Pretreatment and coadministration of oral antidiabetic agents with clomiphene citrate or rFSH for ovulation induction in clomiphene citrate (CC) resistant polycystic ovarian syndrome (PCOS)

By

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Dr. Mosammat Rashida Begum has conducted the research for PhD programme under Dhaka University titled "**Pretreatment and coadministration of oral antidiabetic agents with clomiphene citrate or rFSH for ovulation induction in clomiphene citrate (CC) resistant polycystic ovary syndrome (PCOS)**" very efficiently and successfully under my supervision. She has completed her thesis writing. I have gone through the thesis and it is found very satisfactory.

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Author's Declaration

The work reported in this thesis is my own original research and this thesis has not been submitted as a credit for a degree in any other University.

Signature .....

Date 9.6.13

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Abbreviations used in this Thesis

17-OHP	17Hydroxyprogesterone
ANOVA	Analysis of variance
ART	Assisted reproductive technology
ASRM	American Society of Reproductive Medicine
BIRDEM	Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders
BMI	Body mass index
CC	Clomiphene citrate
DFMO	Difluromethylornithine
DHEA	Dihydroepiandrosterone
DHEAS	Dihydroepiandrosterone sulfate
DHT	Dihydrotestosteron
ER	Estrogen receptor
ESHRE	European Society of Human Reproduction & Embryology
FDA	Food & drug administration
FSH	Follicle stimulating hormone
FSH-GC	Follicle stimulating hormone granulosa cell
GDM	Gestational diabetes mellitus
GLUT-4	Glucose transporter type-4
GnRH	Gonadotropin releasing hormone
GnRHa	Gonadotropin releasing hormone agonists

HAIR-AN	Hyperandrogenism insulin resistance & acanthosis nigricans
hCG	Human chorionic gonadotropin
HDL	High density lipoprotein
HMG	Human menopausal gonadotropin
HOMA	Homeostasis model assessment
ICRC	Infertility Care and Research Center
ICSI	Intracytoplasmic sperm injection
IGF-1	Insulin-like growth factor
IGF-BP	Insulin-like growth factor binding protein
IR	Insulin receptor
IUI	Intra uterine insemination
IVF-	In-vitro fertilization
IVM	In-vitro maturation
LDL	Low density lipoprotein
LH	Luteinizing hormone
LOD	Laparoscopic ovarian drilling
LOS	Laparoscopic ovarian surgery
MRI	Magnetic resonance imaging
NIH	National Institute of Health
OCP	Oral contraceptive pills
ODC	Ornithine decarboxylase
OHSS	Ovarian hyperstimulation syndrome

OMI	Oocyte maturation inhibitor
PA	Plasminogen activator
PGE2	Prostaglandin E2
PGF2	Prostaglandin F2
PGI2	Prostaglandin I2
POCD	Polycystic ovarian disease
POCS	Polycystic ovarian syndrome
RCT	Randomized controlled trial
rFSH	Recombinant follicle stimulating hormone
SHBG	Sex hormone binding globulin
TAS	Transabdominal sonogram
TIC	Theca interstitial cell
TVS	Transvaginal sonogram
USG	Ultra sonogram
VIDAS	Vitek immunu diagnostic assay system
VNTR	Valuable number of tandem repeat
WHO	World Health Organization
WHR-	Waist hip ratio
χ^2	Chi-square

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Abstract:

Objectives:

The objective of this study was to explore the result of pretreatment and concomitant use of metformin with clomiphene citrate (CC) and rFSH for ovulation induction in clomiphene citrate resistant polycystic ovary syndrome (PCOS).

Design:

Randomized controlled trial

Setting:

Outpatient department of Obstetrics and Gynaecology of Dhaka Medical College and Hospital and Infertility Care and Research Centre (a tertiary level infertility care centre) Dhaka.

Participants and intervention:

One hundred and sixty five infertile patients having PCOS with CC resistant (Failure to ovulate after 150mg CC for 5 days for two consecutive cycles), who attended in the outpatient Department of OB/GYN Dhaka Medical College and Hospital and Infertility Care and Research Centre were the target population for this study. Patients were divided into three groups A, B and C. Two groups A and B were allocated for Metformin and C for control which was selected by lottery. Along with metformin group A received CC and group B received rFSH. Group C was treated by only rFSH. Metformin was given 1500 mg daily in divided doses for 4 weeks. After 4 weeks of pretreatment CC or rFSH were added for induction of ovulation along with metformin. Ovulation was monitored and confirmed by TVS, and hormone assessment. Six ovulatory cycles were assessed. Treatment was terminated when no response with maximum dose of CC and rFSH or after six ovulatory cycles and no pregnancy, and after getting pregnancy. Statistical analysis was done by SPSS software by ANOVA, student's t test or χ^2 test as appropriate. A p value of <0.5 was considered as significant

Main Outcome measures:

Main outcome measures were ovulation, pregnancy rate, live birth rate and congenital anomaly.

Results:

Ovulation (89.09%) and pregnancy (54.55%) rate were higher in group B where patients received metformin and rFSH. Ovulation (74.55%) and pregnancy (29.09%) rate were also satisfactory in group C where patient did not receive metformin and received rFSH only. But dose of rFSH requirement was significantly higher (p=0.001) in comparison to group B and days of stimulation was longer than group B. In group A both ovulation and pregnancy rate were much lower than other two groups (27.27% and 12.73% respectively). There were no significant differences in abortion and detectable congenital anomalies between the groups.

Conclusions:

Use of metformin increases the response of ovulation inducing agents and reduces the cost of gonadotropins during induction of ovulation. It can be used safely in PCOS patients who are CC resistant.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction:

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting between 4% and 8% of reproductive aged women [Chang R, 2004], although the prevalence may be as high as 30% in women with secondary amenorrhoea, 75% in women with oligomenorrhoea and 90% in women with hirsutism [Adams, 1986]. About 20% of couples seeking fertility treatment do so due to anovulation and 85% - 90% of those have PCOS [Pincock , 2002]. Although the symptoms and signs of PCOS are very heterogeneous, the syndrome usually presents with any combination of the following- menstrual irregularities (usually oligomenorrhoea or amenorrhoea), signs of hyperandrogenism (hirsutism, acne, alopecia) a characteristic appearance of the ovaries on ultrasound examination and an endocrine disturbance often involving high serum concentrations of LH and androgens.

There is a well established association between PCOS, insulin resistance and hyperinsulinaemia. Insulin resistance is a pivotal defect in PCOS probably counts as one of the most important factor in the battle to control the disorder. This metabolic abnormality leads to a compensatory increase in circulating insulin and this elevated insulin level directly stimulates the ovary to produce excess androgens [Nestler and Jakubowicz, 1996; Nestler et al, 1989]. It also decreases hepatic sex hormone binding globulin (SHBG) [Poretskyl et al, 1999], so increasing biologically available free testosterone concentration in the circulation [Tsilchorzidou, 2004; Carmina, 2003].

This hormonal abnormality leads to oligomenorrhoea and anovulation. The antioestrogen clomiphene citrate (CC) is widely accepted as a first line drug for ovulation induction in PCOS. Some 50-80% of anovulatory patients ovulate and 40-50% conceives on CC at a dose of 50-150 mg/day [Shepard et al, 1979; Lobo et al,1982]. However, in spite of administering high doses of CC, some patients may fail to ovulate, thus they are considered CC resistance [Nestler, 2002]. CC resistance is associated with insulin resistance. As insulin resistance turns out to be one of the primary causes of anovulation in PCOS, we would expect drugs that reverse insulin resistance can also relieve hyperandrogenism, restore normal menstruation and help to eliminate the infertility associated with PCOS. At the top of that list of pharmaceutical agents is metformin, which was developed in 1957 to treat type 2 diabetes mellitus. Metformin reduces insulin resistance of peripheral tissue and allows muscle and adipose cells to take in glucose at normal insulin levels. It inhibits hepatic glucose production [Inzucchi et al, 1998], decreases intestinal absorption and promotes glucose uptake, utilization by peripheral tissues at the post receptor level [Baily and Puah, 1986]. Metformin increases the number of insulin receptors but not insulin concentration and therefore does not cause hypoglycemia in normoglycemic patients. The sum total of these actions is a decrease in insulin levels and reduced insulin level lowers circulating total and free androgen levels with a resulting improvement of the clinical sequeale of hyperandrogenism. Once androgen level falls ovarian response to CC or other stimulating agent increases.

Bangladesh perspective: In Bangladesh there is no definite national statistics of prevalence of infertility. In institution like BIRDEM where patients are referred for special management the incidence of infertility is 10-12%. Among them 20% infertility is due to anovulation and PCOS is occupying 70-80%. In another private infertility institution where 2700-2800 infertile couples are managed yearly, where 20%-25% infertility is due to anovulation and among them 90% is due to PCOS (unpublished data ICRC). Majority of them needed multiple drugs and long time to become ovulatory. There is no such study conducted so far to explore insulin resistance and to correct it. So, purpose of this study is to explore the efficacy of metformin to increase ovarian sensitivity to CC and rFSH in CC resistant PCOS patients of Bangladesh.

Literature Review

1. What is PCOS?

PCOS or polycystic ovarian syndrome is the most common hormonal disorder in women of reproductive age. It affects up to 10% of pre-menopausal women and responsible for 75% of all causes of anovular infertility. In PCOS ovulation fails to occur in a regular basis. Symptoms include irregular menstrual cycles, increased amounts of body hair and a tendency to easily gain weight specially in the trunk and abdomen. Recent research indicates that the underlying defect in PCOS patients may be due to decreased sensitivity to insulin.

2. History

As early as 1844 Chereau described sclerocystic changes in the human ovary [Chereau, 1844] 90 yrs before the classic paper of Stein and Leventhal [Stein and Leventhal, 1935]. In 1935, Stein and Leventhal described the association of enlarged sclerocystic ovaries with amenorrhoea, infertility and hirsutism and called it polycystic ovary syndrome. Today it is one of the most common endocrine disorders with unknown aetiology having polycystic ovaries, obesity, hyperandrogenism, menstrual irregularities and infertility-either singly or in combination.

3. Epidemiology

Polycystic ovarian syndrome is the commonest cause of anovulatory infertility. As there are no well-accepted criteria for diagnosis, the incidence of PCOS is not really known. Based on ultrasound findings the incidence seems to be between 21-22%. But number of women remain asymptomatic and may be missed. Based on symptoms

incidence varies between 5% to 21% (menstrual irregularities) and 3.5-9% (hyperandrogenimia). It is estimated that 40% of women with oligomenorrhoea, 84% of women with hirsutism and 100% of women presenting with severe acne have PCOS as their aetiology [Bernard et al, 2003; Costello and Eden, 2003;Huber-Buchholz et al,1999; Laboureau et al 2002; Balen A, 2000; Conway GS, 2000]. It is the most common reproductive disorder in women of childbearing age and has a prevalence of 4-10% in United States[Knochenhauer et al, 1998; Frank, 1995]. Prevalnce of PCOS depends on the nature of population being assessed. There are racial and ethnic differences in clinical and biochemical features of PCOS but it remain unanswered whether the difference in expression of this syndrome is due to dietary, lifestyle factors or purely genetic familial expression. The prevalence in the British female population is estimated 22% [Clayton et al, 1992].

The prevalence of this syndrome in Caribbean-Hispanic women is twice than that of African-American and Caucasian women. Furthermore, Caribbean-Hispanic women are significantly more insulin resistant than non-Hispanic white women [Dunaif et al, 1993]. Though there has been large scale study to gauge the disease burden of PCOS in Asia, our clinical evidence and a few studies done on South Asian imigrants show a much more severe symptomatology. One study done on South Asian immigrants settled in England revealed a prevalence of 52% in the South Asian women [Rodin et al, 1998]. The WHO global data based on obesity and BMI in adults from different ethnic groups has recently redefined obesity in South Asian women, a BMI >25 is regarded as class I obesity which is considered equivalent to BMI >30 in white Caucasian subjects [Wijeyaratne et al, 2002; Franks, 1989].

4. Aetiology:

The exact aetiology of PCOS is not known. Familial clustering of hyperandrogenimia, anovulation and polycystic ovaries suggests an underlying genetic basis. Three major theories have been proposed to explain the cause of PCOS. First the luteinizing hormone-theca interstitial cell (LH-TIC) theory suggests that the pathophysiologic mechanisms leading to abnormally elevated levels of LH underlay the phenomenon of PCOS. The second theory, the follicle stimulating hormone granulosa cell (FSH-GC) theory suggests that FSH leads to subnormal induction of cytochrome P 450 aromatase in the granulosa cell, leading to elevated androgen level. The third theory relates to the growth factor autocrine paracrine system. In PCOS, there is evidence of an altered IGF/ insulin system and these act as mediators of biologic responses of sclectogenic and atretogenic follicular hormones [Hickey et al, 2006]. Familial clustering of cases of PCOS has been reported by several groups and both autosomal dominant and x- linked mode of transmission have been proposed [Judd et al, 1973; Givens JR, 1989; Yildiz et al, 2003]. At least one group of patients with PCOS has been described as inheriting the disorder by means of an x-linked dominant transmission. There was a 2 fold higher incidence of hirsutism and oligomenorrhoea with paternal transmission but with marked variability of phenotypic expression. Study suggests inheritance in an autosomal dominant fashion with premature balding as the phenotype in males [Hichey et al, 2006].

Postulated genetic defect in insulin resistance and hyperinsulinimia is defective post receptor signal transduction. The phosphorylation of serine and threonine residues on

the insulin receptor reduces signal transmission and excessive serine phosphorylation by a mechanism extrinsic to the insulin receptor has been demonstrated as a possible post receptor defect in patients with PCOS changing signal transduction. Serine phosphorylation of β chain of insulin receptor and at the same time of adrenal and ovarian P 450c 17 enzymes would explain both hyperinsulinemia and hyperandrogenism. The origin and cause of serine phosphorylation is uncertain but presumably it could have a genetic basis. Serine phosphorylation increases 17,20 lyase activity and androgen production. Molecular phenotype of skeletal muscle and ovarian theca cells from women with PCOS also provides some evidence in support of a genetic aetiology.

Genetic aspect: Familial clustering of hyperandrogenism, anovulation and polycystic ovaries suggest an underlying genetic basis. Family members of women with anovulation, hyperandrogenism and polycystic ovaries have an increased incidence of hyperinsulinaemia in females and premature baldness in males [Norman et al, 1996]. The phenotypic expression of PCOS appears to be dependent on racial origin.For example Mexican American and South Asian women with PCOS are more likely to suffer from insulin resistance compared with Caucasians. European and Maori women with PCOS are more likely to present with hirsutism compared with other races [Williamson et al, 2001]

The major mechanisms for several complex human diseases like PCOS are poorly understood. For genetic diseases that are not associated with obvious structural rearrangements of chromosomes, the causative gene(s) can be localized by genetic linkage analysis in families segregating for the disease phenotype. There is not one

homogenous group of PCOS patients, but rather a spectrum of patients sharing for the most part, the same clinical features that have arisen by similar but probably diverse etiopathologic process. It is likely that hyperinsulinaemic hyperandrogenism represent a significant subgroup, present in >50% of patients with hyperandrogenism of chronic anovulation, and this group can be obese and non obese. Thus the PCOS phenotype is complex, and genetic analysis will necessarily require an understanding of the possible physiologic mechanisms of the disease to search for candidate genes.Attempts were made to find out candidate gene(s) that may be involved in the pathways of the above three major theories. But there is difficulty in tracing the responsible gene(s) because of number of reasons:

a) Lack of clear cut definition of phenotype
b) Underlying complexity of the disorder
c) Lack of clear male phenotype
d) Environmental factors influencing the phenotype of PCOS

Mode of transmission:

The different studies on PCOS imply an autosomal dominant mode of inheritance directing clinician to counsel families that theoretically 50% mothers and sister within a family can manifest this disorder. The actual expression is not more than 20%-40%. This may be due to modification by genetic and environmental factors. Envioronmental factors, for example weight gain may trigger the development of PCOS in predisposed women. The environmental factors may vary between populations and may actually themselves include a genetic component [Vink et al, 2005]. Origin of PCOS occur in

utero or in early life. Timing of gestational androgen excess is important as different PCOS –associated characteristics arise when the hormonal insult is administered at various stages of foetal organogenesis. This concept of early origin for PCOS offers a possible mechanism through which environmental conditions during gestation or early life influence gene expression patterns later in life.

Autosomal dominance vs complex pattern of inheritance: Hickey TE suggested an autosomal dominant inheritance with premature balding as the phenotype in males. However the largest twin study on women with PCOS found a high degree of discordance among twins with polycystic ovaries which suggests a more complex pattern of inheritance than an autosomal dominant pattern of inheritance.

X linked dominant transmission: At least one group of patients with PCOS has been described as inheriting the disorder by means of an X-linked dominant transmission. There was a two fold higher incidence of hirsutism and oligomenorrhoea with paternal transmission but with marked variability of phenotype expression [Givens JR,1988].

Possible mechanism of hyperinsulinemia:

There are some postulated theories which suggested responsible factors for hyperinsulinemia.

a) Functional problems in the insulin receptor could be a consequence of insulin receptor gene mutations. There are three categories of peripheral target tissue insulin resistance.

i) Decreased insulin receptor numbers

ii) Decreased insulin bindings

iii) Post receptor failure

However, mutations and structural defects in the insulin receptor gene have not been detected in anovulatory women with PCOS [Urbanek et al, 1999].

b) Defective post receptor signal transduction: The phosphorylation of serine and threonine residues on the insulin receptor reduces signal transmission and excessive serine phosphorylation by a mechanism extrinsic to the insulin receptor has been demonstrated as a possible post-receptor defect in patients with PCOS, changing signal transduction [Li et al, 2002]. Serine phosphorylation of beta chain of insulin receptor and at the same time of adrenal and ovarian P450c17 enzyme would explain both hyperinsulinemia and hyperandrogenism. The origin and cause of serine phosphorylation is uncertain but presumably it could have a genetic basis. Serine phosphorylation increases 17, 20 lyase activity and androgen production. A unique molecular phenotype of skeletal muscle and ovarian theca cells from women with PCOS also provides some evidence in support of a genetic etiology. Increased insulin receptor serine phosphorylation of skeletal muscles was found in 50% of a sample of women with PCOS. The results from microarray analysis of ovarian theca cells and skeletal muscle cultures from women with PCOS support the concept of a unique molecular phenotype.

Insulin receptor gene:

Specific polymorphism in the genes encoding proteins that are involved in the signaling induced by the insulin receptor have been reported to be associated with anovulation, PCO and type 2 diabetes mellitus. The strongest evidence can be found in support of the region near the insulin receptor gene [Urbanek et al, 1999; Tucci et al, 2001] where

an association was demonstrated between a marker that is located 2 megabases centromeric from the insulin receptor.

Follistatin genes and PCOS:

Follistatin is a candidate gene which works in the following ways

1. It is an activin binding protein, which neutralizes the biological activity of activin, and it is expressed in multiple tissues like ovary, pituitary, adrenal cortex and pancreas.
2. Activin modulates the production of androgens by ovarian theca cells.

It helps in:

a) Development of ovarian follicles

b) Secretion of FSH by pituitary

c) Secretion of insulin by pancreatic beta cells.

3. Altered follistatin activity would be expected to effect follicular development, ovarian androgen production, pituitary FSH secretion and insulin release.

All these processes have been shown to be perturbed in PCOS.

Evidences:

Familial clustering of cases of PCOS has been reported by several groups and both autosomal-dominant and X-linked modes of transmission have been proposed [Jadd et al, 1973; Givens, 1989; Yildiz ,2003]. These early studies are limited by their clinical definition only. Hauge et al, 1988 found the prevalence to be much higher amongst the first degree relatives when PCOD is diagnosed by ultrasonography. Jahanfer et al, 1995 examined 34 pairs of female twins, 19 were monozygotic and 15 dizygotic. In the

group of monozygotic twins ultrasonography showed polycystic ovaries in both sisters of 7 pairs, inanother 7 pairs both sisters had normal ovaries and in five pairs only one of the twins was affected. The authors concluded that PCOD is not the result of a single autosomal defect. On the other hand, fasting insulin levels, androgen concentration and body mass index did appear to be under significant genetic influence. Insler V and Lunenfeld B, 2002 concluded from their studies that PCOD will develop when a whole assembly of gene controlling different metabolic functions is defective. When the defect appears only in some genes may exert a compensating effect and clinical sonographic and biochemical features will develop only in part or not at all. The full clinical picture of PCOD develop near puberty and at this sensitive stage the assembly of defective metabolic genes is prompted into extensive function by a variety of triggers like stress, diet and environment.Waterworth et al [Waterworth et al,1997] evaluated 147 individuals in 14 families. Women were considered affected if they had symptoms of menstrual disturbances and polycystic ovaries on ultrasound. The authors found evidence of linkage with the insulin gene variable number of tandem repeats (VNTR) polymorphism. In addition there was an association between the insulin VNTR and preferential transmission of the class III allele of the insulin VNTR from heterozygous fathers to PCOS daughters. Some of the indirect evidences related to PCOS go in favour of its genetic background; a) PCOS predisposes to non-insulin dependent diabetes in later life [Wild et al, 2003],. b) Women with PCOS may also be at an increased risk of having ovarian cancer [Resta et al, 1993],. c) There is an association of PCOS with postmenopausal breast cancer. A positive association has been shown between PCOS and a family history of breast cancer. In a cross-sectional study of 217

women with and without PCOS, the proportion of women with a positive family history of breast cancer was significantly greater in women with PCOS compared with controls [Atiomo et al, 2003].

An overall appraisal of the evidence in support of a genetic basis for PCOS would therefore, suggest that although there is some evidences that genetics underpins PCOS, the evidence is not overwhelming, and several genes or an interaction between several genes and environmental factors may be involved.

5. Pathophysiology

5.1. Follicular dynamics in normal cycle:

Physiologically the human ovaries produce a single dominant follicle that releases its eggs into the oviduct to be fertilized at the end of the follicular phase of the menstrual cycle. The process of follicular development, which is known as folliculogenesis, begins when a cohort of primordial follicles is recruited to initiate growth.

The store of ovarian follicles invested during foetal development is not only non-replenishable but is constantly depleted by the process of degeneration or atresia. Atresia occurs at all stages of follicle development and >90% of oocyets are lost in this way [Baker T, 1982]. From a maximum of approximately 6 million follicles in the two gonads at the seventh month of intrauterine life, only about 2 million survive to reach neonatal life. By the time of menarche, this number has been depleted to only about 400000 viable follicles. By the age 40-44 years only approximately 8000 primordial follicles are left. From early foetal life follicular growth and development, atresia and programmed cell death or apoptosis is a continuous process. Soon after formation of the

primary oocyte it becomes surrounded by a single layer of flattened granulosa cells to mark the development and to constitute the primordial follicle. Germ cells designated to undergo atresia are incompletely surrounded by this mantle of primitive granulosa cells. Further nuclear materials of the primary oocyte are arrested in the dictyotene stage of prophase of the first meiotic division. It will remain in this stage until ready to resume meiosis and potentially develop into a mature oocyte.

According to need of gonadotropins follicular development occurs at two stages

1. Gonadotropin- independent development
2. Gonadotropin- dependent development

Gonadotropin independent development

The preantral follicles are hormone independent and growth and development takes place without gonadotropin support. The initiation of follicular growth is a continuous process and occurs in all ages even during prepubartal and climacteric year. It remains uninterrupted during pregnancy and is not affected by changes in circulating gonadotropin and sex steroid milieu [Peters et al, 1976].

Gonadotropin dependent development

From antral stage onwards follicular growth depends on gonadotropin. So this stage of follicular growth only occurs from puberty onwards, when continuous secretion of gonadotropins occurs. During this stage the morphologic and endocrine dynamics of

folliculogenesis are well defined and are divided into the intervals of recruitment, selection, dominance and ovulation.

Recruitment

In early follicular phase with the fall of oestrogen level suppression of the hypothalamic pituitary axis decreases and FSH level begins to increase. The group of preantral follicles which acquired gonadotropin receptors mustered into next growth phase and is termed as recruitment. Due to action of FSH these follicles begin to grow. The follicle which has lowest threshold for FSH is the first to undergo activation of aromatase system and begins oestradiol production. As folliculogenesis progresses they produce more oestradiol, which inhibts FSH secretion. Some follicles have increased number of FSH receptors and are capable of sustained growth even in the presence of lower FSH concentration [Moon et al, 1978].

Selection

Between 5 and 7 days of normal 28 days cycle a single follicle becomes destined to ovulate and forms the corpus luteum. This is termed as the selection of the dominant follicle. The selected follicle has lowest FSH threshold, greater granulosa cell proliferation and higher oestradiol production. As a result it is less dependent on circulating FSH and can continue to grow in presence of lowest FSH. The rest of the cohort of follicles become atretic as FSH is suppressed below their own threshold level. The selected follicle is termed as graffian follicle. Rising level of oestradiol in conjunction with FSH induce the appearance of LH (luteinizing hormone) receptors on the outer layer of granulosa cells. Granulosa cells that possessed both FSH and LH

receptors have been shown to respond similarly to both hormones in terms of steroid production. Presence of both LH and FSH receptors of granolosa cells may further protect the emerging dominant follicle from declining FSH concentration.

Dominance

The interval of growth preceding ovulation but following selection is called dominance. Follicular growth continues with enlargement of the antrum of the follicle and proliferation of the granulosa and thecal layers. LH and FSH receptors are up regulated by the combined effects of oestradiol and FSH. The granulosa cells secrete increasing quantities of oestrogens, which is essential for preparation and synchronization of the entire reproductive system for ovulation, fertilization and implantation. As the follicle reaches maturity oestradiol output reaches a peak. In such a high oestrogenic environment the pulse frequency of gonadotropin releasing hormone (GnRH) is more rapid and the sensitivity of the pituitary gonadotropin cells to GnRH is greatly enhanced. These events lead to a massive discharge of gonadotropins, principally LH about 24 hours after the oestradiol peak.

Ovulation

At mid cycle a number of physiologic process occur which provoke the final maturational changes within the follicle and induce ovulation. The gonadotropin surge stimulates three major events:

1. Resumption of meiosis
2. Luteinization of the granulosa and theca cells.

3. Follicle rupture with extrusion of a mature oocyte.

Resumption of meiosis:

LH appears to allow the resumption of meiosis with breakdown of the germinal vesicle and subsequent extrusion of the first polar body. This process is not a stimulatory event but rather a release from a prior inhibition. An ovarian peptide termed oocyte maturation inhibitor (OMI) has been isolated from follicular fluid and is proposed as the agent responsible for preventing early maturation of the oocyte [Safriri et al,1982; Adashi EY, 1992]. It is hypothesized that the midcycle rise in the LH inhibits the production or action of OMI, thus allowing maturation to occur at the appropriate time.

Luteinization:

Along with maintaining oestradiol synthesis, LH induces progesterone production. The LH surge is thus accompanied a small but significant rise in progesterone. This rise starts about 12 hours prior to the onset of the LH surge and signals the start of luteinization in those granulosa cells with LH receptors. It has been suggested that the pre-ovulatory rise in progesterone serves to augment the positive feedback effect of oestradiol on the LH surge. Luteinization requires the LH surge for completion.

Follicle rupture and oocyte extrusion:

Follicular rupture and oocyte extrusion occurs approximately 34-36 hours following the onset of LH surge [Pauerstein et al 1978]. Several mechanisms may be involved in this process.

i) Proteolytic digestion of the follicular wall:

In response to the gonadotopin surge the content of the tissue type plasminogen activator (PA) in follicular fluid and granulosa cells increases [Reich et al, 1985]. The increase in PA produces a parallel increase in intrafollicular plasmin. The enzymatic product of PA action on plasminogen seems to decrease the tensile strength of the follicular wall. In addition PA or plasmin causes the activation of latent collagenase and consequently initiates the proteolytic changes leading to ovulation [Yoshimura and Wallach, 1987].

ii) Action of other mediators:

Mature pre-ovulatory follicles under LH stimulation synthesize prostaglandins (PG E_2, PG F_2 alpha, PGI_2). PG F_2 alpha may facilitate the liberation of hydrolyses by ovarian epithelial cells covering the follicle apex, which initiate breakdown of the cell wall. Follicle rupture may be achieved through vascular changes induced by PGI_2 within the follicular wall. [Yoshimura et al, 1986]. Histamine, bradykinin kallikrein also probably modulates ovuation by stimulation of prostaglandin synthesis and ovarian contractibility and activation of collagenase directly or via PA [Irianni and Hodgen, 1992].

5.2 Pathogenesis

Polycystic ovarian syndrome is a heterogenous syndrome characterized by persistent anovulation, oligomenorrhoea or amenorrhoea and hyperandrogenism in the absence of thyroid, pituitary and or adrenal disease. At the level of the ovary, there are recruitment and growth of follicles to the small antral stage with no selection of a dominant

preovulatory follicle. This leads to the accumulation of multiple small antral follicles which give the syndrome its name [Yen, 1980; Frank, 1995; Futterweit, 1995]. There is also evidence of hyperfunctioning of the thecal compartment in addition to the relative hypofunctioning of the granulosa. While many women with PCOS have relatively high circulating levels of luteinizing hormone compared with follicle stimulating hormone this is not so in all women and does not account totally for the observed increase in thecal production of androgens or the relative quiescence and FSH resistance of the granulosa. This complex disorder probably has its origin within as well as outside the hypothalamic-pituitary-ovarian axis. Clinical and basic researches have highlighted the roles, within the reproductive tract, of intraovarian autocrine and /or paracrine regulators. Outside the reproductive axis, metabolic, neuroendocrine and also endocrine regulators probably contribute to the pathogenesis of this disorder [Kazer et al, 1990; Morales et al, 1996; Dunaif, 1997].

5.2.1. *Abnormal folliculogenesis*

PCOS has a multifactorial basis as in the case of all degenerative diseases and has a strong genetic and environmental component to it. Familial pattern is often observed and an autosomal as well as X linked inheritance has been suggested. However, owing to the polygenic pattern of inheritance it has always been difficult to narrow down on the genes responsible for the syndrome.A complete understanding of the underlying pathophysiology for abnormal folliculogenesis in PCOS is still lacking. Because of the heterogenecity in this disorder, there are most likely, multiple underlying pathophysiologic mechanisms.

Several theories have been proposed to explain the pathogenesis of PCOS.

i) An alteration in gonadotropin releasing hormone secretion results in increased leutinizing hormone (LH) secretion.

ii) An increase in insulin secretion and insulin action results in hyperinsulinemia and insulin resistance.

iii) A defect in androgen synthesis

A defect in androgen synthesis that results in increased ovarian androgen production causing hyperandrogenism.

A) Steroidogenic defect:

a) Theca cells

Increased circulating androgen is one of the cardinal features of polycystic ovary syndrome and is present in both ovulatory and anovulatory women. As the adrenal gland and ovary contribute similar amounts of androgen to the circulation there has been considerable debate as to the origin of these androgens. In both glands the majority of the androgen is in the form of androstenedione with testosterone being secreted in lesser amounts and approximately 50% of the circulating testosterone being derived from peripheral conversion of androstenedione. In the ovary theca cell production of progesterone and androgen from cholesterol is primarily under control of luteinizing hormone (LH) and levels of LH have been shown to be elevated in PCOS. For this reason it is considered that LH may be responsible for increased thecal androgen output. Insulin is also known to increase thecal secretion of androgen and it

was therefore suggested that the hyperinsulinemia of PCO may cause hyperandrogenism. Both LH and insulin cause increased thecal androgen production.

b) Stromal cells:

As the stroma in polycystic ovaries is also a source of androgen and the volume of stroma is greatly increased this will be a further contributing factor. Androstenedione production by stromal cells in culture also increased in response to insulin.

c) Granulosa cells:

Experiments were performed in granulosa cells pooled from follicles from normal ovaries and anovulatory polycystic ovaries. Measurement of oestradiol accumulation in the medium revealed that cells from anovulatory PCO were in fact hyperresponsive to FSH compared with cells from normal ovaries. It is primarily women with anovulation who are the most insulin resistant and therefore, have the highest levels of serum insulin to compensate. Granulosa cells respond to insulin by increasing steroidogenesis [Garzo and Dorrington, 1984]. The effects of hyperinsulinemia on the granulosa cell could therefore, contribute to the paradoxical increase in response to FSH by granulosa cells.

Mechanism of anovulation:

Role of FSH:

Although the levels of FSH in anovular PCO are slightly lower than normal early to mid-follicular phase levels they do not appear to be low enough to cause failure of follicle selection. Investigators suggested that the passage of FSH into the follicle may

be impeded by the basal lamina in these follicles. It could be postulated that thickening occurs in response to the increased androgens or the hyperinsulinemia.

Role of paracrine factors:

Research finding showed that granulosa cells from anovular PCO were very responsive to FSH and yet in vivo the follicles did not grow to preovulatory sizes. It appeared likely that an endogenous inhibitor of aromatase was present, the effect of which was removed when the cells were placed in culture. The presence and production of a wide range of other endocrine and paracrine factors has been investigated in an attempt to explain the abnormal steroidogenesis and failure of ovulation seen in PCOS [Frank and Mason, 1991].The possible involvements of these factors are IGF systems. Hyperinsulinemia leads to lowering of IGF-BP, which accounts for increased availability of IGF-I. IGF-I in conjunction with insulin is responsible for increased release of LH mediated thecal androgen, which subsequently causes follicular atresia and apoptosis.

Role of follistatin:

Folistatin the activin binding protein is involved in ovarian steroid, pancreatic insulin and pituitary FSH production and so could have a causative role in all of the defects associated with PCOS.

Role of LH and 'premature luteinization' theory:

Experiments showed that granulosa cells from normal ovaries did not respond to FSH until the follicle was 9.5mm in diameter, where as granulosa cells from anovular PCO were LH-responsive in follicles of 4mm [Willis et al, 1998]. This might be the fact of prematurely initiating events seen normally at the time of the mid-cycle LH surge, namely increased progesterone production and cessation of follicle growth. It was suggested that raised intrafollicular levels of insulin, in combination with the raised circulating levels of LH in these patients, caused this premature luteinization of the follicles.

B) Altered GnRH secretion

LH hypersecretion is a characteristic hallmark of PCOS. LH is secreted in a pulsatile manner. Women with PCOS have an increase in both the LH pulse frequency and amplitude, resulting in increased 24-hour secretion. This increase in LH secretion is thought to occur as a result of increased frequency of hypothalamic gonadotropin releasing hormone (GnRH) pulses. Increased LH in turn leads to an increase in androgen production by the theca cells within the ovary [Ehrmann, 2005; Tsilchorozidou et al, 2004] causing abnormal folliculogenesis and anovulation.

5.2.2. Hyperinsulinemia and insulin resistance

Insulin resistance is defined as a state in which greater than normal amounts of insulin are required to produce a quantitatively normal response. Since an initial report in 1980 of association between PCOS and hyperinsulinemia it has become apparent that women with PCOS are both insulin resistance and hyperinsulinemic in relation to weight matched control [Burghen et al, 1980; Dunaif, 1995]. Insulin resistance in PCOS is due to a post-binding defect in signaling and is associated with constitutive serine phosphorylation of the insulin receptor (IR). Hyperinsulinemia plays a role in the etio-pathogenesis of hyperandrogenism in women with PCOS by increasing ovarian androgen production and decreasing the serum sex hormone binding globulin (SHBG) concentration [Nestler, 1994; Barbieri et al, 1986; Nestler et al, 1991]. Hyperinsulinemia is proposed to stimulate p450c, 17 alpha activities in PCOS [Nestler and Jakubowicz, 1996]. Cytochrome p450c, 17 alpha is a bifunctional enzyme that has both 17 alpha hydroxylase and 17, 20-lyase activity and is key in the biosynthesis of ovarian androgens. In ovarian theca cells p450c, 17 alpha converts progesterone to 17 alpha hydroxy progesterone through its 17, 20 lyase activity. Adrostenedion is then converted to testosterone by the enzyme 17 β reductase.

Hyperinsulinemia can result from any one of the following reasons:

a) increased insulin production by beta cells

b) insulin resistance in target cells, or impaired hepatic insulin clearance.

Beta cell function:

Abnormalities in insulin secretion have been reported in studies of women with PCOS with and without a family history of type 2 diabetes mellitus, hyperinsulinemia and partly due to increased basal insulin secretion [O'Meara et al, 1993]. Fasting hyperinsulinemia is present in obese women with PCOS and is in part secondary to increased basal insulin secretion. Increased beta cell reactivity is also more pronounced in obese patients. Aging causes beta cell failure, leading to glucose intolerance which may result in type 2 diabetes mellitus, and this may explain the high incidence of diabetes in the older population of PCOS patients. The disproportionate beta cell effect is dependent on the insulin gene and its regulatory site. The VNTR (variable number tandem receptors) mini-satellite, which lies in the insulin gene on chromosome 11p 15.5 is directly involved in the regulation of insulin secretion and has been associated with hyperinsulinemia. At this locus there is bimodal distribution, a short sequence of class I alleles with about 40 receptors and a long sequence of class III alleles are associated with PCOS in different populations and most strongly with anovulatory PCOS patients [Franks et al, 1997; Waterworth et al, 1997].

Insulin clearance in PCOS:

Decreased insulin clearance is present in hyperinsulinemic PCOS patients. This may be directly related to hyperinsulinemia, which decreases the number of hepatic receptors for insulin or secondary to hyperandrogenicity, which also decreases hepatic insulin clearance [Peiris et al, 1987].

Target cell insulin resistance:

Insulin resistance can exists when cellular response does not occur following exposure to insulin. The causes for such abnormal cellular response to insulin are impaired insulin receptor binding due to receptor defect (type A syndrome) or autoantibodies to insulin receptor (type B syndrome), or a defect in insulin signaling which is mediated through a tyrosine kinase receptor causing activation of a number of phosphorylation dephosphorylation steps. Molecular scanning techniquesin obese PCOS identified deletion of exon 3 of the insulin receptor gene. The resulting protein product is deformed, lacking 95% of its amino acids including most of its extracellular, transmembrane and intracellular domains [Wertheimer et al, 1994]. Patients with severe insulin resistance syndromes due to insulin receptor mutations or autoantibodies can also be virilized at a young age or may present with PCOS and acnthosis nigricans [Kahn et al, 1976]. However, insulin receptor mutations were not demonstrated in most patients with PCOS. Studies in lymphocytes, adipocytes and peripheral muscle cells have demonstrated that approximately 50% of PCOS patients have a post receptor insulin signaling defect. In those women, the insulin receptor constantly undergoes serine phosphorylation, which in turn decreases the tyrosine kinase activity [Dunaif et al, 1995]. The biochemical effect of serine phosphorylation has been shown to terminate insulin signaling [Theroux et al, 1992]. The result of continuous phosphorylation of the receptor is slightly increased basal insulin activity but with decreased response to insulin administration. It has been assumed that serine phosphorylation of the insulin receptor is important in the pathogenesis of

hyperglycemia-induced insulin resistance. However, the mechanism for the serine phosphorylation defect in PCOS has not been elucidated.

Effect of insulin on the polycystic ovaries:

Insulin receptors are present in the stroma of the ovary and studies in vitro have demonstrated that insulin can directly stimulate steroidogenesis [Nestler and Starauss, 1991]. Decreased ability of insulin to control glucose metabolism is hallmark of insulin resistance. However, in PCOS the resistance of insulin action does not extend to ovarian steroidogenesis. A few hypotheses have been proposed to explain the ability of the polycystic ovary to hyper respond to high insulin levels in the presence of an insulin resistant state. There can be tissue specific responses to insulin action and difference among ovarian stromal cells and other cells exists. Also a selective defect in peripheral and ovarian sensitivity to insulin has been demonstrated in some patients with PCOS who had several metabolic insulin resistances (in terms of glucose metabolism) but maintained mitogenic insulin signaling pathways sensitivity [Dunaif, 1999]. The most convincing hypothesis suggests that the actions of insulin on steroidogenesis may be mediated by insulin like growth factor (IGF) receptors that are present in ovarian tissues. Insulin has been shown to bind to type I IGF receptors at high concentrations and can mimic IGF-I actions [Rechler and Nissely, 1986; Froesch and Zapf, 1985]. It is likely that under physiological conditions IGF-I rather than insulin stimulates steroidogenesis. However, in the presence of hyperinsulinemia insulin may directly act on IGF receptors. Also the affinity of the IGF-I receptor for insulin varies by tissue.

There are also atypical IGF-I receptors that bind IGF-I and insulin with similar affinity. Dimmers of the insulin and IGF-I receptor can assemble together to form a hybrid [Dunaif, 1997; Moxham and Jacobs, 1992]. All these have been proposed as a mechanism for insulin-mediated hyperandrogenism.

Obesity insulinresistance and hyperandrogenemia:

The association between obesity hyperandrogenism and hyperinsulinemia is well recognized. Obesity however, is not a prerequisite for insulin resistance. Hyperinsulinemia in non-obese hyperandrogenic patients, with or without acanthosis nigricans has been described [Dunaif et al, 1995]. Among obese subjects the most unfavourable endocrine profiles have been demonstrated in those with upper-body obesity. Obese PCOS patients without insulin resistance usually have gynecoid obesity [Meirow et al, 1995]. An increased waist-to-hip ratio is associated with increased androgenecity, decreased SHBG level, increased free testosterone level, increased basal and post-glucose load blood insulin levels and diminished insulin sensitivity in vivo [Evans et al, 1983]. Independent of obesity increased waist-to-hip ratio predicts development of diabetes [Ohlson et al, 1985]. The clinical features of PCOS are heterogenous and may change throughout lifespan, starting from adolescence to postmenopausal age. Obesity plays a significant role in determining the severity of clinical manifestations and metabolic disorder. It has been seen that in patients with PCOS the body mass index is correlated with an increased rate of hirsutism, menstrual irregularity and infertility.

Malnutrition: An excess of nutrition is an important cause of insulin resistance. Both excess glucose and excess fat can cause insulin resistance in muscle and fat tissues and

excess fat can cause insulin resistance in liver. High fat feeding and fat infusion cause impairment in glucose transport thereby leading to development of insulin resistance. Studies have shown defects in insulin signaling which results from accumulation of free fatty acids which in turn lead to activation of protein Kinase C. Obesity resulting from excess nutrient intake also causes insulin resistance by an increase in the production of agents that impair insulin action such as TNF alpha and resistin and a decrease in the production of insulin sensitizing compound adiponectin. Both glucose and free fatty acids acutely stimulate insulin secretion but chronic exposure to high levels of either nutrients leads to impairment of beta cell function [Proietto, 2005].In the obese PCOS there is a more marked dysregulation of insulin levels and impairment of insulin sensitivity and therefore higher levels of androgens.

Mechanisms of obesity-induced insulin resistance are not well understood, but appear to involve a decrease in the number of insulin receptors in target tissues and an inhibition of postreceptor events [Flier et al, 1985]. This form of insulin resistance appears to be reversible. It therefore, appears that obesity is an additional, but not essential risk factor predisposing to insulin resistance [Kissebah et al, 1982].

5.2.3 Hyperandrogenism

Androgens are C-19 steroids,the parent nucleus is androsterone. The adrenals and the ovaries are the primary androgen producing glands in the female. In the ovaries androgens are produced in the thecal layer by the action of LH on LH receptors. Insulin also acts on LH receptors and hence hyperinsulinemia seen in PCOS also increased androgen production in females via LH mediated pathway. Androgens produced in the

theca are converted to oestrogens in the granulosa cells by the enzyme aromatase. Only 1% of androgen in the female plasma is free, the rest is either bound to sex hormone binding globulin (SHBG) or loosely bound to albumin. Reduction of SHBG increases the proportion of free hormone responsible for clinical manifestations of hyperandrogenism. Excess androgens itself suppresses SHBG which in turn increases the free level, thus a vicious cycle is established and hyperandrogenism begets hyperandrogenism. The most potent androgen testosterone is 70% SHBG bound compared to 8% of DHEAS and andrstenedione. Hence a slight reduction of SHBG increases free testosterone more than the others and cause significant hyperandrogenism even though the total testosterone is either normal or marginally increased.

The important androgens in the female are DHEA, DHEAS, androstenedione, testosterone (T) and dihydrotestosterone (DHT). The major androgens produced in the ovary are DHEA and androstenedione (and very little testosterone). About 50% of DHEA in females is produced by the adrenals and the remaining 50% by the ovaries. Regarding androstenedione 50% come from adrenals, 25% from ovaries and 25% from peripheral tissues. Regarding testosterone 50% arise from peripheral conversion of androstenedione 25% from adrenals and 25% from ovaries. In chronic hyperandrogenism the principal biochemical abnormality in women affected by PCOS is often attributed to enhanced biglandular androgen production by both the ovaries and the adrenals [Rosenfield, 1997]. Recent investigations have found that hyperandrogenism in PCOS is due to following factors

1. Increased LH acting on LH receptors in thecal cells [Gillign et al, 1994].

2. Hyperinsulinemia acting via LH receptors [Morales et al, 1996]
3. Reduction of SHBG, thus increasing free androgen levels [Hogveen et al, 2002]
4. Obesity (increased peripheral conversion of DHEA to androstenedione and then to testosterone) [Fassnacht et al, 2003].

Link between hyperinsulinemia and hyperandrogenism in PCOS

Hyperinsulinemia evidently plays a role in the etiopathogenesis of hyperandrogenism in women with PCOS by increasing ovarian androgen production and decreasing the serum sex hormone binding globulin (SHBG) concentration [Barbieri et al, 1986; Nestler et al, 1991]. There are different theories supporting the fact that even in insulin resistant women, ovaries and adrenal cortex may remain insulin sensitive and may produce excess androgen in response to excess circulating insulin. These theories are

1. Intrinsic ovarian and adrenal enzyme P450c 17 alpha theory and
2. SHBG and IGF-1 theory.
3. Co-gonadotropin theory

Intrinsic ovarian and adrenal enzyme P450c 17 alpha theories:

Studies on PCOS patients have found a possibility of dysregulation of P450c 17 alpha, the rate limiting enzyme in androgen biosynthesis, in the ovaries and in the adrenal cortex [Rosenfield R, 1999]. Hyperinsulinemia is proposed to stimulate P450c 17 alpha activity in PCOS. Cytochrome P450 17 alpha is a bifunctional enzyme that has both 17 alpha –hydroxylase and 17, 20-lyase activity and is key in the biosynthesis of ovarian androgens. In ovarian theca cells P450c 17 alpha converts progesterone to 17 alpha-hydroxyprogesterone through its 17 alpha hydroxylase activity. Androstenedione is then converted to testosterone by the enzyme 17 β reductase [Nestler et al, 1996].

SHBG and IGF-1 theory:

- Hyperinsulinemia leads to decline in hepatic synthesis of SHBG and IGF BP-1
- Decline in the level of SHBG will lead to excess bioavailability of free androgen, while decline in the level ofIGF BP-1 will help in increasing the level of circulating IGF-1.
- IGF-1 and insulin are structurally and chemically similar.
- Receptors of IGF-1 are present in the ovary (theca cells)
- Elevated level of insulin –through IGF-1 receptors will amplify LH mediated thecal androgen production
- Hence, the ultimate consequence of hyperinsulinemia is hyperandrogenicity.

GF-1 is a growth factor. Its receptors are abundant in ovaries. Insulin is structurally and chemically similar to IGF-1. Insulin can bind to IGF-1 receptors at high concentration and can mimic IGF-1 action [Rechler and Nissely, 1986]. In the presence of hyperinsulinemia, insulin may directly act on IGF receptors and thus stimulate production of excess amount of LH mediated thecal androgens. Insulin also decreases hepatic production of IGF-BP-1 and thus make IGF-1 more biologically available [Leroith et al, 1995], thereby producing more androgens. Furthermore, insulin has been shown to indirectly stimulate ovarian androgen production by augmenting leutinizing hormone (LH)- stimulated ovarian androgen biosynthesis. Thus hyperinsulinemia may both directly and indirectly increases ovarian androgen production.

Co-gonadotropin theory :

Insulin also acts as co-gonadotropin. There areinsulin receptors in the pituitary. Therefore, excess insulin accelerates LH hypersecretion,which in turn will increase ovarian theca-cell androgen production.

5.2.4 Neo-hormones in PCOS

In the recent years it has been discovered that the adipose tissue contains adipocytes, which are secretory cells that produce a variety of proteins with hormonal type functions, which collectively have been called adipocytokines. Three adipose hormones have been described till date, Leptine, Adiponectin and Resistin. Another hormone called Ghrelin secreted primarily in the stomach mucosa and related to obesity and controlling the secretion of growth hormone (GH) has also been studied and its relationship to PCOS elucidated.

Leptin:

The first adipose hormone to be discovered was leptin, a 146 amino acid protein which acts mostly as a signaling factor from adipose tissue to the central nervous system, thus regulating food intake and energy expenditure [Zhang et al, 1994; Tritos and Mantzoros, 1997]. Leptin is not produced exclusively by adipocytes but its circulating levels are strictly correlated to adipose mass and are higher in obese humans [Gong et al, 1996]. Recent data indicates that leptin may directly affects glucose and fat metabolism and because leptin receptors have been identified in the ovaries [Karlsson et al, 1997], it has been proposed that locally acting leptin may be more important than

circulating leptin in the pathogenesis of the polycystic ovary syndrome and type II diabetes [Spicer and Francisco, 1997; Zachow and Magoffin, 1997].

Adiponectin:

Adiponectin is almost exclusively produced in the adipose tissues and has a potent insulin sensitizing action[Hotta et al, 2001; Yamamuchi et al, 2001]. In contrast to leptin the production of adiponectin is decreased in obese subjects making it the only known adipocyte specific hormone that is down regulated in obesity [Steppan and Lazar, 2002]. High adiponectin levels are independently associated with increased insulin sensitivity [Weyer et al, 2001; Philips et al, 2003] and reduced risk of type II diabetes. There is substantial evidence suggesting that adiponectin plays an important beneficial role in insulin sensitivity in humans [Hotta et al, 2000; Weyer et al, 2001; Philips et al, 2003; Spranger et al, 2003; Okamoto et al, 2000]. Low adiponectin may predict women with PCOS who are at risk for developing type II diabetes.

Resistin:

Resistin is expressed by adipocytes and antagonizes insulin action [Steppan et al, 2001]. It is thought to be related to the development of insulin resistance [Azuma et al, 2003] It has been reported that circulating levels resistin are increased in obesity and resistin impairs insulin action on hepatic glucose production and inhibits glucose uptake in skeletal muscle independent of GLUT-4 [Holcomb et al, 2000; Utzschneider et al, 2005].

Ghrelin:

Ghrelin is mainly produced in the stomach and major binding sites of Ghrelinhave been identified in hypothalamic areas. Plasma Ghrelin concentrations have been shown to be lower in obese patients when compared with normal subjects [Tschop et al, 2001; Shiiya et al, 2002]. The mean plasma Ghrelin levels of women with obese PCOS are lower compared with weight matched controlled subjects. PCOS is a pathological condition often associated with obesity. Ghrelin is inversely correlated with parameters of insulin resistance in obese PCOS. It is not yet clear why a negative significant correlation between Ghrelin and insulin resistance was observed in the obese PCOS group but not in obese patients. Based on currently available data it can be speculated that this relationship may occur in the presence of a moderate to severe insulin resistance only [Tschop et al, 2001].

It is still controversial whether Ghrelin stimulates or inhibits insulin secretion [Egido et al, 2002]. Ghrelin secretion however, appears to be downregulated following glucose ingestion [Shiiya et al, 2002] suggesting a negative feedback system among caloric intake, glucose, insulin and Ghrelin. Ghrelin showed a strong negative correlation with andosterone but not with testosterone and other androgens. Therefore, it has been suggested that Ghrelin may regulate glandular steroidogenesis in vivo. In conclusion it appears that Ghrelin like leptin may represent a further endocrine factor involved in the regulation of energy balance and metabolism and in control of steroidogenesis and gonadal function [Arvat et al, 2000].

6. Clinical manifestations

Polycystic ovarian syndrome is a complex disorder characterized by chronic anovulation, infertility, hyperandrogenism menifested by various degrees of hirsutism, obesity, large cystic ovaries and insulin resistance. Menstrual irregularities may single handedly signal this condition in adolescents or it may coexist with other symptoms. The signs and symptoms of this syndrome stem from disruption of normal ovulatory menstrual cycles to continous or irregular heavy bleeding [Yen,1980; Barnes and Rosenfield, 1989; McKenna, 1988; Burghen et al, 1980; Chang et al, 1983; Dunaif et al, 1989].

A) Menstrual dysfunction:

Whatever may be the pathogenic mechanism of PCOS, the ultimate end result is acyclicoestrogen production and progesterone deficiency. In the early phase of menstrual cycle, oestradiol levels in women with PCOS are equal to those of normal women; however mid-cycle elevations of oestrogen and progesterone that normally occur after ovulation are absent. Because of the lack of cyclical progesterone secretion the action of oestradiol on both the hypothalamic-pituitary axis and the endometrium is unopposed. Both progesterone deficiency and acyclic oestrogen production contribute to increased secretion of LH. The effects of unopposed oestrogen on the endometrium may cause it to become hyperplastic, which may cause intermittent and heavy uterine bleeding and increase the long term risk of endometrial cancer. These effects may be compounded, especially in obese patients by increased levels of oestrone converted from androstenedione in adipose tissue. Hyperinsulinemia is a frequently encountered

feature of PCOS.Hyperinsulinemia enhances LH secretion from anterior pituitary, stimulates ovarian androgen production, inhibits sex hormone binding globulin, which in turn leads to rise in levels of free testosterone. Thus a combination of these factors is likely contribute to the endometrial dysfunction.

About 66% women with PCOS have the symptoms and signs of menstrual irregularities. Amenorrhoea occurs in 30% of PCOS and oligomenorrhoea in 90% of these women [Adams et al, 1986; Franks, 1995] and is indicative of anovulation and oligo-ovulation respectively. Polymenorrhoea with very short menstrual cycles is also a less frequently encountered presentation of PCOS and is indicative of anovulatory cycles or ovulation with short luteal phases. Dysfunctional uterine bleeding occurs as an atypical and uncommon presentation of PCOS because of prolonged unopposed oestrogen action on the endometrium.

The majority are oligomenorrhoic having only six to eight spontaneous episodes of vaginal bleeding per year and only a small percentage are totally amenorrhoic [Legro, 2003].

B) Anovulation and infertility:

Chronic anovulation is the commonest feature of PCOS. About 85%-90% of patients of anovulatory infertility are due to PCOS. Infertility is frequently the initial reason the patient seeks medical advice [Pritts, 2002]. High ovarian androgen level prevents normal ovulation from occurring, thus leading to infertility.

C) Obesity:

Obesity is present in at least 30%of women with PCOS, and in some studies the prevalence is as high as 75% [Ehrmann, 2005]. The obesity commonly seen with PCOS is characterized by an increase in the waist circumference (>35 inches). This type of obesity is associated with insulin resistance, glucose intolerance and dyslipidemia. Obesity is also correlated with decreased sex hormone binding globulin, which causes or increases in circulating free testosterone, which may accelerate the symptoms related to androgen excess [Chang, 2004].

Relation of obesity with menstrual irregularities:

Obesity has positive correlation with menstrual irregularities. Forty five percent of amenorrhoeic women are obese while only 9-13% of women with normal menstruation are overweight [Mitchell and Rogers, 1953]. Grossly obese women have a rate of menstrual disturbance 3.1 times more as compared to their normal weight counterparts [Hartz et al, 1979]. Different studies shown that patients having BMI 23.9-28.6 kg/m^2 are 1.32 times more likely and BMI >28.6 kg/ m^2 are 1.75 times more likely to have menstrual disturbances [Lake et al, 1997; Kiddy et al, 1990; Balen et al, 1995].

D) Hirsutism:

Hirsutism is defined as excessive facial or body hair in a female due to excessive androgen production. It is due to increased androgen production either of ovarian or adrenal origin and or an increased sensitivity of the skin to 5 alpha reductase, the

enzyme, which converts testosterone to dihydrotestosterone (DHT) to which the hair follicles are sensitive. Although androgen stimulates sexual hair follicle conversion from lanugo to terminal adult hair growth patterns, once established these patterns persist despite withdrawal of androgen. It is basically an increased activity of the pilosebaceous unit.5 alpha reductase is stimulated by IGF-1 and increased IGF-1 levels in hyperinsulinemia and insulin resistance can lead to hirsutism. Hirsutism is the most common manifestation of androgen excess associated with PCOS [Guzick, 2004]. It affects approximately 10% of reproductive aged women who do not have PCOS and up-to 70% of women with PCOS [Hill, 2003]. Excessive hair distribution is of male pattern with the most common sites being the upper lip, chin, chest, lower abdomen and inner aspects of the thighs. It implies a vellus to terminal hair transition. These hairs are stiff, course, pigmented and long [Azziz, 2003]. Severity of hirsutism is clinically graded by modified Ferriman Galliway scoring, which is based on 9 androgen sensitive skin areas. Graded from 0-4 (0 no hirsutism, 4 severe hirsutism). The sites included are upper lip, chin, chest, upper abdomen, lower abdomen, upper arm, thigh, upper back and lower back. A score >8, means hirsutism.Increased androgen levels are also associated with the increased sebum production and acne, which are frequently seen in women with PCOS [Legro, 2003; Hunter and Carek, 2003].

E) Seborrhoea and acne:

The sebaceous glandular cells have testosterone and DHT receptors at the basement membrane. Thus hyperandrogenemia produces increased secretion and causes the clinical symptoms of seborrhea. Acne is seen in one third of patients with PCOS.

[Simpson and Barth, 1997]. It is a chronic inflammatory disorder of the pilosebaceous unit. Excess secretion of sebum and glandular hypertrophy of the acinar cells in response to hyperandrogenemia is the initiating factor. Propionibacterium acne a commensal thrives on this sebum and produces a chronic inflammatory reaction [Cunliffe and Simpson, 1998]. Subsequent hyperkeratosis and increased viscocity in response to the chronic inflammation cause blockage of the pores and lead to acne formation. Superadded bacterial infection may cause pustule formation, which is sometimes painful. Though acne is due to hyperandrogenism, a strict correlation between blood androgen level and acne is lacking. Local skin sensitivity may have a role to play.

F) Androgen Alopecia:

This is partly genetic and partly androgen dependent. DHEAS and testosterone are the hormones mainly implicated in the process. It is interesting that the same hormones, which cause excess hair growth elsewhere cause alopecia in the scalp. Male alopecia is bitemporal. Female androgenic alopecia starts at the crown and is initiated as widening of the hair parting in the middle and is seen approximately in 8% of women with PCOS [Simpson and Barth, 1997].

G) Acnthosis nigricans:

It is a dermatologic condition in which the skin appears velvety and hyperpigmented is frequently seen in people who are in a state of insulin resistance [Legro, 2003; Murry, 2004]. It is seen in 5% of women with PCOS. It is often found on the nape of the neck,

the axilla, and the area beneath the breasts. This condition is due to epidermal hyperkeratosis and dermal fibroblast proliferation [Chang, 2004]. It is sign of insulin resistance and the term "HAIR-AN" syndrome is used to describe hyperandrogenism, insulin resistance and acanthosis Nigricans. Factors responsible for these findings have not been identified, although the close association with insulin resistance suggests a causal relationship. There is an abnormally increased thickening and pigmentation of the skin and skin fold region, which can be associated with obesity and high insulin levels.

II) Long term health hazards :

Metabolic derangement associated with this condition may predispose to a range of diseases with attendant morbidity and mortality risks. In general, available data support significantly increased rates of type II diabetes mellitus, dyslipidemia and endometrial cancer in PCOS that are not completely explained by obesity [Solomon, 1999].

1) Metabolic syndrome:

Women with PCOS are also at increased risk for the development of the metabolic syndrome [Chereau, 1844; Stein and Leventhal, 1935]. Diagnostic criteria for metabolic syndrome require three or more of the following abnormalities: 1) waist circumference in females greater than 35 inches; 2) fasting serum glucose $\geq$ 110 mg/dL; 3) fasting serum triglyceride > 150 mg/dL; 4) serum high-density lipoprotein cholesterol (HDL-C) < 50 mg/dL; and 5) blood pressure $\geq$ 130/85 mm Hg [Chereau, 1844]. It is well

documented that metabolic syndrome is associated with an increased risk for both type 2 diabetes mellitus and cardiovascular disease.

Women with PCOS have a 11 –fold increase in the prevalence of metabolic syndrome compared with age-matched controls. The risk of metabolic syndrome is high even at a young age, highlighting the importance of early and regular screening. The triglyceride HDL-C ratio may serve as a screening tool and needs to be prospectively validated in this group [Dokras et al, 2005]. Women with PCOS frequently have abnormal lipid profiles, with raised triglycerides and total and low-density lipoprotein (LDL) cholesterol. The effect of PCOS on high-density lipoprotein (HDL) cholesterol is however controversial [Legro et al, 2003]. Disturbed cholesterol and triglyceride metabolism may eventuate in premature cardiovascular disease [Wild, 1997].

2) Cardiovascular disease:

There have been many reports of metabolic abnormalities in women with polycystic ovaries. These include raised serum concentration of triglyceride and LDL cholesterol, lowered serum concentration of HDL cholesterol and evidence of insulin resistance which are all recognized cardiac risk factors [Barbieri, 1991]. Conway et al examined risk factors for coronary artery disease in premenopausal women with PCOS attending an endocrinology clinic and reported hyperinsulinemia and adverse lipid profiles in both lean and obese women with PCOS [Conway et al, 1991].Obese women with PCOS tending to have higher systolic blood pressure measurements as well.Women with PCOS have multiple risk factors for the development of cardiovascular disease, including hyperandrogenemia, insulin resistance and glucose intolerance, obesity, and central fat deposition [Carmina, 2003].They concluded that as hyperinsulinaemic

women with PCOS had an increased risk of developing cardiovascular disease metabolic screening is needed.

3) Impaired glucose tolerance and Type 2 diabetes Mellitus:

As a consequence of insulin resistance, women affected by PCOS often present with abnormalities of glucose metabolism and lipid profile along with an increased risk of type 2 diabetes and cardiovascular disease. Besides insulin resistance, it has been demonstrated that some of these women also have alterations in pancreatic beta cell function. Both disorders are recognized as major risk factors for the development of type 2 diabetes [Pelusi et al, 2004]. The risk of glucose intolerance among PCOS subjects seems to be approximately 5-10 fold higher than normal and appears not to be limited to a single ethnic group. Moreover, the onset of glucose intolerance in PCOS women has been reported to occur at an earlier age than in the normal population, approximately in 3rd and 4th decade of life. However other risk factors such as obesity, a positive family history of type 2 diabetes and hyperandrogenism may also contribute to increasing the diabetes risk in PCOS [Pelusi et al, 2004]. Women with PCOS after becoming pregnant are at an increased risk of gestational diabetes.

4)Cancer risk:

a) *Endometrial cancer:*

The prolonged anovulation state with consequent continued secretion of oestrogen unopposed by progesterone, may enhance the development and growth of endometrium and development of cancer particularly in young women. Hypersecretion of LH, chronic hyperinsulinemia, obesity and increased serum insulin-like growth factor levels may represent risk factors for endometrial cancer [Gadducci et al, 2005]

b) *Ovarian and breast cancer:*

One report showed ovarian cancer risk increased 2.5 fold among women with PCOS [Schildkraut et al, 1996]. But this study is subject to recall bias and unfortunately did not use a histological definition of PCOS, which would have made the results far more valuable.So no association with ovarian cancer has been proved. Although risk factors for breast cancer include infertility, obesity and hyperandrogenism no association of PCOS with breast cancer has been proved.

7. Diagnostic criteria of PCOS

The syndrome is surrounded by controversies regarding both its diagnosis and treatment. Menstrual dysfunction has been one of the most important diagnostic criteria for PCOS. In 1935, Stein and Leventhal first described the association of polycystic ovaries, amenorrhoea, hirsutism and obesity. However, it was not until 1990 that the key features necessary for the diagnosis of polycystic ovarian syndrome were detailed at a conference convened by the National Institute of Health (NIH). Among these key features were hyperandrogenism, menstrual dysfunction and exclusion of other causes of hyperandrogenism, such as congenital adrenal hyperplasia, androgen secreting tumours and hyperprolactinemia. Beyound the NIH criteria of hyperandrogenism and menstrual dysfunction other manifestations are variable and PCOS emerges as a clinically histologically and biochemicallty heterogenous condition. PCOS was redefined at a joint consensus meeting of the American Society of Reproductive Medicine (ASRM) and the European Soceity of Human Reproduction and Embryology

(ESHRE) held in Rotterdam in May 2003.The need to establish universally accepted diagnostic criteria led to Rotterdam meeting, during which experts of PCOS from all over the world arrived at a consensus regarding the diagnosis of the syndrome [Carmina E, 2003; The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004]. This included the presence of two out of the following three criteria: (a) oligo and /or anovulation; (b) hyperandrogenism (clinical and /or biochemical); and polycystic ovaries with the exclusion of other aetiologies. The morphology of polycystic ovaries has been redefined as an ovary with 12 or more follicles measuring 2-9 mm in diameter and /or increased ovarian volume (>10cm^3) [John and William, 2005]. So the diagnostic criteria can be divided into

A. Clinical
B. Biochemical
C. Ultrasound and imaging

7.1 Clinical tests

This essentially includes a detailed history and physical examination. The clinician should have a high degree of suspicion when a woman presents with complaints of infertility. Occasionally the menstrual irregularities may not begin until the woman is in her late teens or early twenties. It is important to remember that some women with PCOS continue to menstruate regularly even though they are anovulatory [Carmina, 2003].

Table 1. Differential Diagnosis for PCOS

Condition	Clinical Feature	Diagnosis
Nonclassical congenital adrenal hyperplasia due to deficiency of 21-hydroxylase	Severe hirsutism, clitoromegaly, regular menses, family history, and short stature	Elevated serum 17-hydroxyprogesterone, androstenedione, and testosterone
Cushing's syndrome	Obesity, hirsutism, acne, menstrual irregularity, moonlike facies, buffalo hump, hypertension, muscle wasting, abdominal striae, and osteoporosis	Elevated 24-hour urinary free cortisol levels
Androgen-producing adrenal or ovarian neoplasms	Rapid onset of symptoms. Most common symptoms are severe hirsutism, clitoromegaly, male-pattern hair loss, acne, and lowering of the voice	Extremely elevated plasma androgen levels; tumor detected by palpation and/or ultrasound
Hyperprolactinemia	Oligomenorrhea or amenorrhea; galactorrhea	Elevated plasma prolactin level
Thyroid disorder	May have irregular menstrual cycles; may have enlarged thyroid gland	Abnormal TSH, T_3 and T_4 levels
Premature ovarian failure	Oligomenorrhea or amenorrhea, signs and symptoms of low levels of estrogen (e.g., hot flashes, vaginal dryness)	Elevated FSH levels, normal or low levels of estradiol

Sources: Chang 2004, Ehrmann 2005, The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004.

The most distinctive clinical feature is hirsutism, the degree of which can vary from mild to severe. The rate of hair growth is important. Generally, in women with PCOS, the hair growth is gradual and progressive. Rapid onset, specifically if associated with signs of virilization (e.g., deepening of the voice, clitoromegaly, temporal hair loss, and balding) is suggestive of an ovarian or adrenal androgen-secreting tumor. A medication history is also important because many drugs may cause hirsutism.

Table 2: Drugs causing hirsutism

Antiseizure	*Corticosteroids*	*Other*	*Phenothiazine*	*Anabolic*
Phenytoin	Betamethasone	*Medications*	*Derivatives*	*Agents*
Valproate	Cortisone	Metoclopramide	Promethazine	Danazole
Valproic	Dexamethasone	Methyldopa		Testosterone
acid	Fludrocortisone	Reserpine		Minoxidil
	Hydrocortisone			
	Methylprednisolone			
	Prednisone			

A history of oily skin and acne are also subtle signs of androgen excess and may be present in women with PCOS [Hill, 2003; Hunter and Carek,2003]

Table 3. Signs of Virilization

Clitoromegaly
Deepening of the voice
Increased muscle mass
Loss of breast tissue
Malodorous perspiration
Temporal hair recession and balding

Sources: Hill 2003; Hunter and Carek 2003.

Points to be taken into consideration

1. Family history of menstrual disturbances
2. Menstrual history

3. History of weight gain and calculate body mass index (BMI)
4. Clinical suggestion of androgen excess

i) Hirsutism

ii) Seborrhoea and acne

iii) Alopecia

5. Acanthosis Nigricans suggesting insulin resistance

Physical Examination

The physical examination should include an assessment of the body mass index (BMI = kg/m2) and the blood pressure. An elevated blood pressure may suggest androgen excess related to congenital adrenal hyperplasia. The amount of excess hair as well as the distribution is to be noted. The Feriman-Gallway scoring system has been used for evaluation of hirsutism but is limited by subjective variability and is thought by many experts to be of little clinical use. Evaluation for signs of virilization is needed (Table 3) Skin changes, such as acne, acanthosis nigricans, and striae is to be noted, which may be a clinical feature of Cushing's syndrome. Other signs and symptoms of Cushing's syndrome include truncal obesity, moon facies, hypertension, spontaneous ecchymosis, buffalo hump, and muscle weakness. A thorough abdominal and pelvic examination should be performed to exclude any masses [Lobo et al, 1982; Inzucchi et al, 1998]. It is also important to check for the presence of galactorrhea by compressing the areola from its outer perimeter toward the nipple.

7.2 Biochemical Tests:

Hypersecretion of insulin:

The potential role of insulin in PCOS was suspected due to the association of acanthosis nigricans in young women with menstrual disturbances. Women with PCOS possess a specific post-receptor binding defect in insulin action, whereby insulin induced tyrosine phosphorylation is replaced by phosphorylation of serine [Haas et al, 2003; Lergo, 2002]. Anovulatory women have resistance to extra-splancnic action of insulin on carbohydrate metabolism and this effect compounded in overweight patients, because obesity by itself increases insulin resistance and hence increases the secretion of insulin [Vrbikova et al, 2001; Glueck et al, 2001; Arslanian et al, 2002; Elter et al, 2002; Pasquali et al, 2000].

Hypersecretion of LH:

About 40% of patients with PCOS have elevated LH levels. There is an increase in both rate and amplitude of LH pulses [Tasdemir et al, 2003].It has been suggested that increased insulin levels may be associated with an increase in pituitary response to GnRH stimulation and hence be responsible for an increase in LH levels [Barbieri, 2003]. Increased LH level in follicular phase impair fertility by either causing anovulation or even in ovulating patients is associated with a decreased rate of conception and increased rate of miscarriage. Altered LH:FSH ratio is diagnostic. If LH:FSH ratio is low then these patients are likely to be CC resistant [Pasquali et al, 2000; Tasdemir et al, 2003; Barbieri, 2003; Conway and Jacobs, 1993].

Elevated prolactin:

About 15% of patients with PCOS have increased prolactin levels. This may be due to the syndrome per se or may be a co-incidental findings. It is necessary to rule out other causes of hyperprolactinemia in these patients.

The goal is to assess the severity and source of androgen excess as well as to rule out other sources of hyperandrogenism such as an adrenal or ovarian tumour, cushing's syndrome or congenital adrenal hyperplasia and to screen for associated endocrine disturbances. Following tests are to be done to assess biochemical alterations.

- *FSH and LH*

  -- FSH is within the reference range or low.

  -- LH is elevated

  -- The LH: FSH ratio is greater than 3 in 40% cases of the patients. In the rest 60% cases LH:FSH ratio may be normal.[John Bonnar, 2001]

- *Thyroid stimulating hormone and total thyroxine :*

  -- Thyroid dysfunction may cause amenorrhoea and hirsutism

  -- Thyroid function tests may be abnormal in approximately 5% of patients with PCOS.

- *Prolatin:*

  -- A small percentage of patients have elevated prolactin levels.

- Total and free testosterone levels:

  -- Free testosterone levels are mainly indicative of ovarian

Hyperandrogenism and are elevated in patients with PCOS.

-- Sex hormone binding globulin (SHBG) is concomitantly low.

-- Dihydro-epiandrosterone sulphate (DHEAS) mainly indicates adrenal contribution of androgens.

Identification of underlying disorders is the primary purpose of laboratory testing and should be individualized to the patient's clinical manifestations. Given a history of chronic anovulation and androgen excess, the major condition that needs to be excluded when making the diagnosis of PCOS is nonclasssical congenital adrenal hyperplasia. To rule out this disorder, a blood sample for 17-hydroxyprogesterone is to be tested. To maximize sensitivity, the sample should be drawn between 7:00 and 9:00 AM. If the result is <2 ng/mL, nonclassical adrenal hyperplasia is safely excluded; if the result is >2 ng/mL, the patient needs further evaluation. In the absence of virilization, there is really no need to draw testosterone or dehydroepiandrosterone sulfate to evaluate for an ovarian or adrenal tumor, although a baseline total testosterone level will be helpful in assessing the treatment that is directed at reducing testosterone levels [Nestler et al, 1989]. A total testosterone level >60 ng/dL is considered elevated. Despite the widespread practice of measuring serum LH and follicle-stimulating hormone (FSH), the circulating levels of these hormones do not contribute significantly to the diagnosis of PCOS. Because of the pulsatile nature of gonadotropin secretion, there is a wide "normal range" for both LH and FSH. Therefore, a single blood sample from a patient with PCOS will frequently be in this normal range.[Nestler et al, 1989; Poresky et al ,1999]

Table 4. Criteria for the Diagnosis of Metabolic Syndrome in Women with PCOS

Risk Factor	Criteria
Waist circumference	35 inches (>88 cm)
Triglycerides	≥150 mg/dL
High-density lipoprotein cholesterol	<50 mg/dL
Blood pressure	≥135/≥85
Fasting and 2-hour glucose utilizing an oral glucose tolerance test	110-126 mg/dL and/or 2-hour glucose 140-199 mg/dL

Menstrual dysfunction that presents with irregular menses, oligomenorrhea, or amenorrhea is highly suggestive of anovulation. Tests for ovulatory function include basal body temperature evaluation, serum progesterone concentrations, or endometrial biopsy. A secretory endometrium is an indication that ovulation has occurred.

7.3 Imaging Findings in PCOS:

With advent of high resolution ultrasonogram (USG) identification of PCOS is simplified and ovarian biopsy is now unnecessary. Overies are described as PCO if there are 10 or more 2-8 mm cysts aggregated around a dense stroma or scattered throughout an increased stroma. Some workers have also used Magnetic Resonance Imaging (MRI) to identify the polycystic ovaries and demonstrate the increased volume accurately. But with the availability of 3-D ultrasound now this is not justified [Morin-Papunen et al, 2000; Woodward and Gilfeather, 1998; Bataglia et al, 1998; Zaidi et al, 1995]. It is essential to be well verse with the pathophysiology of PCOS before we diagnose the disease. It is also essential to understand the histological features of PCOS.

Histology of PCOS:

1. Ovarian hypertrophy
2. Thickened capsule (>100 μ)
3. Increased number of subcapsular follicular cyst.
4. Scarcity of corpus lutea or albicans
5. Hyperplasia and fibrosis of the ovarian stroma
6. Decreased thickness of granulosa layer
7. Atretic pattern of the granulosa layer
8. Increased thickness of the theca interna
9. Premature luteization of theca cells.

The histo-anatomical features can be variable although an increased number of follicles and increased stromal ovarian tissue is a constant feature and can be identified by pelvic ultrasound [Cheung and Chang, 1990].In clinical practice today ultrasonography has replaced laparoscopic evaluationof polycystic ovaries and with the advent of transvaginal probe the size, shape, internal texture and ovarian volume can be accurately studied.

Ultrasound:

The use of ultrasound for stydying the female pelvis, specially the uterus and ovaries was first described by Kratochwil,who first described the ovarian and uterine changes in relation to the menstrual cycle [Kratochwil et al, 1972]. Since then Kratochwil's landmark paper ultrasound scanning has been emerged as the essential method for monitoring utero ovarian activity in all pelvic conditions. The initial debate of the route

of scanning, transabdominal sonogram (TAS) or transvaginal sonogram (TVS) is no longer valid and except for virgins, all ovaries should be scanned transvaginally. The theoretical advantage of full bladder and panaromic view with TAS [Robert et al, 2000] is overcome with the experience of the sonologist. The anatomic structure of the ovaries cannot be adequately assessed with the transabdominal approach in 42% cases [Hull MG, 1989]. Trans vaginal scanning has many advantages over TAS [Pache et al, 1992], which are as follows

- Avoidance of full bladder.
- Bypasses the problems of obesity-associated attenuation and artifacts.
- Higher frequency, hence better resolution
- Better visualization of pelvic organs as nearer the target
- Greater acceptance.

Sonographic criteria for PCO are

1. Multiple (>10) small (2-8mm) peripheral cysts give necklace appearance.
2. A dense core of stroma
3. Enlarged ovaries, >8 ml

On the basis of a transvaginal scan and follicular distribution PCO is of two types [Matsunaga et al, 1985]

A. Peripheral cystic pattern

B. General cystic pattern

It has been shown that these two different morphologies reflect different histologic types and both reflect specific endocrine PCOS patterns [Takahashi et al, 1994].

Ovarian stromal ecogenicity: The identification of hyperechogenic stroma and echodensity is a subjective evaluation and differs from operator to operator.

Ovarian volume: There is a wide volume overlap between normal and PCOS patients suggesting that the discriminative capacity of ovarian volume alone is not sufficient for the ultrasound diagnosis of PCOS.

Even though ultrasound (TVS) has good sensitivity and specificity, there can be no ultrasound cut off levels for number of follicles, ovarian stromal density or ovarian volume due to great variability and hence a specific diagnosis should be made after combining analysis of all the parameters.

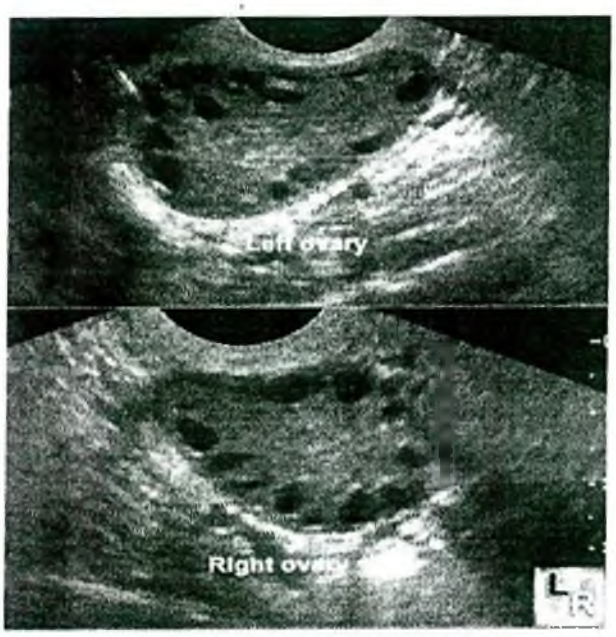


Fig: Sonographic criteria of polycystic varies

Sometimes multiple cysts are present in ovaries of some patients without the evidence of PCOS. In 25% cases ovaries are multicystic due to inappropriate gonadotropin stimulation. Those patients can be identified by certain parameters.

Multifollicular ovaries are seen in various physiological and even pathological situations like

1. Delayed normal puberty
2. Central precocious puberty
3. Hypothalamic anovulation
4. Hyperprolactinemia
5. Early normal follicular predominant phase

Aacording to Adam et al the multifollicular ovaries are different from PCOS by following characteristics [Adam et al, 1985]

1. Follicles are larger
2. Stromal ecodensity is less
3. Ovarian volume is normal
4. Usually a dominant follicle is seen

In 1991 Ardaena had described certain criteria for the ultrasonographic diagnosis of PCOS , which are not very used due to low sensitivity.

External morpgological signs:

- Increased ovarian area and volume
- Increased roundness index (ovarian width/length ratio <1)
- Decreased uterine width/ovarian length ratio.

Internal morphological signs:

- Number of small echoless regions,<10mm in size per ovary (microcysts)
- Increased echogenicity of the ovarian stroma
- Increased surface of the ovarian stroma on a cross-sectional cut

Colour Doppler in PCOS:

Colour Doppler mapping, Doppler wave analysis and power angio analysis has added a new dimension to the pathophysiological information on blood flow dynamics within the female pelvis. Ovarian artery blood flow can be measured and studied at the ovarian hilum. But recently there has been interest in the study of intrinsic blood flow in the ovarian stroma by the small spiral helical vessels supplying the stroma.

Uterine Pulsatility Index:

Women showing less impedance in the helical arteries will tend to hyperstimulate with gonadotrophin therapy. Ovaries showing higher ressitance in the intra ovarian vessels will be resistant to medical treatment and may need laparoscopic drilling and treatment with insulin sensitizing agents.

Magnetic Resonance Imaging (MRI):

With MRI images of the pelvis can be taken in multiple planes and this helps in accurate localization of pelvic viscera and their anatomy and pathology.The external

signs of PCO are very easy to identify by MRI. For stromal assessment ultrasound is better than MRI. MRI does not provide any extra information or details over ultrasound. The main role of MRI is to exclude small virilizing tumour.

In conclusion according to Rotterderm classification [Rotterdam ESHERE/ASRM Consensus, 2004] diagnosis of PCOS can be made by presence of two of the following three features

1. Oligomenorrhoea/ amenorrhoea
2. Clinical and or biochemical signs of hyperandrogenism
3. Polycystic ovaries by ultrasonography
4. FSH:LH ratio >2.5

They should be differentiated from the multicystic ovaries, which are commonly seen around puberty, postpartum period and weight loss related amenorrhoea.

Other tests to follow up long term consequences

A) Endometrial Aspiration:

A total endometrial thickness greater than 4 mm from the second to fifth day of menstruation requires biopsy. If the uterus is normal on examination, for reasons both accuracy and cost effectiveness, the method of biopsy should be office aspiration curettage. Endometrial cavity should be thoroughly curetted in all the directions.

B) Hysteroscopy and D and C:

It is an important diagnostic tool specially in women with dysfunctional uterine bleeding and in whom the baseline endometrium is >4mm is unresponsive to the medical line of treatment and the uterus is not normal on pelvic examination. Hysteroscopy is an endoscopic examination of the uterine cavity to diagnose endometrial pathologies and treat them at the same time. Endometrial pathlogy is identified by histopathological examination [Leon et al, 1999]. Although there is a high rate of spontaneous regression in 80% cases without atypia and over 50% in complex hyperplasia with atypia therapy should be instituted since some patients will progress to cancer nonetheless.

8. Management of PCOS

As the underlying pathology is being unraveled, better treatment strategies have evolved. A great evolution has occurred slowly but steadily in the management over the years.

Aim of treatment of PCOS consists of

- Treatment of menstrual irregularities
- Management of hirsutism and acne
- Reducing the far reaching consequences of insulin resistance and glucose intolerance
- Treatment of infertility

The recent treatment of PCOS is mainly symptom oriented with the objective to

- improve insulin resistance
- antagonize the action of androgens
- correct anovulation for women desiring pregnancy
- maintain a normal endometrium by regulating menses in women not interested in child bearing.

The decision about the use of the therapy will depend on a number of factors

1. Age of the patient
2. Body mass index (BMI)
3. Menstrual history
4. Evidence of hirsutism
5. Interested in childbearing or not

Table 5: Criteria taken into consideration for therapy in PCOS

A. Clinical presentations

- Body mass index (BMI) and waist-hip ratio (WHR)
- Menstrual cyclicity
- Spontaneous ovulation
- Pregnancy
- Acne-severity
- Hirsutism
- Side effects experienced by patient

B. Biochemical findings

- Fasting blood glucose and OGTT
- Insulin levels
- LDL and HDL cholesterol
- Triglyceride levels
- Testosterone and the androgen levels
- Plasminogen activator inhibitor levels (PAI-1)
- FSH and LH levels
- LH : FSH ratio

8.1Treatment of menstrual irregularities

The ultimate goal of treatment include decreasing androgen levels to improve hirsutism, protecting the endometrium, optimizing reproductive function in infertility and reducing the long-term squeal of insulin resistance. The initial therapeutic strategy in the management of PCOS should be directed at management of the patients presenting symptom. There are essentially two causes of the infertility attributable to PCO syndrome. The first is a reduced rate of ovulation and the second is hyper secretion of LH. Once anovulation has been diagnosed and other problems such as tubal occlusion and severe male factor infertility have been excluded the treatment is ovulation induction. An initial therapeutic approach to infertility in women with PCOS

is weight loss through diet and exercise. Huber-Bchholz et al [Huber-Bchholz et al, 1999] observed that lifestyle modification is the best initial management for obese women seeking to improve their reproductive function. The next step is pharmacotherapy.

8.1.1. Weight reduction:

If the patient is overweight or obese (BMI >26kg/m^2) specifically with abdominal obesity (i.e. waist circumference >35 inches) life style modification in the form of moderate caloric restriction and exercise is essential no matter what other intervention is chosen. One study showed that moderate caloric restriction that resulted in a 2%-5% weight loss reduced 21% free testosterone. Nine of 18 women with irregular cycles resumed regular ovulation and 2 of 18 became pregnant [Guzick, 2004]. It has been shown that metformin, a biguanide antihyperglycemic together with a low caloric diet is associated with more weight loss than a low-caloric diet alone [Barbieri, 2003] Weight loss in women with PCOS can lead to spontaneous resumption of menses and ovulation and can lower the circulating androgen and insulin levels [Kiddy et al, 1992; Guzick et al, 1994]. The decrease in free testosterone levels is attributed to an increase in the SHBG levels. These changes have been reported with a weight loss as small as 5% of the initial weight. A 7% reduction in body weight is effective in restoring fertility [Frank et al, 1991]. Although a significant degree of weight reduction is achieved through diet control, maintenance is often difficult and hence lifestyle modification is gaining popularity. Lifestyle modification programme lay emphasis on behavioral management, dietary and exercise interventions. Exercise not only acts through weight reduction, it also prompts the transport of GLUT4 structures to the

outer edge of the cell, which facilitates the entry of glucose into the cell regardless of insulin resistance. Some patients respond positively to even minimal weight loss. The response varies widely however, from patient to patient. In some cases weight loss alone may not suffice, but may increase the efficiency of other therapies. Beneficial effects of weight loss and exercise appear through the following mechanisms

- Reduction in plasma androgen
- Reduction in circulating plasma insulin levels
- Reduction in IGF-1 levels
- Increase in SHBG levels, decrease in free testosterone

More than 90% of obese oligomenorrhoic women may show a dramatic improvement in menstrual pattern with a high spontaneous conception rate and lower miscarriage rate. Surgically induced weight loss is successful in restoring menstruation. Thus diet control and exercise induced weight loss helps to reduce hyperinsulinemia, hyperandrogenism and may spontaneously regularize menstruation.

8.1.2. Insulin sensitizing agents:

Insulin sensitizing agents act by increasing the insulin sensitivity of peripheral tissue and by suppressing hepatic gluconeogenesis. Their role in treating menstrual irregularityis by two mechanisms:

- In hyperinsulinemic women these agents act by both reducing the intraovarian androgen load as well as by initiating weight loss, which in turn improves follicular response and thus ovulatory performance and hence may regularize menstruation.

- Insulin sensitizing agents may provide a useful adjunct to the therapy of endometrial hyperplasia, specially when progestogen therapy alone appears inadequate. In such cases the addition of metformin to progestogen therapy reduces the direct mitogenic effect of hyperinsulinemia, on the endometrium and improves the efficacy of progestogen [Session et al, 2003].

8.1.3. Hormone therapy:

In women with PCOS, supplementation with progesterone or an oral contraceptive pill is enough to cause a withdrawal bleeding and regularize menstruation. Induction of ovulation is not necessary to induce periods unless fertility is desired.

Normal endometrium

a) Unmarried girl and women not desiring pregnancy

- **Oral contraceptive pills:** All the pills are started from day 3-5 of the menstrual cycle for 21 days in each cycle. Pills containing desogestrel or cyproterone acetate are preferable for women who apart from irregular menstruation also have features of hyperandrogenism.

- **Progestogens:** Cyclical progesterone withdrawal is preferred for females who do not tolerate oral contraceptive pills or who do not want to take pills throughout the month and also in women who do not seeking contraception. The most commonly used progesterone

for this purpose is medroxy progesterone acetate. These are used in doses of 15-20 mg a day in 2-3 divided doses for 5 days. These are best given from day 21 to day 25 of each menstrual cycle to induce a withdrawal bleding every 28-30 days. It is very important for these girls to have a withdrawal bleeding every 1-2 months so as to keep the regular endometrial shedding and to prevent endometrial hyperplasia.

b) Women desiring pregnancy

Ovulation induction by various ovulation-inducing agents such as clomiphene citrate, letrozole and gonadotropin is done.

Endometrial hyperplasia

a) Hyperplasia without atypia

Progesterone: Cyclical progesterone therapy is the recommended choice either in last half of cycle or from D5 to D25 of the cycle for 3-6 months. After that patients are resampled and continuous progesterone therapy is recommended if hyperplasia persists. An injectable depot preparation of medroxyprogesterone acetate (Depot Provera) can be given with high efficacy.

Oral Contraceptive pills: Women who want contraception, oral contraceptive pills can be used, which provide the combined benefit of cycle control and contraception.

b) Hyperplasia with atypia

Medical management

i) High dose continuous progesterone therapy

ii) Gonadotropin releasing hormone agonist

iii) GnRHa in combination with high dose progesterone

iv) Danazole and tamoxiphen

Surgical treatment

Hysterectomy and endometrial ablation is restricted for women who completed family

Management of an acute episode of bleeding

Patients presenting with severe bleeding initial management includes the use of prostaglandin synthetase inhibitors with hormones. The most commonly used anti-fibrinolytic agent is intravenous tranexamic acid which is the most potent of all such agents and can cut the blood loss almost to half. Along with this high dose oestrogen is most effective, which stops most uterine bleeding temporarily.For this 25 mg of conjugated oestrogen is used intravenously every four hours.Other hormones, which canbe used are progesterons, which are also used in high doses to help control acute blood loss.Norethisterone acetate appears to be the most potent of all progestogens for this purpose. Apart from these oral contraceptive agents in four times the contraceptive dose have also been used to stop acute episodes of bleeding and are given for one week or till bleeding stops.

8.2. Managemnt of hirsutism:

Hirsutism can range from a cosmetic problem causing psychological consequences to a medical disorder associated with irregular menses, anovulation and hyperandrogenism. Management consists of medical hair removal as well as pharmacological treatment to suppress androgen production and /or action specially in case of moderate to severe hirsutism. [Ina , 2005].

Aim of treatment is to

-- stop development of hair

-- remove the existing hair

Therapy consists of

1. Non-pharmacologic therapy
2. Pharmacologic therapy.

8.2.1. ***Non-Pharmacologic therapy:***

a) Weight loss:

It reduces androgen production and improves insulin sensitivity and can slow the rate of hair growth.

b) Mechanical hair removal:

Mechanical hair removal can be done by

i) Shaving

ii) Tweezing/waxing

iii) Chemical depilation

iv) Electrical depilation

v) Laser therapy

vi) Flornithin hydrochloride

Flornithine hydrochloride: Flornithine hydrochloride (13.9%) also known as difluromethylornithine (DFMO) irreversibly blocks ornithine decarboxylase (ODC) an enzymein the dermal papilla responsible for hair growth [Falsetti et al, 1998]. It reduces the rate of hair growth and makes them softer. Treatment with laser in conjunction with eflornithine cream is superior to laser therapy alone [Fahrettin et al, 2004].

8.2.2. ***Pharmacological therapy:***

The drugs used in the treatment of hirsutism affect different aspects of androgen metabolism.

They may

- Decrease androgen production
- Increase metabolic clearance rate of androgen
- Inhibit androgen receptors
- Inhibit or block enzymes involved in the peripheral production of testosterone or conversion of testosterone to dihydrotestosterone (DHT)
- Increase the amount of sex hormone binding globulin (SHBG).

Suppression of androgen excess alone will not cure hirsutism; an antioestrogen is also required. The response to treatment varies from 20% to 90% depending on the drug, the dosage used and the individual response. The growth cycle of hair is long, 6-24 months

and the conversion of vellus to terminal hair is irreversible. Once hair growth has been stimulated by excessive androgen levels, maintenance of the same growth rate requires much less androgen. Drug treatment is essentially directed toward arresting growth of new hair until the hair that is present enters the catagen stage and is eventually shed from the follicle. Patients must therefore be advised that a response to therapy may not be seen for 6-12 months and that although it is possible to prevent further conversion of vellus to terminal hair little change will be seen in the total number of terminal hairs. As there are wide variation of response, treatment must be individualized and continued beyond the growth cycle of the relevant hairs before benefit can be assessed. The response to therapy varies greatly not only between individuals but also between different sites on the body, depending on the local rate of hair growth. Women who respond to treatment may notice changes in unwanted hair growth by the third month, but most donot until the sixth month. Beneficial effect will be obvious after 12 months, although the effectiveness of treatment increases to a maximum at approximately 2 years.

Usual drugs used in hirsutism:

A) ***Combined oral contraceptive pills:***

The drugs most widely used to suppress ovarian androgen production are combined oestrogen/ progesterone oral contraceptive pills (OCP) [Givens et al, 1974; Jeffcoate W, 1993] and this should be the treatment of choice for mild hirsutism of PCOS.Ovarian androgen secretion is reduced as a result of suppression of gonadotropins. The oestrogen component stimulates SHBG production, further

lowering free androgen levels. In addition to suppression of gonadotropins OCPs induce a decrease in adrenal androgen production [Wiebe and Morris, 1984]. This is obvious therapeutic benefit since at least a third of patients with PCOS have additional adrenal hyperandrogenism. At least three cycles of an oral contraceptive regimen are required to reach an endocrine equilibrium as reflected in levels of luteinizing hormone, follicle stimulating hormone, SHBG and sex steroids [Prelevic et al, 1993]. Treatment with OC containing 2 mg of cyproterone acetate has marked beneficial effects on acne, seborrhoea and hirsutism. The beneficial effect of cyproterone acetate on hirsutism is the result of general reduction of biologically active androgens in circulation and antiandrogen effects at the target organ.

B) ***Antiandrogens:***

Antiandrogens interfere with androgen action at the target organ. They vary in mode of action and either block enzyme reaction thereby limiting the formation of potent androgens or specifically block the androgen receptor, some antiandrogens affect both enzyme reactions and androgen receptor. Following are the antiandrogen used for hirsutism

a) Cyproterone acetate: It has progestational and antigonadotrophic actions in addition to antiandrogen activity [Miller and Jacob, 1986]. The clinical improvement observed with CPA is therefore due to both central antigonadotrophic activity and antiandrogen effect at the end organ. The antiandrogen effect results from inhibition of testosterone and DHT binding to their intracellular receptors; the reduction in concentrations of circulating androgens is the result of inhibition of ovarian androgen production through suppression of LH release. Cyproterone acetate also increases the

metabolic clearance of androgens by hepatic enzyme induction as well as an indirect reduction in the androgen-dependent 5 α-reductase enzyme activity. A reverse sequential regimen is best prescribed in which CPA 25-100 mg daily is given orally on days 5-15 of the cycle and ethinyloestradiol 20-30 μg is given on days D5-D25 of the cycle [Miller and Jacob, 1986; Hammerstein et al, 1975]. Most commonly CPA is used with the combined oral contraceptive pill instead of with ethinylocstradiol alone.

b) Spironolactone: Spironolactone an aldosterone antagonist is also a potent antiandrogen which competitively binds to the androgen receptor within the hair follicle. It possesses antiandrogenic properties and exerts its peripheral antiandrogenic effects in the hair follicle by competing for androgenic receptors and displacing DHT at both nuclear and cytosol rteceptors as well as interfering with translocation of the receptor complex to the nucleus [Shaw, 1991]. It acts by forming a biologically inactive spironolactone –receptor complex. Spironolactone exerts an additional effect on androgen metabolism by interfering with cytochrome P450 mono-oxygenases [Lobo et al, 1985]. This interference results in defective steroidogenesis and a decrease in testosterone production from the ovary. In clinical practice doses of spironolactone range from 50 mg to 200 mg daily; but most patients require at least 100 mg. This doses has been associated with significant improvement in 70-75% of hirsuitc women after 6 months [Crosby and Rittmaster, 1991]. Comparative studies showed it to be as effective as CPA in the management of hirsutism [O'Brien et al, 1991]. The combination of spironolactone with Dianette may have a synergistic effect on hirsutism score [Kelstimur and Sahin,1998].

c) Flutamide: Flutamide is a nonsteroidal compound, which is a pure peripheral androgen antagonist. It is metabolized to hydroxyflutamide, an active metabolite which acts by competitive inhibition of cytoplasmic and nuclear binding of androgens to receptor [Dollery, 1991]. Some data, however, suggests that flutamide may also reduce the synthesis of androgens and or increase their metabolism to inactive molecules. Flutamide induces a significant reduction in total and free testosterone, 5 α – dihydrotestosterone, DHEA, DHEA-S and androstenedione levels. Doses of 250 mg thrice daily resulted in improvement of hirsutism after only 3 months therapy [Marcondes, 1992]. In comparative studies it has been shown to be either better than [Cusan et al, 1994] or similarly effective to spironolacton [Erenus et al, 1994]. It seems to be the fastest of all antiandrogens in decreasing hair diameter.

d) Fenasteride: Fenasteride is a potent competitive 5 α-redcutase inhibitor which does not bind to the androgen receptor [Rittmaster, 1994]. It therefore blocks the conversion of testosterone to the more potent DHT. It induces a decrease in DHEA-S and 5 α-dihydrotestosterone and an increase in testosterone level [Venturoli et al, 1999; Fruzzetti et al, 1995] Comparative randomized trials showed that finasteride 5 mg daily has a clinical effect on hirsutism similar to that of spironolactone and flutamide [Venturoli et al,1999; Moghetti et al, 2000; Wong et al, 1995].

e) GnRH agonist: Gonadotropin releasing hormone agonists such as nafarelin, leuprorelin or buserelin decrease ovarian steroid production by suppressing LH and FSH secretion. This treatment is highly effective in women with PCOS or ovarian hyperthecosis and is particularly indicated in those with concurrent severe premenstrual syndrome [Carr et al, 1995]. Over a period of six months therapy hair growth was

reduced in the majority of patients and skin oiliness was eliminated in all [Falsetti and Pasinetti, 1994].

f) Ketoconazole: Ketoconazole, an imidazole derivative, inhibits ergosterole synthesis in fungi. In larger doses it also interferes with glandular cytochrome P450-linked steroidogenesis. Its principal inhibitory role involves inhibition of the 17,20-desmolase and 11 β hydoxylase steps in steroidogenesis [Venturoli et al, 1990]. Because of its significant ovarian suppressive effects its use has also been suggested for ovarian androgen suppression in hirsutism [Cavalho et al, 1985]. Improvement has been noted with 400 mg per day in PCOS as well as with 1000 mg per day in stromal hyperthecosis. In women with PCOS low-dose ketoconazole attenuated serum androgen levels and inhibited ovarian steroidogenesis in vitro [Gal et al, 1994]. Some 10% of patients may have transient hepatic dysfunction, and although true hepatotoxicity is rare (<1%) long-term use of this drug in patients with PCOS is not recommended.

C) *Metformin:*

Metformin is increasingly used in the treatment of PCOS. It significantly reduces hyperinsulimaemia and hyperandrogenism independent of changes in body weight. It has recently been shown to produce a clinically significant reduction in hair growth compared to placebo [Kelly and Gordon, 2002]. Metformin has also been compared to CPA-EE (Diane-35) combination, for period of 12 months and has been found to be effective for moderate to severe hirsutism in women with PCOS.

8.3: Management of infertility

8.3.1. Ovulation induction:

PCOS is one of the most common causes of infertility in women due to anovulation. The induction of ovulation in women with PCOS is a complex issue, which requires a thorough understanding of the syndrome and a careful and individual assessment of each patient. Different pharmacological agents are used to make the women ovulatory. Some are causing ovulation by increasing production of pituitary gonadotropins, some directly stimulates the ovaries and some reduces the androgen production and make the ovaries sensitive to stimulating agents. Ovualtion inducing agents are

1. Antioestrogens
    i) Clomiphene Citrate
    ii) Tamoxiphene
    iii) Aromatase inhibitor
2. Low dose gonadotropins
3. Pulsatile GnRHa
4. Adjuvant therapies
    1. *Insulin sensitizers*
        a) Metformin
        b) Pioglitazone
        c) Rosiglitazone
        d) Troglitazone

2. *Glucocorticoids*

3. *Bromocriptine*

4. *Ketaconazole*

5. D *chiro-inositole*

8.3.1.1 Antioestrogens

Clomiphene Citrate

Climiphene citrate (CC) alone or in combination with weight loss continues to be the first line of treatment for anovulatory infertility associated with polycystic ovary syndrome. Clomiphene is widely available and relatively well accepted in terms of safety, simplicity, side effects and cost. However, although approximately 70-90% of women ovulate during treatment with CC, only about half of those conceive [Gysler et al, 1982; Lobo et al, 1982; Polson et al, 1989; MacGregor et al, 1968; Gorlitsky et al, 1978; Hammond et al, 1983; Opsahl et al, 1996; Imani et al 1999; Imani et al, 1998]. The reasons for this relatively low pregnancy rate are not clear, but may be related to the high LH levels, the antioestrogenic effects of clomiphene at the level of the endometrium and at the level of the cervical gland secretion and perhaps due to the adverse effects on the oocytes [Homburg et al, 1988].

Mechanism of action:

Clomiphene acts predominantly as an antioestrogen. By binding to the hypothalamic oestrogen receptors, CC displaces endogenous oestrogen from the receptos, leading to a decrease in the negative feedback exerted by endogenous oestrogen and reduced replenishment of oestrogen receptors. As a result gonadotropin releasing hormone (GnRH) secretion appears to increase, followed by GnRH mediated luteinizing hormone

(LH) and follicle stimulating hormone (FSH) secretion. As gonadoropin levels increase, peak ovarian follicular development and oestradiol secretion become evident approximately 5-10 days after the last tablet [Wu and Winkel, 1989]. Rising oestradiol levels then appear to trigger the midcycle LH surge and ovulation.

Dose and administration:

The recommended starting dose is 50 mg/day as almost half of the pregnancies are achieved with this dose [Gysler et al, 1982]. In very few women with extremely sensitive ovaries, the starting dose may be as low as 25 mg/day. The tablets are usually given for 5 days following the onset of a spontaneous or a progesterone induced period. Administration of clomiphene citrate can start at any time from day 2,3,4 or 5 of the cycle as there is no difference in the outcome between these time-points [Wu and Winkel, 1989]. Untill normal ovulation occurs the dose should be increased in each of the next cycles by 50 mg/day up to a maximum dose of 150 mg/day. Maximum recommended dose is 150 mg/day as there is no clear evidence of efficacy at higher doses. Not much in use but probably underestimates is the titrated dose of clomiphene citrate or extended CC therapy in which prolonged administration (7- 10 days) of high doses of clomiphene citrate is used.

Monitoring:

Although the results of large trials suggest that monitoring by ultrasound is not mandatory to ensure good outcome, the practice in many centres is to monitor the first cycle to allow adjustment of the dose in subsequent cycles based on the observed response. In the absence of complete cycle monitoring, a pretreatment ultrasound is

often performed to evaluate ovarian and endometrial morphology, which may be followed by serum progesterone measurement [Legro et al, 2007].

Efficacy:

Approximately 75% to 80% of patients with PCOS ovulate after CC administration [Homburg, 2005; Messinis , 2005]. Although there appears to be discrepancy between ovulation and pregnancy rates life table analysis of the largest and most reliable studies indicates a conception rate of up to 22% per cycle in those ovulating on CC [Eijkemans et al, 2003; Hammond et al, 1982; Kousta et al 1997].

Duration of treatment:

Treatment generally should be limited to six ovulatory cycles [Homburg, 2005; Eijkemans et al, 2003,). Further cycles (Maximum 12 in total) may be considered on an individual basis after discussion with the patient. Cumulative live birth rates vary between 50% and 60% for up to six cycles [Kousta et al, 1997].

Adverse effect:

Hot flushes, headaches and visual complaints are well recognized side-effects during CC treatment, but the drug is generally well tolerated. The multiple pregnancy rate is less than 10% and ovarian hyperstimulation syndrome (OHSS) is rare [Eijkemans et al, 2003]. Anti-oestrogenic effects on endometrium and cervical mucus may occur but appear to represent an idiosyncratic response. There is no clear evidence that the chance of conception is adversely affected in ovulatory cycles [Kolibianakis et al, 2004].

Clomiphene failure:

As the goal of treatment is the induction of ovulation and pregnancy, CC failures must be sub-classified into those who fail to ovulate (ovulation failure) and those who ovulate but fail to conceive (conception failure). While considerable overlap exists between these two sub-groups, the distinction is important as the clinical management of the two problems may be quite different.

Ovulation failure:

The term CC non-responsiveness usually refers to ovulation failure despite maximal conventional doses. Women who fail to ovulate after maximum dose of CC are considered as clomiphene resistant [Nestler et al, 2002]. In women with PCOS, the rates of CC resistance are around 10-30% [Hughes et al 2000]. Several investigators have identified a relationship between CC dose and body weight, body mass index (BMI) or ponderal index [Shepard et al, 1979; Nestler et al, 2002; Imani et al 1998; Dicky et al, 1997; Espinosa et al, 1995]. It has been speculated that the higher esterone concentrations found in obese women necessitate higher CC doses in order to complete with the endogenous oestrogens for hypothalamic receptor sites [Shepard, 1979].

Clomiphene nonresponders tend to have significantly larger ovarian volumes with significantly more intermediate sized follicles than CC responders and normal controls, although considerable overlap exists [Polson et al, 1989; Imani et al, 1998]. Increasing level of LH [Ficicigolu et al, 1996] elevated total or free testosterone [Imani et al 1998;

Ficicigolu et al, 1996; Armstrong et al, 1996; Murakawa et al, 1994], fasting hyperinsulineamia or insulin resistance [Murakawa et al, 1994; Dupon et al, 1973] and decreasing concentrations of sex hormone binding globulin (SHBG) [Dupon et al, 1973] tend to predict poor response to CC. Although CC has been shown to have a beneficial effect on the secretion of FSH and SHBG [Butzow et a,l 1995], the ovary may not be able to respond to an apparently adequate rise in serum FSH [Polson et al, 1989]. Alternatively the beneficial effects may be outweigh by a concomitant increase in the already elevated concentrations of LH and ovarian androgens [Dupon et al, 1973; Butzow et al 1995], thus perpetuating the hyperandrogenic anovulation. In general the more severe the hormonal and ultrasonographic abnormalities the less likely it is that ovulation will be induced with CC.

Conception failure:

In spite of ovulation with CC when patients fail to conceive it is called clomiphene failure or conception failure. Considerable discrepancy exists between ovulation rates 70%-90% [Imani et el, 1998; Messinis, 2005] and conception rates 22% [Eijkemans et al, 2003; Hammond et al, 1983; Kousta et al, 1997]. Among women with PCOS the factors that predict ovulation (obesity, hyperandrogenism and insulin resistance) differ from those that predict conception (age, severity of the menstrual cycle abnormality and other infertility factors).

Approximately 75% of the pregnancies achieved during CC treatment occur within the first three cycles of treatment [Gysler et al, 1982]. Among those who do not conceive within six ovulatory cycles are couples with other infertility factors or PCOS related

factors which may account for these continuing infertility. The antioestrogenic actions of CC may adversely affect vaginal cornification, cervical mucus, and endometrial thickness, thus potentially affecting sperm transport, sperm survival and early implantation. In addition to the potential antioestrogenic effects of CC, ovulation induction in women with PCOS occurs in an environment characterized by high basal LH and androgen concentration both of which may be exacerbated during CC treatment [Kokia et al, 1990; Bateman et al, 1990] and may have a negative impact on outcome. This suboptimal environment may affect oocyte quality and fertilization rates as seen during in vitro fertilization cycles in women with PCOS [Homburg et al, 1993; Urman et al, 1992] or it may increase the likelihood of an early pregnancy loss [Regan et al, 1990].

Alternative therapies:

Antioestrogens other than clomiphene citrate:

Tamoxiphene appears to be as effective as CC for induction of ovulation but is not licensed for that purpose [Messinis and Nillius, 1982]. It can be considered as an alternative to CC in women who suffer intolerable side effects such as hot flushes.

Aromatase inhibitors:

Aromatase inhibitors are antioestrogenic agents which exert their action by inhibiting aromatase enzyme. Administering an aromatase inhibitor early in the follicular phase can induce ovulation by releasing the hypothalamus or pituitary from oestrogen negative feedback on GnRH and gonadotropin secretion leading to an increase in gonadotropin production which would stimulate ovarian follicular development.

Aromatase inhibitors also may act locally in the ovary to increase follicular sensitivity to FSH. This may result from accumulation of intraovarian androgens, which augment follicular FSH receptors expression and amplify FSH effects [Mitwally and Casper, 2000]. Antioestrogenic effect of CC includes long lasting estrogen receptor (ER) depletion. It also appears that CC accumulates in the body because of long half-life (15 days) leading to negative effect on endometrial development and quality and quantity of cervical mucus. But half-life of aromatize inhibitor is only 45 hours so there is no profound antioestrogenic effect on endometrial or cervical mucus [Mitwally and Casper, 2000]. Mitwally and Casper reported the success of aromatase inhibitor in inducing ovulation in women with PCOS [Mitwally and Casper, 2001; Sammour et al, 2001]. Other studies also proved efficacy of aromatase inhibitor in ovulation induction [Mitwally and Casper, 2001; Sammour et al, 76 Suppll; Mitwally and Casper, 2000; Heasley et al, 1987]. Initial preliminary study suggests that letrozole appears to be as effective as CC for induction of ovulation. But the drug is currently not approved for treatment of infertility. Prospective sufficiently powered studies demonstrating efficacy and safety should be awaited before the widespread use of aromatase inhibitors can be recommended. It may however, be considered as an off-label option for some patients after appropriate discussion of risks and benefits.

8.3.1.2. Low dose gonadotropins:

For anovulatory patients who do not respond to other ovulation inducing agents, gonadotropin is the supreme drug for induction of ovulation. For PCOS gonadotropin should be given with caution to avoid clinical hyperstimulation. In the first treatment cycle, a very low dose of HMG, pure FSH or a mixture of these (FSH and LH) should

be given for the first 5 days, gradually increasing the daily doses by 37.5-75 IU every day until the threshold dose is found. Advantage of using gonadotropin is chance of ovulation is more probable. Disadvantages are it requires meticulous judgement for application, dose is very much individualized, expensive, chance of OHSS (sometimes fatal) is more, so needs meticulous monitoring. Chance of multiple pregnancies is also more.

Currently, two low-dose regimens are used:

1.***Step-up regimens:*** Step-up regimens are based upon the principle of a stepwise increase in FSH supply to determine the FSH threshold for follicular development. After commencement of gonadotropin administration, if follicle development is not observed on ultrasound after 1 week, an increase in the dose is recommended. Once follicle growth is observed, the same FSH dose is maintained until follicular selection is achieved. To further reduce the risk of ovarian hyperresponsiveness, the duration of the initial dose of FSH was extended (from 7 to 14 days), and the weekly dose increment was reduced (from 100% to 50% of the dose), leading to the so-called chronic low-dose regimen [Dale et al, 1993; Polson et al, 1987; Sagle et al, 1991; Seibel et al, 1984]

2.***Step-down regimens:*** This regimen is designed to achieve the FSH threshold through a loading dose of FSH with a subsequent stepwise reduction as soon as follicular development is observed on ultrasound[schoot et al, 1992; VAN Dessel et al, 1996; Fauser and Van Heusden, 1997]. Preliminary studies report that both step-up and step-down regimens achieve similar high rates of monofollicular development [Balasch et al, 2001; van Sandbrink and Fauser, 1997]. However, the largest study published so far has shown that the step-up regimen is safer in terms of monofollicular development

[Christin-Maitre and Hugues, 2003]. Moreover, it is widely accepted that monitoring of a step-down cycle may require more experience and skill compared with a low-dose step-up regimen [van Sandbrink et al,1995] . Alternatively, a combined approach of sequential step-up and step-down regimens has been shown to help reduce the risk of overresponse [Hugues et al, 1996; Hugus et al, 2006]

8.3.1.3. Pulsatile GnRHa:

Gonadotropin releasing hormone (GnRH) agonists are synthetic peptide analogues of hypothalamic GnRH. The repeated administration causes pituitary desensitization and induces a reversible state of medical hypophysectomy. The idea of using GnRH agonists in ovulation induction in PCOS stems from the assumption that the endogenous secretion of relatively large amounts of LH may well be the cause of the higher incidence of development of the ovarian hyperstimulation syndrome. Moreover, the high tonic secretion of LH may be deleterious to the quality of the ovum is reduced by GnRHa. Therefore, the use of GnRHa in order to suppress endogenous gonadotropin secretion in PCOS patients and to mimic hypogonadotrophic hypogonadism seemed to be a logical approach for ovulation induction.

8.3.1.4. Combined treatment with GnRHa and gonadotropins:

The use of combined treatment for women with PCOS is indicated in cases who have been found to have persistently raised follicular phase LH levels, proven premature LH surges on hMG treatment alone, an inadequate luteal phase [Heasley et al, 1987], or those who have had two or more early pregnancy losses on clomiphene or hMG. It has been suggested that increased luteinizing hormone (LH) secretion in PCOS may

interfere with fertility. The mechanisms include premature oocyte maturation through inhibition of oocyte maturation inhibitor [Jacobs and Homburg,1990] and deleterious LH effect on granulosa cell steroidogenesis [Willis et al, 1996; Willis et al, 1998]. In addition, elevated LH levels may be associated with an increased pregnancy loss[Balen et al, 1993; Homburg et al, 1988; Regan et al, 1990; Tarlatzis et al, 1995] although more recent data are not consistent with this assumption [Mulders et al, 2003; Rai et al, 2000; Oliveira et al, 2007].

The concomitant use of a GnRH agonist with gonadotropin administration to improve pregnancy rates in patients undergoing ovulation induction has not been firmly established [Dodson et al, 1987; Fleming et al, 1985; Fleming et al, 1988]. Moreover, combined therapy was associated with an increased risk of OHSS [Buckler et al, 1993; Charbonnel et al, 1987; Homburg et al, 1990; Scheele et al, 1993; van der Meer et al, 1996] but there are insufficient data to draw solid conclusions on miscarriage and multiple pregnancy rates [Bachus et al, 1990; Clifford et al, 1996; Homburg et al, 1993]. Therefore, the significantly higher hyperstimulation rate, the associated risk of multiple pregnancies, and the additional inconvenience and cost of concomitant GnRH agonist administration, in the absence of documented increases in pregnancy success, do not justify the routine use of GnRH agonists during ovulation induction with gonadotropins in PCOS patients. The question of whether LH suppression by a GnRH antagonist during gonadotropin-based ovulation induction is of benefit to women with PCOS has not yet been addressed by RCTs.

8.3.1.5. GnRH antagonist

Though GnRHa suppress LH concentration, higher number of follicles during stimulation is an adverse effect. GnRH antagonists have some advantages over agonists. Since the antagonist suppress LH immediately, it can be administered in the late follicular phase when the LH peak is expected thereby reduce premature LH surge in PCOS patients.

Monitoring

Ultrasound assessment of the ovary can be performed at baseline before the initiation of each cycle. Serial ovarian ultrasound is an excellent method of determining follicle growth and development in response to gonadotropin stimulation. In particular, documentation of all follicles greater than 10 mm may be helpful to predict the risk of multiple pregnancies. Adherence to the chronic low-dose regimen of FSH administration in women with PCOS should markedly reduce the likelihood of excessive ovarian stimulation and OHSS. However, before ovulation induction with gonadotropins, it is mandatory to counsel the patient about the risks associated with higher-order multiple pregnancies after polyovulation.

In most previous studies, cycle cancellation has been advised when more than three follicles of 16 mm or larger were observed[Calaf et al, 2003; White et al, 1996; Homburg et al, 1999] to prevent OHSS and multiple pregnancies. In some studies, the limit was four or more follicles >14 mm [Hugues et al, 2006; Kamrava et al, 1982]. Recently, more stringent criteria have been recommended for ovarian stimulation in unexplained infertility: no more than two follicles >14 mm [Farhi et al, 1996] or no more than three or four follicles >10 mm [Dickey et al, 2005; Tur et al, 2001]. In addition, recent data stress the need for taking into account the overall number of

follicles, and cycle cancellation may be considered in the presence of more than three follicles >14 mm. It should be noted that the definition of a monofollicular cycle has usually been a single follicle of ≥16 mm without any information on the number of smaller follicles, except in the study by Leader [Leader, 2006], which defined a cycle as monoovulatory when a single follicle of ≥16 mm was present with no other follicle ≥12 mm. Measurements of circulating estradiol levels have been used to cancel ovulation induction cycles using gonadotropins (due to overresponse or underresponse) or to adjust the dose of gonadotropins used either upward or, more frequently, downward to minimize the risk of multiple pregnancies or OHSS. Although specific normative cut-offs vary, in 2006 the Practice Committee of the ASRM suggested that caution was indicated when a rapidly rising serum estradiol levels or an estradiol concentration in excess of 2500 pg/mL was present during gonadotropin ovulation induction [Practice Committee of ASRM, 2006]. However, in other studies [Dickey et al, 2005; Tur et al, 2001], the cut-off estradiol concentration was much lower, below 1000 pg/mL, which seems to be more realistic according to the number of growing follicles.

It would seem prudent to withhold hCG administration in the presence of more than two follicles ≥16 mm or more than one follicle ≥16 mm and two additional follicles ≥14 mm, to minimize the risk of multiple pregnancies in women with PCOS under the age of 38 without any other infertility factors.

Efficacy

Overall, low-dose regimens result in a monofollicular ovulation rate of approximately 70%, a pregnancy rate of 20%, and a multiple live birth rate of 5.7% [Homburg et al, 1999]. Correspondingly, there is a low incidence of multiple pregnancies (<6%) and OHSS (<1%) [White et al, 1996; van Santbrink; 1995; Balasch et al, 1996; Hamilton-

Fairley, 1991]. These results compare favorably to the unacceptable high risk of multiple follicular development, multiple pregnancies (36%), and severe OHSS (4.6%) reported for conventional dose protocols [Hamilton-Fairley, 1990]

8.3.1.6 Adjuvant

Insulin sensitizers

At least five different modalities have been used to lower insulin levels in PCOS. These include weight loss, diazoxide, metformin, thiazolidinedcones (pioglitazone, rosiglitazone, troglitazone is no longer available for use) and D-chiro inositol. Among all drugs metformin is the most comprehensively evaluated drug. Both metformin and the thiazolidinediones effect reductions in insulin levels but they do so by fundamentally different mechanism. None of the insulin sensitizing drugs have Food and Drug administration (FDA) approval for use in PCOS. Nonetheless the scientific evidence supporting their salutary effects in PCOS is substantial and progressively mounting and their use for this purpose by clinicians is already established. Although troglitazone is effective in resulting ovulation in PCOS due to need of liver transplantation and death from hepatic failure it is withdrawn from the market. Much published data assessing rosiglitazone and pioglitazone and D-chiro inositol in PCOS are not available. Moreover, D-chiro inositol is not yet commercially available.

Metformin

Metformin is a biguanide antihyperglycemic that is approved for the management of type 2 diabetes mellitus. The mechanism by which metformin enhance insulin sensitivity are not fully characterized. At a molecular level, metformin may increase the activity of the enzyme adenosine monophosphate-activated protein kinase [Zhou et al,

2001]. Metformin appears to suppress hepatic glucose output, decreased intestinal absorption of glucose, increased insulin mediated glucose utilization in peripheral tissues and has an antilypolytic affect on fatty acid concentration reducing gluconeogenesis [Bailey et al, 1996]. It does not produce hypoglycemia in either normal subjects or patients with type 2 diabetes. It is rapidly absorbed from the small intestine and without metabolism largely excreted in the urine. It is available in a generic form as 500 mg, 850mg and 1000 mg tablets. The target dose of metformin is in the range of 1500 mg to 2550mg/day [Patance et al, 2000]. Metformin is given with meals to reduce the gastrointestinal side-effects.

The most common side-effects of metformin are diarrhea, nausea, vomiting, flatulence, indigestion and abdominal discomfort. The gastrointestinal side-effects may be caused by high intestinal metfromin concentration that causes build up of lactic acid in the bowel [Wilcock and Bailey, 1994]. A rare problem caused by metformin is lactic acidosis, which is fatal in as many as 30%-50% of cases [Lalau et al, 1995]. Chance of lactic acidosis is increased when patients have renal insufficiency. So it should not be prescribed if serum creatinine level is greater than 1mg/dL. Liver disease, congestive heart failure and previous history of lactic acidosis are other contraindications of metformin therapy. Metformin should be temporarily suspended for all major surgical procedures that involve restriction of fluid intake. Among metformin users in 10% of cases lactic acidosis occurred after the intravenous administration of iodinated contrast agents [Moros and Thomsen, 2001]. So most authorities recommend that metformin should be discontinued 48 hours before any radiologic procedure that involves

intravenous administration of iodinated contrast material. Though some authorities believe that it is safe to give contrast media to person taking metformin as long as renal function is known to be normal [McCartney et al, 1999].

Evidences of use of metformin in PCOS

Number of questions are concerned regarding the use of metformin in PCOS are –can metformin induce ovulation in PCOS with or without insulin resistance? Can it increase sensitivity of ovaries of insulin resistant PCOS patients to other ovulation inducing agents? Can metformin reduce the chance of abortion and development of gestational diabetes mellitus in PCOS patients?

The first study was done by Velazquez et al [Velazquez et al, 1997] to test the hypothesis that androgen reduction follows from reduction of insulin by metformin. Metformin was administered to 26 women with PCOS at a dose of 500 mg thrice daily for 8 weeks and resulted in a significant reduction in total testosterone, free testosterone, free androgen index as well as a significant rise in SHBG in comparison with pretreatment levels. Subjects of this study lost weight, which was a likely contributor to the reduction in insulin secretion. As a result the effect of metformin upon insulin secretion could not be clearly separated from that of weight loss.

To isolate the confounding effects of weight reduction on both insulin secretion and androgen levels Ehrmann et al [Ehrmann et al, 1997] treated 14 obese non diabetic PCOS women with metformin for a 3 months period during which body weight was maintained and compared their ability to respond to oral and intravenous glucose

challenges before and after treatment. They found that both the glucose and insulin response to a oral glucose challenge and the profound insulin resistance of obese women with PCSO were not improved by metformin. These findings were in contrast to those of Nestler and Jakubowicez [Nestler et al, 1996] who found in a study of similar design that the area under the serum insulin curve decreased by 53% after oral glucose administration and was associated with a reduction in both the basal and luprolide stimulated serum 17 hydroxyprogesterone concentration.

Acbay and Gundogdu [Acbay and Gundogdu, 1996] reported that insulin resistance and associated metabolic and hormonal abnormalities did not improve in patients with PCOS who were given metformin for 10 weeks. BMI did not change during the therapy so these patients did not have the beneficial effect of weight loss. However, some studies showed good results with metformin therapy in women with PCOS. One randomized placebo controlled trial showed that metformin therapy administered for 4-8 weeks resulted in decreased levels of insulin, 17-OHP and free testosterone levels and increased SHBG levels without changing in BMI [Balen A, 2000]. In another placebo controlled study metformin improved hyperinsulinemia and reduced androgen levels in non obese women with PCOS within 4-6 weeks [Nestler et al, 1997]. No changes were noted in control group. Improvement in ovulatory rate with metformin have been reported by different authors [Velazquezet al, 1997; Glueck et al, 1999; Moghetti et al, 2000; Vandermolen et al, 2001]. On the contrary another study with 12 weeks metformin therapy did not find any improvement of androgen secretion, menstrual cyclicity and hirsutism [Unluhizarci et al, 1990]. In other 8 trials on non obese PCOS metformin decreased BMI in 4 and did not reduce in 3 trials. The positive result in

reducing androgen level in both the cases indicates that metformin not only acts through weight loss but also stimulates resolution of the symptoms by itself. Why metformin is successful in some studies and not in others is a concern, however, obesity, variation in the dose, genetic background and duration of therapy may be major factors in patient response.

Metformin versus clomiphene citrate for induction of ovulation

Metformin is not an ovulation inducing drug. It is a drug that effects metabolism and acts indirectly to cause ovulation by reducing the circulating concentration of insulin. On the other hand CC is specifically a fertility drug that acts directly to induce ovulation by blocking negative feedback on the hypothalamic pituitary axis. To compare the effects of two drugs in ovulation a head to head trial of metformin and CC is required. But no such study has been reported to evaluate the effects of metformin in PCOS in comparison to CC. In a multicentre study by Nestler et al ovulation rate by metformin was 34% and by CC was 8% approved metformin treatment to be more efficacious [Nestler et al, 1998]. But Legro et al found no superior effects of metformin over CC [Legro et al, 2007]. A recent meta-analysis of 17 rigorously conducted studies with 1639 subjects shows improvement of menstrual cyclicity and ovulation rate with metformin [Creanga et al, 2008]. CC is first acting drug with chance of multiple pregnancy, but metformin takes time to make the women ovulatory resulting in a singleton pregnancy. The ESHRE/ASRM consensus group recommended to use metformin for those patients who are not in a hurry for pregnancy and to use first acting

CC to those who desire pregnancy immediately and for them time is the essence [Thessaloniki ESHRE/ASRM, 2008].

Addition of metformin to clomiphene citrate for ovulation induction

Metformin has been used as an adjuvant agent for ovulation induction in women with PCOS. When metformin used alone 40% patients resumed regular cycles and ovulation [Hearld et al, 2002] and addition of CC in non responders increased ovulation rate to 67%. In another study 28.2% ovulation rate and 4.2% pregnancy rate were achieved with CC in PCOS [Batukan and Baysal, 2001]. But when metformin was added both ovulation and pregnancy rates were increased to 57.9% and 65.2% respectively. The combination of metformin and CC seems to be synergistic. But the ESHERE/ASRM consensus report states that addition of metformin to CC as primary therapy for induction of ovulation has no beneficial effect [Thessaloniki ESHRE/ASRM, 2008]. On the other hand meta-analysis conducted after the consensus paper, which includes the studies cited in the consensus paper as well as the well-designed studies reported that these addition of metformin to CC significantly increased both the ovulation rate and pregnancy rate in women with PCOS. Another positive effect of adding metformin is reduced multiple pregnancy rates.

Pretreatment with metformin before ovulation induction with clomiphene citrate

For women who have no immediate desire for pregnancy consideration should be given to pretreatment with metformin before adding clomiphene as appropriate [Nestler et al, 1998]. This approach offers two advantages. First pretreatment with metformin for 2 or more months may increase the ovarian sensitivity to CC and may be associated with

higher rates of ovulation and live birth. Secondly obese PCOS are less responsive to CC and develop many pregnancy related complications like preeclampsia and diabetes mellitus [Lobo et al, 1982]. Metformin facilitates weight loss and pretreatment for several months can reduce those complications [Pasquali et al, 2000; Dunaif , 2008; Golay, 2008; Harbone et al, 2005; Gambineri, 2004; Barbieri, 2007]. Different studies showed positive effect of pretreatment with metformin. A multicentre randomized double-blind placebo controlled trial [Vandermolen et al, 2001] was conducted in CC resistant PCOS patients. Metformin 500 mg thrice daily or placebo alone was administered for 7 weeks and then metformin or placebo was continued in the anovulatory women, while clomiphene treatment was began at 50mg. With ovulation the dose was not changed but with anovulation it was increased by 50 mg for the next cycle.

Significant improvement in ovulation and pregnancy rates was observed in the women treated with metformin. In the metformin group 75% ovulated in comparison to 27% in placebo group. Pregnancy rate was also higher (58%) in the metformin group, where as only 13% conceived in the placebo group. Another trial [Kocak et al, 2002] compared metformin with placebo before induction with CC. They used 850 mg metformin twice daily during the first cycle and then added 100 mg CC for the subsequent cycle. In addition to a significant decrease in total testosterone, LH level, LH/FSH ratio, insulin resistance and mean BMI, the ovulation rate was significantly higher in the study group than in the control group (77% vs 14%). The pregnancy rate did not differ significantly but the total number of pregnancies in the metformin group was significantly higher.

Metformin as adjuvant to gonadotropin for ovulation induction in CC resistant PCOS

Appropriate randomized, double blind, placebo controlled trial of an insulin sensitizing drug as an adjuvant to gonadotropin for ovulation induction has not yet been reported. One study evaluated the effects of metformin along with FSH in CC resistant PCOS patients. They randomized the patients to receive either no treatment or metformin1500 mg daily for one month prior to ovulation induction [De Leo et al, 1999]. Number of follicles >15 mm on the day of hCG administration was significantly lower in women treated with metformin compared with untreated group (mean 2.5 vs 4.5 follicles respectively). Due to over stimulation hCG was not withheld in any cycle in women treated with metformin compared with 6 cycles withheld in untreated group.

Yarali and colleagues [Yarali et al, 2002] did not observe any improvement in either insulin sensitivity or ovarian response in CC resistant PCOS patients when pretreated with metformin 850 mg twice daily and then induced with recombinant FSH. Although insulin sensitivity did not change during 6 week metformin treatment an increase in spontaneous ovulation rate was observed. Overall ovulation rates and pregnancy rates were higher in the metformin group (94% vs 75% and 31.3% vs 6.3%). This study showed that metformin administration during ovarian stimulation led to higher overall ovulation and pregnancy rates when given to CC resistant PCOS. But other authors

have not observed beneficial effects of the drug despite a treatment period of 10-12 weeks [Ehrmann et al,1997; Acbay and Gundogdu, 1996; Yarali et al, 2002].

Metformin for in-vitro fertilization in PCOS

An abstract of ASRM in 1990 showed that metformin treatment increased the number of mature oocytes retrieved from women with PCOS undergoing gonadotropin stimulated invitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [Duleba et al, 1993]. Metformin treatment 500mg twice daily from day1 of the cycle prior to GnRH suppression significantly increased the number of mature oocytes, fertilization rates and number of embryo produced. Stadtmauer and coworker [Stadtmauer et al, 2001] reported improvement in IVF and pregnancy rates in CC-resistant PCOS patients pretreated with metformin. They administered 1000-1500 mg metformin daily for 1 cycle before induction with gonadotropins. Fertilization and clinical pregnancy rates were higher in patients who received metformin. The author observed better response when metformin was used in combination with rFSH. These studies support the use of metformin as an adjuvant treatment in CC resistant PCOS.

Metformin and early pregnancy loss in PCOS

Women with PCOS are at increased risk of first trimester abortion due to high LH. First trimester abortion are reported to be 30%-50% in women with PCOS [Homburg et al,1988; Regan et al, 1990; Balen et al, 1993; Sagle et al, 1988; Watson et al,1993], which is three fold higher than the rate of 10%-15% reported for normal women [Gray

and Wu, 2000]. In other way 36% -82% of women with recurrent early pregnancy loss are reported to have PCOS [Sagle et al, 1988; Regan et al 1989; Clifford et al, 1994; Liddell et al, 1997]. Hyperinsulinemia has been implicated as an independent risk factor for early pregnancy loss [Fedorcsak et al 2000]. Glueck et al found a very low rate of first trimester spontaneous abortion in patients receiving metformin during pregnancy [Glueck et al, 2002]. Metformin reduces plasminogen activator inhibitor (PAI). PAI has a positive association with bad pregnancy outcome [Glueck et al1999].

Metformin and prevention of gestational diabetes mellitus in PCOS

Obesity, hyperinsulinema and insulin resistance of PCOS are risk factors for developing gestational diabetes [Adams et al, 1986; Nestler et al, 1996; Velazquez et al, 1997; Velazquez et al, 1997; Glueck et al, 1999; Moghetti et al, 2000; Nestler et al 1998; Glueck et al, 1999; Diamanti et al, 1998; Morin-Papunen, 1998; Velazquez et al, 1994; Glueck et al, 2001; Zawadzki et al, 1992; Lobo and Carmina, 2000; Mikola et al, 2001]. When metformin is used in resistant PCOS as adjuvant of ovulation inducing agent it reduces blood glucose level, so there is less chance of pancreatic beta cell exhaustion and development of gestational diabetes mellitus (GDM). Glueck et al found GDM in 31% of patients who did not take metformin vs 3% of patients who took it [Glueck et al, 2002]. It represents a 10 fold reduction of GDM in PCOS patients who took metformin compared with women who did not take it. In another study Glueck et al found GDM in 7% and 30% of patients with and without metformin respectively [Charles et al, 2008]. One Bangladeshi study [Begum et al, 2009] found that GDM was higher in CC resistant PCOS patients who did not continue metformin throughout

pregnancy compared with patients of same category who continued it (30% vs 3.44%). Pregnancy increases requirements for insulin secretion. This along with insulin resistance increases demand on pancreatic beta cells [Antilla et al, 1998; Holte et al, 1998; Kousta et al, 2000; Lanzone et al, 1995, Lesser et al, 1997; Paradisi et al, 1998; Radon et al 1999; Butte, 2000]. Metformin reduces the demand on pancreatic beta cells and effects are maintained throughout pregnancy and may have contributed to reduce the development of GDM.

Safety issue:

Metformin for several reasons is the currently preferred insulin-sensitizing drug for the treatment of infertility in PCOS. The majority of ovulation studies were conducted with metformin so the weight of scientific evidence is greater for that drug. In addition metformin is easily available worldwide with well delineated side effects and toxicities. Among commercially available insulin sensitizing drugs only metformin has a reassuring safety profile for use during pregnancy. Metformin is classified as a category B drug, which means that no teratogenic effects have been demonstrated in animal models. No teratogenic effects or adverse foetal outcome were reported by any author [Glueck et al, 2002; Charles et al, 2008; Begum et al, 2009; Coetzee and Jackson, 1979; Coetzee and Jackson, 1984; Coetzee and Jackson, 1985]

Superiority to reduce hyperinsulinemia in PCOS, hence enhancing ovulation and safety of metformin was proved by many researchers. So metformin can be used safely in PCOS patients who are not responsive to CC. It can eliminate laparoscopic ovarian drilling which is invasive and risky procedure. Its adjuvant use may reduce the cost of

gonadotropins and risk of multiple pregnancies. So before going for laparoscopic ovarian drilling and FSH administration in CC resistant PCOS patients it is a better option of treatment.

8.3.2. Laparoscopic Ovarian Drilling (LOD):

Clomiphene citrate is the first drug of choice in ovulation induction. Tamoxiphene and aromatase inhibitor are other alternatives for induction. When they proved ineffective gonadotropins are the next drug of choice. Gonadotropins are effective ovulation inducing drug but they are expensive, cause hyperstimulation, need meticulous monitoring and sometimes need much drug to cause ovulation. In those cases where patients need much drug to produce egg or tend to be hyperstimulated surgical treatment could be the next step. First described surgical procedure for PCOS is ovarian wedge resection. Stein and Leventhal described resection of half to three quarters of both of the enlarged ovaries of seven patients [Stein and Leventhal, 1935]. Due to parovarian adhesions, ovarian failure and other complications this procedure fell into disrepute. Alternative surgery was described by Gjsnaess in 1984 where ovaries were punctured by electrodiathermy through laparoscope [Gjonnaess, 1984]. This procedure is indicated in women with altered FSH:LH ratios, poor response to ovulation inducing agents and during ruling out other factors causing infertility.

Mechanism of action:

Various theories are proposed in favour of mechanism of action of LOD.

i). Drilling of follicles release androgen rich follicular fluid and also decreases the androgen producing stroma. So as to decrease circulating androgens.

ii). There is transient reduction in inhibin and precipitious fall in LH, which results in increased secretion of FSH and it's expression.

ii). Crowding of cortex decreases, which allows progress of normal follicles to the surface resulting in resumption of normal ovulation.

Efficacy

Cohort studies reported ovulatory rates of 70%-90% and pregnancy rates of 40%-70%. This response is influenced by body weight. Ovulation rate of 96%-97% was achieved in slim and moderately obese and 70% in obese women. In approximately 50% of LOD-treated women, adjuvant therapy will be required. In these women, the addition of CC can be considered after 12 weeks if no ovulation is detected [Bayram et al, 2004]. The addition of FSH should be considered after 6 months [Bayram et al, 2004]. Five RCTs that compared the effectiveness of LOD with that of gonadotropins for women with CC-resistant PCOS did not show a difference in ongoing pregnancy rate or live-birth rate [Farquhqr et al, 2007; Bayram et al, 2004; Lazovic et al, 1998; Farwuhar et al, 2002; Kaya et al, 2005; Vegetti et al, 1998]. In one of these trials [Bayram et al, 2004] if ovulatory cycles were not established 8 weeks after surgery or the woman became anovulatory again, then CC was given in increasing doses. Multiple pregnancy rates were significantly higher in the gonadotropin arms of the five trials compared with LOD (odds ratio [OR]) 0.13; 95% confidence interval [CI], 0.03–0.98). On the other hand, miscarriage rates did not differ between the LOD group and gonadotropin-treated women (OR 0.61; 95% CI, 0.17–2.16). No cases of OHSS were observed in either of

the two most recent studies [Bayram et al, 2004; Farquhar et al, 2002]. The best results by this procedure can be obtained in CC resistant women who are slim, anovulatory and who have raised LH [Gjonnaess,1994; Donesky and Adashi, 1995].

Safety

Immediate complications of the surgery are rare. Out of 778 cases of LOS, two cases with hemorrhage requiring laparotomy and one case with bowel perforation have been reported [Cohen and Audebert, 1989]. Long-term adverse events potentially include adhesion formation and premature menopause. Only two second-look laparoscopy studies have been done. In one study, out of 17 cases there were two with severe adhesion formation [Gurgan et al, 1992]. In a second study of eight patients, all of the women had ovarian adhesions on second look after LOS despite the application of an adhesion barrier to one ovary as part of a study protocol [Greenblatt and Casper, 1993]. Premature ovarian failure is a concern with ovarian drilling, especially when a large number of punctures is used. However, long-term follow-up of women with PCOS treated by LOS has been reassuring in this respect [Amer et al, 2002; Kaaijk et al, 1999].

8.3.3. Ovulation induction and intrauterine insemination (IUI).

The principle of the management of anovulatory infertility in women with PCOS is to induce regular unifollicular ovulation with a view to minimizing the risk of ovarian hyperstimulation syndrome and multiple pregnancies. ART could be considered in women with PCOS who have failed to conceive in spite of repeated ovulatory cycles IUI is generally considered to be an intermediate step of low to moderate complexity before the application of more sophisticated ART like IVF.

8.3.4. Invitro fertilization (IVF):

IVF is not the primary treatment of infertility in women with PCOS. When all steps of treatment fail then IVF remains the option of treatment. When several ovulatory cycles have failed to achieve pregnancy either spontaneously or through IUI then IVF is indicated. Sometimes another cause of infertility like tubal or male factor defect may co-exist in addition to anovulation, IVF is indicated for them.

8.3.5. Invitro maturation (IVM):

In vitro fertilization protocols use gonadotropins to stimulate the ovaries and generate multiple follicles. However, some women are extremely sensitive to stimulation with exogeneous gonadotropins and are at risk of developing ovarian hyperstimualtion syndrome (OHSS), and the women with PCOS are at increased risk of developing this complication. So the recovery of immature oocytes followed by in vitro maturation (IVM) would be an attractive option to eliminate IVF complication, avoiding side effects of exogeneous gonadotropins, avoiding the risks of OHSS and also cutting the costs.Women with PCOS and who have a previus history of a hyperstimualtion reaction during conventional controlled ovarian stimulation have been regarded as particularly good candidates for IVM.

Hypothesis

Pretreatment and concomitant use of metformin with clomiphene citrate and rFSH improve ovulatory and pregnancy outcome in CC resistant PCOS patients.

Objective of the study

General objective:

The objective of the study was to explore the result of pretreatment and concomitant use of metformin with clomiphene citrate and rFSH in CC resistant PCOS patients.

Specific objectives:

1. To identify the ovulation and conception rate in CC resistant PCOS patients with pretreatment and concomitant use of metformin with clomiphene citrate and rFSH.
2. To identify any untoward effect to mother.
3. To identify any untoward effect to foetus
4. To explore the miscarriage rate in the groups.
5. To find out the live born baby rate in the groups.

CHAPTER TWO

METHODOLOGY

Methodology

Study design:

Randomized controlled trial

Setting:

Outpatient Department of Obstetrics and Gynaecology of Dhaka Medical College and Hospital and Infertility Care and Research Centre, Dhaka, a tertiary level infertility care centre.

Study period: December 2004- August 2009

Study population:

One hundred and sixty five infertile patients having PCOS with CC resistance who attended the outpatient Department of Dhaka Medical College and Hospital and Infertility Care and Research Centre were the target population for this study.

Patient selection:

PCOS were diagnosed by revised Rotterdam criteria which included two of the following three findings i) oligo or anovulation ii) clinical and or biochemical signs of hyperandrogenism and iii) polycystic ovaries by ultrasonography [Rotterdam ESHERE/ASRM consensus, 2004].

Infertility evaluation included

- A medical history of both partner, physical examination of woman and if necessary that of man.
- Husband's semen analysis.
- Documentation of ovulation by mid cycle transvaginal ultrasonography and mid-luteal serum progesterone.
- Ultrasonography to exclude or identify any pelvic pathlogy.
- Hormone assessment to identify hyperandrogenism and to exclude hypothyroidism and hyperprolactinemia.

Serum FSH, LH, TSH, Prolactin, DHEAS and free testosterone were assayed by VIDAS. We did not assay 17 OH progesterone as we intended to exclude the cases having high DHEAS. We also did not assay SHBG as our ultimate objective was to find out clinical improvement not biochemical parameters. Oral glucose tolerance test was done to exclude Diabetes mellitus. Fasting serum insulin level was measured to identify insulin resistance. PCOS patients who did not produce any mature follicle with 150 mg clomiphene citrate daily for 5 days for two consecutive cycles were considered as clomiphene citrate resistant [Nestler et al, 2002] and were recruited for the study. Insulin resistance was identified by calculating fasting glucose insulin ratio and Homeostasis Model Assessment (HOMA). HOMA was calculated by the following formula:- fasting insulin (μIU/mL) X fasting glucose (mmol/L) X 18/22.5 [Carmina and Lobo, 2005]. Insulin resistance was considered when glucose: insulin ratio was <6.4 or HOMA was >47 or by combination of both [Carmina and Lobo, 2004].

Inclusion criteria:

PCOS patients who did not produce any mature follicle with 150 mg clomiphene citrate daily for 5 days for two consecutive cycles.

Exclusion criteria:

Patients having hypothyroidism, hyperprolactinemia, Diabetes mellitus, associated endometriosis and PID, suspected or proven tubal factor infertility and partners' abnormal semen parameters were excluded from the study

Protocol:

Patients were divided into three treatment groups, group A, group B and group C by lottery. Two groups (group A and B) were allocated to Metformin and one (group C) was for control and had no metformin. All patients having BMI >25kg/m^2 were advised for life style modification in the form of caloric restriction and exercise. Along with metformin group A received CC and group B received rFSH. Group C were treated by only rFSH. Metformin 500 mg three times daily (1500 mg) was administered for 4 weeks. After 4 weeks same dose was continued for another 6 months along with scheduled clomiphene citrate 150 mg daily for 5 days (D3-D7 of the cycle) (group A) or rFSH (group B) 75 IU every alternate day starting from D3 of the cycle (then daily if necessary after first monitoring on D12) till maturity of follicles or maximum 15 doses of rFSH. Group C was without metformin and was scheduled for only rFSH 75 IU

every alternate day starting from D3 of the cycle (then daily if necessary after first monitoring on D_{12}) till maturity of follicles or maximum 15 doses of rFSH.

The dose and duration was adjusted by monitoring the response by transvaginal ultrasonography. Ovulation was induced by intramuscular injection of human chorionic gonadotropins (10,000 IU) when follicle/follicles became ≥17 mm in size. Ovulation was confirmed by a) transvaginal ultrasonography by observing i) collapse of the follicle ii) fluid in POD, iii) triple lined endometrial development and b) D_{21} serum progesterone level. A D_{21} serum progesterone level of ≥5 ng/ml was considered ovulatory [Mitwally et al, 1999]. Six ovulatory cycles were assessed.

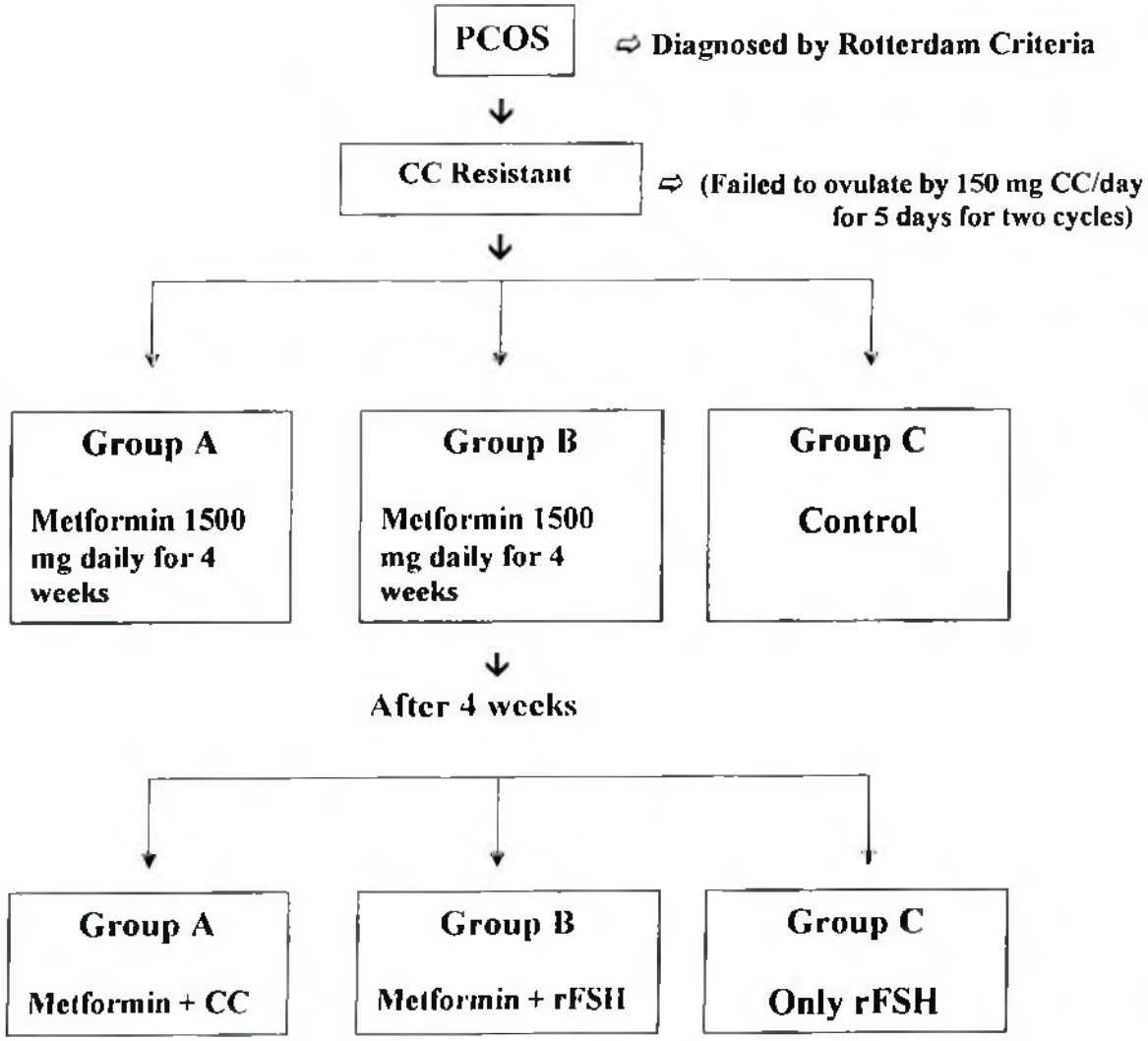
Treatment was terminated

- When no response with maximum dose of CC
- When no response with maximum dose of rFSH
- After six ovulatory cycles with no pregnancy
- After +ve pregnancy test.

After getting pregnancy patients were followed up for abortions and live birth rate.

Study protocol was approved by Bangladesh Medical Research Council (BMRC). Statistical analysis was done by SPSS software by ANOVA, student's t test or χ^2 as appropriate. A p value of <0.5 was considered as significant.

Flow Chart of Methodology



CHAPTER THREE

RESULTS

Results:

One hundred and eighty one subfertile PCOS patients were recruited for this study between December 2004 and August 2009. There were 16 dropouts, 6 from group A, 5 from group B, and 5 from group C. Finally 165 patients were analyzed (55 in each group) for this study.

Table 1: Comparison of demographic data

Characteristics	Group A		Group B		Group C		Sig
	Mean	± SD	Mean	± SD	Mean	± SD	
Age Yrs	26.96	± 4.05	26.84	± 5.13	27.15	±4.20	.830
Duration of marriage Yrs	7.23	±2.52	6.27	±3.00	6.35	±2.99	.524
Duration of infertility Yrs	6.13	±2.38	5.06	±3.04	4.72	±2.78	.603

ANOVA

Table 2: Type of infertility and previous pregnancy outcome

	Group A		Group B		Group C	
	No	%	No	%	No	%
Type of infertility						
Primary	52	94.55	44	80	41	74.55
Secondary	03	05.45	11	20	14	25.45
Previous pregnancy outcome						
MR	2	3.64	3	5.45	3	5.45
Abortion	1	1.81	5	9.09	5	9.09
Abortion + MR	0	00	0	00	3	5.45
Abortion + IUD	0	00	0	00	3	5.45
Live birth	0	00	0	00	0	00
Live birth + MR	0	00	3	5.45	0	00

MR- Menstrual regulation

Table 1 and 2 shows the demographic parameters, type of infertility and previous pregnancy outcome of three groups, which was comparable. There was no significant difference between the groups regarding age, duration of marriage and duration of infertility ($p > 0.05$). In all three groups majority were primary subfertility cases (94.55%, 80%, 74.55% respectively). In secondary infertility cases previous pregnancy was ended in MR, abortion, IUD and live birth. There were only 3 live births in group B. No other patients out of 28 secondary infertility had any live birth children.

Table 3: Comparison of baseline hormonal and metabolic parameters

Parameters	Group A		Group B		Group C		Sig
	Mean	± SD	Mean	± SD	Mean	± SD	
Body mass index(kg/m^2)	27.71	±3.61	28.36	±4.54	28.98	±3.19	.554
FSH (mIU/mL)	6.82	±1.33	6.87	±1.50	6.68	±1.50	.469
LH (mIU/mL)	11.00	±3.57	9.61	±3.45	10.07	±4.99	.666
E2 (pg/mL)	62.87	±18.64	67.29	±16.25	75.76	±25.95	.338
Prolactin (ng/mL)	20.48	±4.16	21.32	±5.77	21.67	±6.10	.394
TSH (μ IU/mL)	3.81	±1.21	3.37	±1.25	2.91	±1.25	.493
Free Testosterone (ng/mL)	1.05	±.47	1.39	±.72	1.34	±.79	.604
DHEAS (μg/dl)	159.02	±55.03	154.98	±57.71	129.16	±57.16	.699
Fasting sugar (mmol/L)	5.21	±.53	5.41	±.67	5.00	±.58	.424
Fasting insulin (μ IU/mL)	16.18	±2.19	16.95	±1.70	16.58	±1.56	.311
Glucose :insulin ratio	5.89	±1.01	5.79	±.92	5.49	±.87	.205
HOMA	67.81	±11.96	73.38	±12.38	66.69	±9.16	.572

Table 3 shows the baseline hormonal and metabolic parameters. All were comparable between the groups and there were no significant difference in baseline parameters ($p > 0.05$). All patients were insulin resistant, which was assessed either by fasting glucose: insulin ratio or HOMA or by both. Mean glucose insulin ratio was 5.89 ± 1.01, 5.79 ± .92 and 5.49 ± .87 in group A, B and C respectively. Range of the ratio was 3.6-

8.3, 3.41-8.03 and 3.69-8.16 in group A, B and C respectively. Considering <6.4 insulin resistance by glucose: insulin ratio a few patients 14 (25.45%) in group A, 9 (16.36%) in group B, and 6 (10.91%) in group C were not detected as insulin resistance. But after calculating HOMA they were found insulin resistant.

So by HOMA 165 (100%) patients were insulin resistant considering the value <47 normal. Range of HOMA was 49.92-94.24, 51.52-98.80, 50.40-85.12 in group A, B and C respectively. After treatment we did not seek for observing HOMA and metabolic changes. We observed only ovulatory and pregnancy outcome.

Table 4 shows the response of drugs in terms of follicular development, oestradiol production, endometrial development and progesterone production. These were calculated only among the ovulatory patients. There was no significant difference in the number of follicles developed.

Table 4: Response to stimulation in terms of follicular maturity and endometrial development

	Group A		Group B		Group C		Sig
	Mean	±SD	Mean	±SD	Mean	±SD	
Number of follicles (n)	2.62	± 1.00	2.69	±1.05	2.35	±1.04	0.314
Size of the follicles (mm)	14.45	±3.80	19.82	±2.17	17.40	±4.25	0.591
Day needed for follicular maturity (days)	13.73	±1.58	14.08	±1.67	16.07	±2.35	0.277
Serum E2 level (pg/mL)	391.33	±146.18	371	±187.76	370.63	±165.4	0.497
Endometrial thickness (mm)	10.27	±1.90	10.62	±1.60	10.68	±2.00	0.74
Serum P4 level (ng/mL)	25	±5.42	28.49	±6.85	25.87	±6.19	0.375
FSH dose needed (IU)	NA	NA	Mean 310.91	Range 225-375	Mean 593.18	Range 375-1125	0.001

ANOVA

After gaining follicular maturity there were also no difference in size of the follicle, endometrial development, oestradiol and progesterone production. Day needed for follicular maturity was higher in group C mean 16.07 days with a range of 12-19 days. In group A less number of patients (only 15) were responded to treatment but except one all of their follicles matured within 14 days. On the other hand in rFSH group (Group C) more patients responded to treatment and many of them needed >14 days even up to 19 days. Dose of rFSH requirement was significantly higher in group C where the patients did not receive metformin, 310.91 vs 593.18 IU in rFSH + metformin vs rFSH alone, p= 0.001 (Table 4).

Table 5: Ovulation and pregnancy in three groups

Outcome	Group A N=55		Group B N=55		Group C N=55	
	N	%	N	%	N	%
Ovulation	15	27.27	49	89.09	41	74.55
Pregnancy	7	**12.73**	**30**	**54.55**	**16**	**29.09**
1st cycle	2	28.57	12	40	5	31.25
2nd cycle	1	14.29	9	30	4	25
3rd cycle	0	00	3	10	3	18.75
4th cycle	0	00	6	20	2	12.5
5th cycle	3	42.86	0	00	0	00
6th cycle	1	14.29	0	00	2	12.5

Table 6: Pregnancy outcome in three groups

Outcome	Group A (n=7)		Group B (n=30)		Group C (n=16)	
	N	%	N	%	N	%
Abortion	1	14.28	2	10	3	18.75
Blighted ovum	1	14.28	2	6.67	1	6.25
Ectopic pregnancy	0	00	1	3.33	0	00
Congenital anomaly	0	00	0	00	0	00
Multiple pregnancy	0	00	1	3.33	0	00
Live birth	5	71.43	24	80	12	75
	Mean	± SD	Mean	± SD	Mean	± SD
Birth weight (kg)	2.82	±.230	2.85	±.241	2.82	±.245

Table 5 and 6 shows the ovulatory and pregnancy response in the groups. Both ovulation and pregnancy rate was higher in group B where patient received metformin and rFSH combination. Table 4 shows pregnancy outcome in the groups. Majority pregnancy occurred within first few ovulatory cycles. There was no congenital anomaly in any group. Only 1 set of twin was in group B. Abortion and blighted ovum were similar in all three groups. Regarding adverse effect of metformin to mothers and foetuses we did not find any untoward effects to mother and foetus.

Table 7: Difference in ovulation and pregnancy rate among the groups

Outcome	N % Group A	N% Group B	Sig
Ovulation (% within total number)	15 27.27	49 89.09	0.001
	Group B	Group C	
	49 89.09	41 74.55	0.02
	Group C	Group A	
	41 74.55	15 27.27	0.001
Pregnancy (% within total number)	Group A	Group B	
	7 12.73	30 54.55	0.001
	Group B	Group C	
	30 54.55	16 29.09	0.01
	Group C	Group A	
	16 29.09	7 12.73	0.02
Abortions and blighted ovum (% within number of pregnancy)	Group A	Group B	
	2 28.57	5 16.67	0.10
	Group B	Group C	
	5 16.67	4 25.00	0.10
	Group C	Group A	
	4 25.00	2 28.57	0.10

Results are expressed from Chi square calculation

Table 7 shows significant differences in ovulation and pregnancy between the groups. Both ovulation and pregnancy were significantly higher in group B comparison to group A and C $p<0.05$. Differences in pregnancy rate were also highly significant between group A and B. There were no significant differences in abortion between the groups.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Discussion

Insulin resistance is pivotal defect in polycystic ovary syndrome (PCOS), which leads to compensatory increase in circulating insulin, and evidences suggest that this elevated insulin level directly stimulates the ovary to produce excess androgens [Nestler et al, 1996; Nestler et al, 1989]. Since both obese and lean women with PCOS have insulin resistance, it is safe to assume that women with PCOS are insulin resistant [Chang et al, 1983; Nestler et al, 1997]. Various researchers suggested that drugs which reverse insulin resistance relieve hyperandrogenism, restore normal menses and help eliminate infertility associated with PCOS. Different drugs like metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositole have been used in PCOS as insulin sensitizing agents, with metformin as the most comprehensively evaluated drugs. None of other drugs have Food and Drug Administration (FDA) approval for use in PCOS. So metformin remains in the top of the list, which reduces insulin resistance of peripheral tissue and allows muscle and adipose cells to take in glucose at normal insulin levels. The drug also reduces intestinal absorption of glucose and decreases the production of glucose by the liver, without causing hypoglycemia.

Most studies or case reports of metformin [Pasquali et al, 2000; Velazquez et al, 1997; Glueck et al, 1999; Moghetti et al, 2000; Vandermolen et al, 2001; Nestler et al, 1998; Morin-Papunen et al, 1998; Velazquez et al, 1994; Sarlis et al, 1999; Kolodziejczyk et al, 2000; Seale et al, 2000] have demonstrated that metformin administered at a dose of 500 mg three times daily increases menstrual cyclicity, improves spontaneous ovulation and promotes fertility.

The first published experiment that used metformin to treat PCOS patients administered 500 mg three times a day after meal to 26 moderately obese women for 8 weeks [Velazquez et al, 1994]. The drug significantly reduced serum LH and increased serum FSH and sex hormone binding globulin (SHBG) and decreased free and total testosterone. None of them had a normal menstrual cycle before entering the study but after taking metformin three became pregnant and seven out of seven women who took the drug for more than 8 weeks resumed normal menstrual cycle. In a subsequent study 21 out of 22 women treated with metformin for 6 months resumed normal menstrual cycles and four became pregnant.

The first placebo controlled clinical trial that looked at metformin for the treatment of PCOS used 500 mg three times daily for 4-8 weeks in 24 obese women. The drug caused insulin level to drop, decreased GnRH simulated LH release and free testosterone. It also increased SHBG levels. Another placebo controlled study using metformin 500 mg three times daily in lean women with PCOS yielded similar result [Nestler et al, 1997]. Unfortunately not all the evidences on metformin is so positive. In a placebo controlled 16 week trial, French investigators concluded that metformin had no additional benefit over diet in improving hyperinsulinemia and hyperandrogenism by using 850 mg metformin twice daily in very obese women (35.2 kg/m2) [Crave et al, 1995]. Another similar study using 850mg metformin three times daily in markedly obese women (39kg/m2) found that metformin did not significantly reduce hyperinsulinemia or hyperandrogenism [Ehrmann et al, 1997]. These studies suggest that the drug will not benefit PCOS patients suffering from morbid obesity. But in spite of these negative findings many subsequent studies showed that the drug improves

insulin metabolism, hormone parameters or both. In present study we did not assess the improvement of menstrual cyclicity, insulin metabolism and hormonal parameters. Our aim was to detect any changes in the ovulation and pregnancy rate. Regarding ovulation every study to date has shown that giving metformin 500 mg three times daily to lean and moderately obese women restore normal menstrual cycles and improves spontaneous ovulation [Glueck et al, 1999; Moghetti et al, 2000; Nestler et al, 1998; Morin-Papunen et al, 1998; Sarlis et al, 1999; Kolodziejczyk et al, 2000; Seale et al, 2000]. When the drug was taken with clomiphene citrate it enhances the likelihood of successful ovulation induction.

Different single blind or double blind placebo controlled study was conducted to see the efficacy of metformin in PCOS. Some of them were clomiphene resistant and others were not screened for the presence of insulin resistance but only needed to fulfill the entrance criteria of oligomenorrhoea and biochemical hyperandrogenism. In one such study metformin or placebo was given for 5 weeks to women with PCOS, 34% in the metformin group ovulated in comparison to only 4% in the placebo group [Nestler et al, 1998]. The women who did not ovulate were given clomiphene citrate 50mg/d for 5 days while continuing to take metformin or placebo. In the metformin clomiphene group 90% ovulated while only 8% ovulated in the metformin placebo group. Therefore metformin increased both the spontaneous and clomiphene induced ovulation rates by greater than eight and 11 fold respectively. Cochrane review concluded that ovulation was improved in women taking metformin alone, rather than placebo or no treatment (OR=2.12, 95% CI 1.50-3.0, p=0.0001). The analysis of ovulation rates took into consideration of 13 studies in which 875 patients participated. Separate analyses of the

data relating to obese (BMI > 30 kg/m^2) and non-obese (BMI < 30 kg/m^2) women revealed that ovulation rates were improved in both groups [Tang et al, 2010].

It has been suggested that a longer treatment period might result in greater ovulatory improvements with metformin [Harborne et al 2003]. In support of this, a retrospective study looking at the effects of short- and long-term metformin treatment on menstrual cyclicity in women with PCOS found that the response rate was 40% higher for women who were treated with metformin for more than six months, compared to those treated for between three and six months [Essah et al, 2006].

In contrast to these positive reports some studies failed to show the positive effects of metformin in CC resistant PCOS. CC resistant PCOS patients were initially given either placebo or 500 mg of metformin three times daily for 3 months. The researchers added 100 mg of CC to this regimen for 5 days for one cycle for women who did not ovulate after taking metformin or placebo alone. But none of the women ovulated in the treatment cycle [Ng et al, 2001]. In another double blind randomized trial with CC resistant PCOS patients ovulation occurred 41% in metformin/CC group and 25% in placebo/CC group (p=0.63). They treated the patients either by metformin 500 mg three times daily or by placebo for 3 months. Then added CC 50-100 mg for 5 days for three cycles with metformin and placebo. Pregnancy occurred 25% in metformin and 14% of placebo group (p=0.59). This study suggested that metformin may be beneficial to a subset but not all patients with PCOS [Sturrock et al, 2002].

Ovulation rates with single therapy metformin have not been found to be superior to those with single therapy clomiphene [Lergo et al, 2007; Palomba et al, 2007; Palomba et al, 2005; Zain et al, 2009]. The Cochrane review involving 2470 women found clomiphene to be superior to metformin for ovulation induction (OR=0.48 95% CI 0.41-0.57). Statistically significant superiority of clomiphene was shown in the two studies that involved obese (BMI > 30 kg/m^2) patients [Tang et al, 2010].

With regard to combined metformin and clomiphene treatment, one study found that addition of metformin to clomiphene increased the cumulative ovulation rate from 49.0% to 60.4% (p=0.003, combination vs clomiphene) [Lergo et al, 2007]. Also the Cochrane study involving 2668 patients of eleven studies mentioned that combining metformin and clomiphene therapy was found to result in higher ovulation rates than with clomiphene alone (OR=1.76 95% CI 1.51-2.06 p<0.00001) [Tang et al, 2010]. Another Cochrane review concluded that combined clomiphene and metformin treatment was more effective for ovulation induction in women with PCOS than treatment with clomiphene alone [Sinawat et al, 2008]. It added that it is not clear whether short-course (<4 weeks) or long-course (≥4 weeks) treatment with the combination is more effective [Sinawat et al, 2008].

Life style modification in the form of moderate caloric restriction and exercise is essential no matter what other intervention is chosen. Moderate caloric restriction that resulted in a 2%-5% weight loss reduced 21% free testosterone [Guzick, 2004]. It has been shown that metformin together with a low caloric diet is associated with more

weight loss than a low-caloric diet alone [Barbieri, 2003]. Weight loss in women with PCOS can lead to spontaneous resumption of menses and ovulation and can lower the circulating androgen and insulin levels [Kiddy et al, 1992; Guzick et al, 1994]. A 7% reduction in body weight is effective in restoring fertility [Frank et al, 1991

In our study we identified CC resistant PCOS patients and divided into three groups.

Two groups received metformin 1500 mg daily in divided doses for 4 weeks. One group did not receive metformin. One group received CC 150mg daily for 5 days and another group received rFSH 75 unit every alternate day or daily according to response from D3 of cycle till getting optimum sized follicle or by using maximum 15 doses of FSH. All patients having BMI >25kg/m^2 were advised for life style modification in the form of caloric restriction and exercise irrespective of drug therapy. Only 27.27% patients of group A ovulated and 12.73% got pregnant. In group B 89.09% patient ovulated and 54.55% got pregnant. Both ovulation and pregnancy rate was lower in our series (metformin + CC) than shown by Sturrock et al, 2002. But when we compared ovulation and pregnancy rate between group A (metformin + CC) and group B (metformin + rFSH) the second group (Group B) shows significant improvement both in ovulation and pregnancy. rFSH is much more effective drug in terms of ovulation in comparison to CC. So it is not logical to compare the effect of rFSH and CC. That is why we also compared the effect of rFSH with or without metformin. In metformin treated group 89.09% and control group 74.55% patients ovulated. Pregnancy rate was higher in metformin treated group (54.55% vs 29.09%). In some patients of group C duration of stimulation was longer and it might affect the quality of eggs for fertilization or implantation. It may be the cause of less pregnancy rate in group C in

spite of satisfactory ovulation. Although there is no significant difference in ovulation among group B and C the amount of rFSH needed to achieve optimum effect was much higher in group C (310.91 vs 593.18 IU in metformin vs control group respectively p=<0.05). Duration of stimulation was also higher in control group (14.08 ±1.67 vs 16.07 ±2.35 in metformin vs control group).

Women with PCOS tend to have a hyperstimulatory response to FSH. But when patients are pretreated with metformin chances of developing hyperstimulation is less. In one study 26.3% patients developed OHSS who was not treated with metformin vs 16.6% who were treated with metformin. The study evaluated CC resistant women with PCOS and randomized to receive either no treatment or metformin (1500mg/d) for one month prior to ovulation induction with rFSH. It reported that no cycles with hCG were withheld in women treated with metformin because of excessive follicular development compared with 6 cycles withheld in untreated women [De Leo et al, 1999].

In our series we used rFSH for induction in patients either receiving metformin or no metformin. In metformin treated group both ovulation and pregnancy rate were higher than the control group though the difference is not statistically significant. But to achieve the goal rFSH requirement was much higher in control group (p=0.000). Moreover, who did not ovulate in metformin group by D16 developed follicles up to 14 mm size. In contrast in control group those who did not ovulate only one developed follicles of 14 mm size by D16. No patient in either group developed ovarian

hyperstimulation syndrome. Though follicular recruitment was higher in control group due to slow stimulation most of them became atretic and finally optimum sized follicles were not much in number (2.62 ± 1.00, 2.69 ±1.05, 2.35 ±1.04 in group A,B and C respectively). After getting metformin in group A 27.27% patients ovulated with same dose of CC by which they did not ovulate before. Though there was no marked increment, it indicates that metformin increased the ovarian sensitivity to some extent. So it is evident that even after metformin treatment ovulation rate was not very high with CC only. We did not find any congenital defect in any group. There was 1 set twin in group B and no multiple pregnancies in group A or C. It is usually said that multiple pregnancy rate is higher with FSH. But in our experience we found that slow induction by FSH produces less number of follicles. So chance of OHSS and multiple pregnancies should not be higher if stimulation is very controlled.

Now question may arise should CC resistant patients be treated with metformin? Or should all PCOS patients be treated with metformin? If we consider anovulatory infertility then we must use some ovulation inducing agents. Metformin though is not an ovulation inducing agent, reverts metabolic derangements and increases the sensitivity of ovary either to endogenous or exogenous gonadotropins. Most of PCOS patients (75%-80%) ovulate easily with CC at a dose of 50mg -150mg/d for 5 days. Who do not ovulate with CC can be treated by metformin to ameliorate metabolic effects and to increase ovarian sensitivity to ovulation inducing agents.

The strong association between hyperinsulinemia and anovulation would suggest that a reduction of insulin concentrations could be of great importance. Vast majority of published series shown restoration of ovulation in 78-96% of patients after using

metformin [Nestlet et al, 1997; Velazquez et al,1997; Moghetti et al, 2000]. In a RCT performed in CC resistant infertile patients with PCOS compared with placebo, metformin caused markedly improved ovulation and pregnancy rates with CC treatment [Vndermolen et al, 2001]. Nestler et al in 1998 shown 19/21 ovulation with CC+metformin in comparison to 2/25 in placebo+CC. But Ng et al, 2001 did not demonstrate any superiority of metformin over placebo in CC treated patients. When women with CC resistant PCOS were administered FSH with or without pretreatment with metformin for 1 month in a RCT, those receiving metformin developed significantly less large follicles and had fewer cycle cancellation due to excessive follicular development. The reduction of insulin concentrations induced by metformin seemed to favour a more orderly follicular growth in response to exogenous gonadotropins for ovulation induction [De Leo et al, 1999].

A number of studies showed beneficial effect of metformin in reducing hyperinsulinemia, BMI, androgen level, restoring menstruation, ovulation and increasing pregnancy [Nestlet et al, 1996; Nestlet et al, 1997; Velazquez et al, 1997; Glueck et al, 1999; Moghetti et al, 2000; Vandermolen et al, 2001; Nestler et al, 1998; Heard et al, 2002; Batukan et al, 2001; Kocak et al, 2002; Stadtmauer et al, 2001; Rosenfield et al, 1994; White et al, 1995]. Other studies found no beneficial effect by using metformin alone or with CC or FSH regarding ovulation or pregnancy [Ehrmann et al, 1997; Acbay and Gundogdu, 1996; Unluhizarci et al, 1990; Yarali et al, 2002; Kolodziejczyk et al, 2000]. Why metformin is successful in some studies and not in others is a concern. Obesity, variation in the dose, genetic background and duration of therapy may be major factors in patient response. So in light of these studies, can

metformin be used as an adjunctive therapy in ovulation induction? As there is variation of result depending upon variation of doses, obesity and genetic factors the result of these studies support the use of metformin as an adjunctive treatment in CC resistant PCOS.

Based mostly on results from two large, randomised, controlled trials [Lergo et al, 2007; Moll et al, 2006] it was concluded that, with regard to ovulation induction, no advantage of adding metformin to clomiphene in women with PCOS was apparent except in women with high BMI (>35 $kg/m^{2)}$ and in those with clomiphene resistance, and that clomiphene should remain the first-line treatment for ovulation induction in most anovulatory women with PCOS. It was also advised that metformin use for PCOS should be restricted to women with glucose intolerance [Tariatzis et al, 2008].

Other benefits of using metformin in PCOS are reducing early pregnancy loss and gestational diabetes mellitus. Women with PCOS are at increased risk of first trimester abortion due to high LH. First trimester abortion are reported to be 30-50% in women with PCOS [Kokia et al, 1990; Ehrmann et al, 1997; Balen et al, 1993; Sagle et al, 1988; Watson et al, 1993], which is three fold higher than the rate of 10-15% reported for normal women [Gray and Wu, 2000; Regan et al, 1989]. In other way 36-82% of women with recurrent early pregnancy loss are reported to have PCOS [Sagle et al, 1988; Regan et al, 1989; Clifford et al. 1994; Liddell et al, 1997]. Hyperinsulinemia has been implicated as an independent risk factor for early pregnancy loss [Fedorcsak et al, 2000]. Glueck et al found a very low rate of first trimester spontaneous abortion in patients receiving metformin during pregnancy [Glueck et al, 2002]. Metformin reduces

plasminogen activator inhibitor (PAI). PAI has a positive association with bad pregnancy outcome [Glueck et al, 1999].

But one systematic review and meta-analysis of RCTs demonstrates no statistically significant benefit of pregestational metformin administration on the abortion risk in PCOS patients who received the drug as monotherapy or combined with other fertility drugs. They concluded that at the moment, there is no type I clinical evidence to suggest that metformin administration reduces the risk of abortion in women with PCOS [Palomba et al, 2009].

In this study blighted ovum and spontaneous miscarriage rate was 28.57%, 16.67% and 25% in group A, B and C respectively. Other benefit is reduction of GDM by using metformin in PCOS. Obesity, hyperinsulinema and insulin resistance of PCOS are risk factors for developing gestational diabetes [Adams et al, 1986; Nestler et al, 1996; Velazquez et al, 1997; Velazquez et al, 1997; Glueck et al, 1999; Moghetti et al, Nestler et al, 1998, Glueck et al, 1999; Diamanti-Kandarakis et al, 1998; Morin-Papunen et al, 1998; Velazquez et al, 1994; Glueck et al, 2001; Zawadzki et al, 1992; Lobo and Carmina, 2000; Mikola et al, 2001].

Some studies showed that the use of metformin for women with anovulatory PCOS had no benefit with respect to enhancing either fertility or live-birth rates [Thessaloniki ESHRE/ASRM sponsored concensus, 2008; Tang et al, 2010] and its routine use is not recommended. Similarly, a recent meta-analysis showed metformin had no effect on the miscarriage risk in PCOS patients when administered before pregnancy [Palomba et al, 2009]. One small RCT did, however, report a reduced rate of severe pregnancy

complications when metformin was taken through pregnancy [Vanky et al, 2004], although a subsequent larger multicenter trial by the same group found no benefit of metformin in reducing pregnancy complications or altering fetal weight [Vanky et al, 2010].

Another metaanalysis did not find significant difference in pregnancy rates and live-birth rates for metformin and clomiphene-treated patients (clinical pregnancy rates: 36.7% versus 35.7%, p=0.85 (metformin versus clomiphene); live birth rates: 30.3% versus 30.8%, p=0.79 (metformin versus clomiphene) [Jhonson, 2011].

Pregnancy and live-birth rates differed significantly between metformin and placebo groups only in women in the high BMI group (Pregnancy rates: 41.4% versus 26.0% metformin versus placebo); live-birth rates: 36.5% versus 22.1% (metformin versus placebo, p=0.04) [Morin-Papunen, 2010]. Though the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group (Bart et al, 2012) concluded that there is no evidence for improved live-birth rates or decreased pregnancy complications with the use of metformin either before conception or during pregnancy (level A).

According to guidelines on fertility published by the National Institute for Health and Clinical Excellence (NICE) in 2004, women with PCOS who have not responded to clomiphene and who have a BMI greater than 25 kg/m^2 should be offered metformin plus clomiphene because combined therapy increases ovulation and pregnancy rates [NICE clinical guidline 11, 2004].

When metformin is used in resistant PCOS as adjuvant of ovulation inducing agent it reduces blood glucose level, so there is less chance of pancreatic beta cell exhaustion and development of gestational diabetes mellitus (GDM). Glueck et al found GDM in 31% of patients who did not take metformin vs 3% of patients who took it [Glueck et al, 2002]. It represents a 10 fold reduction of GDM in PCOS patients who took metformin compared with women who did not take it. In another study Glueck et al 2008, found GDM in 7% and 30% of patients with and without metformin respectively. Begum et al, 2009 found that GDM was higher in CC resistant PCOS patients who did not continue metformin throughout pregnancy compared with patients of same category who continued it (30% vs 3.44%). Glueck et al 2008, also showed GDM in 6% pregnancies in women in whom metformin was continued throughout pregnancy, vs. 14.3% pregnancies where metformin was stopped at a median of 13 weeks (P=.16).

Pregnancy increases requirements for insulin secretion. This along with insulin resistance increases demand on pancreatic beta cells [Anttila et al,1998; Holte et al,1998; Koustaet al, 2000; Lanzone et al, 1995; Lesser et al, 1997; Paradisi et al, 1998; Radon et al, 1999; Butte et al, 2000, Coetzee et al, 1979; Coetzee et al, 1984]. Metformin reduces the demand on pancreatic beta cells and effects maintained throughout pregnancy and may have contributed to reduce the development of GDM. In a meta-analysis in which pregnancy outcomes in women with PCOS were compared with controls, 15 studies were included, involving 720 women presenting with PCOS and 4,505 controls [Boomsma et al, 2006]. Women with PCOS demonstrated a

statistically significantly higher risk of developing GDM (odds ratio [OR] 2.94; 95% confidence interval [CI], 1.70–5.08), pregnancy-induced hypertension (OR 3.67; 95% CI, 1.98–6.81), preeclampsia (OR 3.47; 95% CI, 1.95–6.17), and preterm birth (OR 1.75; 95% CI, 1.16–2.62). Their babies had a statistically significantly higher risk of admission to a neonatal intensive care unit (OR 2.31; 95% CI, 1.25–4.26) and a higher perinatal mortality (OR 3.07; 95% CI, 1.03–9.21), unrelated to multiple births [Boomsma et al, 2006]. For present study we did not look for GDM in our study population.

Regarding safety issue major contraindications to metformin include organic or functional renal failure with serum creatinine level higher than 1.4 mg/dL, alcoholism, hepatic disease and chronic cardiopulmonary dysfunction. The main adverse effects are transient nausea and diarrhoea. As many as 20% of patients experience GI side effects but the symptoms appear to be dose related and can be minimized by gradually increasing the dose and taking the medication with food [Glueck et al, 2002; Mikola et al, 2001; Buchanan et al, 2000; Sills et al, 2000]. We excluded the patients with high SGPT and patients who complained of weakness vertigo or intolerance.

The majority of ovulation studies were conducted with metformin so the weight of scientific evidence is greater for that drug. In addition metformin is easily available worldwide with well delineated side effects and toxicities. Among commercially available insulin sensitizing drugs only metformin has a reassuring safety profile for use during pregnancy. Metformin is classified as a category B drug, which means that no teratogenic effects have been demonstrated in animal models. No teratogenic effects

or adverse foetal outcome were reported by any author [Glueck et al, 2002; Charles et al, 2008; Begum et al, 2009; Coetzee et al, 1979; Coetzee et al, 1984; Coetzee et al, 1985]. Out of 41 live born babies no one had any detectable congenital defect in our series.

The limitation of this study was that the study was not blinded. So there might be some biasness. To avoid biasness the data collection, patient examination and sonographic monitoring of three groups were done by three different skillful observers following the same principle. All laboratory investigations were done from same pathological laboratory to avoid interlaboatoty variations.

Finally it should be noted that metformin is licensed for the treatment of type 2 diabetes only [Pfizer Ltd, 2012]. Therefore the prescriber takes full responsibility when prescribing metformin for PCOS.

Conclusion

By HOMA test this small study shows that all CC resistant patients are insulin resistant. Though to draw firm conclusion the result of this small study is not enough and large studies with metaanalysis are needed. Metformin as insulin sensitizer can be used safely in PCOS patients if they are not responsive to maximum dose of CC. It can eliminate laparoscopic ovarian drilling which is invasive and risky procedure. Its adjuvant use reduces the cost of gonadotropins and risk of multiple pregnancies. So before going for laparoscopic ovarian drilling and rFSH administration in CC resistant PCOS patients it is a better option of treatment. If patient is not responsive to mximum dose of CC then metformin can be used for few weeks prior to re-administration of CC and can be continued till pregnancy.

CHAPTER FIVE

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বাংলাদেশ চিকিৎসা গবেষণা পরিষদ
Bangladesh Medical Research Council
BMRC

Ref: BMRC/ERC/2001-2004/ 613 Date: 04/07/04

Ethical Review Committee

Dr. Mosammat Rashida Begum
Asstt. Professor
Deptt. of Obst. & Gynae.
Dhaka Medical College
Dhaka.

Subject: Ethical Clearance

With reference to your application on the above subject, this is to inform you that your Research Proposal entitled **"Pretreatment and co-administration of oral antidiabetic agents with Clomiphene Citrate (CC) or rFSH for ovulation induction in Clomiphene Citrate (CC) resistant Polcystic Ovary Syndrome (PCOS)"** has been reviewed and approved by the Ethical Review Committee of Bangladesh Medical Research Council (BMRC).

You are requested to please note the following ethical guidelines as mentioned at page 2 (overleaf) of this memo.

(Prof. Harun-Ar-Rashid)
MD, MSc, MPH, PhD, FRCP Edin
Director 04/7/04

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THE ETHICAL GUIDELINES TO BE FOLLOWED BY THE PRINCIPAL/ CO-INVESTIGATORS

- ❒ The rights and welfare of individual volunteers are adequately protected.
- ❒ The methods to secure informed consent are fully appropriate and adequately safeguard the rights of the subjects (in the case of minors, consent is obtained from parents or guardians).
- ❒ The Investigator(s) assume the responsibility of notifying the Ethical Review Committee if there is any change in the methodology of the protocol involving a risk to the individual volunteers.
- ❒ To immediately report to the Ethical Review Committee if any evidence of unexpected or adverse reaction is noted in the subjects under study.
- ❒ This approval is subject to P.I.'s reading and accepting the BMRC ethical principles and guidelines currently in operation.