INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of child-bearing age. It is typically identified during the early reproductive years or adolescence. Worldwide, PCOS affects 6–10% of women ***(Fauser et al., 2012)***.

The main underlying endocrinopathies related to PCOS are hyperandrogenism and impaired glucose tolerance secondary to insulin resistance. The precise pathogenesis of PCOS is yet to be determined. Autoimmunity has been proposed in the pathogenesis of PCOS since early 1990s as several authors have reported association between PCOS and certain autoimmune disorders, including autoimmune thyroiditis, Graves' disease, chronic lymphocytic thyroiditis, diabetes mellitus, and even autoimmune oophoritis and premature ovarian failure (POF). The link between PCOS and autoimmunity has been the question of recent studies and reviews ***(Hassan et al., 2014)***.

The association of ovarian autoimmunity with PCOS has been previously evaluated and the detection of autoantibodies directed against various ovarian targets strongly supports the hypothesis of an autoimmune aetiology of PCOS. However, studies of anti-ovarian antibodies (AOA) that were performed so far yielded conflicting results and until now, neither the specificity nor the diagnostic significance of these antibodies

has been unanimously established. One of the reasons for these discordances is the diversity of the detection methods as well as the heterogeneity of the patient and control groups in the different studies ***(Forges et al., 2004; Petrikova et al., 2012)***.

AIM OF THE WORK

The aim of this thesis is to investigate the association between PCOS and AOA in a sample of Egyptian women with PCOS compared to healthy controls.

POLYCYSTIC OVARY SYNDROME

A. Definition

Polycystic ovary syndrome (PCOS), is a highly prevalent heterogeneous syndrome of clinical and/or biochemical androgen excess, ovulatory dysfunction and polycystic ovaries (PCO) ***(Goodarzi et al., 2011)***. Polycystic ovary syndrome was first reported in modern medical literature by Stein and Leventhal who in 1935, described seven women suffering from amenorrhea, hirsutism, and enlarged ovaries with multiple cysts ***(Sirmans and Pate, 2014)***.

Currently, three different definitions of PCOS exist. The three recognized sets of criteria for PCOS diagnosis include the National Institute of Health (NIH) (1990) criteria, the Rotterdam (2003) criteria, and the Androgen Excess and Polycystic Ovary Syndrome Society (2006) criteria. The prevalence of PCOS depends upon the diagnostic criteria used. Rotterdam is the most inclusive, while the NIH criteria are the most strict ***(Burks and Wild, 2014)***. Importantly, other conditions are known to cause or to mimic the features of PCOS, and must be excluded prior to diagnosis. These include congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumours for hyperandrogenism and raised prolactin or insufficient luteinising hormone for anovulation ***(Norman et al., 2007)*** (Table.1).

The NIH (1990) Criteria:

The 1990 NIH–Conference of PCOS concluded that the major criteria for PCOS should include ***(Artini et al., 2010):***

- Hyperandrogenism and/or hyperandrogenemia.
- Oligo-ovulation.
- Exclusion of other known disorders that mimic the features of PCOS.

The Rotterdam (2003) Criteria:

In 2003, a consensus workshop sponsored by European Society of Human Reproduction and Embryology (ESHRE)/ American Society for Reproductive Medicine (ASRM) in Rotterdam indicated PCOS to be present if any 2 out of 3 criteria are met excluding other endocrinopathies ***(Rotterdam ESHRE/ASRM 2004)****:*

- Oligo- and/or anovulation
- Clinical and/or biochemical hyperandrogenism
- Polycystic ovaries (by ultrasound)

Androgen Excess-PCOS Society (2006) Criteria:

Finally, in 2006, the Androgen Excess-PCOS Society suggested a tightening of the diagnostic criteria to all of the following ***(Teede et al., 2010):***

- Clinical and / or biochemical hyperandrogenism.
- Oligoovulation / anovulation and/or PCO.
- Exclusion of other entities that would cause excess androgen activity.

Table (1): All possible phenotypes of PCOS ***(Goodarzi et al., 2011)***.

Potential phenotypes	Hyperandrogenemia	Hirsutism	Oligoanovulation	Polycystic ovaries	NIH 1990 criteria	Rotterdam 2003 criteria	AE-PCOS 2006 criteria
A	✓	✓	✓	✓	✓	✓	✓
B	✓	✓	✓	–	✓	✓	✓
C	✓	–	✓	✓	✓	✓	✓
D	✓	–	✓	–	✓	✓	✓
E	–	✓	✓	✓	✓	✓	✓
F	–	✓	✓	–	✓	✓	✓
G	✓	✓	–	✓	–	✓	✓
H	–	✓	–	✓	–	✓	✓
I	✓	–	–	✓	–	✓	✓
J	–	–	✓	✓	–	✓	–
K	✓	✓	–	–	–	–	–
L	–	–	–	✓	–	–	–
M	–	–	✓	–	–	–	–
N	–	✓	–	–	–	–	–
O	✓	–	–	–	–	–	–
P	–	–	–	–	–	–	–

B. Epidemiology:

Worldwide, PCOS affects 6–10% of women according to 1990 NIH criteria and even more individuals according to the broader Rotterdam criteria, which makes it one of the most common human disorders and the single most common endocrinopathy in women of reproductive age. Several types of women have an increased risk of PCOS, including those with clinical hyperandrogenism (namely, hirsutism, acne or alopecia), menstrual dysfunction, PCO, hyperinsulinemia from adiposity-dependent insulin resistance and a family history of PCOS ***(Azziz et al., 2004)***.

C. Pathogenesis:

Polycystic ovary syndrome is a heterogeneous neuroendocrine disorder with different degrees of reproductive and metabolic dysfunctions ***(Alemzadeh and Kansra, 2011)***. It is a multifactorial genetic trait with influence from both heritable and nonheritable factors. Inherited gene variants may include those regulating the secretion and action of gonadotropins and insulin, weight and energy regulation, and androgen biosynthesis and action ***(Baptiste et al., 2010)*** (Fig.1).

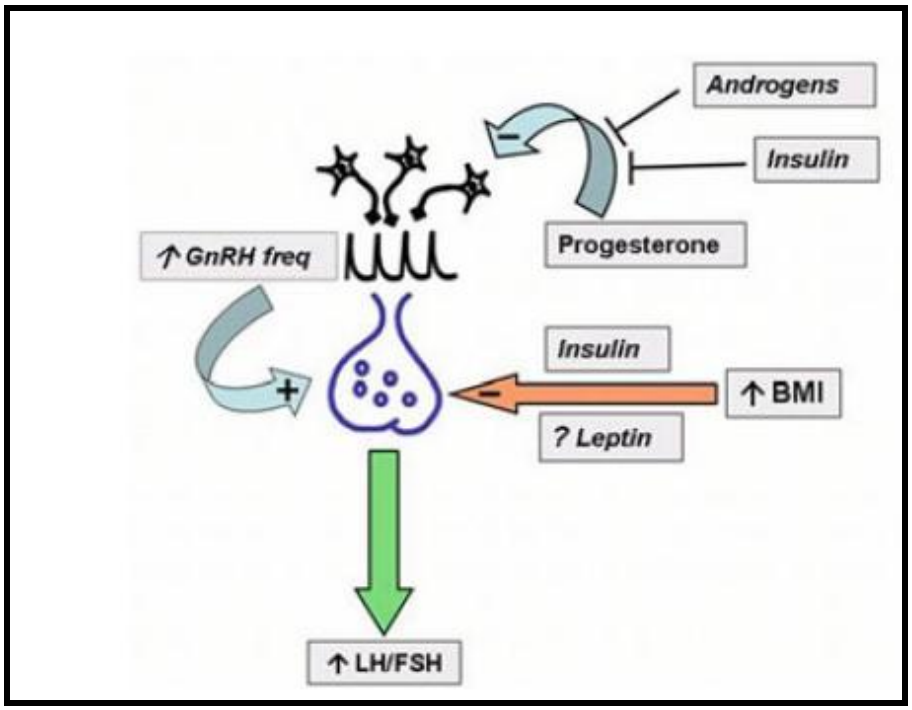

Fig. (1): The neuroendocrine abnormalities in PCOS ***(Burks and Wild, 2014)***.

1- Insulin Action Abnormalities:

About 50–70% of women with PCOS have insulin resistance beyond that predicted by their body mass index ***(DeUgarte et al., 2005)***. Women with PCOS seem to have a level of peripheral insulin resistance that is much like that of women with type 2 diabetes, which is characterized by a 35–40% decrease in insulin-mediated glucose uptake. This causes compensatory hyperinsulinemia, which drives many of the phenotypic features of PCOS ***(Norman et al., 2007)***.

Insulin resistance might contribute to hyperandrogenism and gonadotropin abnormalities through several mechanisms.

High concentrations of insulin reduce circulating sex hormone-binding globulin (SHBG) values, thereby increasing the bioavailability of testosterone, and might also serve as a co-factor to stimulate adrenal and ovarian androgen biosynthesis, thereby contributing to abnormal gonadotropin concentrations ***(Azziz, 2003)***. Also, Insulin resistance in the disorder is characterised by selective-tissue insulin sensitivity, in which some tissues seem highly resistant (ie, skeletal muscle) and others sensitive (ie, adrenal and ovary). In affected tissues, metabolic pathways are generally resistant to stimulation by insulin, but mitogenic or steroidogenic pathways are not ***(Diamanti-Kandarakis and Papavassiliou, 2006)***.

2- Androgen abnormalities:

60–80% of women with PCOS have high concentrations of circulating testosterone and about 25% have high concentrations of dehydroepiandrosteronesulfate (DHEAS) ***(Kumar et al., 2005)***. Both insulin and luteinizing hormone (LH), alone and in combination, exacerbate androgen production ***(Homburg, 2008)*** (Fig.2).

Polycystic ovaries have a thickened thecal layer, and thecal cells derived from these ovaries secrete excessive androgens in vitro under basal conditions or in response to LH stimulation. The expression and activity of many steroidogenic enzymes is increased in thecal cells from patients with the disorder, and this hyperactivity might be caused by a

disturbance of intracellular signalling pathways ***(Escobar-Morreale et al., 2005)***.

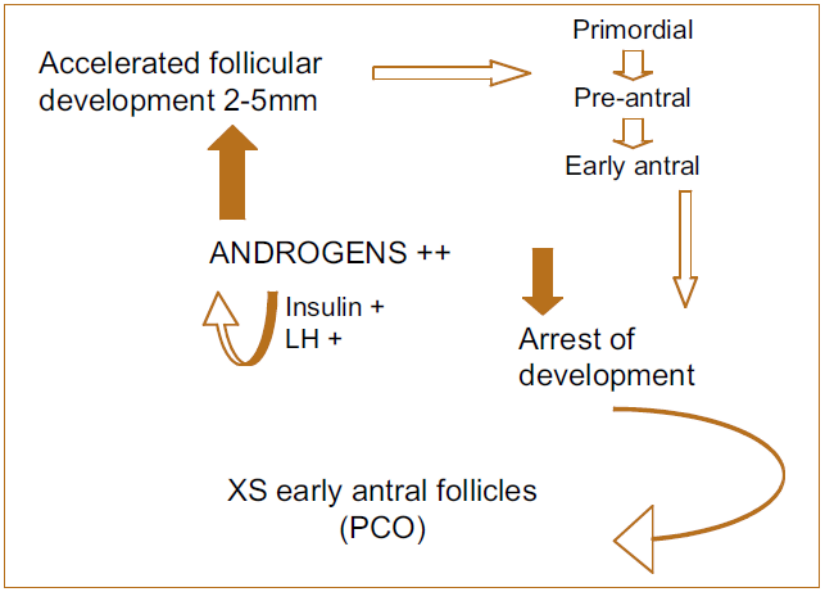

Fig. (2): The key role of excessive androgen production in the polycystic ovary ***(Homburg, 2008)***.

3- Ovulatory dysfunction:

Approximately six to eight times more pre-antral and small antral follicles are present in the polycystic ovary of PCOS women compared with the normal ovary. They arrest in development at a size of 2–9 mm, and a total ovarian volume >10 cc is often witnessed ***(Homburg, 2008)***.

In PCOS, ovarian hyperandrogenism, hyperinsulinemia from insulin resistance and altered intraovarian paracrine signaling can disrupt follicle growth. The consequent follicular arrest in PCOS is accompanied by menstrual irregularity, anovulatory subfertility and the accumulation of small antral

follicles within the periphery of the ovary, giving it a polycystic morphology ***(Huang et al., 2010)***.

Several studies have reported a positive correlation between follicle number and serum testosterone and androstenedione concentrations in these women. Although the increase in follicle numbers in PCO might be due to a trophic effect of androgens on primate follicular cells, it might also be that the follicles of a polycystic ovary grow very slowly, creating a stockpiling effect. This slow growth might be mediated by deficient growth signals from the oocyte or by the inhibitory effect of excess Anti-Mullerian hormone produced by granulosa cells of ovarian follicles ***(Jonard and Dewailly, 2004; Welt et al., 2006)***.

The frequent occurrence of associated insulin resistance and hyperinsulinemia in PCOS further exacerbates ovarian follicular arrest. Hyperinsulinemia amplifies LH-stimulated and insulin-like growth factor 1 (IGF-1)-stimulated androgen production, elevates serum free testosterone levels through decreased hepatic SHBG production, enhances serum IGF-1 bioactivity through suppressed IGF-binding protein production and promotes premature follicle luteinization ***(Balen et al., 2005)***. Granulosa cells from women with PCOS also seem to be selectively insulin resistant, whereby insulin-stimulated glucose metabolism is impaired but insulin-stimulated steroidogenesis is normal, suggesting that deficient energy mobilization within the follicle contributes to anovulation ***(Rice et al., 2005)***.

4- Gonadotropin abnormalities:

Women with PCOS display abnormal patterns of gonadotropin pulsatility, which is characterised by excessive secretion of LH but normal secretion of follicle-stimulating hormone (FSH). This pattern of secretion gives rise to an abnormal circulating LH to FSH ratio in some patients, mostly those of normal weight ***(Norman et al., 2007)***.

Increased LH pulse frequency in PCOS, from enhanced hypothalamic gonadotropin releasing-hormone (GnRH) pulsatile release, occurs owing to reduced steroid hormone negative feedback on LH secretion because of androgen excess. Other neuroendocrine abnormalities in women with PCOS include exaggerated LH responsiveness to GnRH ***(Blank et al., 2006)***.

5- Genetic factor:

Polycystic ovary syndrome is more prevalent among family members than in the general population with 20–40% of first-degree female relatives of women with PCOS affected by the syndrome ***(Azziz et al., 2004)***. A heritable component to hyperandrogenemia, insulin resistance and insulin secretion exists in families of women with PCOS, as evidenced by elevated DHEAS levels in first-degree male relatives and an increased prevalence of insulin resistance, endothelial dysfunction and metabolic syndrome ***(Coviello et al., 2009)***.

Polycystic ovary syndrome appears to be inherited as a common complex disorder, similar to type 2 diabetes mellitus and inflammatory bowel disease, where in several genetic variants are present that each contribute a moderate effect, combined with risk-increasing lifestyle and environmental factors ***(Goodarzi et al., 2011)***. In recent decades, many researchers have focused on the virulence gene association studies, which are principally related to reproductive hormones, insulin resistance, and chronic inflammation ***(Chen and Shi, 2010)***.

6- Environmental factors:

Lifestyle profoundly affects the phenotypic expression of PCOS. Weight gain worsens metabolic and reproductive abnormalities of PCOS, as evidenced by increased total and abdominal obesity as well as insulin resistance, menstrual irregularity and hyperandrogenism in women with the most severe PCOS phenotype. Conversely, weight loss in women with PCOS lowers circulating androgen and insulin levels, while improving hirsutism, menstrual and ovulatory dysfunction as well as dyslipidemia. A sedentary lifestyle alone also contributes to metabolic dysfunction in PCOS because moderate-intensity exercise without weight loss improves insulin resistance and decreases body adipose tissue ***(Dewailly et al., 2006; Moran et al., 2009)***.

D. Diagnosis:

1- Clinical assessment (Teede et al., 2010):

PCOS has many signs and symptoms;

- **Menstrual disorders:** PCOS is mostly associated with oligomenorrhea or amenorrhea.
- **Infertility:** results directly from chronic anovulation.
- **High levels of masculinizing hormones:** lead to acne, hirsutism and androgenic alopecia.
- **Metabolic features:** This appears as a tendency towards central obesity and other symptoms associated with insulin resistance.

2- Laboratory investigations:

Recommendations for testing include total and free testosterone, (DHEA-S), prolactin, and thyroid stimulating hormone (TSH) ***(Sheehan, 2004)***. Irregular ovulation should be confirmed by either overt or biochemical confirmation of oligoanovulation, particularly as 40% of hirsute patients with 'regular' episodes of vaginal bleeding are actually oligoanovulatory, as evidenced by low midluteal serum progesterone levels ***(Azziz, 2003)***.

Principal disorders to exclude are steroid 21-hydroxylase deficient non classic adrenal hyperplasia (NCAH), hyperprolactinemia

and thyroid dysfunction. The last two are excluded by serum prolactin and TSH measurements, although PCOS can still be diagnosed once prolactin levels and or thyroid dysfunction is normalized, if PCOS-related signs and symptoms persist. Non classic adrenal hyperplasia can be excluded by obtaining a follicular (preovulatory) phase serum 17-hydroxyprogesterone (17-OHP) level (the immediate steroid precursor for steroid 21-hydroxylase) displays the most useful laboratory tests in the evaluation of PCOS ***(Goodarzi et al., 2011)*** (Table 2).

Table (2): The most useful laboratory tests in the evaluation of PCOS ***(Goodarzi et al., 2011)***.

Laboratory test	Usefulness
TSH Prolactin	To rule out thyroid dysfunction and hyperprolactinemia If present, reassess for PCOS once resolved
17-OHP (measured in the follicular phase)	If 17-OHP >6 nmol/l, perform an ACTH stimulation test An ACTH-stimulated 17-OHP >10 nmol/l is diagnostic of 21-OH deficient NCAH*
Total and free testosterone	To assess for hyperandrogenemia if no clinical evidence of hyperandrogenism (that is, hirsutism) is present Total testosterone >7 nmol/l or DHEAS >16 μmol/l should prompt evaluation for an androgen-secreting neoplasm; however, clinical presentation is more useful than androgen levels for predicting the presence of an androgen-secreting neoplasm
Luteal phase (day 22–24) progesterone	To assess ovulation in patients with hirsutism who report 'regular menses'
1 mg DST or 24 h urinary free cortisol	To screen for Cushing syndrome if clinical stigmata are present

*The ACTH stimulation test is performed with 250 μg of corticotropin, followed by measurement of 17-hydroxyprogesterone 60 min later. Abbreviations: ACTH, adrenocorticotropic hormone; DHEAS, dehydroepiandrosterone sulfate; DST, dexamethasone suppression test; 21-OH, 21-hydroxylase; 17-OHP, 17-hydroxyprogesterone; NCAH, nonclassic adrenal hyperplasia; PCOS, polycystic ovary syndrome.