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HORMONE IMBALANCE IN POLYCYSTIC OVARIAN SYNDROME

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Abstract: Polycystic Ovarian Syndrome (PCOS) is a disease that is characterized by an increased Gonadotropin-Releasing-Hormone (GnRH) pulsatile frequency, causing an altered LH/FSH ratio. More precisely an increased Luteinizing Hormone (LH) secretion compared to a decreased Follicle-Stimulating Hormone (FSH) secretion leads to the development of hyperandrogenism and to a low-level concentration of estrogens and therefore decreased negative estrogenic feedback in the control axis. The purpose of this review is to connect the physiological Hypothalamic-Pituitary-Ovarian (HPO) axis with said pathology and the ensuring discussion about the possible mechanisms of pathogenesis and guidelines for relieving associated symptoms.

Keywords: Polycystic Ovarian Syndrome, hyperandrogenism, hyperestrogenism, infertility, hirsutism.

Introduction

Polycystic Ovarian Syndrome (PCOS) is extremely frequent in the general population (4-20% of women of fertile age are affected), yet it is still not perceived as a major health problem although, in addition to anovulation, hyperandrogenism, and polycystic ovaries, PCOS can lead to insulin resistance, Luteinizing Hormone (LH) hypersecretion, dyslipidemia, hirsutism, type 2 diabetes and/or infertility (Deswal et al, 2020). Despite the prevalence, the ethiology of PCOS is poorly known, the disease is incurable, and the treatment is symptomatic. Due to the extremely varied symptomatology, several diagnostic criteria have been established over time: *National Institute of Health (NIH) criteria* (Nicolaides et al, 2020), *Rotterdam criteria* (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) and *AE-PCOS Criteria* (Azziz et al, 2006).

The purpose of this review is to emphasize the most important mechanisms of estrogen secretion regulation in women, insisting on the biochemical mechanisms underlying the hormonal imbalance that occur in PCOS and some therapeutic possibilities for the treatment of the associated symptoms.

Biochemical aspects of estrogen secretion

The estrogen secretion underlies the Hypothalamic-Pituitary-Ovarian (HPO) axis. The HPO axis starts in the hypothalamus, where production of Gonadotropin-Releasing-Hormone (GnRH) takes place especially in the preoptic and anterior nuclei of the hypothalamus (Lee et al, 2021). The GnRH will use the hypothalamic hypophyseal portal system to reach the hypophysis, where it will act on gonadotroph cells. Depending on the frequency of release of GnRH, the women can either have FSH or LH release. High frequency of GnRH release will cause more LH release compared to FSH, while lower frequency of GnRH release will stimulate more FSH release compared to LH (Stamatiades and Kaiser, 2018).

The FSH and LH will then enter the bloodstream and act on the primordial follicles of the ovary. FSH is especially important as it causes not only proliferation of granular cells (follicular cells), but it also causes development and formation of thecal cells. This newly formed layer can be divided into theca interna and theca externa. Regulation of estrogen and progesterone production by the hypothalamic-pituitary axis is presented in **Figure 1**.

The theca interna is especially important because it causes formation of progesterone and other similar hormones, which can be used by the granular cells to produce estrogens. Another important effect of FSH is that it causes increased expression of estrogen receptors to which estrogen will bind and cause an increased expression of LH receptors. The combination of increased LH receptors, increased estrogen receptors and FSH causes increased proliferation of granular cells, which will cause more estrogen secretion. Once the estrogen reaches a specific threshold, it will cause a switch from negative feedback of estrogen to positive feedback of estrogen (Orisaka et al, 2021).

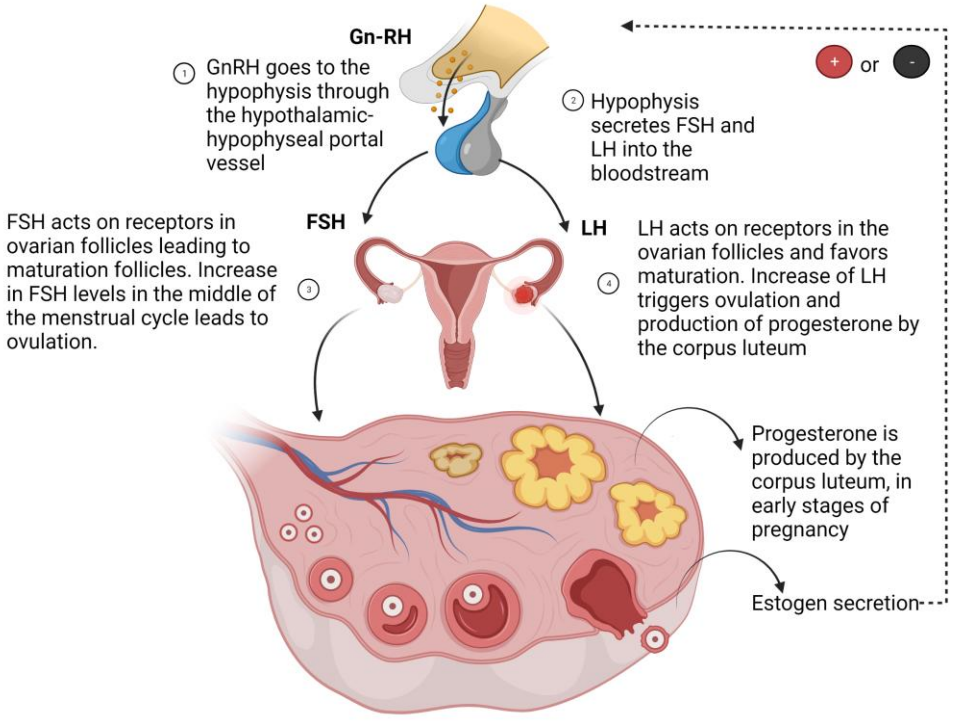

Fig. 1. Regulation of estrogen and progesterone production (FSH - Follicle-Stimulating Hormone, LH - Luteinizing Hormone, GnRH - Gonadotropin-releasing hormone) (da Costa CS et al., 2021)

In addition to the above-mentioned process, androgens are very important for the development of primordial follicles to primary follicles and from primary follicles to secondary follicles. They exert these effects through Insulin-like Growth Factor-1 (IGF-1) which is produced by androgens binding to the androgen receptor (AR). Androgens seem also to facilitate further development to the Graafian follicle by increasing sensitivity to FSH, via increased production of FSH receptors. (Sanchez-Garrido and Tena-Sempere, 2020).

Although a lot of primordial follicles will start to develop during the follicular phase, only one primordial follicle will become a mature Graaf follicle. Although it is not clear how this is happening, one of the theories is that the increase in estrogen will cause a reduced secretion of GnRH due to the negative feedback. The predominant follicle will be able to keep producing more estrogen with such a low level of GnRH but the other follicles, which were not as developed will not produce enough estrogen to survive, and therefore involute (Ding et al, 2021). Another hormone which inhibits follicular differentiation is the Anti-Müllarian Hormone (AMH). AMH is secreted in high amounts by tertiary and Graaf follicles, and will further inhibit the FSH and estrogen release, accentuating the abovementioned process (Rudnicka et al, 2021). It is important to mention that the release of this hormone is constant and does not vary throughout the ovarian cycle. It will vary however throughout the life of the female, being at its peak at around 25 years and minimal at menopause. AMH in fact seems to represent the available follicles in the ovary and is therefore often used to denote fertility. High levels of AMH however are not always representative for increased fertility, as they will be elevated in PCOS, even though the patients are infertile (Moolhuijsen and Visser, 2020).

Recent research has suggested that both negative and positive feedback act via an indirect pathway. Experiments on transgenic mice have shown that there are specific neuron groups residing in the hypothalamus, which via connections with GnRH neurons can cause both positive and negative feedback. The neuronal groups responsible for negative feedback in these mice are located in the arcuate and median eminences although the group of neurons behind negative feedback doesn't seem to have been fully discovered. The positive feedback neurons are located in the Anteroventral Periventricular nucleus (AVPV), median preoptic, periventricular preoptic and suprachiasmatic nucleus. The first three nuclei form the RP3V group while the suprachiasmatic nucleus is responsible for connecting the estrogen feedback to the circadian rhythm (Moenter et al, 2020). In addition, a connection between the circadian rhythm and the estrogen feedback in humans could not be established while in other species like sheep and primates, circadian rhythms don't seem to be relevant in the cyclical changes of estrogen feedback (Webster and Smarr, 2020).

There are two types of Estrogen Receptors (ER), Erα/β, one that acts through an E-dependent pathway and an Estrogen Response Elements (EREs)–independent pathway. The former pathway mentioned acts through activation of the ERE to alter the transcription of specific genes. The ERE-independent pathway exerts its effect by influencing protein function which in turn influences gene transcription at non-ERE sites (Chen et al, 2022). Recent data agree that the ERα that act through the ERE-independent pathway are responsible for partially causing the negative feedback, that means that although this type of receptor causes negative feedback, alone it is not able to cause the strength of the negative feedback noticed in the normal organisms. To

cause the normal strength of the negative feedback, it is noticed that the ERα that acts through both the ERE-dependent and ERE-independent pathways is needed in every healthy organism. The ERα that act through the ERE-dependent pathway are responsible for the positive feedback and in part for the negative feedback (Moenter et al, 2009).

In the last decade, a lot of steps forward in understanding the positive feedback of estrogen were done. After each menstruation, the GnRH released in a pulsatile manner causes release of FSH and LH. The FSH acts on follicular cells in the follicles of the ovary. Those types of cells are very important as they are responsible for the production of estrogens. With each GnRH release from the hypothalamus the number of follicular cells increases and so does the amount of estrogen. These estrogens will act on the ERα, causing the increased transcription of the progesterone receptor gene, thereby increasing the amount of progesterone receptors on the cell membranes of the gamma-aminobutyric acid (GABA) and glutamatergic (Glu) neurons. Estrogens also act on astrocytes which cause the release of Ca^{2+} ions from the smooth endoplasmic reticulum, thus starting progesterone synthesis. By binding to the progesterone receptors, the progesterone causes the release of GABA and Glu, causing the LH surge (He et al, 2017).

Although a lot of receptors are responsible for the synthesis of progesterone in the astrocytes, the main receptor is the ERα (Sinchak et al, 2020). During the negative feedback, GnRH neurons have a pulsatile secretion, secreting GnRH for a few minutes every 1h to 3h. The advantage of such a pulsatile secretion is that the gonadotroph cells in the adenohypophysis will not suffer a downregulation of the GnRH receptors and therefore lose responsiveness to GnRH. During the positive feedback we have a change in secretion from only a pulsatile secretion to a pulsatile secretion superimposed with a continuous secretion. The reason behind this change is an increase of estrogen over a specific threshold for several hours (Herbison, 2018).

A specific group of GnRH neurons seems to be involved in the change of secretion. This group is composed of a sub-population of GnRH neurons located in the rostral preoptic area around the organum vasculosum of the lamina terminalis. The reason behind their involvement is still unknown. A hypothesis is that this subgroup is involved in the formation of the positive feedback due to specific properties, whereas another hypothesis is that they just receive a different type of signal causing them to react differently. As a result, the properties of this subgroup are unknown. As an example, it is not known if this subgroup is only responsible for the surge or if they also exhibit pulsatile secretion and are therefore involved in negative feedback too (Constantin, 2017).

Hormone imbalance in PCOS

The most important underlying reason for the pathogenesis of PCOS is genetics. The women suffering from PCOS have an increased frequency of release for GnRH and hyperandrogenism (Witchel et al, 2019). Hyperandrogenism is an essential characteristic of PCOS, being caused in women by the increase in serum values of androstenedione and testosterone, and in the case of 50% of women by the increase in dehydroepiandrosterone (DHEA) (Manu et al, 2022).

A high GnRH release frequency will lead to an increased release of LH and a decreased release of FSH and, in some patients the cells responsible for producing LH react strongly to GnRH leading to an excess LH production (Escobar-Morreale, 2018). As high quantities

of LH and a low FSH are released, the ovarian follicles will not develop, leading to lack of estrogen production. The cells in the follicles responsible for estrogen production are the follicular cells and the thecal cells (Richards et al, 2018). The thecal cells produce androgens, which will be used to produce estrogens. Due to the low production of FSH however, there will be a lack of granulosa cells which will lead to hyperandrogenism. The hyperandrogenism will lead to the partial development of the follicles, but due to the lack of FSH, estrogens are not produced. Furthermore, due to high levels of 5α-reduced androgens, the aromatase activity is reduced further inhibiting estrogen production. Due to the lack of ovulation overall, there will be more follicles, which combined with the partial development of the follicles due to hyperandrogenism will lead to very high levels of AMH. The increased AMH levels will lead to further inhibition of FSH release (Fujibe et al 2019).

Some comorbidities that will exacerbate the symptoms of PCOS are obesity and insulin resistance. Obesity will also allow for the transformation of androgens to estrogens since adipose tissues also contain aromatase. Insulin resistance is defined as resistance of the insulin receptors to the hormone because of increased levels of free fatty acids, increased levels of adipokines with the exception of adiponectin and inflammation. Obesity will increase the levels of adipokines, thereby increasing resistance and increase the levels of free fatty acids. The increased levels of free fatty acids favor insulin resistance directly and indirectly; directly by inhibiting insulin signaling and indirectly by favoring inflammation. Inflammation will lead to increased levels of IL1 which will lead to insulin resistance (Moghetti and Tosi, 2021).

It should also be noted that hyperandrogenism itself can cause insulin resistance. In addition to that, elevated levels of insulin found in patients suffering from insulin resistance, can exacerbate the already present hyperandrogenism by stimulating thecal cells to produce even more androgen (Unluhizarci et al, 2021).

The high levels of androgens and the elevated insulin levels due to insulin resistance will lead to a decrease in the production of Sex Hormone Binding Globulin (SHBG) which, as a result, leads to increased levels of androgens in the blood, and thus will exacerbate the effects of androgens. Since the effects of androgens are enhanced, insulin resistance will be increased (Zhu et al, 2019). High androgen levels in obese women suffering from PCOS, will lead to the production of estrogens in the adipose tissue due to the presence of aromatase in the adipocytes. Basic mechanism of PCOS pathogenesis and pathophysiology are presented in **Figure 2**.

Another secondary effect of hyperandrogenism in PCOS is hirsutism, while the hyperestrogenism will be responsible for the hyperplasia of the endometrium. Insulin resistance and obesity are responsible for the cardiovascular problems and the high risk for developing diabetes encountered in these patients (Spritzer et al, 2022; Hill et al, 2021).

Main biochemical pathways in PCOS

The main biochemical pathway responsible for the PCOS pathogenesis involves estrogen production in the ovaries. This pathway, at the base of the two-cell theory, necessitates both theca and follicular cells. In both thecal and follicular cells, we have LH receptors and Low-Density Lipoproteins (LDL) receptors. LH secretion in women determines synthesis of steroid hormones from the ovaries (Coutinho and Kauffman, 2019).

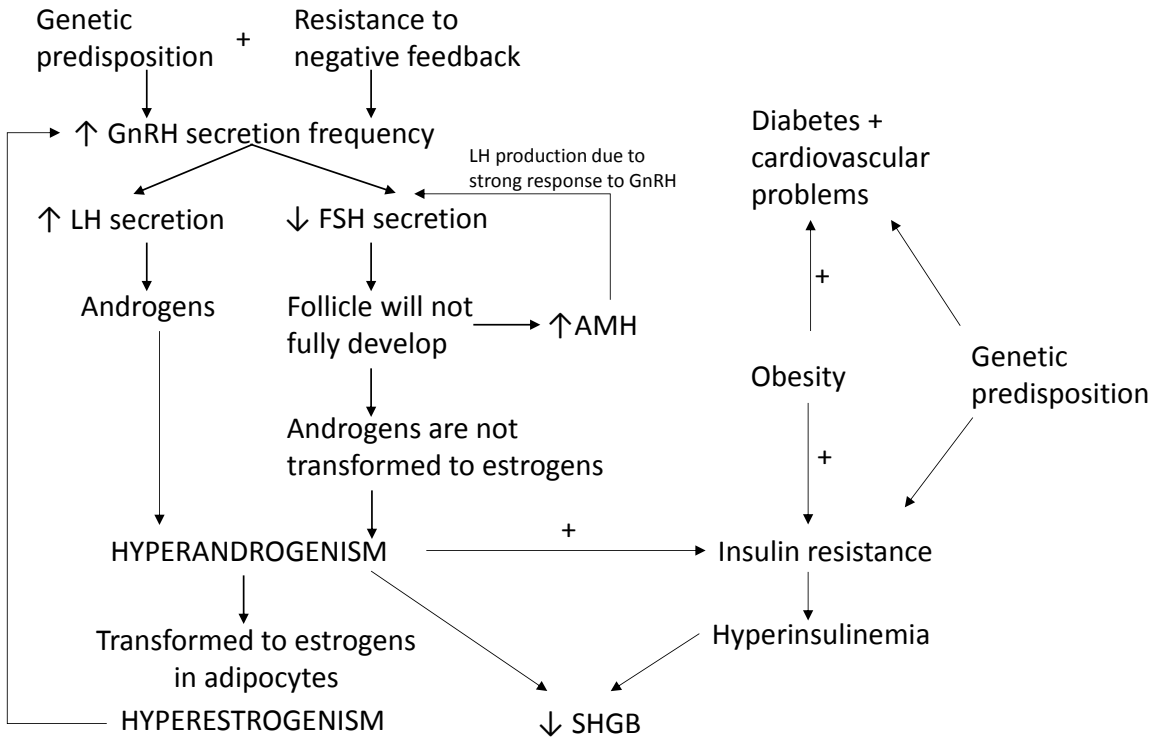

Fig. 2. Basic mechanism of PCOS pathogenesis and pathophysiology (FSH - Follicle-Stimulating Hormone, LH - Luteinizing Hormone, GnRH - Gonadotropin-releasing hormone, SHBG - Sex Hormone Binding Globulin, AMH - Anti-Müllarian Hormone)

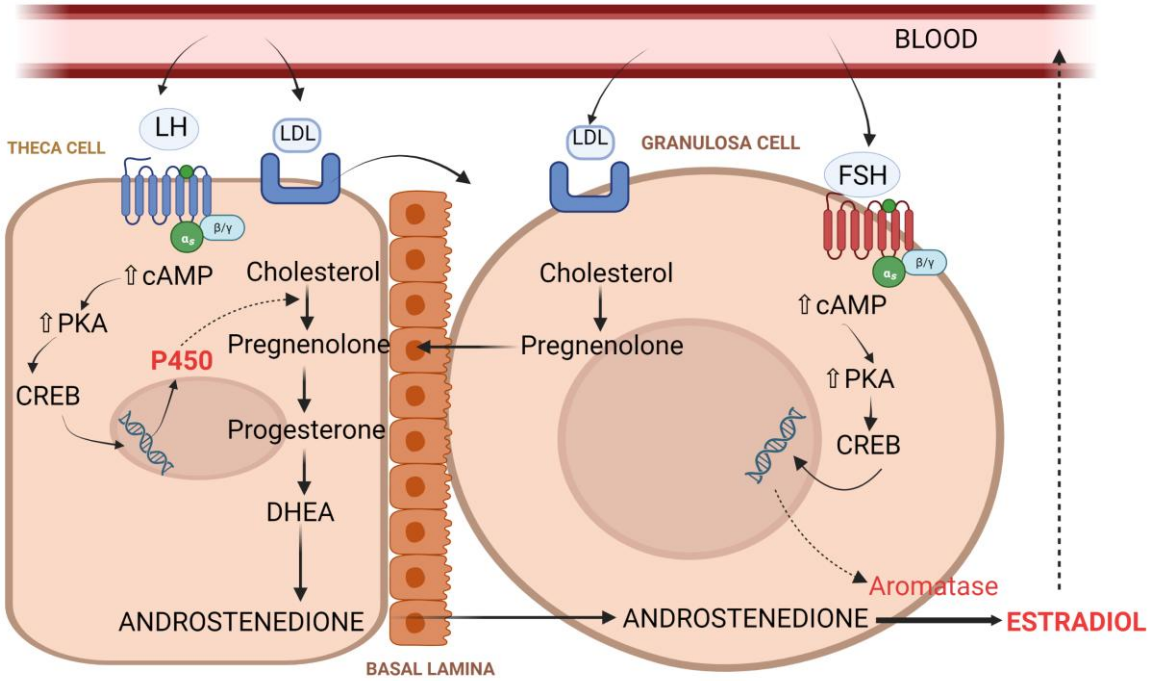

Fig. 3. Two cell theory of estrogen production (FSH - Follicle-Stimulating Hormone, LH - Luteinizing Hormone, LDL - Low Density Lipoprotein, cAMP - cyclic adenosine monophosphate, PKA - protein kinase A, CREB - cAMP response element-binding protein, P450 - Cytochromes P450, DHEA - dehydroepiandrosterone dehydroepiandrosterone) (adapted from Orisaka et al., 2021)

In the follicular cells LH leads to the production of progesterone. This newly formed progesterone will diffuse to the theca cells, where it will be added to the progesterone produced there. Progesterone will be transformed to androgens, which will diffuse to the follicular cells again, where the aromatase will transform it to estrogens. Here, by increasing the levels of cAMP, FSH activates protein kinase A (PKA) which will lead to the production of aromatase (Casarini and Crépieux, 2019). Two cell theory is presented schematically in **Figure 3**.

Depending on the concentration of androgens, these hormones will preferentially be transformed to estrogens or potent androgens thanks to the 5α- reductase. If the concentration is low, androgens will be transformed to estrogens thanks to the aromatase. If the concentration of androgens is increased, they will preferentially be transformed to potent androgens thanks to the 5α-reductase. To be noted that 5α-reduced androgens inhibit aromatase activity (Hammes and Levin, 2019).

Pharmacological alternatives to treat and relieve symptoms associated with PCOS

Considering that the symptoms of PCOS are varied (hirsutism, infertility, menstrual cycle irregularities, insulin resistance), treatment must be individualized according to the patient.

Thus, depending on the visible signs of PCOS, there are multiple pharmacological recommendations. For example, to limit hirsutism, the use of spironolactone and metformin is recommended (Onalan et al, 2005). For ovulation induction, the use of clomiphene and metformin (alone or in combination) is recommended, and depending on other comorbidities, rosiglitazone **(**Moll et al, 2006; Ganie et al, 2020). For the treatment of obesity-related insulin resistance and oligomenorrhea, the most recommended agent is also metformin (Patel, 2018). Since the disease seems to have genetic origins, the treatment itself is not curative but symptomatic.

Another pharmacological intervention to regulate the hormonal imbalance is represented by the use of oral contraceptives, if the patient can tolerate the side effects (nausea, headaches, thrombophlebitis, etc.) (Podfigurna et al, 2020).

Another aspect to consider in hyperandrogenism is that it is responsible for decreasing the levels of SHBG, whose level is low in the case of insulin resistance (Qu and Donnelly, 2020). Thus, by correcting the hyperinsulinemia, the level of SHBG returns to normal, managing to bind sex hormones, bringing back the free form of these hormones to normal concentrations.

A simpler representation of the agents used in PCOS is illustrated in **Table 1**.

Conclusions

PCOS is a complex pathology extremely frequently encountered among the female population, with multiple causes, leading to important hormonal changes and serious consequences upon the health and quality of life (diabetes, insulin resistance, hirsutism, infertility), whose treatment remains only symptomatic.

Considering the complex clinical picture, with multiple tissue modifications, but also the not to be neglected the psychological impact on the affected females, this article insists on understanding the mechanisms behind the various symptoms. One future goal is to understand the importance of individualized treatment and the early detection of risk groups.

Table 1. First and second-line agents for PCOS symptoms

First-line agents	Mechanism/Effect	Second-line agents	Mechanism/Effect	References
Clomiphene	SERM function, for infertility	Acarbose	reducing insulin resistance;	Moll et al., 2006
Metformin	Reduces insulin resistance and the manifestations related to hyperinsulinemia	Estroprogestative combinations	Reduce hirsutism	Onalan et. al, 2005
Thiazolidinediones	Similar to metformin	5-alpha-reductase inhibitors (finasteride)	Reduce hirsutism	Patel, 2018
Spironolactone	Anti-androgenic effect	Antiandrogens (flutamides)	Reduce hirsutism	Onalan et al., 2005
		Aromatase inhibitors (letrozole)	Infertility treatment	
		Anorexigenic agents (sibutramine)	Reduce hirsutism and insulin resistance	

Conflict of interest

The authors declare that there are no conflicts of interest related to this article.

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