed on the literature used in this thesis and advice is given to adjust the diagnostic approach toward PCOS.

Chapter 1: PCOS?

'... it's always kind of been in the back of my head or maybe I have it. But never really done anything about it, and then my periods became a lot lighter ... I never had a regular period and that's when I thought maybe I need to get something sorted about this, you know. I thought because it wasn't an ailment it's not like I'm ill from it, I never thought the doctors would really be that bothered.'
- Participant 11, age 26 on having PCOS.

(Retrieved from Hadjiconstantinou et al., 2017).

Polycystic ovarian syndrome (PCOS) was first described by Stein and Leventhal in 1935. It is a complex heterogeneous disorder that affects the ovaries and hormonal regulation of a woman's body. Globally, women between the age of 18 to 44 years are affected by PCOS and the estimated prevalence lies between 6% and 26% (Manisha et al., 2020; Deswal et al., 2020). Excessive hair growth, infertility, acne, insulin resistance, obesity, and absence of menstruation are known issues with the disorder (Cahill, 2009). Infertility in PCOS women has a prevalence between 70 and 80% (Melo et al., 2015). Obesity is also common comorbidity in PCOS with a prevalence of 40-80% (Sam & Dunaif, 2003; Sam, 2007). For the absence of menstruation, the demographics are a bit different, as absence of menstruation is divided into two groups: oligomenorrhea (irregular menstruation) and amenorrhea (no menstruation). Within these groups, women with PCOS have an 85-90% prevalence of oligomenorrhea and 30-40% of the women with PCOS have amenorrhea (Hart et al., 2004). Moreover, PCOS patients are known to have higher rates of endometrial cancer, an increased chance of developing type-2 diabetes and cardiovascular disease (Taylor, 1998). The origins of these conditions lie within the three diagnostic criteria, namely: anovulation, clinical indications of hyperandrogenism – excess of androgens in the blood, and formation of multiple cysts in the ovaries (polycysts) (Azziz, 2006).

The National Institute of Health introduced initially two criteria required for a diagnosis in 1990, which included only the presence of anovulation and hyperandrogenism (Zawadski & Dunaif, 1992). Later in 2003, during the PCOS consensus workshop organized by The European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) in Rotterdam, these criteria were expanded by including the polycystic ovaries. However, the Androgen Excess Society wanted to centre PCOS as an androgenic disorder, limiting the possible phenotypes. This was not accepted and to this day, the Rotterdam criteria are still used.

Thus, the current criteria are anovulation, hyperandrogenism and polycystic ovaries. Here, anovulation is defined as a physiological defect where follicles in the ovaries fail to select a dominant follicle (Franks et al., 2000), resulting in the follicles not releasing an egg. This leads to limited menstruation and infertility problems. Hyperandrogenism can be recognized by measuring the concentration of free, unbound testosterone in the blood. For free unbound testosterone this normal range lies between 0.13-1 ng/dL in reproductive-aged women. A concentration of total testosterone exceeding 200ng/dL can be found in hyperandrogenic women, whereas the normal healthy range lies between 8-60ng/dL of total testosterone (Mayo Clinic Laboratories, 2022). Androgen excess manifests itself usually as hirsutism, which is a condition where hair growth occurs in places that is uncommon in women. For example, beard growth or dark coarse hair on the arms and legs. Polycystic ovaries are characterized by enlarged ovaries accompanied by numerous begin-stage follicles, with more than 12 follicles per ovary (Rotterdam, 2003).

Currently, PCOS does not appear to have a clear cause, resulting in no cure for PCOS. However, there are available treatments that are prescribed on a symptom-oriented basis. Lifestyle modifications are introduced first to see if they improve the patient's symptoms. These lifestyle modifications can include a change in diet, an increase in exercise or both, which aim to improve ovulatory function and reduce the androgenic excess (Lass et al., 2011). Besides lifestyle modifications, medical treatment is provided for severe symptoms that the change in lifestyle cannot solve. Oral contraceptive pills (OCP) can be prescribed as a treatment for hyperandrogenism and menstrual cycle irregularities (Costello et al., 2007). OCP block the LH and FSH pathway, which prevents the settlement of PCOS symptoms, OCP are not sufficient, hormonal medication in the form of oestrogen receptor blockers is prescribed (Ibáñez et al., 2017). For ovulation induction, clomiphene citrate is prescribed, which induces ovulation as it contributes to oestrogen uptake by follicles. Metformin is also used to treat infertility in women with PCOS and sometimes clomiphene citrate and metformin are used together (Ndefo, 2013). It is also known that not all PCOS patients react to this treatment, resulting in lasting infertility (Jin & Xie, 2017).

Although there are some treatments available, the syndrome is still in discovery and the original cause seems to be different per phenotype. This contributes to unfitted medications for PCOS patients and uncertainties in their reproductive future. That is why this thesis will unravel the diagnostics of PCOS and investigate the cause-effect of the syndrome, so a better starting point can be utilized for further research.

Chapter 2: Anovulation

Menstrual cycles of normal length, between 21-35 days are considered to represent women’s wellness. Regular menstruation is seen as evidence of ovulation, which is an indication of fertility. Regular ovulation occurs in the ovaries, where a follicle is selected to mature into a dominant follicle to get released for fertilization in the ovarian tubes. Anovulation is the result of not releasing a dominant follicle in the ovarian tube. This can result in irregular menstruation cycles, known as oligomenorrhoea, or sometimes an absence of menstruation, amenorrhoea. In clinically normal menstrual cycles, anovulation occurs in one-third of the cases when looking at a random population (Prior et al., 2015), meaning that it is normal that sometimes no ovulation occurs during a cycle. As hormones are responsible for ovulation, hormonal imbalance and anovulation are possibly linked.

Endocrine physiology and ovulation

The hypothalamus, anterior pituitary and ovaries are part of the endocrine system in the body. They are responsible for the production of the majority of the reproductive hormones in men and women. Figure 1 shows this hormone production in women. The hypothalamus produces gonadotropin-releasing hormone (GnRH), which has positive feedback on the anterior pituitary. GnRH activates the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the anterior pituitary, creating this positive feedback. LH stimulates cells present in the follicles to initiate the production of androgens. During this production, also called steroidogenesis, hormones such as dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), androstenedione and testosterone are produced. Oestrogen and progesterone are also produced after stimulation of LH and FSH. These androgens are required for the maturing process of the follicle, to prepare it for ovulation. During ovulation, the oocyte is released from the follicle into the fallopian tube ready for fertilization.

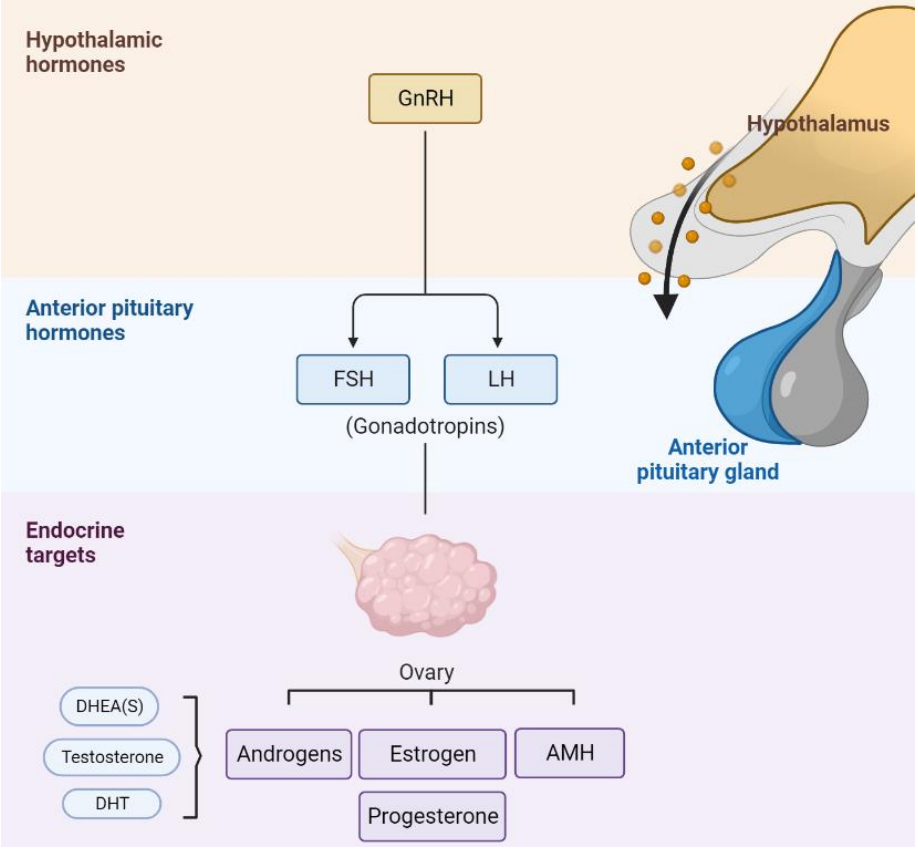

Figure 2: Hormone production in women. GnRH: gonadotropin-releasing hormone; FSH: follicle stimulating hormone; LH: luteinizing hormone; DHEA(S): dehydroepiandrosterone (sulphate); DHT: dihydrotestosterone. Figure made with BioRender.

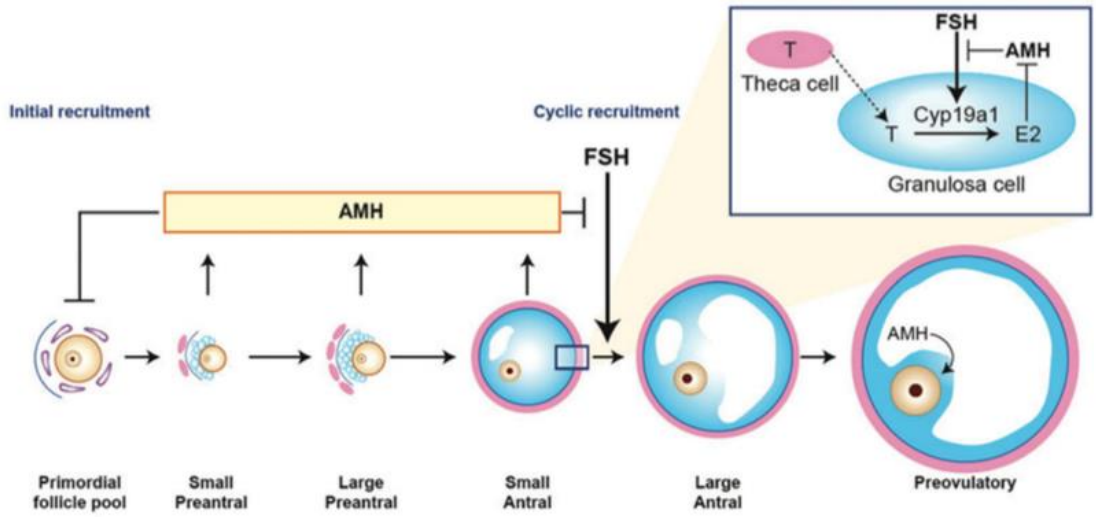

Figure 1: Schematic view of follicle development. AMH is produced by the small, large preantral, and small antrall follicles. AMH inhibits initial recruitement and FSH. FSH stimulates cyclic recruitement, which leads to one follicle selection for the maturation process. In the follicle testosterone (T) is converted by CYP19A1 into oestradiol (E2), which inhibits AMH. Figure retrieved from Dumont et al. (2015).

FSH regulates the development, growth, pubertal maturation, and reproductive processes. The main relevant function of FSH is to stimulate the growth of follicles in the ovaries. Follicles are made up of an oocyte surrounded by theca cells and granulosa cells. Each follicle has to go through certain phases to reach the final phase: the preovulatory follicle (Dumont et al., 2015). Within the follicles, the granulosa cells are responsible for the production of anti-Müllerian hormone (AMH). Especially during the preantral phase, the phase before follicle selection and maturation, AMH is produced. This

hormone is involved in the development of the small follicles and selection of follicles into a dominant phase (Dumont et al., 2015). AMH inhibits FSH in a limited manner, resulting in enough FSH necessary for a follicle to enter the antral phase where it can develop into a preovulatory follicle (see Figure 2) (Durlinger et al., 2001). The slight excess of FSH activates aromatase, also known as CYP19A1, which converts androgens into oestradiol, required for ovulation. Oestradiol on itself inhibits AMH when present in excess, limiting the inhibitory function of AMH on FSH, and stopping the recruitment of follicles (Dumont et al., 2015). It can thus be said that multiple factors are involved in the ovulation process in women.

Causes of anovulation

Hypogonadism is a disorder originated in the hypothalamus/pituitary axis. This disorder characterizes by a lack of LH and FSH production in the anterior pituitary gland and is most common in women who are underweight or with an excessive exercise regime. The production of GnRH is also affected by this. Since the hormones LH, FSH, and GnRH are less expressed, it can also lead to anovulation or a limited ovulation cycle in women. However, when women, affected by anovulation, go to the doctor for a diagnosis, hypogonadism will be screened as well.

Increased follicle recruitment leads to anovulation

PCOS is the most common disorder associated with decreased fertility due to anovulation (Hamilton-Fairley & Taylor, 2003), giving rise to doctors also checking for PCOS when non-ovulating women ask for help. The cause of anovulation in this disorder is found in the development of follicles and the production of hormones by these follicles.

AMH

Franks et al. (2008) investigated the AMH expression in antral follicles obtained from women with normal ovaries, polycystic ovaries without anovulation (PCO-AO) and polycystic ovaries with anovulation (PCO+AO) and revealed that the tissue samples of PCO+AO individuals had a lower percentage of AMH present in the primordial and transitional follicles as seen in Figure 2. This result sounds a bit counter-intuitive, but it could be explained by lower concentrations of AMH that were found in the early stages of the follicles. These low AMH concentration results in less inhibition of the primordial follicle recruitment, letting more primordial follicles enter the development process and entering the growing phase. Since these follicles develop into primary and secondary follicles, the AMH production increases more, as there are more follicles present. A higher concentration of AMH would then be the result. So, due to AMH's inhibitory actions on FSH and thus aromatase, the selection and maturation process of the selected follicle, under the influence of oestradiol, cannot occur (Durlinger et al., 1999; Durlinger et al., 2001), resulting in lack of ovulating follicles.

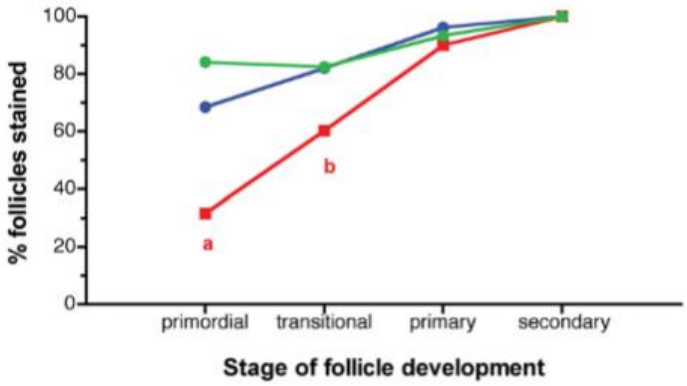

Figure 3: Percentage of AMH staining in pre-antral follicles per follicle development in three groups: normal (blue), PCO+AO (red) and PCO-AO (green). Primordial and transitional follicles of PCO+AO group were significantly lower with p=0.0015 (a) and p=0.0017 (b) compared to the ovulatory groups. Retrieved from Franks et al., 2008.

Other studies also show that PCOS women have increased values of AMH (Dewailly et al., 2011; Tal et al., 2014; Pellat et al., 2010). In particular, the study by Pellat et al. (2010) discovered that anovulatory PCOS patients have an 18 times higher concentration of serum AMH compared to ovulatory PCOS patients. Tal et al. (2014) investigated this as well and saw the same difference in AMH serum concentrations in PCOS patients (see Figure 4). AMH is thus an important factor within the scope of PCOS.

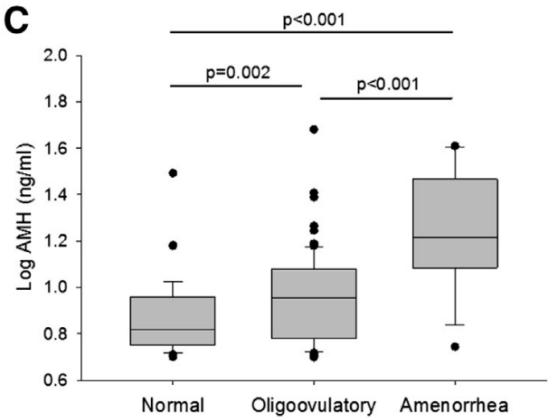

Figure 4: AMH serum concentration in PCOS patients with different manifestations of symptoms. Oligoovulatory: infrequent mentrual cycle; amenorrhea: no ovulation. Graph shows a significant difference between groups, where the amenorrhea group has the highest AMH serum concentration compared to the rest. Retrieved from Tal et al., 2014.

Androgens

Androgens are also involved in increased follicle recruitment, resulting in anovulation. Sen et al. (2014) discovered that androgens contribute to increased expression of protein FSH-receptor, which in turn increases the sensitivity of the follicles towards FSH, thereby promoting preantral follicle growth and its progression to antral follicles.

Besides an increased sensitivity to FSH by androgens, an increase in testosterone levels has been found to aid in follicle recruitment (Vendola et al., 1998). A study on healthy rhesus monkeys was performed. They were treated with testosterone for 3 or 10 days. They found that these androgen treatments stimulated early stages of primate ovarian follicle growth independently from the cycle stage or gonadotropins. In Figure 5 this increase in follicle number can be appreciated. Especially the small preantral and antral follicles together with the granulosa and theca cell proliferation were increased. Although the study did not cover ovulation occurrence, they speculated that ovulation would be less likely to happen when these follicles were present. This also gives the reason why women with ovulation issues are checked for androgen excess for diagnostic purposes for PCOS.

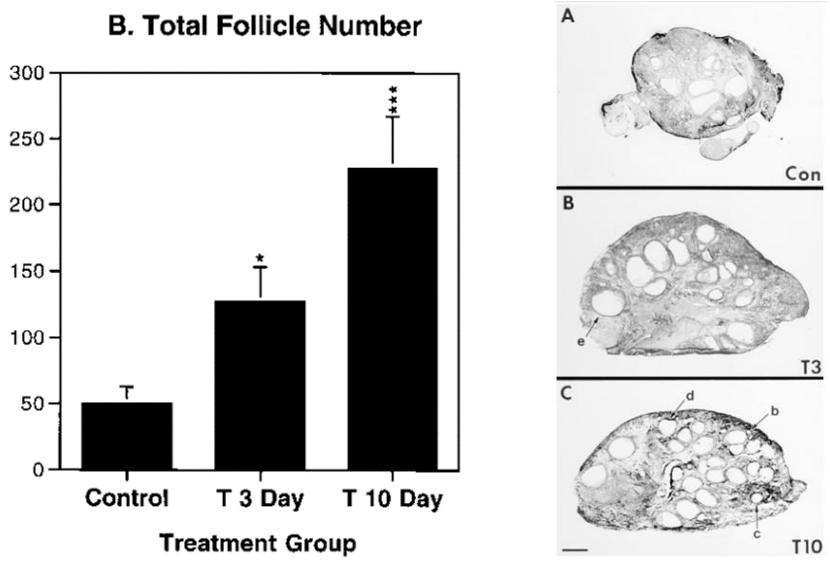

Figure 5: Total follicle number in rat ovaries per treatment group; control (no T), T3 Day (T treatment for three days), and T10 Day (T treatment for ten days). Significant growth in both T3 and T10 groups are observed. Retrieved from Vendola et al. (1998)

A review by Rosenfield and Ehrmann (2016) concludes that androgens are essential in follicular maturation, but in excess, they can hinder ovulation. This is modulated by the homologous desensitization of theca cells to LH. When a high concentration of LH is present, the androgenic response to high LH levels is reduced. Androgen excess causes thus premature luteinization, hindering ovulation, as it affects the indication of which follicle will mature into a dominant follicle.

In conclusion, the production of follicles in women with PCOS is increased, limiting the ovulation process in the ovaries. Together with the increased proliferation of granulosa cells, the expression of hormones such as testosterone and AMH is increased. These hormones contribute both to further increase in growth of preantral follicles and inhibit FSH and aromatase and with that limit the recruitment cycle of dominant follicles, respectively. This leads to the first phenotype of PCOS, anovulation and the presence of increased androgens.

Chapter 3: Hormonal problems in PCOS

The endocrine system manages the production of hormones by multiple organs. The hypothalamus, pineal and pituitary glands are the start and centre of the system providing the body with the necessary hormones. A healthy body is in a hormonal balance, where from each hormone enough is produced and is counteracted by its inhibitor. When the balance is interrupted, one or more hormones or inhibitors involved are produced in excess. Identifying the role of androgens and their imbalance is vital to the discussions surrounding PCOS diagnostics.

One of these imbalances presents itself as hirsutism. This is a condition where hair growth occurs in places that is uncommon in women. For example, beard growth or dark coarse hair growth on legs, belly, and arms. Hirsutism is present in 10% of women (Escobar-Morrealle et al., 2012) and is associated with hyperandrogenism – an excess of androgens in the blood. However, there are women with hirsutism that have normal androgen levels. This is known as idiopathic hyperandrogenism or idiopathic hirsutism (Azziz et al., 2000).

In the matter of how hyperandrogenism is involved in PCOS, an understanding of androgen hormones and their production is important. After elaborating on androgens, the chapter will discuss and identify the causes of hyperandrogenism and how that can result in the pathophysiology of PCOS.

Androgens

Androgen production in women takes place in the ovaries and the adrenal glands. The synthesis of androgens occurs in the theca cells of the follicles present in the ovaries. The adrenal glands are also partly responsible for androgen production as precursor androgen molecules get synthesized in the blood. The five androgens are dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), androstenedione and testosterone. DHEA and DHEAS and androstenedione are noted as pro-androgens and have therefore no biological activity in the body, whereas DHT and testosterone do have an androgenic effect in the body.

The production of DHEAS occurs in the adrenal zona reticularis. and is converted into androstenedione in circulation by other enzymes. DHEA is produced merely by the adrenal zona reticularis and accounts for 50% of the production by the adrenal gland. The other half is produced by theca cells (20%) and is converted from DHEAS in circulation (30%). Androstenedione is equally produced by both the adrenal zona reticularis and the ovaries (Davison & Bell, 2006).

The follicles in the ovaries exist out of theca and granulosa cells. The theca cells convert cholesterol into androgens under the influence of LH. The granulosa cells are promoted by FSH and transform the androgens into oestradiol. Each androgen molecule has its location and ratio of production, which contributes to the total androgen concentration in the body.

Testosterone is a reactive androgen and is usually bound to sex hormone-binding globulin (SHBG) by around two-thirds of the total concentration in the body. The other third is bound to albumin, which leaves between 0.5-1% of free testosterone in the body of women (Davison & Bell, 2003). The biological activity of testosterone occurs through binding to the androgen receptor (AR) in target tissues. Eventually, testosterone will be converted into oestradiol by aromatase in the peripheral tissue or granulosa cells (Kempegowda et al., 2020) or it is bound to albumin or SHBG for transportation to target tissues. Together testosterone and oestradiol affect the reproductive hormones of the female reproductive system.

Steroidogenesis

The biosynthesis of androgens has in general the same process within the ovaries and adrenal glands, with cholesterol being the precursor of all androgens. The production of androgens in the ovaries only occurs when the follicle is in a growing process, as it then produces the involved enzymes and receptors to execute steroidogenesis (Baird et al., 1976; Palermo, 2007). LH receptors (LH-R) are not expressed in undifferentiated progenitor theca cells (Magoffin & Weitsman, 1994), leaving the theca cell differentiation independent of gonadotropin. LH binding to LH-R initiates the conversion of cholesterol into androstenedione and testosterone. Because LH is released in a pulsating manner by the anterior pituitary, the pulse frequency in this is directly linked to the number of androgen hormones produced. Each spike of LH is followed by an increase in androgens (Baird et al., 1976; Palermo, 2007).

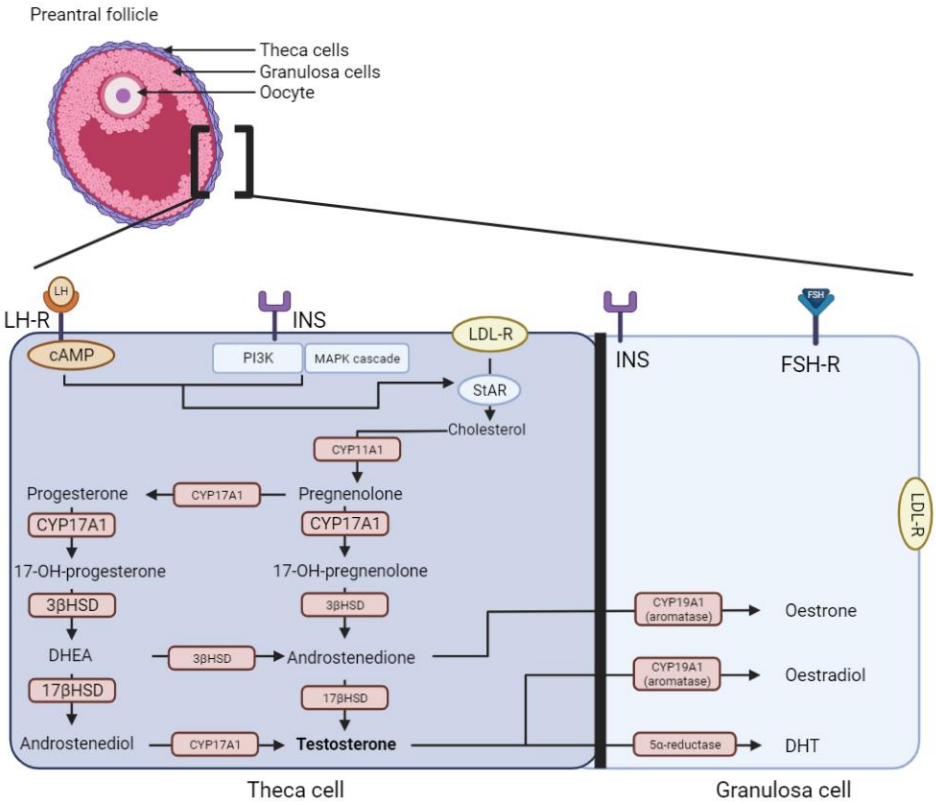

Figure 6: Steroidogenesis (androgen production) in theca and granulosa cells of human ovaries. Figure based on Walters & Handelsman (2018) and E. Diamanti-Kandarakis et al. (2008). LH-R: Luteinizing hormone receptor; INS: insulin receptor; FSH-R: follicle stimulating hormone receptor. Figure is made with BioRender.

The conversion of cholesterol into androgens is modulated by three enzymes: cholesterol side-chain cleavage cytochrome P450 (CYP11A1), 17α-hydroxylase (CYP17A1) and 3β-hydroxysteroid dehydrogenase (3βHSD). First, the steroidogenic acute regulatory protein (STAR) is activated via the PI3K-Akt pathway, where cAMP accompanies cholesterol into the mitochondria and initiates steroidogenesis with the enzyme CYP11A1. CYP11A1 is required to convert cholesterol into pregnenolone, which is then metabolized to DHEA by CYP17A1 and can be further metabolized into androstenedione by 3βHSD, or by 17βHSD into androstenediol (Turcu et al., 2014). For the final conversion into testosterone, 17βHSD catalyses the metabolization of androstenedione into testosterone, or androstenediol is metabolized into testosterone by CYP17A1. Testosterone is transported into the granulosa cell, where it is converted into DHT, by 5α-reductase or into oestrone and oestradiol via aromatase. Testosterone is also transported to target tissues via albumin or SHBG. There it will bind to the androgen receptor in the cell, initiating various reactions. In Figure 6 you can appreciate the process of steroidogenesis in a follicle.

Hyperandrogenism

The cause of hyperandrogenism is difficult to pinpoint, as the process of androgen production can be influenced at many points. As discussed earlier, the production of androgens is influenced by LH and FSH, which on their part are influenced by GnRH. Research suggests that an increased pulse frequency and the amplitude of GnRH and LH can contribute to more androgen production (Cho et al., 2006). A higher concentration of LH contributes to more activation of the cAMP-dependent pathway, which increases the expression of steroidogenesis enzymes StAR and CYP17. This cascade is one of the many starting points of hyperandrogenism, but more factors are involved.

Causes of hyperandrogenism

Hyperandrogenism is an increased concentration of free testosterone in the body. The cause of this increased concentration has different origins. One of the important proteins involved in the testosterone balance in the blood is the sex hormone binding globulin (SHBG). As previously mentioned, this protein is bound to most of the testosterone in the blood and transports it to the target tissues.

In 1974, this protein already showed a decrease in hirsute women, with no relationship to PCOS. The lower concentration of SHBG was accompanied by an increase of 17OH-androgens as seen in Figure 7. The origin of hyperandrogenism can be found in the production of more androgens and less SHBG. Moreover, androgen excess itself also contributes to a reduced level of SHBG via downregulating the expression of the transcription factor hepatocyte nuclear factor 4 alpha (HNF-4α) involved in the expression of

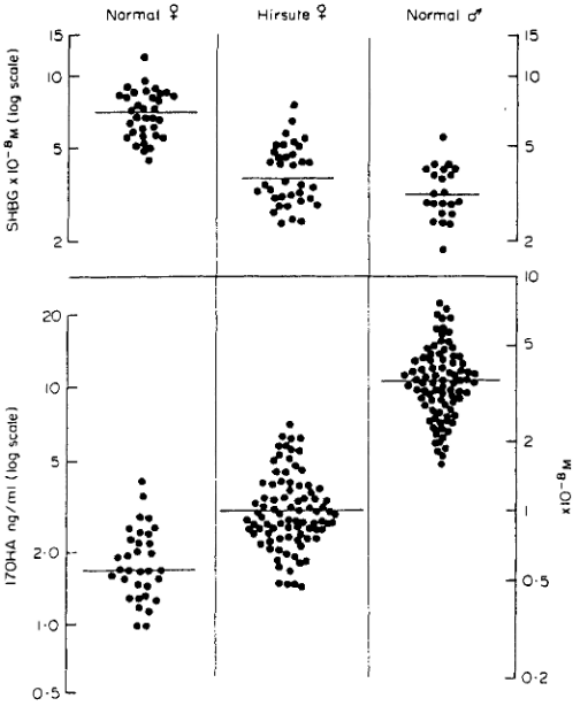

Figure 7: Concentration of SHBG and 17OH Androgens in the blood of healthy women, hirsute women, and healthy men. Concentration of SHBG in hirsute women are decreased and the concentration of 17OH Androgens are increased.

SHBG (Zhu et al., 2019).

Variants of other CYP genes involved in steroidogenesis are also known to influence this via over- or under-expression (Ashraf et al., 2019). Wickenheisser et al. (2004) found that the promotor function of the cytochrome p450 was increased in PCOS patients. According to Ashraf et al. (2019), there are more genetic influences, but this research will not go further into detail about the genetic influences on hyperandrogenism and PCOS.

As seen in Figure 4, the insulin receptor is involved in the production of testosterone via the activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) enzyme. This pathway is activated by insulin and affects cell growth and cell survival. When this pathway is interrupted, insulin resistance, metabolic disorders, and diabetes are known side effects. Insulin resistance (IR) and the resulted hyperinsulinemia are speculated to be a key factor in the pathogenesis of PCOS (Marshall & Dunaif, 2012), as the insulin receptor is the start of the PI3K-Akt pathway. Moreover, various in vitro studies have shown that insulin and the insulin-like growth factor I (IGF-I) stimulate the production of androgens in human theca cells (Bergh et al., 1993; Cappel et al., 2005; Bleach et al., 2021) or vice versa (Ashton et al., 1995; Vendola, 1998). Yet, a full understanding of IGF-I in PCOS remains unknown.

Insulin influences androgen production

Insulin has also an effect on the androgen concentrations in the blood, but merely indirect. Insulin affects the expression of transcription factor HNF-α in the liver, limiting the final expression of SHBG, which contributes to a lower SHBG concentration in the blood and thus more free testosterone (Zhu et al., 2019). Malini & Roy (2021), 800 women who originated from an infertility clinic and were diagnosed with PCOS, were investigated the influence of insulin on LH, testosterone and SHBG production. This study identified that insulin influences the relationship between LH, testosterone and SHBG. Increased concentrations of LH and testosterone levels were observed in PCOS patients. Decreased SHBG levels combined with increased levels of insulin can explain a higher concentration of free testosterone in PCOS patients. This decreased level of SHBG was also observed in hirsute women as previously mentioned, suggesting a relationship between these findings and that PCOS and hirsutism are possibly linked.

Besides insulin on its own, insulin resistance and hyperinsulinemia (increased insulin concentration in the blood) have also been reported as regulators of steroidogenesis in ovaries. Insulin resistance entails the loss of affinity of the insulin receptor to insulin, creating an abundance of insulin that is not used by tissue and resulting in hyperinsulinemia, an increased concentration of insulin in the blood. Insulin resistance and hyperinsulinemia are known side effects of obesity. Obesity is also a known comorbidity of PCOS and many studies include this separate group in their studies (). However, this thesis will not focus on this group.

A study on only PCOS women and hyperinsulinemia by Tosi et al. (2012) investigated the concentrations of androgens and GnRH under the influence of high concentrations of insulin or saline treatment. The patients were all given a GnRH agonist to provoke a higher production of androgens. Treatment with dexamethasone was given to exclude adrenal production of androgens. In Figure 8 the increased concentrations of multiple androgens can be observed. A significantly higher concentration of androstenedione and progesterone was measured. These findings suggest that hyperinsulinemia and insulin have a stimulatory effect on ovarian steroidogenesis and thus causing hyperandrogenism. It is

important to note that this research only investigated PCOS patients, limiting the study as it could not compare data to healthy individuals.

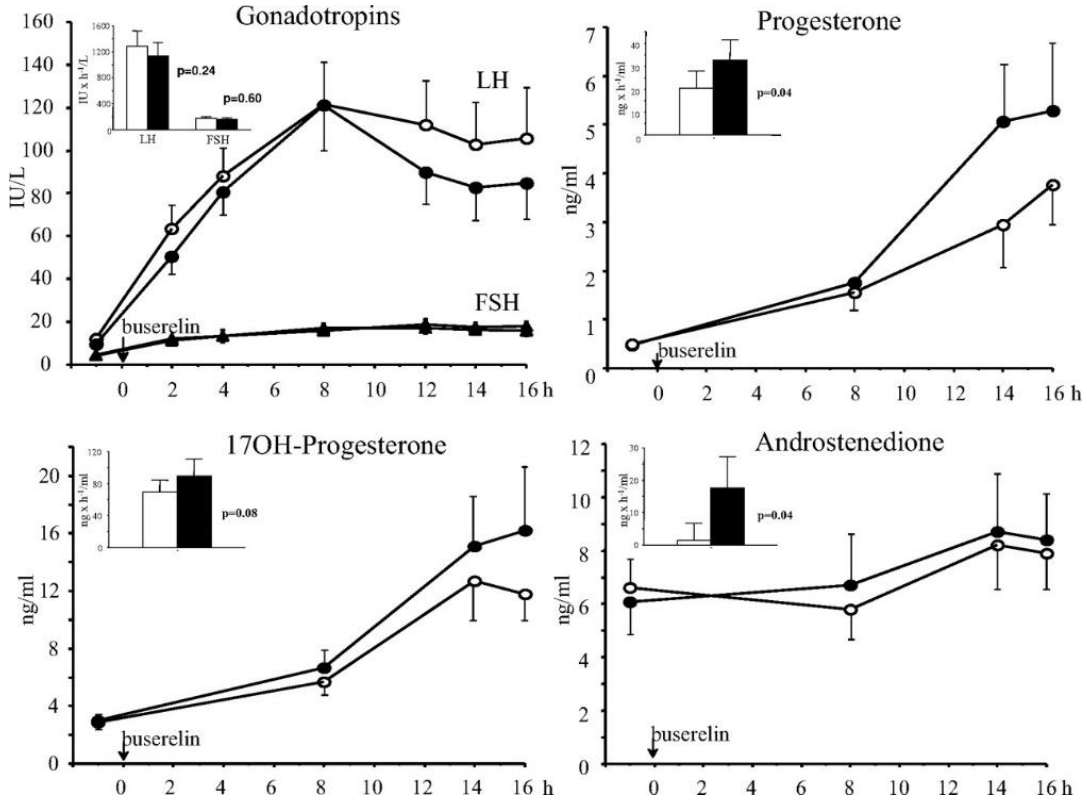

Figure 8: Concentration of gonadotropins, progesterone, 17OH-progesterone, and androstenedione under the influence of insulin or saline after treatment with GnRH agonist (buserelin). White (saline), Black (insulin). Progesterone and androstenedione concentrations are significant between insulin and saline treatment. Retrieved from Tosi et al. (2012)

Hyperandrogenism and cystic ovaries

Hyperandrogenism is one of the necessary criteria for a PCOS diagnosis (Rotterdam, 2003). Approximately 60-80% of all PCOS patients suffer from hyperandrogenism (Ibañez et al., 2017). It can therefore be said that androgens do play an important role in PCOS and its diagnosis. An androgen excess has been reported to increase follicle growth of preantral and small antral follicles. The binding of androgen-to-androgen receptors (ARs) is known to limit the degeneration of follicles as well. This physiology leads to excessive growth of follicles, limiting the further development of dominant follicles and thus anovulation. Moreover, an increased number of follicles and the anti-apoptotic characteristics of androgens induce follicles to remain in the ovaries, resulting in the formation of multiple small cysts, which are known as polycystic ovaries.

In summary, hyperandrogenism has different causes with a metabolic origin. The steroidogenesis can be affected due to genetic influences or influences from circulation such as insulin, insulin resistance or decreased levels of SHBG. It is important to note that hyperandrogenism lies mostly at the root of other symptoms in PCOS, suggesting that PCOS is an androgenic disorder. However, before these speculations, polycystic ovaries as the last criteria should be discussed first.

Chapter 4: Cysts

7% of women worldwide will develop an ovarian cyst at a moment in their lives (Farghaly, 2014). Ovarian cysts can come with heavy periods, bloating, pelvic pain, and difficulty to get pregnant unrelated to fertility problems. Women with these symptoms will undergo an ultrasound, which will detect the shape, size, location, and mass of the cysts. Besides an ultrasound, extra tests are performed. To rule out ovarian cancer or a hormonal problem, blood is tested on a cancer-specific antigen or tested on certain hormones. At last, a pregnancy test is done to rule out that the symptoms originate from a pregnancy (Iglesia, 2022).

Cysts have two origins: pathological and functional cysts. Pathological cysts formation is not related to hormones and the menstrual cycle. These cysts form from abnormal cell growth from ovarian cells. So, for this thesis, pathological cysts will not be discussed further, as these types of cysts are not involved in the PCOS characteristics (Mobeen & Apostol, 2022).

Functional cysts are known to have a hormonal origin and are dependent on the menstrual cycle. Functional cysts resemble the follicles present in the ovaries. Multiple different types of cysts can form in the ovaries: neoplastic cysts, theca lutein cysts and follicular and corpus luteal cysts. Neoplastic cysts originate from an overgrowth of cells within the ovary, which can be present in a benign (harmless) and malignant (harmful) state. Theca lutein cysts have a follicle as origin and are a result of overstimulation by the hormone human chorionic gonadotropin (hCG) (Mobeen & Apostol, 2022). At last, there are follicular and corpus luteal cysts, which can develop during a normal menstrual cycle. They generate when a follicle fails to undergo atresia during ovulation (Mobeen & Apostol, 2022). Progressive accumulation of follicular fluid and expansion of the antrum is observed. Together with the increase of the follicle, the granulosa cell layer undergoes apoptosis. Lastly, the wall of the follicle will become thin and in combination with an increased atretic granulosa cell layer, a cyst can form (Chang et al., 2013)., which causes a formation of a smooth, thin-walled follicle. These cysts continue to grow since the hormonal stimulation is not at rest.

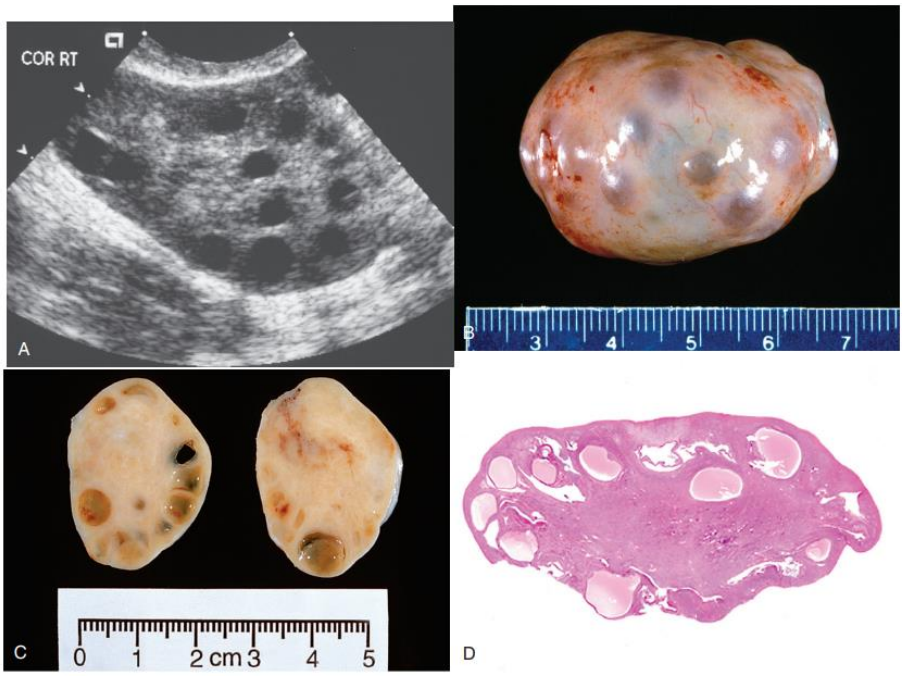

Figure 9: Polycystic ovaries displayed in four different visualizations. A: ultrasound of polycystic ovaries; B: Gross appearance of polycystic ovaries. Cysts can be observed under the capsule; C: Cross section of B, shows the subcortical cystic follicles; D: Retrieved from Meserve & Crum (2018)

The latter type of cysts is usually seen in women with PCOS. For a diagnosis of polycystic morphology (PCOM), the ovaries have to be enlarged and characterized by numerous antral follicles of which more than 12 follicles per ovary or an ovary volume over 10 ml (Rotterdam, 2003). Although, with the newer technology at hand, more cysts can be found, so the threshold for PCOM is changed to 25 cystic follicles instead (Goodman et al., 2015). In Figure 9 polycystic ovaries can be seen from different perspectives.

Cause of follicular cysts in PCOS

Increased concentration of androgens causes polycystic ovaries. A study was performed on 17 transsexual men[1] (Pache et al., 1991). They were treated with testosterone either orally or via intramuscularly injections. The ovaries retrieved from these transsexual women after hysterectomy were compared with non-treated individuals. These comparisons showed that in treated transsexual women the ovarian volume was significantly increased ($p<0.0001$). Moreover, the number of follicles, including healthy, cystic atretic and atretic follicles, were increased in treated transsexual women. In Figure 10 an ovary with (atretic) cystic follicles can be observed. This study suggests that androgens in the long run with increased concentrations affect follicle growth, which relates to the androgen excess present in PCOS.

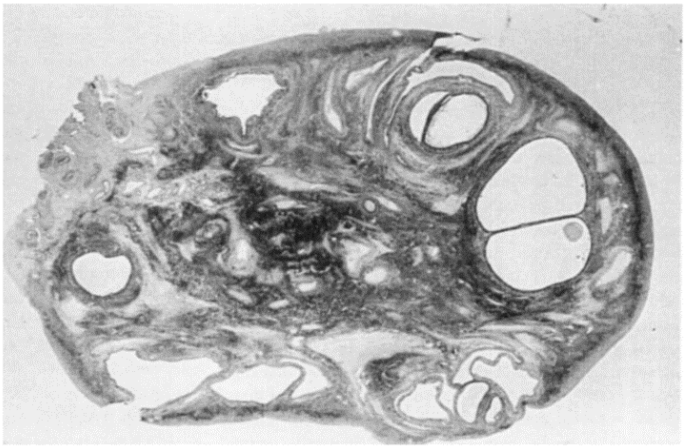

Figure 10: Ovary retrieved via hysterectomy from a transsexual women after ~2 year treatment with testosterone. Atretic and cystic follicles can be observed. Retrieved from Pache et al (1991).

Other studies suggest that the PI3K-Akt pathway is involved in the formation of cystic follicles. Restuccia et al. (2012) reported that PKBβ (also known as PI3K) knockout mice (PKBβ KO) develop ovarian cysts that are associated with increased steroidogenesis in theca cells and hyperinsulinemia. The PKBβ is involved in insulin signalling and is activated in theca cell after stimulation of LH. Suggesting that a defect in steroidogenesis is eventually also responsible for the formation of cysts.

All three criteria involved in a PCOS diagnosis are now discussed. For a diagnosis, a patient is only required to have two of the three criteria as already mentioned in Chapter 1. In Table 1 the following phenotypes of PCOS can be appreciated: Complete PCOS with all criteria, classic PCOS including hyperandrogenism and ovulatory dysfunction, and ovulatory PCOS accompanied by hyperandrogenism and polycystic ovaries. A conclusion can already be made upon the fact that hyperandrogenism on its own can cause PCOM, but not in all cases, which also accounts for ovulatory dysfunction.

[1] Transsexual men are people living as a man today but were born a woman. In the study of Pache et al. (1991) there were named transsexual women, but are officially called transsexual men now.

In summary, PCOM has a hyperandrogenic origin, where an increased concentration of androgens causes the formation of multiple cysts and together with the limited atresia of the follicles, the follicles will develop into multiple small cysts.

Table 1: PCOS phenotypes according to the Rotterdam criteria (2003). HA: hyperandrogenism; OD: ovulatory dysfunction; PCOM: Polycystic ovarian morphology.

	HA	OA	PCOM
Phenotype A (Complete)	+	+	+
Phenotype B (Classic)	+	+	-
Phenotype C (Ovulatory)	+	-	+

Chapter 5: Cause-effect in PCOS

All criteria for a PCOS diagnosis have been discussed; anovulation, hyperandrogenism, and polycystic ovaries. All criteria were mentioned with an androgen excess origin. Based on this research it can thus be hypothesized that PCOS' origin lies in the androgen excess caused by different factors. Figure 11 shows an indicative diagram of how the pathophysiology of PCOS develops due to hyperandrogenism and the factors involved.

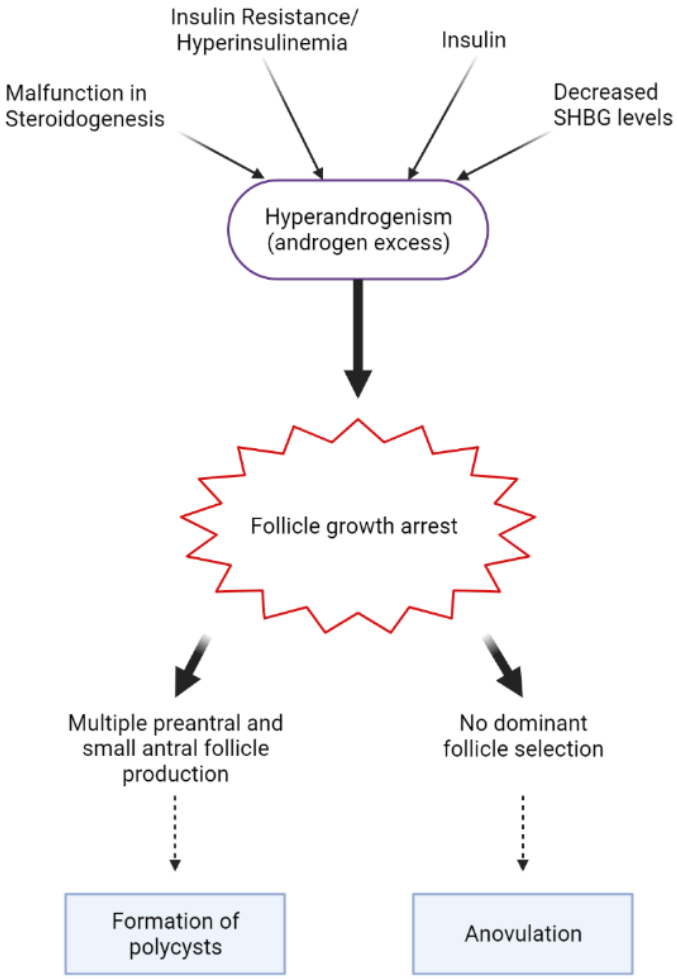

Figure 11: Indicative diagram of the pathophysiology of PCOS where hyperandrogenism is the origin. Hyperandrogenism can be caused by a malfunction in steroidogenesis, insulin resistance and its accompanying hyperinsulinemia, insulin, and/or a decreased level of sex hormone binding globulin (SHBG). Figure is made with BioRender.

However, for a diagnosis of PCOS, only two of the three mentioned criteria are required. According to the literature found in this thesis, hyperandrogenism must lay at the roots of PCOS. Yet, a fourth phenotype is also known within the scope of PCOS. This is non-hyperandrogenic PCOS and patients show both anovulation and formation of polycysts but without an androgen excess. Since the origin of this phenotype does not comply with the 'standard' image, it is peculiar that this phenotype is taken up in the diagnostic criteria for the syndrome. For a complete picture, Table X is updated into Table 2.

Table 2: PCOS phenotypes according to the Rotterdam criteria (2003). HA: hyperandrogenism; OD: ovulatory dysfunction; PCOM: Polycystic ovarian morphology.

	HA	OA	PCOM
Phenotype A (Complete)	+	+	+
Phenotype B (Classic)	+	+	-
Phenotype C (Ovulatory)	+	-	+
Phenotype D (Non-hyperandrogenic)	-	+	+

How can non-hyperandrogenic PCOS develop?

For patients with non-hyperandrogenic PCOS, the underlying cause is less clear. With this comes uncertainty in how to handle the disorder in the scope of medication or other remedies. Non-hyperandrogenic PCOS is a phenotype that is present in differing frequencies. Papers have mentioned a frequency within their cohort studies of 51.6% (Elasam et al., 2022), 3.6% (Sachdeva et al., 2019), and 5.2% (Ekwutosi et al., 2012). Figure 12 shows a distribution of the phenotypes based on a referral and unselected population. Here phenotype D accounts for 20% of an unselected population (Mumusoglu & Yildiz, 2020).

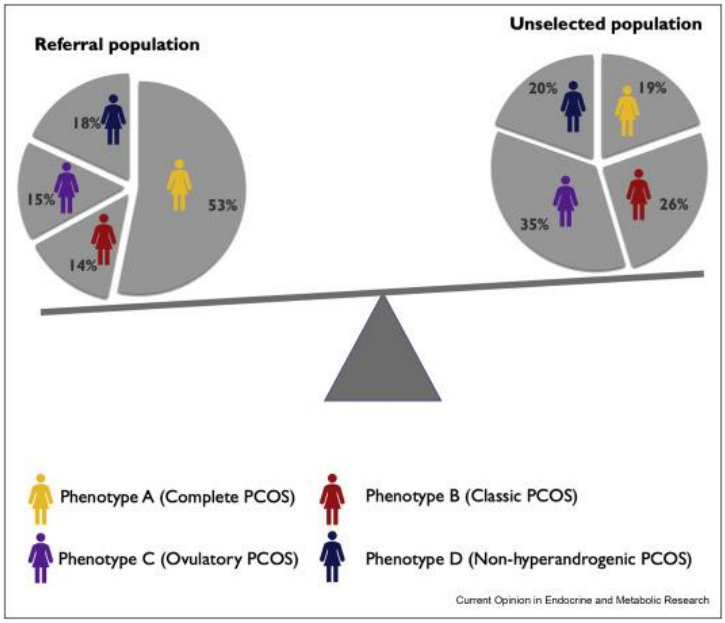

Figure 12: Prevalence of PCOS phenotypes in referral and unselected populations. Where 20% in the unselected population accounts for non-hyperandrogenic PCOS. (Retrieved from Mumusoglu & Yildiz, 2020).

The origin of the patients might be the cause of this disparity. Elasam et al. only investigated Sudanese women and the other papers had a more western cohort. The general 20% can then be accounted for as a 'middle ground' in an unselected population. A higher incidence of phenotype D was also found in India (Gupta et al., 2019). Though, a study performed in a Black vs. White women cohort was performed, where they found no direct evidence of different hormone levels (Ladson et al., 2011). It is

important to note that in that study the participants were recruited from the United States. Women from India and Sudan have different lifestyles compared to women in the United States. So further research has to be performed on this subject.

Besides ethnicity, the subject of insulin is also important. Insulin resistance and hyperinsulinemia are common research topics when talking about PCOS. This is mainly because PCOS patients have a higher risk of developing type 2 diabetes mellitus (Taylor, 1998). As earlier discussed, insulin and insulin resistance are factors involved in the androgen excess and thus hyperandrogenism. However, Moghetti et al. (2013) investigated the different phenotypes of PCOS and its relationship to insulin resistance by measuring it with a glucose clamp, which can measure the secretion and resistance of insulin in the body. They found out that the insulin action was diminished in classic and ovulatory PCOS but not in non-androgenic phenotypes and healthy women (see Figure 13). A higher insulin action means lower insulin resistance. This significant finding shed light upon the metabolic background of PCOS phenotypes, where non-hyperandrogenic PCOS behaves as a separate group within the syndrome. Phenotype D of PCOS might not be caused by insulin.

A recent study on IGF-I production in 3D follicle mouse models showed that IGF-I excess can lead to inhibitory follicle growth (Dai et al., 2022). They exposed mouse secondary follicles with three different concentrations of IGF-I: 0 ng/mL, 5 ng/mL, 10 ng/mL, and 50 ng/mL. The highest concentration of 50 ng/mL showed an inhibitory effect on follicle growth and overall IGF-I has an anti-apoptotic effect. These findings can explain the follicle growth arrest and the formation of follicles. Since these findings are not related to an increased concentration of androgens, non-hyperandrogenic PCOS could be explained. However, the study was performed in a mouse model, so further research on IGF-I has to be performed via a human research model.

It can be speculated that another mechanism is involved in the development of polycysts and interfering with the ovulation process in the ovaries causing the non-hyperandrogenic PCOS.

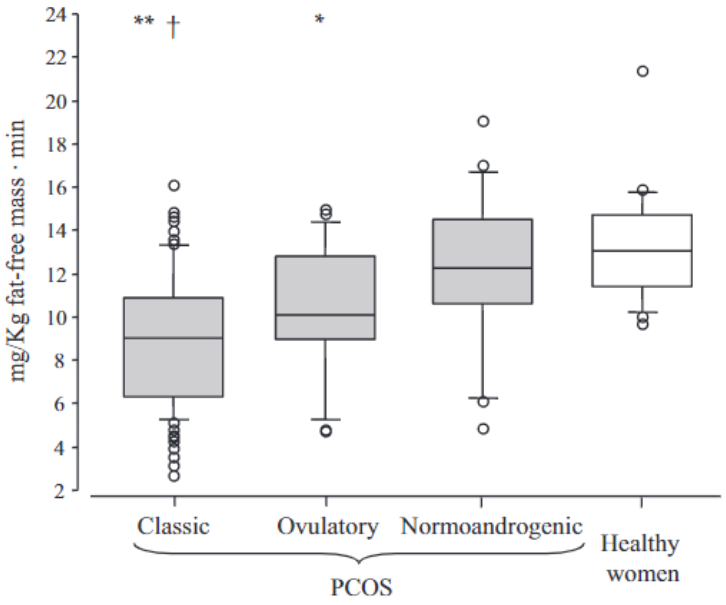

*Figure 13: Box plots of the glucose clamp values measured in the women with PCOS given per phenotype. Data are compared with reference values for healthy control subjects. *P=0.013 vs healthy control subjects; **P .001 vs healthy control subjects; †P<0.001 vs normoandrogenic phenotype. Here normoandrogenic equals non-hyperandrogenic. (Retrieved from Moghetti et al., 2013).*

Discussion

Polycystic ovarian syndrome (PCOS) knows four different phenotypes, which all require two out of three criteria. These criteria are anovulation, hyperandrogenism and polycystic ovarian morphology. The origin of the pathophysiology of each criterion can be found within these phenotypes as this literary study has shown. Anovulation is merely caused by an excess of androgen or AMH due to increased follicle recruitment, causing preantral and small antral follicles to not differentiate into dominant follicles necessary for ovulation. This eventually leads to anovulation. Hyperandrogenism has a more diverse origin, where influences within the androgen production are at fault, having a genetic cause or the influences from circulation cause the androgen excess. Here, a lower concentration of SHBG can increase the free testosterone concentration. Besides, insulin and insulin resistance affect the production of androgens as well, via stimulating the PI3K-Akt pathway and thus the steroidogenesis in theca cells. Polycystic ovarian morphology is the last criterion that was discussed, which is mainly caused by androgenic excess and insulin resistance. Though, more studies have to be done to find more underlying mechanisms involved.

These findings all point towards a metabolic origin that causes this pathophysiology. However, these findings contradict the present phenotypes, as only phenotypes A-C are affected by hyperandrogenism, and phenotype D (non-hyperandrogenic PCOS) is not. It is peculiar that most papers cover the involvement of androgen excess and insulin in the PCOS pathophysiology, whereas in phenotype D no androgen excess is present. This study introduced some underlying causes of non-hyperandrogenic PCOS, including ethnicity and different metabolic origins. So, there is little to no explanation for the pathophysiology of non-hyperandrogenic PCOS.

It is important to note that this literary research has its limitations. When looking into data from research papers regarding PCOS, findings on obesity were always included. This was not discussed in detail, though PCOS is known to be more common in women with PCOS, besides being present in normal-weight women (Barber et al., 2006). Besides obesity, menopause is also a key factor in hormone-related disorders, since the hormone balance shifts when women enter this phase. However, our study did not include argumentation regarding this group as those women do not have the same hormonal levels as pre-menopausal women and can have ovulatory dysfunction due to menopause, not caused by PCOS underlying causes (Roeca et al., 2018).

Oestrogen production and action were also not mentioned. Androgen production does not stop at testosterone as already mentioned. Testosterone is converted into oestrogen: oestradiol and oestrone, which also affect the body. Studies have indicated that besides androgen and androgen receptors, oestrogen and its receptors are involved in the development of PCOS (Walters, 2020; Walters & Handelsman, 2018; Xu et al., 2021). Oestrogen receptors are indicated to regulate follicular maturation and stimulation of granulosa cell growth. Oestrogen antagonists are used as a treatment to initiate ovulation in women with PCOS (Steckler et al., 2007). It is thus an important factor involved in its pathophysiology besides androgens. So, for a full picture of the metabolic background of PCOS, the oestrogen receptor has to be investigated as well.

In conclusion, non-hyperandrogenic PCOS does not belong under the PCOS syndrome umbrella. Given the fact that no influence of androgens causes the pathophysiology of this phenotype, it cannot be scaled under PCOS. Besides, the Androgen Excess Society rewrote the diagnostic criteria for PCOS in 2006, excluding non-hyperandrogenic PCOS, which was not accepted. Maybe, with the current knowledge, these criteria can be adopted now, so patients with this non-hyperandrogenic PCOS can

get the treatment that fits best to their pathophysiology. Also, the current research on hyperandrogenic PCOS can be more skewed into their pathophysiology, having more uniform cohorts.

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