INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder among reproductive-aged women associated with anovulation, infertility and hyper-androgenism ***(Lim et al., 2013)***. It is estimated that 4–7% of reproductive-aged women have PCOS according to the National Institutes of Health (NIH) criteria of hyperandrogenism and anovulation ***(Azziz et al., 2004)*** or 15–18% according to the European Society of Human Reproductive and Embryology/ American Society for Reproductive Medicine (ESHRE/ASRM) criteria of two of the three features of (i) anovulation; (ii) hyperandrogenism; and (iii) polycystic ovaries on ultrasound ***(March et al., 2010; Mehrabian et al., 2011)***. Transvaginal ultrasound examination typically reveals ovaries that are modestly enlarged and contain numerous small follicles aligned in the periphery ***(March et al., 2010)***.

Women with PCOS have increased risk of metabolic syndrome ***(Hudecova et al., 2011)***, type 2 diabetes ***(Moran et al., 2010)***, and cardiovascular diseases including coronary heart disease and stroke ***(de Groot et al., 2011)***.

A large proportion of women with PCOS are overweight, obese or centrally obese. Excess body weight worsens certain features of PCOS including hyperandrogenism ***(Liou et al.,***

2009), menstrual disturbances ***(Liou et al., 2009)***, infertility ***(Brassard et al., 2008)***, insulin resistance ***(Kaya et al., 2009)*** and dyslipidaemia ***(Lim et al., 2013)***.

Ghrelin is a recently discovered peptide hormone produced by oxyntic cells in the gastric mucosa ***(Kojima et al., 1999)*** with important effects on energy balance, food intake and weight regulation ***(Otto et al., 2005)***. Ghrelin is a strong secretagogue of growth hormone ***(Ghigo et al., 2005)***. Low ghrelin levels were found in conditions of positive energy balance such as obesity ***(Tschop et al., 2001)*** and, accordingly, studies have reported low ghrelin levels to be associated with insulin resistance and diabetes ***(Poykko et al., 2003)***.

These biological functions of ghrelin lead to speculation about its possible role in the pathogenesis of PCOS ***(Pagotto et al., 2002)***. A significant correlation between androgen levels and ghrelin was found in some studies ***(Panidis et al., 2005)***, thus further supporting the relationship between android characteristics in PCOS and metabolic ***(Diamanti-Kandarakis et al., 2007)*** and cardiovascular risk factors ***(Rizzo et al., 2007)***.

AIM OF THE WORK

The aim of this study is to compare ghrelin levels in women with polycystic ovary syndrome and healthy subjects and to evaluate the relationships between circulating ghrelin and the clinical and biochemical manifestations in women with polycystic ovary syndrome.

FOLLICULOGENESIS

In biology, folliculogenesis is the maturation of the ovarian follicle, a densely-packed shell of somatic cells that contains an immature oocyte. Folliculogenesis describes the progression of a number of small primordial follicles into large preovulatory follicles that enter the menstrual cycle. Contrary to male spermatogenesis, which can last indefinitely, folliculogenesis ends when the remaining follicles in the ovaries are incapable of responding to the hormonal cues that previously recruited some follicles to mature. This depletion in follicle supply signals the beginning of menopause ***(Hirshfield, 1991)***.

The primary role of the follicle is oocyte support. From birth, the ovaries of the human female contain a number of immature, primordial follicles. These follicles each contain a similarly immature primary oocyte. After puberty and commencing with the first menstruation, a clutch of follicles begins folliculogenesis, entering a growth pattern that will end in death or in ovulation (the process where the oocyte leaves the follicle) ***(Craig et al., 2007)***.

During post-pubescent follicular development, and over the course of roughly a year, primordial follicles that have begun development undergo a series of critical changes in character, both histologically and hormonally. Two-thirds of the way

through this process, the follicles have transitioned to tertiary, or antral, follicles. At this stage in development, they become dependent on hormones emanating from the host body, causing a substantial increase in their growth rate ***(McGee and Hsueh, 2000)***.

With a little more than ten days until the end of the period of follicular development, most of the original group of follicles died (a process known as atresia). The remaining cohort of follicles enter the menstrual cycle, competing with each other until only one follicle is left. This remaining follicle, the late tertiary or pre-ovulatory follicle, ruptures and discharges the oocyte (that has since grown into a secondary oocyte), ending folliculogenesis ***(Matzuk et al., 2002)***.

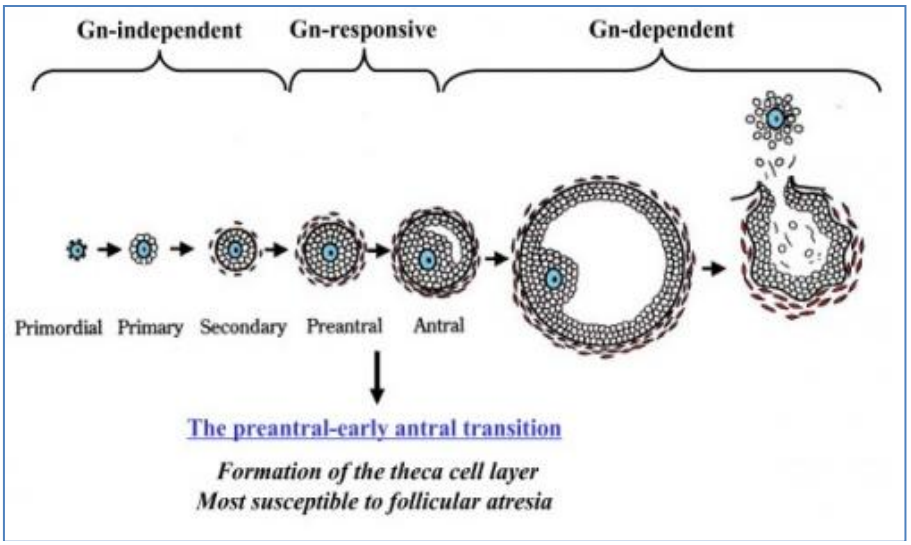

Fig. (1): Diagram of folliculogenesis, starting from pre-antral (late secondary) ***(Orisaka et al., 2009)***.

Phases of follicular development

Folliculogenesis lasts for approximately 375 days. It coincides with thirteen menstrual cycles. The process begins continuously; meaning that at any time the ovary contains follicles in all stages of development, and ends when a mature oocyte departs from the preovulatory follicle in a process called ovulation ***(Richards et al., 2002)***.

The growing follicle passes through the following distinct stages that are defined by certain structural characteristics (unfamiliar terms will be defined in their respective sections). In a larger perspective, the whole folliculogenesis, from primordial to preovulatory follicle, belongs to the stage of ootidogenesis of oogenesis ***(Fortune, 2003)***.

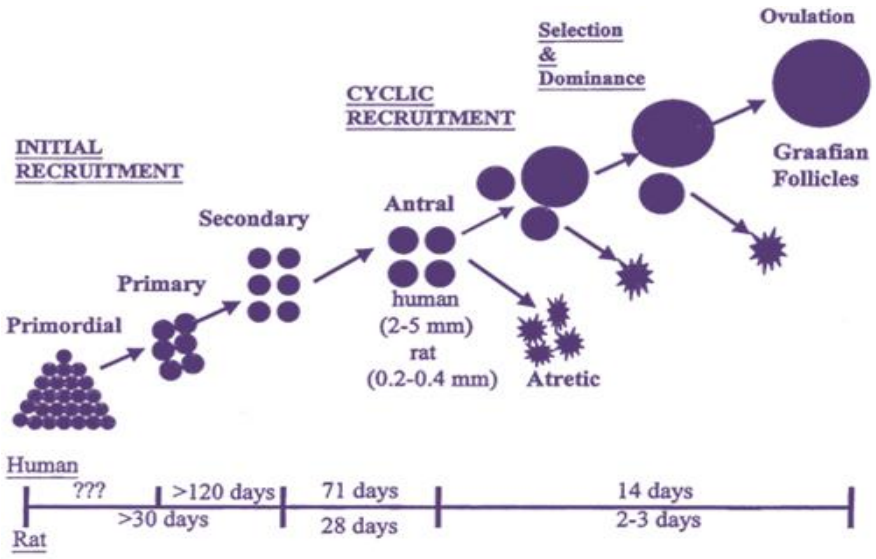

Fig. (2): Phases of follicular development ***(McGee and Hsueh, 2000)***.

Ovulation and the corpus luteum

By the end of the follicular, or proliferative, phase of the thirteenth day of the menstrual cycle, the cumulus oophorus layer of the preovulatory follicle will develop an opening, or stigma, and excrete the oocyte with a complement of cumulus cells in a process called ovulation ***(Pache et al., 1990)***.

The oocyte is now called the ovum and is competent to undergo fertilization. The ovum will now travel down one of the fallopian tubes to eventually be discharged through menstruation, if not fertilized by a sperm cell, or implanted in the uterus, if previously fertilized. The fully developed oocyte (gamete) is now at the behest of the menstrual cycle. The ruptured follicle will undergo a dramatic transformation into the corpus luteum, a steroidiogenic cluster of cells that maintains the endometrium of the uterus by the secretion of large amounts of progesterone and minor amounts of estrogen ***(Albrecht et al., 1997)***.

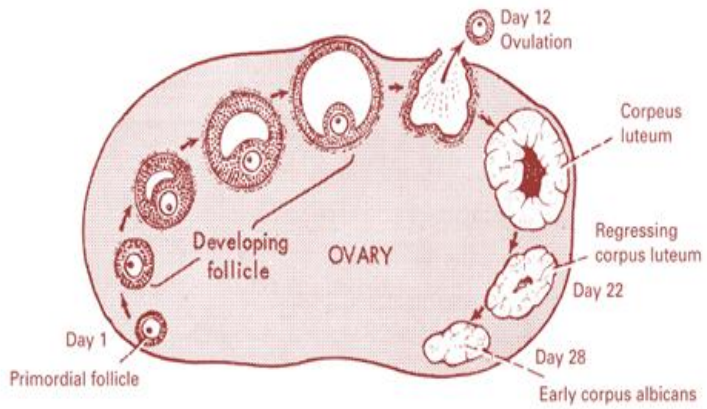

Fig. (3): Ovarian cycle ***(Hodgen, 1982)***.

POLYCYSTIC OVARY SYNDROME

Introduction

The presence of male secondary sexual characteristics in women has been recognized from ancient times, but it was not until 1921 when ***Achard and Thyers*** reported the association of hyperandrogenic symptoms with abnormalities in glucose metabolism, highlighting the presence of polycystic ovaries (PCO) in some of their patients (***Escobar-Morreale et al, 2005)***.

In 1935, *Stein and Leventhal* published a paper on their findings in seven women with amenorrhea, hirsutism, obesity and a characteristic polycystic appearance to their ovaries, which was the first description of a complex phenotype today known as the polycystic ovary syndrome (PCOS) ***(Ehrmann, 2005)***.

Stein and Leventhal described seven patients (four of whom were obese) with amenorrhea; hirsutism; and enlarged polycystic ovaries. They reported the results of bilateral wedge resection, removing one-half to three-fourths of each ovary; all seven patients resumed regular menses, and two became pregnant. They developed the wedge resection after they observed that several of their amenorrheic patients menstruated after ovarian biopsies and reasoned that the thickened tunic was

preventing follicles from reaching the surface of the ovary ***(Speroff and Fritz, 2005)***.

PCO is diagnosed by the presence of 12 or more follicles in each ovary measuring 2 to 9 mm in diameter and/or increased ovarian volume >10 cm^3; calculated using the formula (0.5 x length x width x thickness), better by transvaginal approach and the presence of a single PCO is sufficient to provide the diagnosis ***(Balen et al., 2003)***.

PCOS is the most common endocrine disorder affecting 5-10% of women of reproductive age ***(Asuncion et al., 2000)***, accounting for at least 75% of cases with anovulatory infertility ***(Hull, 1987)***. It is characterized by a heterogeneous group of disorders that occur in varied combinations including clinical [oligomenorrhoea/ anovulation, hirsutism, acne and elevated body mass index (BMI)], biochemical (elevated circulating androgens and/or LH and evidence of insulin resistance) and/or ultrasound features of polycystic ovaries ***(Amer et al, 2007)***.

Diagnostic criteria for PCOS

PCOS is a disorder that was eventually recognized as affecting as many as one in 15 reproductive-aged women and to have significant reproductive, metabolic and dermatological consequences ***(Azziz, 2004)***.

Several factors contribute to difficulties in the diagnosis of PCOS, presenting signs and symptoms are heterogeneous and vary over time, in addition, a precise and uniform definition of the syndrome has been lacking ***(Ehrmann, 2005)***.

PCOS is a heterogeneous condition, the pathophysiology of which appears to be both multifactorial and polygenic. The definition of the syndrome has been much debated. Key features include menstrual cycle disturbance, hyperandrogenism and obesity. There are many extra-ovarian aspects to the pathophysiology of PCOS, yet ovarian dysfunction is central ***(Balen et al, 2003)***.

National Institutes of Health (NIH) 1990 Criteria for PCOS

The definition of PCOS most commonly used today arose from the proceedings of an expert conference sponsored by the NIH in April 1990. The proceedings summarized the results into the following major research criteria (in order of importance):

1) Hyperandrogenism and/or hyperandrogenemia.

2) Oligo-ovulation.

3) Exclusion of known disorders, such as Cushing's syndrome, hyperprolactinemia and congenital adrenal hyperplasia (CAH) ***(Zawadzki and Dunaif, 1992)***.

A fourth criterion, PCO on ultrasound (US), was considered particularly controversial. In essence, the results of this expert conference identified PCOS as a disorder of ovarian androgen excess ***(Azziz, 2006)***. At that time, and not by consensus, the majority of participants believed that the presence of PCO by US was suggestive but not diagnostic of PCOS ***(Azziz, 2004)***.

Rotterdam 2003 Criteria for PCOS

Another expert conference was organized in Rotterdam in May of 2003, sponsored in part by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). The proceedings of the conference noted that PCOS could be diagnosed, after the exclusion of related disorders, by two of the following three features:

1) Oligo or anovulation.
2) Clinical and/or biochemical signs of hyperandrogenism.
3) PCO by US

Rotterdam 2003 criteria did not replace the NIH 1990 criteria, because all women who were diagnosed by the NIH 1990 criteria would also meet the Rotterdam definition ***(Azziz, 2006)***.

Rather, Rotterdam 2003 criteria expanded the definition of PCOS, adding two additional phenotypes as PCOS, including women with:

1) PCO and clinical and/or biochemical evidence of androgen excess, but without ovulatory dysfunction.

2) PCO and ovulatory dysfunction with no signs of androgen excess ***(Azziz, 2006)***.

The negative impact of labeling these new phenotypes as PCOS can be significant, because these criteria increase the phenotypic heterogeneity of the disorder, their use will decrease the ability of genetic and other molecular studies to detect a common underlying abnormality ***(Van Der Meer et al., 1998)***. Women with these new phenotypes are at increased risk for metabolic and cardiovascular consequences, as are patients with more classic forms of PCOS ***(Azziz, 2006)***.

To clarify the whole phenotypic spectrum of PCOS, ***Diamanti-Kandarakis and Panidis (2007)*** reported their results of prospective study. In this study they included 634 Greek women with PCOS (18 to 35 years) diagnosed with PCOS by ESHRE/ASRM criteria and 108 healthy controls with comparable BMI. The PCOS group was then subdivided into two main phenotypes, ‘‘classic (NIH) PCOS’’ and ‘‘nonclassic (ESHRE/ASRM) PCOS.’’ These phenotypic groups were then

further divided into subphenotypes based on their androgen and ovulatory status and the presence or absence of PCO ***(Diamanti-Kandarakis and Panidis, 2007)***.

In addition to the epidemiologic data obtained, an evaluation was completed between the hormonal and metabolic abnormalities of each subphenotype. The classic phenotype for PCOS was the most frequent compared with the nonclassic phenotype ***(Diamanti-Kandarakis and Panidis, 2007)***.

In women with classic PCOS, follicular number was positively related with insulin resistance and biochemical hyperandrogenemia ***(Porter, 2008).***

Androgen Excess Society (AES) 2006 Criteria for PCOS

AES created a commission of experts charged to revise all the published data and provide an evidence-based definition of PCOS, in order to simplify and standardize the clinical diagnosis of the disorder and attempt to provide more solid bases for future clinical and epidemiological studies ***(Azziz et al., 2006)***.

AES 2006 criteria for diagnosis of PCOS require the presence of hyperandorgenism, clinical (hirsutims) and/or biochemical, and either oligo-anovulation or PCO, after exclusion of related disorders ***(Galluzzo et al., 2008)***.

This definition then recognized one additional phenotype above that noted by the NIH 1990 criteria, namely that of women with PCO, hyperandrogenism, and apparently normal ovulation ***(Barbieri and Ehrmann, 2008)*.**

In spite of considerable evidence supporting the direct or indirect role of insulin resistance in the genesis of PCOS, the NIH, Rotterdam and AES criteria all tend to consider the syndrome as having a mainly hyperandrogenic profile ***(Galluzzo et al., 2008)***.

Prevalence

PCO are commonly detected by US or other forms of pelvic imaging, with estimates of the prevalence in the general population being in the order of 20–33% ***(Polson et al., 1988; Clayton et al., 1992; Farquhar et al., 1994[1,2]; Michelmore et al., 1999)***. However, not all women with PCO demonstrate the clinical and biochemical features which define the syndrome of PCO ***(Balen, 1999)***.

Estimates of the prevalence of PCOS are greatly affected by the nature of the population which is being assessed. Populations of women who are selected on the basis of the presence of a symptom associated with the syndrome (e.g. hirsutism, acne and menstrual cycle disturbances) would be

expected to demonstrate a prevalence greater than that which exists in the general population ***(Balen and Michelmore, 2002)***.

The highest reported prevalence of PCO was 52%, amongst South Asian immigrants in Britain, of whom 49.1% had menstrual irregularity ***(Rodin et al., 1998)***. It was also demonstrated that South Asian women with PCO had a comparable degree of insulin resistance to that of established type 2 diabetes mellitus ***(Balen and Michelmore, 2002)***.

Pathogenesis

PCOS has been studied intensely, although the exact etiology is still unknown ***(Homburg, 2008)***. The heterogeneity of the disorder has led to multiple mutually inconsistent theories regarding its etiology, including:

1. Primary defect in insulin action and/or secretion leading to hyperinsulinemia.
2. Primary neuroendocrine defect leading to an exaggerated leutinzing hormone (LH) pulse frequency and amplitude.
3. Primary defect in ovarian/adrenal androgen biosynthesis resulting in hyperandrogenism.

(Tsilchorozidou et al., 2004)

The serine phosphorylation hypothesis may explain two major features of PCOS which are hyperandrogenemia and