INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders. It is a complex, heterogeneous disorder of uncertain etiology, but there is strong evidence that it can, to a large degree, be classified as a genetic disease ***(Fauser et al., 2011).*** Genetic and environmental contributors to hormonal disturbances combine with other factors, including obesity ***(Diamanti-Kandarakis et al., 2006).*** Ovarian dysfunction and hypothalamic pituitary abnormalities contribute to the etiology of PCOS ***(Doi et al., 2005).***

It produces symptoms in approximately 5% to 10% of women of reproductive age (12-45 years old). It is thought to be one of the leading causes of female subfertility ***(Goldenberg and Glueck, 2008).***

Its prevalence has increased with the use of different diagnostic criteria and has recently been shown to be 18% (17.8 ± 2.8%) in the first community-based prevalence study based on current Rotterdam diagnostic criteria ***(March et al., 2010).***

Clomiphene citrate is a first line pharmacological treatment of ovulatory dysfunction associated with PCOS, because it is easily administered, relatively safe, and inexpensive ***(Norman et al., 2004).***

Clomiphene citrate is a selective estrogen receptor modulator (SERM) that increases production of gonadotropins

by inhibiting negative feedback on the hypothalamus. It is used in the form of its citrate to induce ovulation ***(Shelly et al., 2008).***

Clomiphene Citrate is known to be both an estrogen agonist and antagonist; however, its agonist properties manifest only when endogenous estrogen levels are extremely low ***(Schorge et al., 2008).***

Clomiphene citrate administration leads to depletion of estrogen receptors at the level of the pituitary and hypothalamus, interrupting the negative feedback that estrogen normally produces. As a result, GnRH secretion is improved and stimulates pituitary production of follicle-stimulating hormone (FSH), which in turn drives follicular growth and maturation with emergence of 1 or more dominant follicles ***(Schorge et al., 2008).***

In the study of ***(Brown et al., 2009)***, Clomiphene was effective in increasing pregnancy rate compared to placebo (Odd's ratio, OR 5.8, 95%confidence interval, CI 1.6 to 21.5).

The standard effective dose of CC ranges from 50 mg/d to 250 mg/d, although doses in excess of 100 mg/d are not approved by the US Food and Drug Administration (FDA) and add little to clinical pregnancy rates ***(Dodge et al., 1986).***

About 52% of women ovulate in response to treatment with 50 mg. Those who do not ovulate with 50 mg CC may

ovulate at higher doses using a step-up regimen with doses escalating 50 mg with each anovulatory cycle (22% with 100mg, 12% with 150mg, 7% with 200mg, and 5% with 250mg) ***(Gysler et al., 1982).***

Badawy at al. (2009) tested a novel protocol of luteal phase administration of CC for ovulation induction in women with polycystic ovary syndrome (PCOS), and concluded that early administration of CC in patients with PCOS will lead to more follicular growth and endometrial thickness, which might result in higher pregnancy rates.

Metformin is a biguanide, has been used to treat insulin resistance in women with PCOS with the goals of reducing ovarian androgen production. It lowers elevated blood glucose levels in patients with diabetes mellitus, but does not reduce blood glucose values in non-diabetics. Insulin resistance in PCOS involves a post-receptor defect in the insulin signal transduction chain between the receptor kinase and glucose transport ***(Ciaraldi et al., 1992).***

Metformin increases the binding of insulin to its receptor and enhances the post-receptor action, women with PCOS might benefit from metformin therapy. In addition to improving insulin sensitivity, metformin has weight-reducing effects and has also been shown to be safe during embryogenesis and pregnancy ***(Denno and Sadler, 1994).***

AIM OF THE STUDY

The aim of the present study was to compare late luteal phase administration of clomiphene citrate to the conventional combined administration of clomiphene citrate and metformin in early follicular phase as ovulation induction in women with clomiphene citrate resistant polycystic ovary syndrome.

POLYCYSTIC OVARIAN SYNDROME

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders. PCOS is a complex, heterogeneous disorder of uncertain etiology, but there is strong evidence that it can, to a large degree, be classified as a genetic disease ***(Fauser et al., 2011).***

It affects 5–10% of women in their reproductive years. PCOS is associated with 75% of all anovulatory disorders causing infertility, with 90% of women with oligoamenorrhea, more than 90% with hirsutism and more than 80% with persistent acne ***(Homburg, 2008)***.

It is important to appreciate that PCOS is a syndrome not a disease, reflecting multiple potential etiologies with variable clinical expressions ***(Rosenfield, 2008)***.

The inclusion of ultrasound morphology of the ovaries as a further potential criterion to define PCOS was proposed by the Rotterdam 2003 consensus conference, which established that at least two of the following criteria – oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (PCO) at ultrasound – are sufficient for the diagnosis. The fundamental role of hyperandrogenism has been pointed out ***(Azziz et al., 2006).***

Several new studies in PCOS claimed that Polycystic ovarian morphology (PCOM) at ultrasound should be currently

used in the diagnosis of PCOS and the usage of serum AMH level as a marker of polycystic ovaries in PCOS. In 2011, ***Dewailly and his colleges*** hypothesized that the previously proposed threshold value of 12 (in Rotterdam criteria) as an excessive number of follicles per ovary is no longer appropriate because of current technological developments. They concluded that the former threshold of FNPO (Follicle Number Per Ovary) >12 is no longer valid. Also, a serum AMH >35 pmol/l (or >5 ng/ml) appears to be more sensitive and specific than a follicle count per ovary >19 and should be therefore included in the current diagnostic classifications for PCOS ***(Dewailly et al., 2011).***

In 2014, ***Dewailly and his colleges*** published a new one in which that The Task Force recommends using FNPO for the definition of PCOM setting the threshold at =25, but only when using newer technology that affords maximal resolution of ovarian follicles (i.e. transducer frequency = 8 MHz). If such technology is not available, we recommend using ovarian volume more than 10 cm3 rather than FNPO for the diagnosis of PCOM for routine daily practice but not for research studies that require the precise full characterization of patients. The Task Force recognizes the still unmet need for standardization of the follicle counting technique and the need for regularly updating the thresholds used to define follicle excess, particularly in diverse populations. Serum AMH concentration generated great expectations as a surrogate marker for the

follicle excess of PCOM, but full standardization of AMH assays is needed before they can be routinely used for clinical practice and research. Finally, the finding of PCOM in ovulatory women not showing clinical or biochemical androgen excess may be inconsequential, even though some studies suggest that isolated PCOM may represent the milder end of the PCOS ***(Dewailly et al., 2014).***

Recently, the prevalence of PCOS was 16.6% according to the Rotterdam criteria. When replacing the criterion for polycystic ovaries by antral follicle count (AFC) > 19 or AMH > 35 pmol/l, the prevalence of PCOS was 6.3 and 8.5%, respectively ***(Lauritsen et al., 2014)***.

PCOS compromises other pathological conditions that strongly modify the phenotype and play a dominant role in the pathophysiology of the disorder, including insulin resistance and hyperinsulinemia, obesity and metabolic disorders, all favouring, together with androgen excess, an increased susceptibility to develop type 2 diabetes mellitus (T2DM) and, possibly, cardiovascular diseases (CVD). PCOS by itself may also have some genetic component as documented by familial aggregation and genetic studies ***(Ehrmann, 2005).*** All the clinical features may change throughout the lifespan, starting from adolescence to postmenopausal age. Therefore, PCOS should be considered as a lifespan disorder, although the specific phenotype of PCOS in postmenopausal women is still poorly defined ***(Pasquali et al., 2006).***

The clinical evaluation should include a complete medical history and physical examination, and consideration of differential diagnosis ***(Pasquali et al., 2006).*** Past medical history should also include knowledge of prior ovarian surgery that could impact current hormonal and menstrual status, and prior records of an abdominal procedure may provide information as to the appearance of the uterus and/or ovaries. A complete history of prior therapies must be documented, including topical treatments for acne and hirsutism that are likely to influence the appearance of the skin over time. Acne is known to be caused by certain medications, including azathioprine, barbiturates, corticosteroids, cyclosporine, disulfiram, halogens, iodides, isoniazid, lithium and phenytoin. Finally, a list of all cosmetic therapies is necessary for the interpretation of physical findings; topical and other treatments of hirsutism and acne will in fact influence the clinical manifestations of these conditions ***(Taylor, 2007).***

Several studies have documented an increased risk of PCOS in sisters and daughters of women with PCOS, so the history provides an opportunity to identify new cases of PCOS. Hirsutism, acne, menstrual irregularity, early cardiovascular disease, obesity and T2DM are all potential indicators of a familial tendency towards the PCOS. A family history of infertility and/or hirsutism may also indicate disorders such as non-classic congenital adrenal hyperplasia. The presence of symptoms that are very different from those of other family

members may increase the level of concern for a more pathologic explanation for the menstrual defects or the androgenic symptoms. The family history of metabolic defects and CVD is also an opportunity to quantify the risk of the patient ***(Escobar-Morreale et al., 2005).***

The joint meeting of the ASRM and ESHRE in Rotterdam in 2003 was a key to the agreement of a refined definition of polycystic ovarian syndrome (PCOS)

The definition required two out of three of the following criteria:

A. Oligo- and/or anovulation.

B. Hyperandrogenism (clinical and/or biochemical).

C. Polycystic ovaries by ultrasound.

A. Oligo- and/or anovulation:

The menstrual irregularity of PCOS typically manifests in the peripubertal period, although some women may apparently have regular cycles at first and subsequently develop menstrual irregularity in association with weight gain. Menses irregularities include mild or severe oligomenorrhea (cycle length more than 35–40 days) or amenorrhea (no cycles for 6 or more consecutive months) ***(Venturoli et al., 1992).***

In addition, anovulation is very common in the presence of mild oligomenorrhea, but also when normal cycles are present ***(Chang, 2007)***. Some cycles may be associated with dysfunctional bleeding. Endometrial atrophy may be present in some women with PCOS who have prolonged amenorrhea, which may be related to androgen excess. Chronic anovulation is one of the most important criteria in the diagnosis of PCOS. However, occasional ovulation may occur, particularly in women with less severe oligomenorrhea. Ovulation can be easily detected by measuring progesterone levels in the luteal phase, at approximately days 20–22 after cycle onset. Appropriate hormone levels suggesting an adequate luteal phase are 6–8 ng/mL ***(Chang, 2007).***

In women presenting while taking OC, blood testing or pelvic ultrasounds should not be performed until they have discontinued OC use for at least three months. Moreover, weight- and exercise-related causes, hyperprolactinemia, subclinical or overt thyroid dysfunction, particularly in young women, should be investigated. Premature ovarian failure should also be suspected in adult women with unexplained amenorrhea ***(Pasquali et al., 2006).***

B. Hyperandrogenism (clinical and/or biochemical):

In women with polycystic ovary syndrome (PCOS), hyperandrogenism is clinically manifested by hirsutism, acne and androgenic alopecia, and it contributes to chronic

anovulation and menstrual dysfunction. Biochemically, hyperandrogenism is established by elevated circulating levels of serum total or unbound testosterone, androstenedione and an increased free androgen index (FAI). In prospective studies, hyperandrogenism appears to affect about 1 in 10 women of reproductive age, although racial differences or the selection criteria of patients can increase its prevalence up to 30% ***(Diamanti-Kandarakis et al., 1990).***

I- Clinical Evaluation of Hyperandrogenism:

- **Hirsutism:**

Hirsutism in women is defined as the growth of terminal hair in an adult male distribution. Terminal hairs are pigmented, coarse and greater than 1 cm in length. Common presentations are excess facial hair (especially the upper lip and chin), hair on the chest between the breasts and hair on the lower abdomen. Excessive terminal hair affecting only the lower legs and forearms does not constitute hirsutism as these areas are normally covered by a mixture of terminal and vellus hairs ***(Azziz et al., 2006).***

Hirsutism is usually of benign etiology and must be differentiated from virilisation, which includes increased muscle mass and libido, breast atrophy, clitoromegaly and deepening of the voice. Hirsutism is affected by familial and racial factors. Hirsutism has frequently been used as the primary clinical indicator of androgen excess. A score has been

validated to reflect the extent of hirsutism by Ferriman–Gallwey. The **Ferriman–Gallwey score** is a method of evaluating and quantifying hirsutism in women. The method was originally published in 1961 by D. Ferriman and J.D. The original method used 11 body areas to assess hair growth, but was decreased to 9 body areas in the modified method: Upper lip, Chin, Chest, Upper back, Lower back, Upper abdomen, Lower abdomen, Upper arms, Forearms (deleted in the modified method), Thighs, Legs (deleted in the modified method) ***(Ferriman et al., 1961).***

In the modified method, hair growth is rated from 0 (no growth of terminal hair) to 4 (extensive hair growth) in each of the nine locations. A patient's score may therefore range from a minimum score of 0 to a maximum score of 36. In Caucasian women, a score of 8 or higher is regarded as indicative of androgen excess. With other ethnic groups, the amount of hair expected for that ethnicity should be considered. The method was further modified in 2001 to include a total of 19 locations, with the 10 extra locations being: sideburns, neck, buttocks, inguinal area, perianal area, forearm, leg, foot, toes and fingers. Each area has its own specified definition of the four-point scale ***(Goodman et al., 2001).***

- **Acne:**

Acne is an inflammatory disorder of the hair follicle and its associated sebaceous and apocrine gland. Androgens have a

direct effect on sebaceous glands, increasing both gland cell division and sebum production.

Still, acne is by any means less frequent than hirsutism as the sole clinical manifestation of androgen excess, affecting between 20–40% of patients without hirsutism and menstrual irregularities ***(Azziz et al., 2006).*** In a large series of PCOS, women (4.6%) had only acne, while hirsutism was associated with acne in 12.6% of patients ***(Slayden et al., 2001).***

- **Male-Pattern Alopecia:**

The sole presence of androgenic alopecia as an indicator of hyperandrogenism has been less well studied. The majority of women with a primary complaint of alopecia suffer from polycystic ovaries. However, alopecia is a relatively poor marker of androgen excess, unless present in the oligo-ovulatory patient. The loss of scalp terminal hair is usually progressive and therefore probably under diagnosed in women with PCOS. The presence of alopecia requires a familial predisposition to baldness and an associated increase in circulating androgens, consequently not all women with an excess of circulating androgens will suffer from androgenic alopecia ***(Cela et al., 2003).***

II- Biochemical Features of Hyperandrogenism:

- **Ovarian Hyperandrogenism:**

The ovary and adrenal cortex share the bulk of the steroid biosynthesis pathways. Androgen is produced in the

ovary by the theca interna layer of the ovarian follicle, whereas the zona fasciculata of the adrenal cortex synthesises adrenal androgens. The enzymes utilised in the formation of androstenedione from the initial substrate, cholesterol, are similar in both glands and are under the endocrine control of LH in the ovary and adrenocorticotrophic hormone (ACTH) in the adrenal glands. The ovary is considered the main source of excess androgens in women with PCOS, although excess adrenal androgen production may also occur. Ovarian theca cells produce androgen under the influence of LH ***(Balen, 2004).***

A certain amount of intraovarian androgen is essential for normal follicular growth and for the synthesis of oestradiol. Nonetheless, when the synthesis of androgens is not co-ordinated with the needs of a developing follicle, and is in excess, poor follicle maturation and increased follicular atresia results. In the normal ovary, LH acts on the theca–interstitial–stromal cells, whereas FSH acts on granulosa cells. According to the 'two-gonadotrophin, two-cell theory' of oestrogen biosynthesis, the thecal compartment secretes androgens in response to LH, and the produced androstenedione is converted in the granulosa cell to oestrogens by the action of aromatase, which in turn is under the influence of FSH. In PCOS, the ovarian theca cells are increased in number, and they have increased steroidogenic capacity ***(Azziz et al., 2006).***

- **Adrenal Hyperandrogenism:**

Among PCOS women, a significant percentage ranging between 20% and 30% demonstrates adrenal androgen excess which is detectable by increased serum levels of DHEAS and androstenedione, and adrenal hyperresponsivity to ACTH stimulation test ***(Azziz et al., 2006).***

Measurements of serum DHEAS have been generally used for the assessment of adrenal androgen excess because DHEAS is secreted by 97–99% in the adrenals. DHEAS secretion declines with increasing age and is also partly influenced by a genetic component. The incidence of increased DHEAS serum levels in PCOS women range between 20 and 33% among black and white women ***(Kumar et al., 2005).***

Androstenedione is a weak androgen which is secreted by both the ovary and the adrenals. Although it has an adrenal component, its value in the estimation of adrenal androgen excess is unclear, although, it may be the sole androgen in excess in approximately 10% of PCOS women ***(Knochenhauer et al., 1998).*** Adrenal androgen excess appears to be due to adrenocortical steroidogenic abnormalities, abnormalities in the metabolism of adrenal products or to alterations in the responsivity of adrenals to ACTH as women with PCOS present a generalized hypersecretion of adrenocotrical products in response to ACTH stimulation ***(Moran et al., 2004).***