EFFECT OF GLUCOPHAGE (METFORMIN HYDROCHLORIDE) TREATMENT IN IMPROVING FERTILITY OF POLYCYSTIC WOMEN

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CERTIFICATE

This thesis submitted by Ms Asma Fazal is accepted in its present form by the Department of Animal Sciences as satisfying the thesis requirement for the degree of Doctor of Philosophy in Reproductive Physiology.

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IN THE NAME OF ALLAH

THE MOST MERCIFUL AND
THE
MOST COMPASSIONATE

TO

MY PARENTS

MY HUSBAND
IRFAN

MY LOVING KIDS
MBI
&
NOOR

THEIR LOVE IS THE SOURCE OF INSPIRATION
WHICH HELPED ME IN COMPLETION OF THIS THESIS

CONTENTS

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List of Abbreviations

A androstenedione
ACTH adrenocorticotropic hormone
AUC area under curve
BMI body mass index
CC clomiphene citrate
CV--Coefficient of Variation
DHEA dehydroepiandrosterone
DHEAS dehydroepiandrosterone sulphate
E2 estradiol
FAI free androgen index
FFA free fatty acid
FSH follicle stimulating hormone
GDM gestational diabetes mellitus
G:I ratio glucose insulin ratio
GnRH gonadotrophin releasing hormone
HDL high density lipoprotein
HCG human chorionic gonadotrophin
HMG human menopausal gonadotrophin
17-OHP 17-hydroxyprogesterone
G :I ratio. glucose insulin ratio
IFG impaired fasting glucose
IGT impaired glucose tolerance
IVF in vitro fertilization
IVGTT intravenous glucose tolerance test
LDL low density lipoprotein
LH luteinizing hormone
NIDDM non insulin dependant diabetes mellitus
NPY neuropeptide Y
OGTT oral glucose tolerance test
PAI-1 plasminogen activator inhibitor
PCO polycystic ovaries
PCOS polycystic ovary syndrome
QUICKI quantitative insulin sensitivity check index
SE standard error
SHBG sex hormone binding globulin
T testosterone
TA total area
Trigly triglycerides
VLDL very low-density lipoprotein
W:H ratio Waist to hip ratio

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ABSTRACT

In this study patients treated for three or six months with glucophage (metformin) and conceived after glucophage alone or with combination treatment of glucophage and ovulation induction medicine were of younger age than those who did not conceive. Younger patients after six months treatment with glucophage conceived without even taking ovulation induction medicines. The frequency of conceptions in both primary and secondary infertile patients in this study was more after six months glucophage treatment. Secondary infertile patients responded the best to glucophage treatment and to lesser dose of ovulation induction. The mean BMI, waist to hip ratio and subscapularis skin fold thickness indicated insulin resistance. After three months treatment with glucophage in conceived patients there was highly significant decrease ($P<0.001$) in mean Fasting Blood Glucose and Fasting Insulin. The patients who were given treatment for six months had highly significant decrease($P<0.001$) in mean Fasting Blood Glucose and Fasting Insulin in conceived and not conceived patients, however highly significant rise ($P<0.001$) in QUICKI and significant rise ($P<0.05$) in Glucose Insulin ratio was seen in conceived patients. When comparison was made Fasting blood glucose levels decreased highly significantly ($P<0.001$) in patients who took glucophage for six month compared to patients who took glucophage for three months. These results showed that three or six month treatment with glucophage resulted in improvement in insulin resistance in these patients. The results showed that in Polycystic patients the high Serum Leptin Levels did not show any significant decrease when glucophage was given for three months but the significant decrease was seen only when glucophage drug was given for six months in conceived group of patients. Serum LH and testosterone levels decreased highly significantly($P<0.001$) where as significant increase ($P<0.05$) in Serum Estradiol levels was observed in patients who were given six months glucophage treatment compared to patients who were given three months glucophage treatment. The systolic and diastolic blood pressure decreased highly significantly ($P<0.001$) both in conceived and not conceived patients of three months and six months glucophage treatment. Serum cholesterol levels decreased significantly ($P<0.01$) in conceived patients of three months glucophage treatment, however highly significant decrease ($P<0.001$) was seen in conceived patients of six months glucophage treatment. In this study the overall conception after three months treatment with glucophage was highly significantly low ($p=0.0041$) compared to those who were given glucophage for six months. Clomiphene Citrate and Human Menopausal Gonadotrophins in combination with

glucophage has proved to be a better medicine for the treatment of infertility in PCOS. This also depends on the age of the patient as younger patients responded to initial (lesser dose) medication while older patients required higher dose of treatment. The overall conception after six months treatment with glucophage was significantly high than who were given glucophage for three months. Live birth rate and single births were significantly higher in patients who continued glucophage during pregnancy duration. Abortions, still births and threatened abortions were higher in patients who did not continue gucophage during the pregnancy.

CHAPTER-1
INTRODUCTION

Prevalence

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of fertile age. It is the most common endocrinopathy in women and the most common cause of anovulatory infertility, affecting 5-10% of the Italian and American population (Vincenzo et al, 2003; Myers et al, 2005). In another study an overall 4.0% prevalence of PCOS in women from the U.S. (4.7% in White women and 3.4% in Black women) was found (Knochenhauer et al, 1998), and a 6.8% prevalence of PCOS in the Greek island of Lesbos was found (Diamanti-Kandarakis et al, 1999). 6.5% prevalence of PCOS was found in Caucasian women from Madrid, Spain (Asuncion et al, 2000). Polycystic ovary syndrome is the most common form of female infertility in the U.S. In addition to poor conception rates, pregnancy loss rates are high (30-50%) during the first trimester (Jackubowiez et al, 2002).

Definition of polycystic ovaries

Histologically, the major diagnostic macroscopic features of PCOS are bilateral enlargement, thickened ovarian capsule, multiple follicular cysts (usually ranging between 2-8 mm in diameter) and an increased amount of stroma (Goldzieher and Green, 1963). Transvaginal ultrasound is currently the gold standard for diagnosis of polycystic ovaries (Tasoula et al, 2004) The ultrasound definition of PCO is the presence of either multiple cysts (ten or more) from 2-8 mm in diameter distributed evenly around the ovarian periphery with an increased amount of stroma, or (less commonly) multiple small cysts 2-4 mm in diameter distributed throughout abundant stroma (Adams et al, 1985).

The number of peripherally distributed follicles > 10 was considered the most sensitive feature of PCO, while stromal brightness had the best specificity to detect PCO (Atiomo et al, 2000). By a 2003 international consensus conference, diagnosis is made by two out of three criteria: chronic oligoovulation or anovulation after excluding secondary causes, clinical or biochemical evidence of hyperandrogenism (but not necessarily hirsutism due to inter-patient variability in hair follicle sensitivity), and radiological evidence of polycystic ovaries (Lanham et al, 2006).

Criteria of the US National Institutes of Health **Polycystic ovary syndrome** Presence of menstrual abnormalities and anovulation Presence of clinical and/or biochemical hyperandrogenaemia Absence of hyperprolactinaemia or thyroid disease Absence of late-onset congenital adrenal hyperplasia Absence of Cushing's syndrome **Polycystic ovaries** Presence of polycystic ovaries on ultrasound examination Absence of menstrual or cosmetic symptoms Absence of biochemical hyperandrogenaemia **Idiopathic hirsutism** Presence of excess hair growth Absence of biochemical hyperandrogenaemia **Proposed criteria (European Society of Human Reproduction and Embryology and American Society for Reproductive Medicine)*** Polycystic ovary syndrome is diagnosed if there are any two of the Following: Presence of polycystic ovaries on ultrasound examination Clinical or biochemical hyperandrogenism Menstrual dysfunction with anovulation
*As concluded at a ESHRE/ASRM-sponsored symposium on PCOS; 1 May 2003; Rotterdam, The Netherlands

Clinical Features of PCOS

The clinical features include menstrual abnormalities, hirsutism, acne, alopecia, anovulatory infertility and recurrent miscarriages (Tasoula et al, 2004). Infertility was included in the original description of PCOS (Stein and Leventhal, 1935). The prevalence of infertility, caused mainly by anovulation, in PCOS women varies between 35 and 94% (Goldzieher and Green, 1963; Franks, 1995; Guzick, 1998). The cause of infertility in patients with PCOS is generally lack of ovulation because of a failure of follicles to develop 10 mm. Most cycles are anovulatory and induction of ovulation is essential (Robert et al, 2004). Some studies have also described an increased miscarriage rate in PCOS, the mechanism of which is poorly understood. It has been suggested that high follicular phase concentrations of LH have a deleterious effect on rates of conception and miscarriage (Homburg et al, 1988; Balen, 1993).

Endocrine and Metabolic Features

The endocrine features include elevated androgens, luteinizing hormone, oestrogen and Prolactin levels. The metabolic aspects of this syndrome include insulin resistance, obesity, lipid abnormalities and an increased risk for impaired glucose tolerance and type 2 diabetes mellitus (type2 DM) (Tasoula et al, 2004)

Endocrine Features.

- **Inappropriate gonadotrophin secretion (FSH and LH)**

An inappropriate gonadotrophin secretion is associated with the classic form of PCOS.

Compared with the follicular phase of the normal menstrual cycle, women with PCOS exhibit a disproportionately high LH secretion with relatively constant low FSH secretion (MacArthur et al, 1958; Yen et al, 1970). The prevalence of increased serum LH in PCOS ranges from 30% to 90 % (Conway et al, 1989; Franks 1989). It has been suggested that gonadotrophin defects, particularly an excess of serum LH, is a predominant finding in hyperandrogenic women, whether they be adolescents or older perimenopausal women (Apter et al, 1994; Taylor, 2000).

The underlying cause of this pattern of gonadotrophin secretion is linked to an accelerated gonadotrophin releasing hormone (GnRH) pulse generator activity and heightened pituitary response to GnRH. LH and FSH synthesis and secretion are highly dependent on the pattern of the GnRH stimulus, with rapid frequencies favoring LH and slower pulses FSH synthesis and secretion(Clarke et al, 1984; Waldstreicher et al, 1988) but the LH pulse frequency in PCOS women is not influenced by BMI (Morales et al, 1996; Arroyo et al, 1997).

- **Ovarian steroidogenesis**

Chronic LH stimulation in PCOS induces sustained hypersecretion of androgens by the theca compartment (Yen et al, 1970). Theca cells are shown to secrete abnormal amounts of steroids in culture, both before and after LH stimulation (Gilling-Smith et al, 1994). Insulin also augments ovarian androgen production. It has been shown that insulin acts alone or synergistically with LH to increase androgen production in the ovary (Barbieri et al, 1986). The thecal cell hyperresponse to LH enhanced by insulin accounts for androgen excess.

Granulosa cells are hyperresponsive to FSH, but hyperestrogenism is prevented by a compensatory reduction in FSH levels (Rosenfield, 1999).

The ensuing hyperandrogenism contributes to hyperestrogenemia and is accompanied by ovulatory dysfunction and apparent resistance to estrogen/progesterone-dependent feedback restraint of LH secretion, another typical feature of women with PCOS (Arroyo et al, 1997; Homburg R, 1998; Pastor et al, 1998; Eagleson et al, 2000; Van Dam et al, 2002).

- **Metabolic Features**

A number of features that are often, but not always, present in PCOS may provide mechanistic clues: obesity, peripheral insulin resistance, and chronic hyperinsulinemia (Dunaif A, 1997; Gambineri et al, 2002)

1)Obesity

More than 30% of women with PCOS are obese (body mass index >30 kg/m^2), reflecting primarily visceral adiposity (Gambineri et al, 2002). Intra-abdominal obesity is frequently accompanied by insulin resistance and compensatory hyperinsulinemia (Carey et al, 1996). The cause of obesity in the polycystic ovary syndrome remains unknown, but obesity is present in at least 30 percent of cases; in some series, the percentage is as high as 75 (Azziz et al, 2001). Women in the United States with the polycystic ovary syndrome generally have a higher body weight than their European counterparts (Franks S, 1989; Conway et al, 1989; Azziz et al, 2001; Carmina et al, 2003). This fact has been cited as an explanation for the increase in the incidence of the polycystic ovary syndrome in the U.S. population — an increase that parallels the increase in obesity (Mokdad et al, 2003).

Increased adiposity, particularly visceral adiposity that is reflected by an elevated waist circumference (>88 cm [35 in.]) or waist-to-hip ratio, has been associated with hyperandrogenaemia, insulin resistance, glucose intolerance, and dyslipidemia (National Cholesterol Education Program, 2002). PCOS women tend to have an increased waist-hip ratio, (WHR) i.e. abdominal (visceral) obesity (Rebuffe-Scrive et al, 1989; Bringer et al, 1993). The waist girth and the subscapularis skin fold measure two different types of fat, the predominantly visceral and the subcutaneous truncal fat respectively (Bouchard et al, 1993), both types being independently associated with insulin resistance (Ross et al, 1996). In a study of 72 women with PCOS mean BMI (Kg/m2) was 26.2, WHR was 0.85 and subscapularis skin fold thickness was 27.2 mm (Gennarelli et al, 2000).

Recent studies show that in healthy individuals subcutaneous truncal–abdominal fat is highly correlated with the level of insulin resistance even more so than is intraperitoneal fat (Abate

et al, 1995). In addition, it has been suggested that the thickness of subscapularis skin fold could help to identify women at risk of non-insulin dependent diabetes mellitus NIDDM (Peiris et al, 1989). The coefficient of variation for waist girth was 2.9%, whereas it was somehow higher for the subscapularis skin fold (8.5%). The latter value is not far from that of previous investigations on different populations (Peiris et al, 1989).

2)Hyperinsulinemia

Hyperinsulinemia drives ovarian (over) production of androgens and simultaneously inhibits hepatic sex hormone-binding globulin (SHBG) synthesis, potentially enhancing androgen availability to target tissues (Dunaif A, 1997; Poretsky L, 1991).

Insulin resistance and its compensatory hyperinsulinemia contribute to the anovulation, hyperandrogenism, infertility and early pregnancy loss suffered by women with PCOS (Cheang et al, 2006).

3)Insulin resistance

Insulin resistance is an important pathophysiological feature of the polycystic ovary syndrome (PCOS).

Clinical and biochemical findings suggesting insulin resistance as given by De Leo et al, 2003.

Parameter		References
Obesity	(Legro et al, 1999; Acien et al,1999; Morin Papunen et al,2000; Barbieri, 2000)	
Waist-to-hip ratio	> 0.85	(Gennarelli et al, 2000; Barbieri, 2000)
Subscapularis skin fold	> 50 mm	(Gennarelli et al, 2000)
Fasting insulin	>30 mU/liter	(Acien et al,1999; Gennarelli et al, 2000)
G:I ratio	< 4.5	(Legro et al,1998)
Amenorrhea		(Robinson et al, 1993)

Clinical assessment of insulin resistance can be done by QUICKI (quantitative insulin sensitivity check index) test which is quantitative, non invasive, simple, economical, reproducible, has ability to measure glucose tolerance, and correlates with the clamp technique. The fasting Glucose Insulin ratio may be a useful as a screening test for insulin resistance in obese white PCOS patients. A fasting G: I ratio below 4.5 predicted insulin resistance (De Leo et al, 2003)

4) Glucose tolerance

Several studies have shown that approximately 20–40% of adult and adolescent PCOS women, including both lean and obese patients, suffer from abnormal glucose metabolism, i.e. either impaired glucose tolerance (IGT) or Type 2 Diabetes Mellitus (Ehrmann et al, 1999; Palmert et al, 2002).

According to the study, PCOS was a more important risk factor for glucose intolerance than was ethnicity or race (Legro et al, 1999).

Fasting glucose has been suggested as a screening parameter for abnormal glucose metabolism. However, several studies demonstrate that a substantial proportion of PCOS women with Insilin Glucose Tolerance or even Type 2 Diabetes Mellitus show normal fasting glucose concentrations (Ehrmann et al, 1999; Legro et al, 1999; Palmert et al, 2002).

5) Leptin and PCOS

Leptin, a 167 amino-acid protein transcribed from the ob gene, was discovered by Zhang et al. 1994. In obese, hyperphagic, homozygote ob/ob mice, two mutations of the ob gene was demonstrated to lead to a lack of leptin (Zhang et al, 1994).

Leptin, a peptide secreted by fat cells in adipose tissue, acts on the neurons in the central nervous system and is involved with regulation of eating behaviour and energy balance. It is the product of the 'ob' gene and a deficiency in the protein or mutation in the gene leads to obesity (Caro et al, 1996)

Leptin, the obesity protein is produced by the adipose tissue (Zhang et al, 1994). High levels of leptin may suppress neuropeptide Y in the hypothalamus, leading to the high GnRH and LH levels seen in PCOS. In a study in California Fasting (0800 h) leptin levels in thirty three PCOS women (24.1 $\pm$ 2.6 ng/mL) did not differ from those of Normal Control (21.5 $\pm$ 3.5 ng/mL) and were positively correlated with BMI with no difference in the relationship between BMI and leptin levels for the two groups (r 5 0.81, P , 0.0001 for PCOS and NC together) Leptin levels for PCOS and Normal Control correlated positively with fasting and 24-h mean insulin levels (r 5 0.81, ($P < 0.0001$) for both PCOS and NC) and were positively correlated with BMI ($r = 0.81$) and percent body fat ($r = 0.91$) for the two groups (both $P < 0.0001$)(Laughlin et al, 1997). Serum leptin concentrations in women with PCOS have been reported to be higher in study of (Brezechffa et al, 1996). However, leptin levels are directly related to obesity, so it is very important to exclude the effect of BMI on the leptin levels, as mean leptin levels are significantly higher in the obese groups (Orabi et al, 1999). Numerous studies (Morin-Papunen et al, 1998, 2000; Pasquali et al, 2000; Koivunen et al, 2001) show metformin treatment is associated with a decrease in leptin levels. Fremark and Bursy showed

that in obese adolescent girls with hyperinsulinaemia, metformin lowered BMI and serum leptin levels (Fremark and Bursy, 2001). There was a strong correlation between serum leptin concentrations and Body Mass Index and Waist to Hip Ratio in PCOS (Spritzer et al, 2001)

6) Lipids and PCOS

Women with PCOS have lower HDL and /or HDL2 levels, higher Triglycerides and low-density lipoprotein (LDL) levels than age-, and weight-matched control women (Wild et al, 1985, Conway et al, 1989). To a large extent, lipid profiles in PCOS are found to be related to the degree of insulin resistance / hyperinsulinemia, independent of androgen levels and BMI (Norman et al, 1995). Alterations of lipids may be partly responsible for the increased incidence of hypertension, coronary heart disease and thrombosis in PCOS women (Wild et al, 1985; Talbott et al, 2000).

TREATMENT OF PCOS

1) GLUCOPHAGE (METFORMIN HYDROCHLORIDE)

It is an oral antihyperglycemic drug used in the management of type 2 diabetes. Metformin, which also reduces hyperinsulinemia, might be effective in treating obese, infertile women with the polycystic ovary syndrome. In a clinical trial reported in 1998, 32 women were randomly assigned to receive either metformin or placebo pills. Clomiphene was added in the second cycle if there was no ovulation. Overall, 89% of the women who were treated with metformin ovulated, either spontaneously or in response to clomiphene, as compared with 12% of the women who were treated with placebo, either alone or with clomiphene (Nestler et al, 1998). Metformin probably improves ovulation in women with PCOS by reducing gluconeogenesis, improving insulin sensitivity, and reducing ovarian androgen production (Shaw et al, 2005).

2) CLOMIPHENE CITRATE

The number of drugs have been used to induce ovulation in women with the polycystic ovary syndrome, clomiphene citrate is a simple, tried-and-true treatment. Clomiphene is an orally active, antiestrogenic substance that promotes the release of follicle-stimulating hormone from the pituitary gland, thus stimulating the development of ovarian follicles and ovulation (Clark and Markaverich, 1981). Early experience with clomiphene in the treatment of infertile, anovulatory women (about half of whom were likely to have had the polycystic

ovary syndrome on the basis of the presence of hyperandrogenemia) yielded a cumulative pregnancy rate of 56% after six cycles of treatment (Hammond et al, 1983). More recently, a cumulative pregnancy rate of 73% was reported when treatment with clomiphene citrate was repeated for up to nine ovulatory cycles (Imani et al, 1999). Clomiphene has its drawbacks, however. It is associated with a multiple pregnancy rate of 5 to 10 % (Asch and Greenblatt, 1976). It does not address the underlying abnormalities in the polycystic ovary syndrome, including hyperinsulinemia and hyperandrogenism.

3) CLOMIPHENE CITRATE AND METFORMIN COMBINATION

In Clomiphene Citrate (CC) resistant women, the combination of CC plus metformin is the preferred treatment option before starting with Laproscopic Ovarian Drilling or FSH. Metformin leads to a reduced risk of Ovarian Hyper Stimulation Syndrome (Etelka Moll et al, 2006). It is well known that interventions that improve insulin resistance and reduce hyperinsulinemia, such as weight loss, in women with this syndrome also reduce hyperandrogenemia and induce ovulation in many cases (Hoeger, 2006). A meta-analysis of 13 randomized trials (Lord et al, 2003) comparing metformin with placebo, or metformin plus clomiphene with clomiphene alone, in women with the polycystic ovary syndrome concluded that metformin increased the ovulation rate by a factor of approximately four. Of note, pregnancy rates did not differ significantly between the metformin groups and the placebo groups, although the pregnancy rates for metformin plus clomiphene were significantly higher than for clomiphene alone (Lord et al, 2003). More recently, a clinical trial in Italy, in which 100 infertile, nonobese women with the polycystic ovary syndrome were randomly assigned to receive either metformin or clomiphene, showed similar rates of ovulation in the two groups, although the pregnancy rate in the metformin group was twice that in the clomiphene group (Palomba et al, 2005).

4) GONADOTROPHINS

Metformin also appeared to improve the outcomes of ovulation induction therapies when combined with clomiphene and gonadotrophin (Nestler et al, 1998; De Leo et al, 1999; Vandermolen et al, 2001; Costello and Eden, 2003; Lord et al, 2003). After ovulation induction with Human Menopausal Gonadotrophins the cumulative conception and live birth rates in the first course of therapy and after 12 cycles of treatment were, respectively, 73.2

and 62.4% in PCOS patients, the miscarriage rates for all courses of treatment were 15.5% in PCOS patients (Adam et al, 1994).

EFECT OF GLUCOPHAGE ON ANTHROPOMETRIC PARAMETERS

{BMI (Body Mass Index), WHR (Waist-Hip ratio) and Subscapularis Skin Fold Thickness}

The changes in body mass in l patients receiving the 8-month metformin treatment (n=83), revealed that there was a highly significant reduction (3.8%) from a mean BMI of 37.2 kg/m2, with 95% confidence limits of 35.9 –38.5 at the start (T0) to a mean BMI of 36.1 (95% confidence limits, 34.7, 37.4) after eight months of treatment (by repeated measures ANOVA, $P< 0.0001$) (Harborne et al, 2005). **A** nother study showed that after 14-wk treatment of PCOS Patients (n=45) the BMI decreased significantly in the metformin group. There was no change seen in the WHR (Fleming et al, 2002). In Italy a group of 20 women with PCOS, were given metformin 850 mg twice daily) (12 PCOS and 8 controls, respectively) for the following 6 months plus they were advised hypocaloric diet. During the 6-month pharmacological treatment, both PCOS and controls treated with metformin similarly and significantly decreased body weight (PCOS, P , 0.05; controls, P , 0.001) and BMI (Kg/m^2) decreased from 39.8$\pm$ 7.9 to 36.4$\pm$7.4 (PCOS, $P < 0.001$). In all groups, there was a significant reduction in waist circumference after the first month of hypocaloric diet. Metformin therapy further reduced mean waist-hip ratio during the 6-month treatment in PCOS from 0.87 $\pm$0.07 to 0.86$\pm$ 0.07 but it was not statistically significant (Pasquali et al, 2000).

In Chinese PCOS women n=8 BMI decreased significantly after three months Metformin treatment from 24.1 to 23.0 (P<0.01) (Ng et al, 2001).

EFECT OF GLUCOPHAGE ON BIOCHEMICAL PARAMETERS

1) FASTING GLUCOSE, INSULIN, GLUCOSE INSULIN RATIO, QUICKI

The biguanide metformin inhibits hepatic glucose production and enhances peripheral tissue sensitivity to insulin, resulting in a reduction in insulin secretion (Nagi and Yudkin, 1993; DeFronzo et al, 1991).

Different parameters in women with PCOS (n = 45), aged 21–36 yr, were checked at baseline, after four weeks, eight weeks and twelve weeks. There was no difference in the levels of fasting Glucose mg/dl from 83.0 baseline levels to 81.5 after twelve weeks. Fasting Insulin levels (uU/ml) also showed no significant difference from baseline levels (20.0) to at twelfth weeks (20.0). Similarly Fasting Glucose/Insulin ratio did not show any significant change from baseline levels (4.0) to after twelve weeks (4.4) of treatment with Metformin. QUICKI showed no significant change from baseline to post treatment values. (Eisenhardt et al, 2006). Metformin exert beneficial effects on insulin sensitivity (Lord et al, 2003).

In PCOS patients, metformin has been shown to be beneficial in reducing hyperinsulinaemia and hyperandrogenaemia while facilitating normal menses and pregnancy (Velazquez et al, 1994, 1997; Morin-Papunen et al, 1998; Nestler et al, 1998; Glueck et al, 1999; Moghetti et al, 2000).

After four weeks of treatment of polycystic patients (n=26) with 500mg Glucophage 3 times daily follicular phase fasting serum insulin concentrations decreased significantly in the metformin group from 206 $\pm$ 27 to 79 $\pm$ 14 pmol/L ($P < 0.001$) (Jakubowicz et al, 2001).

Although metformin treatment did not result in a (significant) decrease of insulin resistance, it did cause a significant decrease in androgen serum concentrations and improved the endogenous gonadotrophin–oestrogen balance (De Leo et al, 1999; Yarali et al, 2002; Van Santbrink et al, 2005). Metformin in PCOS causes a reduction in insulin and Testosterone and improvement in menstrual cyclicity (Velazquez et al, 1994).

2) LEPTIN

Numerous studies (Morin-Papunen et al, 1998, 2000; Pasquali et al, 2000; Koivunen et al, 2001) show metformin treatment is associated with a decrease in leptin levels. Metformin may have beneficial effects not only to control glycemia but also to correct eating behavior in obese type 2 diabetic patients with the difficulty in controlling their appetites. The improvement was related to the reduction of insulin resistance and serum leptin levels (Komori et al, 2004). Fremark and Bursy showed that in obese adolescent girls with hyperinsulinaemia, metformin lowered BMI and serum leptin levels (Fremark and Bursy, 2001). After 14-wk treatment of PCOS Patients (n=45), the circulating leptin concentration declined in the metformin-treated group from 41.1ng/ml to 37.3 ng/ml ($P < 0.05$) (Fleming et al, 2002).

3) REPRODUCTIVE HORMONES

Whereas reproductive and cyclic abnormalities improved strikingly after treatment, the positive effects of treatment were independent of changes in body weight or BMI and were not correlated to hormonal improvements (Moghetti et al, 2000; Eisenhardt et al, 2006). There are two studies to mention that show a direct effect of metformin in reducing androgen production in theca cells (Mansfied et al, 2003; Attia et al, 2003). In Chinese PCOS women (n=8) after Metformin treatment for three months the serum FSH (IU/l) levels had no significant decrease from (6.7 vs. 5.4). The serum LH (IU/l) levels did not show significant decrease (10.0 vs. 9.7). Serum Testosterone levels (nmol/l) decreased highly significantly (1.8 vs.1.2) $P<0.05$ (Ng et al, 2001). After 14-wk treatment of PCOS Patients (n=45) with Metformin, responders to metformin treatment showed significantly lower Testosterone (2.5 nmol/liter *vs.* 3.5 nmol/liter; 95% CI = 0.07 and 2.1, respectively; $P = 0.04$) (Fleming et al, 2002). The failure of metformin to influence circulating SHBG concentrations beyond placebo or control is another surprising observation that has been recorded previously (Dunaif et al, 1996).

4) BLOOD PRESSURE

Moghetti et al, (2000) and Nestler et al, (1996) showed a significant reduction for metformin in both systolic blood pressure and diastolic blood pressure.

5)LIPIDS

Harborne et al, (2005) studied the effect of two doses 1500mg and 2550 mg of metformin and found significant reduction in in total cholesterol, however, no dose effect was noted.

GLUCOPHAGE AND INDUCTION OF OVULATION

Ovulation was achieved in 46% of those who received metformin alone. Where metformin and clomifene were compared with clomifene alone, ovulation occurred in 76% of women receiving metformin and clomifene, compared with 42% of those receiving clomifene alone (Jonathan et al, 2003). Ovulatory response to clomiphene citrate is enhanced by the addition of metformin in clomiphene-resistant and obese PCOS patients (Nestler et al, 1998; Lord et al, 2003). Ovarian induction improves both ovulation and pregnancy rates in both unselected and clomiphene citrate-resistant PCOS women (Costello and Eden, 2003; Lord et al, 2003, 2004; Kashyap et al, 2004). Patients with PCOS undergoing gonadotrophin ovarian iduction or in vitro fertilization usually show an increased response to gonadotrophins and consequently

produce large numbers of follicles and oocytes with high serum estradiol (E_2) levels, resulting in an increased risk of ovarian hyperstimulation syndrome (OHSS) (Aboulghar and Mansour, 2003; Yarali and Zeyneloglu, 2004). The reduction of insulin secretion by metformin would increase the ovulatory response to clomiphene. Metformin has effects on spontaneous and clomiphene-induced ovulation in the polycystic ovarian syndrome (Nestler et al, 1998). Metformin administration restores ovulatory menstrual cycles and improves fertility in anovulatory PCOS women (Lord et al, 2003, Palomba et al, 2005).

GLUCOPHAGE AND PREGNANCY

Traditionally, the group of oral hypoglycemic agents has been regarded as teratogenic and therefore contra-indicated in pregnancy. Although an earlier animal study showed an increased risk of teratogenicity with use of oral hypoglycemic agents in pregnancy (Schardein, 1993; Shepard et al, 1995) an increasing amount of data support the safe use of metformin throughout pregnancy. In a prospective pilot study of 22 non-diabetic PCOS women taking metformin 1.5–2.55 g/day throughout pregnancy, no birth defects have occurred (Glueck et al, 2001). In a study of 154 infants whose mothers had PCOS and took metformin during pregnancy had no adverse outcome (Glueck et al, 2002). In a study by Jakubowicz et al, (2002) in Venezuella 96 nondiabetic women with the polycystic ovary syndrome who became pregnant in Caracas were screened. Those who either did not receive metformin at the time of conception or during pregnancy (control group; n = 31) or became pregnant while taking metformin and continued taking metformin at a dose of 1000–2000 mg daily throughout pregnancy (metformin group; n = 65). When metformin was administered throughout pregnancy to women with the disorder, the rate of early pregnancy loss was decreased dramatically, compared with women who had not received metformin (8.8% *vs.* 41.9%).

CHAPTER-2

SUBJECTS AND METHODS

Study Population

A total of 315 infertile women were screened and interviewed in this study. The study population included women treated for infertility during a period of three and half years at the Pakistan Institute of Medical Sciences Islamabad and Noor specialized clinic Islamabad. The study was sponsored by Higher Education Commission of Pakistan therefore patients did not pay any cost for clinical tests. None of the patient had received any drugs known to interfere with hormonal concentrations for at least 3 months before the study. Patients were instructed not to modify their eating habits throughout the study. All the patients were advised for hysterosalpingography before the study so that any tubal defect could be ruled out and 69 patients have had this investigation. Out of these one had unilateral tubal defect and two had bilateral tubal blockage. These patients were excluded from the study. Their husbands were advised to have semen analysis. Forty male partners had low sperm count and thirty five had motility problems for which they received medicines. The study protocol was approved by the Pakistan Institute of Medical Sciences Ethics Committee, and written informed consent was obtained from all subjects.

Inclusion criteria

All the study participants were subjected to the 'Revised 2003 consensus' on diagnostic criteria for PCOS, implying that at least two of the three following criteria were ful- filled: polycystic ovaries, hyperandrogenism (clinical and/or biochemical) and oligo- and/or anovulation (The Rotterdam ESHRE/ASRM sponsored PCOS workshop group, 2004).

- All patients hadd isturbed ovulatory function with chronic oligomenorrhea (cycle length > 35 d; less than nine cycles per year) or amenorrhea (cycle length >12 wk)
- Typical appearance of polycystic ovaries by ultrasound according to the criteria of the Rotterdam consensus meeting 2003 which all patients fulfilled. (The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004).

Exclusion criteria

- Patients with thyroid problem, tubal defects or on previous medications were excluded from the study.
- Patients with azospermic husbands were excluded from the study.

Study Design

These patients were divided into two groups:

Group A (Those who gave consent (n=170) to take glucophage for three months)

Group B (Those who gave consent (n=145) to take glucophage for six months)

In both groups the **Glucophage** drug (oral biguanide) by Merck was started in low dose to avoid its side effects. Initially the patients were advised to take 500 mg tablet daily i.e half tablet in morning and half in the evening for the first week and in the next week 500 mg tablet twice i.e one in the morning and one in the evening. In the third week the dose was increased to 500 mg three times daily. In Group A 16 patients discontinued the treatment in a period of one month this included two patients with tubal defects and in Group B 11 patients discontinued the treatment after a period of 15 days, this included one patient with tubal defect. In Group A total number of patients who continued with the treatment of glucophage for three months was 154 a nd in Group B 134 patients continued with the treatment for six months. All the patients were well settled with the treatment and had no side effects. Patients used to visit every month and they were asked to mate with their husbands in the midcycle. They were told to take early morning body temperature by thermometer and any rise in one degree during the mid of cycle was considered as sign of ovulation. Fifteen patients reported achievement of regularity of cycle after three months in Group A. Later on they were given combination treatment of glucophage with ovulation induction medicines and were advised regular monitoring with ultrasound for ovulation. A few patients had regular ultrasound monitoring in the mid of cycle so complete data regarding ovulation was not available. In Group B twenty nine patients reported to have normal menstruation. When they were given ovulation induction medicines along with Glucophage all the patients either reported menstruation or in some if no periods at the end of month were investigated for pregnancy. If they missed the next period their urine and blood HCG was done and ultrasonography was done to assess any sac or formation or fetal pole. When they were given ovulation induction medicines along with Glucophage all the patients either reported menstruation or in some if no periods at the end of month were investigated for pregnancy.

Routine ultrasonography was done for nine months in these patients to assess any complication of pregnancy. Patients conceived either after three months or six months of glucophage treatment or after combination treatment of glucophage with ovulation induction medicines (clomiphene citrate and human menopausal gonadotrophin) at the end of three or six months of glucophage treatment were classified as class a, b, c and d. The ovulation induction medicines used were

- clomiphene citrate 50 mg(Cerophene-by Hilton pharma- Antioestrogenic drug) 1 tablet once daily for five days starting on 2nd day of menstrual cycle
- clomiphene citrate 100 mg(Cerophene-by Hilton pharma- Antioestrogenic drug) 2 tablet of 50 mg once daily for five days starting on 2nd day of menstrual cycle
- injection of Human Menopausal Gonadotrophin (inj HMG MASSONE- by Instituto Massone /Excel Health care. Contains 75 iu FSH and 75 iu LH) daily for 4 days starting on 7th day of cycle to 10th day of cycle.

Class a: comprises of those patients who conceived after taking glucophage 500 mg (Glucophage by Merck –Germany) 1 tablet three times a day for three or six months respectively. In Group A none of the patient conceived while in Group B 19 patients conceived. The patients who did not conceive were given further treatment of glucophage in combination with ovulation induction medicines (Clomiphene citrate 50 mg).

Class b: comprises of those patients who conceived after combination treatment of glucophage 500 mg 1 tablet three times a day and clomiphene citrate 50 mg, 1 tablet once daily for five days starting on 2nd day of menstrual cycle. In Group A eleven patients conceived while in Group B twenty seven patients conceived. The patients who did not conceive were given further treatment of glucophage in combination with ovulation induction medicines (Clomiphene citrate 100 mg).

Class c: comprises of those patients who conceived after combination treatment of glucophage 500 mg 1 tablet three times a day and clomiphene citrate 100 mg (50 mg tablet)2 tablet daily for five days starting on 2nd day of menstrual cycle. In Group A twenty two patients conceived while in Group B sixteen patients conceived. The patients who did not conceive were given further treatment of glucophage in combination with ovulation induction medicines (Clomiphene citrate 100 mg and Human Menopausal Gonadotrophins).

Class d: consists of those patients who conceived after combination treatment of glucophage 500 mg 1 tablet three times a day and clomiphene citrate 100 mg (50 mg tablet) 2 tablets

daily for five days starting on 2nd day of menstrual cycle and injection of Human Menopausal Gonadotrophin daily for 4 days starting on 7th day of cycle to 10th day of cycle. In Group A twenty three patients conceived while in Group B twenty four patients conceived. Only one patient developed mild ovarian hyper stimulation symptoms but did not need hospitalization. The patients who did not conceive were given no further treatment. This included ninety eight patients in Group A, and forty eight patients in Group B. In Group A out of ninety eight patients who did not conceive, sixty five had signs of ovulation on ultrasound and in Group B fourty three patients had ovulation and regular menstrual cycle. These women were advised hysteroslpingography and out of them only six patients had unilateral tubal defects and twenty nine had their husbands still with low sperm count and husbands of two patients in Group B had erection problems. Rest one hundred nine patients were diagnosed for idiopathic or unexplained infertility.

The patients who conceived at the time of their conception were given the option to continue with low dose of glucophage 500 mg ½ tablet three times a day throughout their pregnancy period. Of the total conceived patients those who signed the consent to continue the treatment throughout their pregnancy are placed in **group I.**

The patients who did not give the consent to continue glucophage throughout their pregnancy duration are placed in **group II.** The patients who continued Glucophage during pregnancy had no severe complications like nausea or other gastric symptoms as they were already taking the drug.

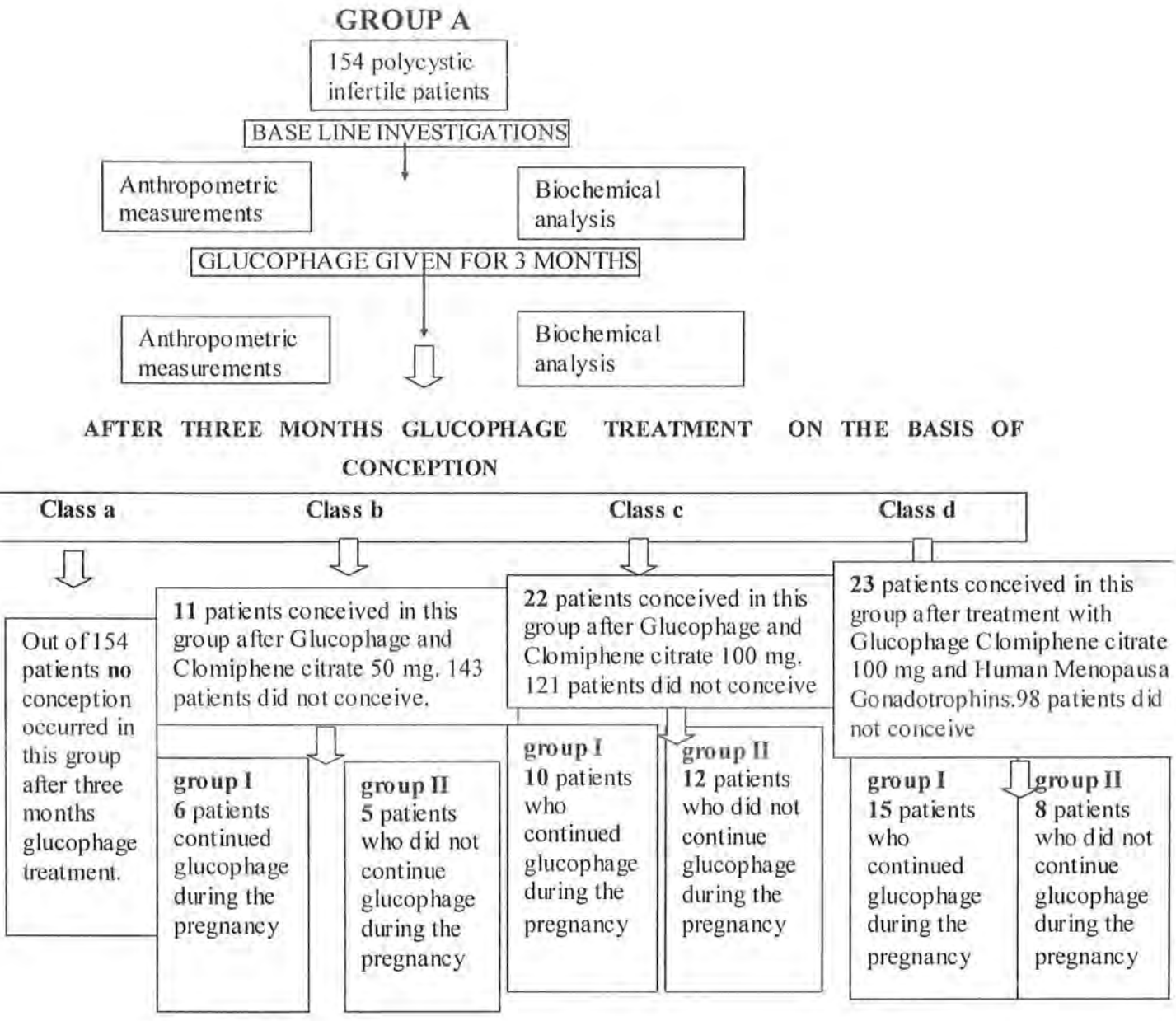

Fig.1 Group A Patients who were given three months glucophage treatment and later on were given ovulation induction medicines, according to their pattern of conception were classified as **a, b, c** and **d** and those who took glucophage during their pregnancy duration were placed in group I and those who did not take glucophage during their pregnancy duration were placed in group II.

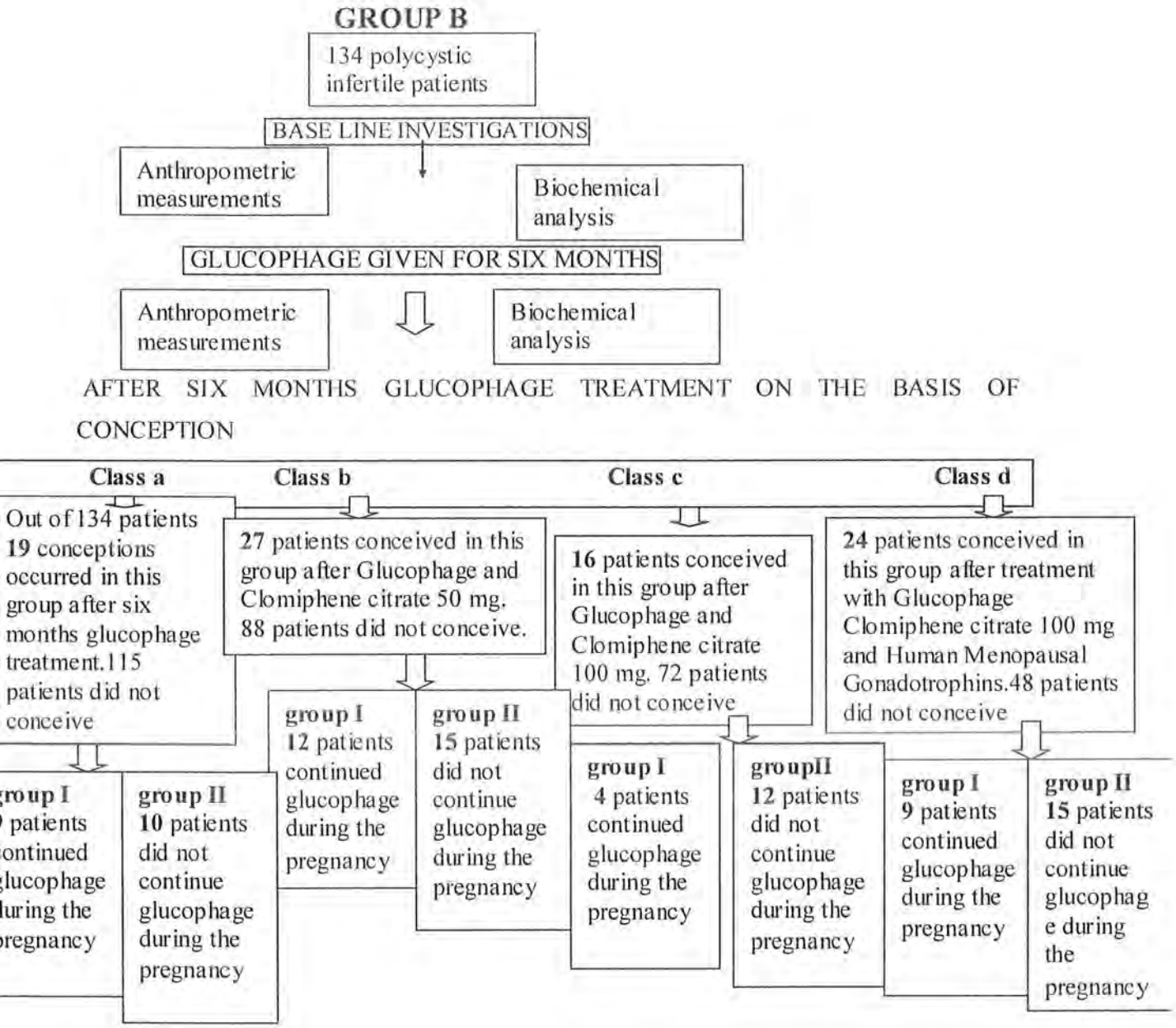

Fig.2 Group B Patients who were given six months glucophage treatment and later on were given ovulation induction medicines, according to their pattern of conception were classified as **a, b, c** and **d** and those who took glucophage during their pregnancy duration were placed in group I and those who did not take glucophage during their pregnancy duration were placed in group II

Assessment Program

All patients underwent clinical and hormonal assessments. These included anthropometric/physical measurements of height, weight (BMI), and waist/hip ratio, Subscapularis skin fold thickness as well as measurements of Blood pressure and Ultrasound assessments. Circulating blood samples taken before start of glucophage therapy in Group A and B and after glucophage therapy for three months in Group A and six months in Group B. Blood samples taken were tested for Insulin, Glucose, LH, FSH, Estrogen, Progesterone, Prolactin Testosterone, , total Cholesterol and Leptin after an overnight fast. Blood samples were drawn from an antecubital vein. All samples were obtained about 10 ml between 08:00 and 10:00 A.M on day 2^{nd} to 5^{th} day of the menstrual cycle in women who had menstruation that month or in amenorrheic women after progesterone withdrawl bleeding on 2^{nd} to 5^{th} day.

During the study, the conception, live birth, abortion, single birth and multiple birth rates were evaluated in each woman. The **conception rate** was defined as the percentage of conceptions in the group per total conceptions. A rising human chorionic gonadotropin and the sonographic evidence of intrauterine gestational sac were considered criteria to define a conception.

Abortion rate was defined as a percentage of miscarriage during the first 12 weeks of gestation per total pregnancies. **Live-birth rate** was obtained after a 9-month extension of the follow-up period and was defined as a percentage of women with baby alive per women who gave birth to babies either alive or dead.

Still birth was defined as babies who had intra uterine deaths.

Single birth rate was defined as percentage of single births per total births of fetuses.

Multiple birth rate was defined as percentage of multiple births per total births of fetuses.

Post natal deaths were defined as deaths of babies within one month after delivery of baby.

Threatened abortion cases were those patients who complained of bleeding per vaginum during the first 12 weeks of gestation but became normal after treatment and had no complete abortion.

Preterm delivery cases were those patients who delivered before term that is before 36 weeks of gestation.

Postterm delivery cases were those patients who delivered after 40 weeks of gestation.

Techniques

1. Anthropometric measurements

- **Waist :Hip ratio**

Waist and hip circumferences were measured to the nearest centimeter with a soft tape according to World Health Organization criteria. Waist circumference was obtained as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the gluteal region. Waist to hip ratio >0.85 is indicative of insulin resistance (Gennarelli et al, 2000; Barbieri RL, 2000).

- **Subscapularis skin fold thickness**

Subscapularis skin fold thickness was measured with a Lange-Caliper (Beta Technology, Cambridge, MA) to the nearest 0.1 mm. It is measured below inferior angle of the scapula, at 45° to the vertical, along the natural cleavage lines of the skin. (Ada et al, 2005).Subscapularis skin fold thickness >50 mm is indicative of insulin resistance. (Gennarelli et al, 2000)

- **BMI (Body Mass Index)**

BMI was calculated using the equation (weight in kg divided by height in m^2) with normal ranging between 17–25.9, overweight between 26–30, and obese over 30.1 (Diamanti-Kandarakis et al, 1999).

2. Ultrasonography

Ultrasound was performed by the transvaginal method using a vaginal probe (Sonoline Sienna Ultrasound Imaging System, Transducer 6.5EV13+; 6.5 MHz; Siemens, Erlangen, Germany). In each ovary, ovarian volume, greatest number of follicles in any one ovarian plane, and the diameter of the largest follicle were calculated. In pregnant women routine ultrasound examination was performed by abdominal probe to determine any complication of pregnancy.

3. Biochemical Parameters

After an overnight fast 20 ml blood sample was drawn from an antecubital vein for determination of plasma Glucose, Insulin, Leptin, LH, FSH, Estradiol, Progesterone, Prolactin and total Testosterone Levels. All samples were obtained between 08:00 and 10:00 A.M. Centrifugation was done at 3000 revolutions per minute and serum obtained was frozen in aliquots at -20^{0}C till the tests were performed.

- **Fasting Blood Glucose** Glucose was measured by the glucose oxidase technique (PAP method) by Merck Microlab 300. Normal range for fasting blood glucose is 55-115 mg/dl (Barham and Trinder, 1972).F asting glucose levels were classified by the revised 1997 American Diabetes Association (ADA) criteria. Normal fasting glucose (NFG) was less than 110 mg/dl (6.06 mmol/liter), impaired fasting glucose (IFG) was 110–126 mg/dl (6.06–6.94 mmol/liter), and type 2 diabetes (fasting DM) was 126 mg/dl or more (6.94 mmol/liter).

- **Fasting Insulin** Serum insulin levels were measured by ELISA with intra and interassay CVs of 5.3% and 5.6%, respectively. The thresh- hold value to define hyperinsulinaemia was arbitrarily established at 23 µIU/ml, taking into consideration the upper normal limit for insulin (25 µIU/ml).Normal range 2-25 µIU/ml. Fasting Insulin >30 mu/liter is indicative of insulin resistance (Acien et al, 1999; Gennarelli et al, 2000)

- **Fasting Glucose: Insulin ratio** It was calculated by dividing fasting serum glucose by fasting serum insulin (Spritzer *et al.*, 1998). G:I <4.5 is indicative of insulin resistance (Legro et al, 1998).
- **QUICKI** We performed a sensitivity analysis of glucose and insulin by Quantitative Insulin sensitivity check index QUICKI. The formula for calculation of QUICKI is = $1/[\log(I_0) + \log(G_0)]$, where I_0 is the fasting insulin, and G_0 is the fasting glucose. (Arie Katz et al, 2000)**Serum Cholestrol** was m easured by photometric determination by Merck Microlab 300 (Deeg and Ziegenhorn, 1983).

- **Serum FSH and LH** concentrations were measured by ELISA (IBL). The respective intra- and interassay CVs were 7.46% and 5.89% for FSH and 5.8% and 7.16% for LH. FSH value in follicular phase of cycle is 2-10mIU/ml.LH value in follicular phase is <20 mIU/ml
- **Serum Prolactin** levels were determined by ELISA (IBL) with intra- and interassay CVs of 4.3% and 6.83% respectively. (Normal range 2.39-25.15 ng/ml).
- **Serum Progesterone** level were determined by ELISA (IBL) with intra and interassay CVs of 5.4% and 9.96% respectively. Normal value in follicular phase is (0.2-1.4 ng/ml)
- **Serum Leptin** levels were measured by ELISA (IBL) with intra and inter assay CVs of 5.95% and 11.55% respectively. Normal value is 7.36±3.73 ng/ml.
- **Serum Estradiol (E_2)** was measured by ELISA (IBL) with intraassay CVs of less than 6.8% and interassay CV of 7.25%. Normal value in premenopausal women is 13-191 pg/ml
- **Serum Testosterone** concentrations were measured with ELISA (IBL) with Intra- and inter-assay CV of 4.16% and 9.93% respectively. Normal value in females is 0.26-1.22 ng/ml.

ELISA (Enzyme Linked Immuno Sorbent Assay)

Principle

To detect antigen A, purified antibody specific for antigen A is linked chemically to an enzyme. The samples to be tested are coated onto the surface of plastic wells to which they bind nonspecifically; residual sticky sites on the plastic are blocked by adding irrelevant proteins (not shown). The labeled antibody is then added to the wells under conditions where nonspecific binding is prevented, so that only binding to antigen A causes the labeled antibody to be retained on the surface. Unbound labeled antibody is removed from all wells by washing, and bound antibody is detected by an enzyme-dependent color-change reaction. This assay allows arrays of wells known as microtiter plates to be read in fiberoptic multichannel spectrometers, greatly speeding the assay. Modifications of this basic assay allow antibody or antigen in unknown samples to be measured

Table.1 Biochemical tests with their normal values and units.

Test	unit/normal value
FASTING INSULIN	2-25 µIU/ml
FASTING LEPTIN	7.36 ± 3.73ng/ml
FSH	2-10 mIU/ml
LH	<20 mIU/ml
ESTRADIOL	13-191 pg/ml
PROGESTRONE	0.2-1.4 ng/ml
PROLACTIN	2.39-25.15 ng/ml
TESTOSTERONE	0.26-1.22 ng/ml
CHOLESTROL	180 mg/dl

Values as given by IBL

µIU/ml—micro international unit per milliliter

ng/ml---nanogram per milliliter

mIU/ml---milliinternational unit per millileter

mg/dl---milligram per deciliter

Procedure

a) The first step was coating of wells with antibody:

100 ml of diluted (with buffer A) antibody was added to each well. The antibody is directed against the antigen to be determined. Then I covered the plate with plastic film or aluminium foil. Incubation was done overnight at +4°C.

b) Washing: The plate was emptied by inversion and the plate w as tapped against a few layers of soft tissue paper to remove any residual liquid. The plate was washed by filling the wells by immersion in buffer B. It was drained to empty the plate. Repeat washing was done two more times.

c) Incubation with test samples: 100 ml of sample was added and/or standard, diluted in buffer B, to each well. The plate was covered and allowed to stand at room temperature for about two hours.

d) Incubation with peroxidase-conjugated antibody: 100 ml of peroxide conjugated antibody was added to each well. The plate was covered and allowed to stand at room temperature for 1 hour. The peroxidase-conjugated antibody was directed against the antigen to be determined.

e) Washing as described in step (b).

f) Color development: 100 ml of chromogenic substrate was added to each well. The plate was covered and allowed to stand at room temperature for 15 minutes or until color had developed. The plate was protected from light during this period.

g) Stopping the reaction: The reaction was stopped by adding 150 ml of 1M sulfuric acid to each well. For quantitation purposes, it was important that each well had been incubated with color reagent for exactly the same length of time.

h) Measuring the absorption. The absorption was read in a suitable photometer or ELISA plate reader (set at 492 nm) within 3 hours of color development. The standard curve was plotted on semilogarithmic paper with A492 as ordinate and log10 concentration as abscissa.

Statistical Analysis

For data analysis means±S.E were calculated. Two way analysis of variance and t.tests were applied for the comparison of means. Probability <0.05 was considered as the level of significance.

PERFORMA

NAME----------
AGE----------
PARITY----------
INFERTILITY(DURATION)----------
BLOOD PRESSURE----------

HISTORY
Medication----------
Diabetes Mellitus----------
Infertility----------
Hypertension----------
Heart disease----------
Thyroid disease----------

SYSTEMIC EXAMINATION
Cardio vascular system----------
Respratory system----------
Gastrointestinal tract----------
Central Nervous system----------

*systemic examination was performed in routine clinical practice.

ANTHROPOMETRIC MEASUREMENTS

	Baseline value	after 3 months	after 6 months
BMI			
Waist-Hip ratio			
Subscapularis skin fold thickness			

BIOCHEMICAL PARAMETERS

	Baseline value	after 3 months	after 6 months
Fasting Blood Glucose			
Fasting Insulin			
Fasting Leptin			
FSH			
LH			
Estradiol			
Progesterone			
Prolactin			
Testosterone			
Cholesterol			

Glucophage Dosage----------
Any side effects----------
Tubal Patency Test report -----

Semen Analysis of Husband---

CHAPTER-3

RESULTS

The number of female infertile patients screened and interviewed for this study was 315.Through ultrasonography and biochemical tests these women were diagnosed for polycystic ovarian disease. These patients were divided into two groups, those who gave consent (n=170) to take glucophage for three months (**Group A**) and others (n=145) consented to take glucophage for a period of six months (**Group B**). In Group A 16 patients discontinued the treatment in a period of one month and in Group B 22 patients discontinued the treatment after a period of 15 days. In Group A total number of patients who continued with the treatment of glucophage for three months is 154 and in Group B 134 patients continued with the treatment for six months. Patients in Group A and B were further divided into class a, b, c, d according to treatment by which they conceived. **Class a** patients were those who conceived with glucophage therapy alone. **Class b** patients were those who conceived with combination treatment of glucophage and Clomiphene citrate 50 mg. **Class c** patients were those who conceived with combination treatment of glucophage and Clomiphene citrate 100 mg. **Class d** patients were those who conceived with combination treatment of glucophage, Clomiphene citrate 100 mg and Human Menopausal Gonadotrophins.

Chapter 3.1

Mean Age of the Patients

Mean Age of the total patients who conceived and who did not conceive

The number of total patients and their mean ages (±S.E) (years) of all the infertile polycystic patients at the time of presentation who conceived and those who did not conceive after initial treatment with glucophage for three months in Group A and for six months in Group B and also with later on treatment with ovulation induction medicines is given in Table 2a. Patients in Group A and B were of comparable mean age. In Group A mean age of the patient who conceived were of younger age (26.5±0.137 years) than those who did not conceive (31.08±0.22 years).The former patients were significantly younger than the latter patients ($t_{(152)}$= 4.64;P<0.001). In Group B the mean age of the patients who conceived (27.7±0.32 years) were highly significantly younger than those who did not conceive (32.6±0.42 years) ($t_{(132)}$= 6.62;P<0.001). The results showed that patients younger in age responded to the treatment in terms of conception which was not observed in the case of older infertile patients.

Mean age of the patients in different classes

The mean ages (±S.E) (years) at which the patients conceived with different treatment regimes are given in Table 2b.

Group-A

No patient conceived in **class a**, but they conceived in **class b** at mean age 28.1±0.32 years. Those who conceived in **class c** were comparatively older than the former group (29.5±0.54 years). Older patients (30.2±0.63 years) in **class d** conceived showed significant difference ($t_{(32)}$ =2.21; P<0.05) in mean age compared to those who conceived in **class b** (28.1±0.32 years).

Group B

Similar picture as with Group A was observed that older patients required a higher dose of treatment or more than two combinations of treatment dose. The older patients of **class d** (29.7±0.38 years) highly significantly ($t_{(41)}$ =7.14; P<0.001) differ in age compared to younger patients (24.2+0.33 years) of **class a.**

Table2a: Mean age and age range (years) of the patients at the time of presentation in Group A (who took glucophage for three months) and in Group B (who took glucophage for six months) and later were given further combination treatment of glucophage and ovulation induction medicines as a result of which they conceived or not conceived.

	Group A	Group B
Total patients	29.7±2.9	29.5±3.81
	(23-37)	(23-36)
Conceived	26.8±0.45	27.7±0.32
	(23-31)	(23-30)
Not conceived	31.4±0.54***	32.6±0.42***
	(28-37)	(29-36)

Conceived vs not conceived P<0.001***
(n) age range of patients

Table 2b: Mean age and age range (years) of the conceived patients at the time of presentation in Group A and Group B who were divided into a, b, c and d classes according to their pattern of conception

Conceived Patients

Classes	a	b	c	d
Group A	-	28.1±0.32	29.5±0.54	30.2±0.63*
	-	(23-29)	(28-31)	(29-31)
Group B	24.2±0.33	27.4±0.45	29.3±0.64	29.7±0.38***
	(23-25)	(26-28)	(28-30)	(28-30)

Class b vs Class d in Group A P<0.05*
Class a vs Class d in Group B P<0.001***
Values are given as Mean ± SEM
(n)= age range of patients
Class a Patients –those who conceived with glucophage therapy alone.
Class b Patients –those who conceived with combination treatment of glucophage and Clomiphene citrate 50 mg.
Class c Patients –those who conceived with combination treatment of glucophage and Clomiphene citrate 100 mg
Class d Patients –those who conceived with combination treatment of glucophage, Clomiphene citrate 100 mg and Human menopausal gonadotrophin.

Primary and Secondary infertility

Primary Infertility

Total number of Primary infertile patients was 174. Of these 89 (57.7%) gave consent to take glucophage treatment for three months and 85 (64.4%) patients agreed to take glucophage treatment for six months. Patients in Group A and Group B were treated with ovulation induction medicine (Clomiphene citrate; injection human menopausal gonadotrophin HMG) in combination with glucophage. The results of conception after these treatments are given in Table-3a. In Group A only 25 (28.08%) primary infertile patients conceived but in Group B, 64 (50.5%) patients conceived. This showed that higher number of patients in Group B conceived significantly ($P<0.001$) compared to Group A. A mirror image of this was observed in the case of patients who did not conceive that 64 (71.9%) patients in Group A and 42 (49.4%) patients in Group B showed highly significantly ($P<0.001$) lesser number of patients who did not conceive in Group B compared to Group A.

Secondary Infertility

Total number of Secondary infertile patients was 114. Of these 65 (42.2%) gave consent to take glucophage treatment for three months and 49 (36.5%) patients agreed to take glucophage treatment for six months (Table-3a). In Group A only 31 (47.6%) patients conceived but in Group B, 43 (87.7%) patients conceived. Patients in Group B conceived significantly higher ($P=0.000$) in number compared to Group A.The number of patients who did not conceive was 34 (52.3%) in Group A and 6 (12.3%) in Group B, which was highly significantly ($P=0.000$) lesser number of patients who did not conceive in Group B compared to Group A.

Table 3a: Number and percentage of Primary and Secondary infertile patients conceived / not conceived after treatment with Glucophage for three months in Group A and six months in Group B.

	Primary infertility n=174		Secondary infertility n=114	
	Group A	Group B	Group A	Group B
Total -number	89	85	65	49
%	57.7	64.4	42.2	36.5
Conceived-number	25	64	31	43
%	28.08	50.5***	47.6	87.7***
Not-conceived number	64	42	34	6
%	71.9	49.48***	52.3	12.3***

Conceived and Not- Conceived patients Group A vs Group B

Primary infertility P<0.001***

Secondary infertility P<0.001***

Classification of Group A and B (a, b, c and d)

The Primary and secondary infertile polycystic patients in Group A (who took glucophage initially for three months) and Group B (who took glucophage initially for six months) were classified into class a, b, c and d according to their pattern of conception as given in Table 3-b.

Primary Infertility

In **class a** of Group A no conception occurred while in Group B 4 (4.7%) conceptions occurred, which indicated that six months of treatment is more beneficial as compared to three months treatment in terms of conception.

In **class b** of Group A out of 89 patients, 6 (6.74%) patients conceived and out of 81 patients, 7 (8.64%) patients conceived in Group B.

In **class c** of Group A out of 83 patients, 10 (10.84%) patients conceived and out of 74 patients, 11 (14.6%) conceptions occurred in Group B.

Class d patients responded the best in terms of conception to this treatment regime as out of 74 patients, 10 (13.51%) conceptions were in Group A and out of 63 patients, 21 (33.3%) conceptions were in Group B.

Secondary infertility

In **class a** of Group A no conception occurred and out of 64 patients, 15 (30.6%) conceptions took place in Group B. This showed that six months treatment with glucophage resulted in higher number of conceptions indicating this treatment was beneficial.

In **class b** out of 65 patients, 18 (27.69%) conceptions occurred in Group A and out of 34 patients, 20 (58.8%) conceptions were in Group B. Maximum number of conceptions took place with this treatment regime .

In **class c** of Group A, out of 47 patients, 8 (17.02%) conceptions and in Group B 5(35.71%) conceptions occurred out of 14 patients.

In **class d**, 5 out of 39 patients (1.28%) conceived in Group A and out of 9 patients, 3 (6.12%) conceptions were observed in Group B.

Table 3b: Number of Primary and Secondary infertile patients conceived in classes a, b, c and d after treatment with Glucophage for three months (Group A) and six months (Group B) and further treatment with Ovulation Induction Medicines (Clomiphene Citrate and Human mnopausal gonadotrophin).

Conceived Patients	Primary Infertility				Secondary Infertility			
Classes	a	b	c	d	a	b	c	d
Group A								
number	-	6	9	10	-	18	8	5
%	-	(6.74)	(10.84)	(13.51)	-	(27.6)	(17.02)	(1.28)
Group B								
number	4	7	11	21	15	20	5	3
%	(4.7)	(8.64)	(14.8)	(33.3)	(30.6)	(58.8)	(35.7)	(33.3)

Class a Patients –those who conceived with glucophage therapy alone.
Class b Patients –those who conceived with combination treatment of glucophage and Clomiphene citrate 50 mg.
Class c Patients –those who conceived with combination treatment of glucophage and Clomiphene citrate 100 mg
Class d Patients –those who conceived with combination treatment of glucophage, Clomiphene citrate 100 mg and Human menopausal gonadotrophin.

Chapter 3.2

Anthropometric parameters: Group A

Patients: Conceived in Group A

Mean (±SE) of anthropometric parameters before and after glucophage therapy administered for three months in conceived patients is given in Table 4. Number of patients conceived was fifty six.

Waist hip ratio before glucophage treatment was 0.87 ± 0.01cm but after three months treatment with glucophage mean waist-hip ratio (0.82 ± 0.01cm) decreased highly significantly ($P<0.001$). Similarly, Subscapularis skin fold also decreased significantly ($P<0.05$), with three months glucophage treatment (51.8±0.43mm) compared to before treatment (53.2+0.44mm). There was no appreciable difference in BMI.

Patients: Not Conceived in Group A

Mean (±SE) of anthropometric parameters before the start of glucophage therapy and after the glucophage therapy given for three months in patients who did not conceive is given in Table 4. Number of patients who did not conceive was ninety eight.

Mean waist to hip ratio before glucophage treatment was 0.87 ± 0.01cm but after three months treatment with glucophage mean waist-hip ratio (0.82 ± 0.01cm) decreased highly significantly ($P<0.001$). Compared to before treatment there was no appreciable difference in Subscapularis Skin Fold thickness and BMI ($P>0.05$).

Table 4. Anthropometric parameters in conceived and not conceived polycystic patients before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for three months in Group A.

Group A Patients	conceived patients n=56		not-conceived patients n=98	
Anthropometric parameters	Pretreatment (Baseline)	Posttreatment (after 3 months)	Pretreatment (Baseline)	Posttreatment (after 3 months)
Waist-Hip ratio (cm)	0.87±0.01	0.82± 0.01***	0.88+0.01	0.83+ 0.009***
Subscapularis skin fold thickness (mm)	53.2± 0.44	51.86±0.43 *	52.5± 0.43	51.47±0.35
BMI (Kg/m^2)	33.9±0.70	32.9 ±0.66	30.7±0.42	30.8 ±0.48

Values are given as Mean ± SEM

*P<0.05
**P<0.01
***P<0.001

Biochemical Parameters: Group A

The biochemical parameters were Fasting Glucose, Insulin, Glucose Insulin ratio, QUICKI, Leptin, Systolic Blood Pressure, Diastolic Blood Pressure, serum Cholesterol, FSH, LH, Estradiol, Progesterone, Prolactin and Testosterone.

Patients: Conceived in Group A

Mean (±SE) of biochemical parameters before the start of glucophage therapy (pretreatment) and after the glucophage therapy (post treatment) given for three months in patients who conceived is given in Table-5. Number of patients conceived was fifty six. Mean fasting Blood Glucose, Insulin, Systolic Blood Pressure, Diastolic Blood Pressure, serum Cholesterol, LH and Testosterone levels decreased highly significantly ($P<0.001$) after treatment with glucophage for a period of three months. However, Glucose Insulin ratio, QUICKI, Mean Fasting Leptin , Serum FSH , Estrogen ,Progesterone and Prolactin levels showed no significant ($P>0.05$) difference after treatment with glucophage for three months.

Patients: Not Conceived in Group A

Mean (±SE) of biochemical parameters before the start of glucophage therapy and after the glucophage therapy given for three months in patients not conceived is given in Table 5. The number of patients was ninety eight.

Mean fasting Blood Glucose, Systolic Blood Pressure, Diastolic Blood Pressure and Serum LH levels decreased highly significantly ($P<0.001$), however serum Insulin levels decreased significantly ($P<0.05$) after treatment with glucophage for a period of three months. Mean serum Leptin, Cholesterol, FSH, Estrogen, Progesterone, Prolactin and Testosterone levels showed no significant difference ($P>0.05$) between before and after treatment with glucophage for three months.

Table 5. Biochemical parameters in conceived and not conceived polycystic patients before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for three months in Group A.

GROUP A	connceived patients n=56		not conceived patients n=98	
Biochemical parameters	Pretreatment Baseline	Posttreatment after 3 months	Pretreatment Baseline	Posttreatment after 3 months
Blood Glucose(mg/dl)	123.4 ± 1.04	112.0 ± 1.10 ***	124.0± 0.96	114.4±1.23 ***
Insulin(μIU/ml)	30.9 ±0.71	28.4±0.53 ***	30.4± 0.55	28.9 ± 0.47 *
Glucose Insulin ratio	3.99±0.12	4.18± 0.09	3.97+ 0.09	4.15+ 0.11
QUICKI	0.27±0.004	0.28±0.003	0.28±0.005	0.28±0.002
Leptin(ng/ml)	17.8 ± 0.65	16.5 ± 0.48	17.1±0.54	16.8 ± 0.44
B.P Systolic(mmHg)	135.1±1.14	131.3±0.97 **	135.3±0.66	129.1± 1.08 ***
B.P Diastolic(mmHg)	85.4±0.88	81.9±0.81 **	83.6± 0.66	80.2±0.55 ***
Cholestrol(mg/dl)	173.0±3.6	160.5± 2.7**	164.2± 3.1	160.4±2.8
FSH(mIU/ml)	11.0 ± 0.39	10.8± 0.37	11.0±0.30	11.1±0.27
LH(mIU/ml)	12.8± 0.30	11.9±0.26 ***	12.3±0.19	10.4±0.31***
Estradiol(pg/ml)	147±5.83	153.6±5.6	144.9±4.56	154.6±4.23
Progestrone(ng/ml)	0.83± 0.03	0.87±0.03	0.84± 0.024	0.85± 0.02
Prolactin(ng/ml)	11.7±0.36	11.8±0.35	11.9±0.24	11.6± 0.23
Testosterone(ng/ml)	2.11±0.09	1.45±0.05 ***	2.08±0.07	2.15±0.13

Values are given as Mean ± SEM
*P<0.05
**P<0.01
***P<0.001

Patients Conceived vs. not conceived in Group A

Mean Waist to hip ratio after treatment with glucophage for three months in patients who conceived was (0.82± 0.01cm) and in those who did not conceive was (0.71±0.009 cm) as given in Table 4. This showed that patients who did not conceive had highly significant decrease in this ratio compared to patients who conceived ($t_{(152}$=5.78; P<0.0005).

BMI after treatment showed significant decrease ($t_{(152)}$ = 1.84; P<0.05) in those who did not conceive (30.7±0.42 kg/m^2) compared to those who conceived (32.9±0.66 kg/m^2)vas given in Table-4.

Serum LH after treatment showed highly significant decrease in level ($t_{(152)}$ =2.631; P<0.005) in patients who did not conceive (10.4±0.31 mIU/ml) compared to patients who conceived (11.9±0.26 mIU/ml) as given in Table 5.

Serum testosterone after treatment in patients who did not conceive (2.15±0.13 ng/ml) showed highly significant decrease in level ($t_{(152)}$ =3.89; P<0.0005) as compared to those who conceived (1.45±0.05 ng/ml) as given in Table-5.

Anthropometric parameters: Group B

Patients: Conceived in Group B

Mean (±SE) of anthropometric parameters before the start of glucophage therapy and after the glucophage therapy given for six months in patients who conceived is given in Table 6. Number of patients conceived was eighty six.

Waist hip ratio before glucophage treatment was (0.87 ± 0.01cm) but after six months treatment with glucophage mean waist-hip ratio (0.75 ± 0.01cm) decreased highly significantly ($P<0.001$) . Similarly, Subscapularis skin fold also decreased highly significantly ($P<0.001$) with six months glucophage treatment (50.5±0.45mm) compared to before treatment (52.8+0.42mm). Basal metabolic index (BMI) before (33.1±0.59kg/m^2) and after treatment (31.2±0.53kg/m^2) showed highly significant decrease ($P < 0.001$) after glucophage treatment

Patients: Not- conceived in Group B

Mean (±SE) of anthropometric parameters before the start of glucophage therapy and after the glucophage therapy given for six months in patients who did not conceive is given in Table 6. The number of patients was forty eight.

Mean waist to hip ratio before glucophage treatment was 0.89 ± 0.01cm but after six months treatment with glucophage mean waist-hip ratio (0.81 ± 0.01cm) decreased highly significantly ($P<0.001$). Similarly, Subscapularis skin fold also decreased hi ghly significantly ($P<0.001$) with six months glucophage treatment (50.9±0.47mm) compared to before treatment (53.5±0.53mm). Compared to before treatment there was no appreciable difference in BMI.

Table 6.The Anthropometric parameters in conceived and not conceived polycystic patients before the start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for six months in Group B.

Group B Patients	conceived patients n=86		not-conceived patients n=48	
Anthropometric parameters	Pretreatment (Baseline)	Posttreatment (after 3 months)	Pretreatment (Baseline)	Posttreatment (after 3 months)
Waist-Hip ratio (cm)	0.87±0.01	0.75± 0.01***	0.89±0.01	0.81 ±0.01***
Subscapularis skin fold thickness (mm)	52.8± 0.42	50.5±0.45 ***	53.5± 0.53	50.9± 0.47 ***
BMI (Kg/m^2)	33.1±0.59	31.2± 0.53 ***	31.4±0.56	30.5±0.55

Values are given as Mean ± SEM

***P<0.001

Biochemical parameters: Group B

Patients: Conceived in Group B

Mean (±SE) of biochemical parameters before the start of glucophage therapy and after the glucophage therapy given for six months in patients who conceived is given in Table 7. Number of patients conceived was eighty six.

Mean fasting Blood Glucose, Insulin, Systolic Blood Pressure, Diastolic Blood Pressure, Cholesterol, Serum LH and Testosterone levels decreased highly significantly ($P<0.001$) however Fasting Leptin level decreased significantly ($P<0.05$) after treatment with glucophage. QUICKI and Estradiol levels increased highly significantly ($P<0.001$) and Glucose insulin ratio increased significantly ($P<0.05$) after treatment with glucophage. Other parameters such as Mean Serum FSH, Progesterone and Prolactin levels showed no significant difference ($P>0.05$) after treatment with glucophage.

Patients: Not- Conceived in Group B

Mean (±SE) of biochemical parameters before the start of glucophage therapy and after the glucophage therapy given for six months in patients who did not conceive is given in Table 7. Number of patients not- conceived was forty eight.

Mean fasting Blood Glucose, Systolic Blood Pressure, Diastolic Blood Pressure, serum Insulin and LH levels decreased highly significantly ($P<0.001$) however Serum Cholesterol level decreased significantly ($P<0.05$) after treatment with glucophage for a period of six months. Glucose Insulin ratio, QUICKI, serum Leptin, FSH, Estradiol, Progesterone, Prolactin and Testosterone levels showed no significant difference ($P>0.05$) after treatment with glucophage.

Table7. Biochemical parameters in conceived and not conceived polycystic patients before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for six months in Group B.

GROUP B	connceived patients n=86		not conceived patients n=48	
Biochemical parameters	Pretreatment Baseline	Posttreatment after 6 months	Pretreatment Baseline	Posttreatment after 6 months
Blood Glucose(mg/dl)	120.7 ± 0.73	104.6±0.89 ***	121.1± 0.83	114.8±1.23 ***
Insulin(μIU/ml)	31.0 ±0.61	26.8±0.69 ***	30.6± 0.75	29.2± 0.68 **
Glucose Insulin ratio	3.97±0.09	4.41± 0.13 *	3.99±0.12	4.18± 0.09
QUICKI	0.27±0.001	0.29±0.002 ***	0.27±0.004	0.28±0.003
Leptin(ng/ml)	17.5± 0.56	15.8 ±0.48 *	17.2 ±0.72	16.8 ±0.68
B.P Systolic(mmHg)	135.6±0.77	116.9±0.79 ***	135.9± 1.00	130.8±0.79 ***
B.P Diastolic(mmHg)	84.8±0.74	76.8±0.59 ***	85.1±0.97	79.3±0.58 ***
Cholestrol(mg/dl)	173.2±2.98	157.2± 1.98 ***	165.4± 4.93	153.1±3.18 *
FSH(mIU/ml)	11.1 ± 0.33	10.7± 0.25	11.2 ±0.43	10.6±0.37
LH(mIU/ml)	12.6± 0.20	7.3±0.22 ***	12.6±0.30	8.41±0.39 ***
Estradiol(pg/ml)	141.0±4.98	170.4±3.5 ***	149.7±6.54	158.7±5.29
Progestrone(ng/ml)	0.85± 0.02	0.84±0.024	0.86± 0.03	0.86± 0.03
Prolactin(ng/ml)	11.8±0.26	11.7±0.26	11.4±0.38	11.5± 0.37
Testosterone(ng/ml)	2.07±0.07	0.70±0.06 ***	2.03±0.10	2.56±2.56

Values are given as Mean ± SEM
*P<0.05
**P<0.01
***P<0.001

Patients Conceived vs. Not conceived in Group B

Anthropometric parameters (given in Table-6)

Mean Waist to hip ratio after treatment with glucophage for six months in patients who conceived was (0.75± 0.01cm) and in those who did not conceive was (0.81±0.01 cm). This showed that patients who conceived had highly significant decrease in this ratio compared to patients who did not conceive ($t_{(132}$=3.0; P<0.001).

Biochemical parameters (given in Table-7)

Mean Fasting blood glucose after treatment in patients who conceived (104.6±0.89 mg/dl) and those who did not conceive (114.8±1.23 mg/dl) showed significant decrease in level in patients who conceived ($t_{(132)}$=4.81; P<0.001).

Mean Fasting Insulin after treatment in patients who conceived (26.8±0.69 µIU/mg) and those who did not conceive (29.2±0.68 µIU/mg) showed highly significant decrease in level in the former in those patients who conceived($t_{(132)}$=1.75;P <0.05).

Mean Systolic Blood Pressure after treatment in patients who conceived was116.9±0.79 mmHg and showed highly significant decrease in level after treatment with glucophage ($t_{(132)}$ =8.79; P<0.0005) compared to those who did not conceive (130.8± 0.79 mmHg).

Mean Serum LH also showed significant decrease in level ($t_{(132)}$ =1.80; P<0.05) in conceived patients (7.3±0.22 mIU/ml) compared to those who didnot conceive (8.41±0.39 mIU/ml).

Group A versus Group B

Anthropometric and Biochemical measurements (given in Tables 4, 5, 6 and 7)

Patients conceived in Group A and B

The number of patients conceived in group A was fifty six and number of patients conceived in Group B was eighty six. After treatment with glucophage in Group A for three months and for six months in Group B, anthropometric and biochemical parameters were compared. Patients in Group B showed highly significant difference in some variables compared to Group A. A highly significant decrease was found in levels of fasting blood Glucose ($t_{(140)}$=3.71; P<0.0005); Serum LH levels ($t_{(140)}$=9.58; P<0.0005) and testosterone levels ($t_{(140)}$=6.816; P<0.0005) in Group B Patients compared to Group A Patients. Significant increase in Serum Estradiol levels was observed in Group B patients compared to Group A patients ($t_{(140)}$=1.84; P<0.05).

CONCEIVED PATIENTS : DISTRIBUTION INTO CLASSES

The conceived patients of Group A and B were further subdivided into classes a, b, c and d. The Anthropometric and Biochemical parameters were studied in these patients before and after three months treatment with glucophage (Group A) as given in Figure 1 and before and after six months treatment with glucophage (Group B) as given in Figure 2.

GROUP A

1. Anthropometric parameters

Anthropometric parameters of patients treated for three months with Glucophage in class, b, c and d is given in Table 8.

Class a : No patient in this group as no conception took place in this group after three months glucophage therapy.

Class b : Patients conceived on combination treatment of glucophage and clomiphene citrate 50 mg after initial 3 months glucophage therapy.

Mean Waist hip ratio, Subscapularis skin fold thickness and BMI showed no significant difference ($P>0.05$) in pre and post treatment with glucophage.

Class c : Patients conceived on combination treatment of glucophage and clomiphene citrate 100 mg after initial 3 months glucophage therapy.

Mean Waist to hip ratio, Subscapularis skin fold thickness and BMI showed no significant difference ($P>0.05$) between before and after treatment with glucophage.

Class d : Patients conceived on combination treatment of glucophage and clomiphene citrate 100 mg and HMG after initial 3 months glucophage therapy.

Mean Waist to hip ratio, Subscapularis skin fold thickness and BMI showed no significant difference ($P>0.05$) in pre and post treatment with glucophage.

Table 8. Anthropometric parameters in conceived polycystic patients of classes a, b, c and d before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for three months in Group A.

GROUP A	class b n= 11		class c n= 22		class d n=23	
ANTHROPOMETRIC PARAMETERS	Pre treatment (baseline)	Post Treatment (after 3months)	Pre treatment (baseline)	Post Treatment (after 3 months)	Pre treatment (baseline)	Post treatment (after 3 months)
Waist to hip ratio (cm)	0.90± 0.02	0.84± 0.02	0.85± 0.02	0.83± 0.02	0.87± 0.02	0.79±0.01**
subscaapularis skin fold thickness(mm)	53.2± 1.09	52.1± 0.99	52.7± 0.72	51.4± 0.67	53.3± 0.66	33.7±0.72
BMI (Kg/m2)	31.3± 0.89	29.7± 0.93	33.8± 1.3	33.6±1.2	35.3± 0.90	33.7±0.93

Values are given as Mean ± SEM
*P<0.05
**P<0.01
***P<0.001
Class a: no patients

GROUP A

2. Biochemical parameters

Biochemical parameters of patients treated for three months with Glucophage in class b, c and d is given in Table 9.

class a

There is no patient in this class.

class b

Mean Fasting Blood Glucose showed highly significant decrease ($P<0.001$) and Serum FSH showed significant rise ($P<0.05$) in levels after treatment with glucophage. All other parameters showed no significant difference in levels after treatment with glucophage for three months.

class c

Mean Fasting Blood Glucose showed highly significant decrease ($P<0.001$) in levels and systolic blood pressure showed significant decrease ($P<0.05$) after treatment with glucophage for three months. All other parameters showed no significant difference ($P>0.05$) after treatment with glucophage for three months.

class d

Mean Fasting Blood Glucose showed highly significant ($P<0.001$) decrease in level, however Systolic and Diastolic Blood Pressure showed significant decrease in level ($P<0.05$) after treatment with glucophage for three months. No significant difference ($P>0.05$) was observed in all other parameters after treatment with glucophage for three months.

Table 9. Biochemical parameters in conceived polycystic patients of class a, b, c and d before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for three months in Group A.

GROUP A	class b n= 11		class c n= 22		class d n=23	
BIOCHEMICAL PARAMETERS	Pre treatment (baseline)	Post Treatment (after 3 months)	Pre treatment (baseline)	Post Treatment (after 3 months)	Pre treatment (baseline)	Post treatment (after 3 months)
Blood Glucose (mg/dl)	117.1 + 1.41	105.3+2.57***	123.2 ± 1.7	113.3 ± 1.57***	126.6 ± 1.49	114±1.58 ***
Insulin (µlU/ml)	30.8 + 1.51	29.4+ 1.29	32.0± 1.23	31.0± 1.03	29.9±1.05	29.7± 1.0
Glucose Insulin ratio	3.84+ 0.03	3.96+ 0.12	3.84+ 0.03	3.96+ 0.12	4.23+ 0.09	4.12+ 0.08
QUICKI	0.27±0.006	0.28±0.003	0.278±0.006	0.28±0.003	0.30±0.008	0.29±0.007
Leptin (ng/ml)	20.8± 0.56	19.9± 0.77	17.7±1.11	17.6± 0.88	16.4±1.07	16.5± 0.84
B.P Systolic (mmHg)	130± 2.13	126.6 ± 2.0	136.1± 1.7	131.3 ± 1.4 *	136.7± 1.8	133.4 ± 1.5
B.P Diastolic (mmHg)	81.3 6± 1.1	79.09± 1.13	86.1±1.4	81.9±1.13 *	86.8±1.4	83.3±1.29
Cholestrol (mg/dl)	156.6± 9.6	144.4+ 6.9	174.2± 5.6	166.3± 3.9	179.7±4.89	162.7±3.63
FSH (mIU/ml)	9.87 ± 3.3	11.4+ 2.2 *	11.5 ±0.6	10.5± 0.6	11.03±0.66	10.8±0.64
LH (mIU/ml)	10.9 ± 1.24	10.3 ± 1.42	13.7 ± 0.4	11.1 ±0.5	12.8± 0.46	12.03±0.42
Estradiol (pg/ml)	152±4.98	146.9±3.5	150.4±9.1	164.7±9.4	141.3±10.2	146.2 ±4.8
Progestrone (ng/ml)	0.91 ± 0.02	0.90 ±0.024	0.83 ± 0.05	0.84 ±0.05	0.77±0.05	0.85±0.05
Prolactin (ng/ml)	12.48± 10.02	12.46± 7.15	10.9± 0.6	11.1±0.6	12.03±0.47	12.1±0.50
Testosterone (ng/ml)	2.46± 0.75	2.15± 0.61	2.03± 0.11	2.26± 0.14	2.03±0.14	2.1±0.14

class a- there is no patient in this class.

Values are given as Mean ± SEM
*P<0.05
**P<0.01
***P<0.001

Group B

1) Anthropometric parameters

Anthropometric parameters of patients treated for six months with Glucophage in class a,b,c and d is given in Table 10.

class a

Nineteen patients conceived. Mean Waist to hip ratio showed significant decrease ($P<0.001$) after treatment with glucophage. Subscapularis skin fold thickness and BMI showed no significant difference ($P>0.05$) after treatment with glucophage.

class b

Mean Waist to hip ratio ($P=0.0087$) and Subscapularis skin fold thickness ($P=0.0425$) showed significant decrease in levels after treatment with glucophage. BMI ($P=0.4335$) showed non significant difference after treatment with glucophage.

class c

Mean Waist to hip ratio decreased highly significantly ($P<0.001$) however, Subscapularis skin fold thickness and BMI showed significant decrease ($P<0.05$) in levels after treatment with glucophage.

class d

Mean Waist to hip ratio a nd Subscapularis skin fold thickness showed significant decrease in levels. BMI showed non significant difference after treatment

Table 10. Anthropometric parameters in conceived polycystic patients of class a, b. c and d before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for six months in Group B

GROUP B	class a n= 19		class b n= 11		class c n= 27		class d n=24	
ANTHROPOMETRIC PARAMETERS	Pre treatment (baseline)	Post treatment (after 6 months)	Pre treatment (baseline)	Post treatment (after 6 months)	Pre treatment (baseline)	Post treatment (after 6months)	Pre treatment (baseline)	Post treatment (after 6 months)
Waist to hip ratio (cm)	0.90± 0.02	0.82± 0.01***	0.86± 0.01	0.77± 0.0***	0.89± 0.01	0.77± 0.02***	0.87±0.02	0.71±0.02***
subscaapularis skin fold thickness(mm)	53.2± 0.44	51.86± 0.43	52.7± 0.73	50.9± 0.47*	53.9± 0.77	51.27±0.60**	50.8± 0.80	47.8±0.80
BMI (Kg/m2)	33.9±0.70	32.9± 0.66	32.6± 1.17	31.5±0.92	35.6± 0.96	33.0±0.90*	30.3± 0.99	28.0±0.90

Values are given as Mean ± SEM
*P<0.05
**P<0.01
***P<0.001

GROUP B

2) Biochemical parameters

Biochemical parameters of patients treated for six months with Glucophage in class a, b, c and d is given in Table 11.

class a

Mean fasting Blood Glucose, Insulin, Systolic Blood Pressure, Diastolic Blood Pressure , Serum LH and Testosterone showed highly significant decrease ($P<0.001$) in levels after treatment with glucophage . Serum Leptin and C holesterol levels also showed significant decrease ($P<0.05$). Serum Estradiol levels increased highly significantly ($P<0.001$) after treatment with glucophage. However, Glucose Insulin ratio; QUICKI; Serum FSH, Progesterone and Prolactin levels showed no significant difference ($P>0.05$) after treatment with glucophage .

class b

Mean Fasting Blood Glucose, Insulin, Systolic Blood Pressure, Diastolic Blood Pressure, Serum LH and Testosterone levels showed highly significant decrease ($P<0.001$) after treatment with glucophage for a period of six months. Serum Estradiol levels increased highly significantly after treatment with glucophage. ($P<0.001$). Fasting Leptin and Cholesterol levels showed significant decrease($P<0.05$). However, Glucose Insulin ratio, QUICKI , F asting Leptin , Serum FSH, Progesterone and Prolactin levels showed no significant difference ($P>0.05$) after treatment with glucophage .

class c

Mean fasting Blood Glucose, Systolic Blood Pressure, Diastolic Blood Pressure , Serum LH and Testosterone levels showed highly significant decrease ($P<0.001$) and Cholesterol showed significant decrease ($P<0.05$) in level after treatment with glucophage for a period of six months. Serum Estradiol levels increased highly significantly ($P<0.001$), however Glucose Insulin ratio increased significantly ($P<0.05$) after treatment with glucophage. Serum Insulin, QUICKI, Leptin, FSH, Progesterone and Prolactin levels showed no significant difference ($P>0.05$) after treatment with glucophage.

class d

Mean fasting Blood Glucose, Insulin, Systolic Blood Pressure, Diastolic Blood Pressure, Serum LH and Testosterone levels showed highly significant decrease ($P<0.001$) after treatment with glucophage for a period of six months. Serum Estradiol levels increased highly significantly

Table 11. **Biochemical parameters in conceived polycystic patients of class a, b. c and d before start of Glucophage therapy (Pretreatment) and after giving Glucophage (Post treatment) for six months in Group B**

GROUP B	class a n= 19		class b n= 11		class c n= 27		class d n=24	
BIOCHEMICAL PARAMETERS	Pre treatment (baseline)	Post treatment (after 6 months)	Pre treatment (baseline)	Post treatment (after 6 months)	Pre treatment (baseline)	Post treatment (after 6months)	Pre treatment (baseline)	Post treatment (after 6 months)
Blood Glucose (mg/dl)	118±1.03	98.8±1.67 ***	123.5± 1.2	105.7±1.7 ***	117.3± 1.13	105.8±1.79 ***	126.6 ± 1.49	114±1.58 ***
Insulin (μIU/ml)	31.3 ± 1.37	24.6± 1.16 ***	30.7 ± 1.1	25.1± 1.6 ***	30.2 ±1.36	27.7±1.34	29.9±1.05	29.7± 1.0
Glucose Insulin ratio	3.86+ 0.07	4.01+ 0.09	4.02+ 0.09	4.2+ 0.09	3.87+ 0.09	4.01+ 0.08 *	4.23+ 0.09	4.12+ 0.08
QUICKI	0.28±0.006	0.29±0.007	0.28±0.005	0.29±0.006	0.28±0.006	0.29±0.002	0.30±0.008	0.29±0.007
Leptin (ng/ml)	17.0 ± 1.25	13.6 ± 0.91 *	17.3 ± 1.0	15.0± 0.82	18.2± 1.0	17.2±1.0	16.4±1.07	16.5± 0.84
B.P Systolic (mmHg)	135.1±1.14	131.3±0.97 ***	135.5± 1.07	116.7±1.42 ***	137.1±1.64	118.7±1.54 ***	136.7± 1.8	133.4 ± 1.5
B.P Diastolic (mmHg)	85.4±0.88	81.9±0.81 ***	85.2 ± 1.337	7.7 ± 1.04 ***	84.0±1.7	77.3 ±1.43 ***	86.8±1.4	83.3±1.29
Cholestrol (mg/dl)	173.0±3.6	160.5± 2.7 **	171.1± 5.5	154.4±3.18 **	179.6±5.5	161.8±4.2 **	179.7±4.89	162.7±3.63
FSH (mIU/ml)	11.0 ± 0.39	10.8+ 0.37	11.0± 0.55	11.2 ± 0.49	10.5 ±0.65	10.9±0.55	11.03±0.66	10.8±0.64
LH (mIU/ml)	12.8± 0.30	11.9±0.26 ***	12.6± 0.29	6.9 ±0.40 ***	12.9±0.46	6.7±0.38 ***	12.8± 0.46	12.03±0.42
Estradiol (pg/ml)	147±5.83	153.6±5.6	134.6±8.4	171.7±6.3 ***	138.7±12	181.8±5.8 ***	141.3±10.2	146.2 ±4.8
Progestrone (ng/ml)	0.83± 0.03	0.87±0.03	0.84± 0.05	0.83±0.05	0.86±0.05	0.82 ±0.04	0.77±0.05	0.85±0.05
Prolactin (ng/ml)	11.7±0.36	11.8±0.35	12.2±0.4	12.2± 0.4	11.7±0.73	11.1± 0.69	12.03±0.47	12.1±0.50
Testosterone (ng/ml)	2.11±0.09	1.45±0.05 **	2.2±0.12	0.53±0.04 **	2.18±0.15	0.62± 0.05 ***	2.03±0.14	2.1±0.14

Values are given as Mean ± SEM
*P<0.05
**P<0.01
***P<0.001

(P<0.001) after treatment with glucophage for six months. Other parameters showed non significant difference (P>0.05) after treatment with glucophage.

Comparison of Anthropometric and Biochemical parameters of three months treatment (Group A) with six months treatment (Group B) in different classes is given in Table 10 and 11.

Group A vs. Group B

class b

The number of patients conceived in group A was eleven and number of patients conceived in Group B was twenty seven. After treatment with glucophage in Group A for three months and six months in Group B, mean anthropometric and biochemical parameters were compared. Patients in Group B after treatment with glucophage showed highly significant difference in some variables compared to Group A. A highly significant decrease was found in levels of Waist to Hip ratio ($t_{(36)}$=1.75;P=<0.05); Systolic Blood Pressure ($t_{(36)}$=2.89;P=<0.005); Serum LH levels ($t_{(36)}$=1.86;P=<0.05) and Serum Testosterone levels ($t_{(36)}$=2.49;P=<0.01). Highly significant increase in serum Estradiol levels was observed in Group B patients compared to Group A patients ($t_{(36)}$=2.53; P=<0.01).

class c

The number of patients conceived in group A was twenty two and number of patients conceived in Group B was sixteen. Patients in Group B after treatment with glucophage showed highly significant difference in some variables compared to Group A. A highly significant decrease was found in levels of Fasting Blood Glucose ($t_{(36)}$=2.23;P=<0.02); Systolic Blood Pressure ($t_{(36)}$=4.28;P=<0.0005); Diastolic Blood Pressure ($t_{(36)}$=1.79;P=<0.05); Serum LH levels ($t_{(36)}$=5;P=<0.0005) and Serum Testosterone levels ($t_{(36)}$=8.31;P=<0.0005).

class d

The number of patients conceived in group A was twenty three and number of patients conceived in Group B was twenty four. Patients in Group B after treatment with glucophage showed highly significant difference in some variables compared to Group A. A highly significant decrease was found in levels of Waist to Hip ratio ($t_{(45)}$=3;P=<0.002); Subscapularis Skin Fold thickness ($t_{(45)}$=2.44;P=<0.01); BMI ($t_{(45)}$=2.66;P=<0.005); Systolic Blood Pressure ($t_{(45)}$=5.16;P=<0.0005);

Diastolic Blood Pressure ($t_{(45)}$=3.45;P=<0.0005); Serum LH levels ($t_{(45)}$=4.53;P=<0.0005) and Serum Testosterone levels ($t_{(45)}$=8.44;P=<0.0005).

Chapter 3.3

Conceptions in Group A and B

The total number of patients in Group A and B were 154 and 134 respectively as given in Table 12 a. The total number of patients conceived in Group A was 56 (36.36%) and those who did not conceive were 98(63.6%). In Group B the total number of patients conceived was 86(64.18%) and those who did not conceive were 48(35.82%). The overall conception in Group A (30.36%) are highly significantly low (p=0.0041) compared to those in Group B.

In **class a** of Group A there was no conception out of 154 patients after three months of treatment with glucophage but in Group B 19 patients out of 134 patients (22.09%) conceived with glucophage after six months of treatment (table 12 b). Group A and Group B patients were given treatment with a combination of Glucophage and Clomiphene citrate 50 mg in **class b** as a result of which 11 patients out of 154 patients (7.14%) from Group A and 27 patients out of 115 patients (23.4%) from Group B conceived. In **class c** the remaining patients were treated with Glucophage and Clomiphene citrate 100 mg combination resulting in conception of 22 patients out of 143 patients (15.38%) in Group A and 16 patients out of 88 patients (18.1%) in Group B. Lastly in **class d** treatment of Glucophage, Clomiphene citrate 100 mg and Human Menopausal Gonadotrophins combination was given to patients who had not yet conceived with previous treatment. In Group A 23 patients out of 121 patients (19.0%) and in group B 24 patients out of 72 patients (33.3%) conceived with this treatment.

Outcome of pregnancy in Group A

The outcome of pregnancy in the form of live births among patients who conceived after initial treatment of these patients with Glucophage for three months and later on treatment with ovulation induction medicines is given in Table 13. Group A was divided into group I and group II .Patients in group I continued with Glucophage treatment throughout their pregnancy period (their number was 31). Patients in group II did not give consent to continue with glucophage treatment during pregnancy (their number was 25). Conception rate in group I and group II did not show any

Table 12a: The total number and percentage of patients in Group A (patients who took glucophage for three months initially) and Group B (patients who took glucophage for six months initially) and later were given ovulation induction medicine as a result of which they conceived or did not conceive.

		Group A	Group B
Total		154	134
Conceived	n	56	86
	%	(36.36)	(64.18)***
Not conceived	n	98	48
	%	(63.6)	(35.82)***

Conceived and Not-conceived patients of Group A vs Group B P<0.001***

Table 12b: The number and percentage of conceived patients in different classes of Group A (who were given glucophage for three months) and Group B (who were given glucophage for six months) and were given further combination treatment of Glucophage and Ovulation Induction Medicines.

Conceived Patients				
Classes	a	b	c	d
Group A				
n	0	11	22	23
%	(0)	(7.14)	(15.38)	(19.0)
Group B				
n	19	27	16	24
%	(14.17)	(23.4)	(18.1)	(33.3)

(n) = number of patients

significant difference. Live births in group I were 100% as compared to 91.3% in group II. Significantly higher single births rate was found in group I than in group II ($P<0.05$). There was no significant difference in multiple births in group I and II. There was not a single case of abortion and stillbirths in group I, but 20 % abortions and 8.6 % stillbirths were observed in group II patients. Abortions were significantly higher in group II ($P<0.05$) but stillbirths were not significantly higher ($P>0.05$) in group II. Post natal deaths ($P<0.05$) and preterm delivery rate was significantly higher in Group II ($P<0.05$). There was no significant difference in threatened abortions and post term delivery in Group I and II.

Outcome of Pregnancy in Group B

The outcome of pregnancy in the form of live births among patients who conceived after initial treatment of these patients with Glucophage for six months and later on treatment with ovulation induction medicine is given in Table 14. Group B was divided into group I and group II .Patients in group I continued with Glucophage treatment throughout their pregnancy period (their number was 34) Patients in group II did not give consent to continue with glucophage treatment during pregnancy (their number was 52). Conception rate did not show significant difference in group I and II. Live birth rate was significantly higher in group I than group II ($P=0.044$). Significantly higher single births were found in group I than in group II ($P=0.027$). There was no significant difference in multiple births in group I and II. There was not a single case of abortion and stillbirths in group I, but 19.2% abortions and 9.62% stillbirths were observed in group II patients. Abortions and still births were significantly higher in group II ($P=0.007$ and 0.062). Threatened abortions were significantly higher in group II, 11.5% ($P=0.043$) while none of the case occurred in group I. Preterm delivery ($P=0.104$) and post term delivery ($P=0.108$) when compared in group I and II showed no significant results.

Comparison of outcome of pregnancy in Group A and B

When comparison was made of outcome of pregnancy in Group A and B no significant difference was found in all the parameters.

Table 13: Parameters representing outcome of pregnancy in patients conceived after giving initial Glucophage therapy for three months (Group A) and six months (Group B) and further treatment with Glucophage and Ovulation Induction Medicines (Clomiphene Citrate and Human Menopausal Gonadotrophin) in Group I patients who continued glucophage during their pregnancy duration and in Group II who did not continue glucophage during their pregnancy duration.

	Group A		Group B	
Parameters	Group I	group II	group I	group II
Conceptions	31	25	34	52
conception rate	55.35	44.64	39.50	60.40
Total births	33	23	35	45
Live births	33	21	35	40
Live birth rate	100	91.3	100	88.8 *
Single birth	29	15	33	34
Single birth rate	87.87	65.2 *	94.2	75.5 *
Multiple births	2	3	1	3
Multiple birth rate	6.45	13.04	2.85	6.6
Abortions	-	5	-	10
Abortion rate	-	20	-	19.24
Still births. n (%)	-	2 (8.6)	-	5 (9.62)
Post natal deaths. n (%)	-	4 (17.3)	1(2.85)	4(8.8)
Threatened abortions. n (%)	3(9.67)	6(24)	-	6(11.53)
Preterm delivery. n (%)	1(3.22)	6(24) *	1(2.85)	6(13.3)
Post term delivery. n (%)	1(3.22)	-	2 (5.71)	-

n= number of patients
(%) = percentage of patients
*P<0.05
**P<0.01
***P<0.001

CHAPTER-4

DISCUSSION

Polycystic ovarian infertile patients were studied before treatment and after treatment with Glucophage (Metformin hydrochloride) for three months and six months. Later on these patients were treated with ovulation induction medicines (Clomiphene citrate and Human Menopausal Gonadotrophins).The effects of treatment with glucophage were also looked at outcome of pregnancy and Anthropometric and Biochemical parameters.

AGE

Treatment with glucophage resulted in conceptions of comparatively younger women (27.09±1.0 years) than older women (29.04±0.66 years).Younger patients after six months treatment with glucophage conceived even w ithout taking ovulation induction medicines. Imani et al (1999) observed that those who conceived were younger (27±4 years) compared with clomiphene citrate failure or those who did not conceive (29±4 years). This study revealed that predictive power of age was highest. Fauji et al (1997) showed no effect of age on ovulation or the treatment cycles. There was no difference of age in the subjects who conceived after ovulation induction with clomiphene citrate and those who conceived spontaneously. Taylor and Braude (1994) stated that if the female partner is young (<30 years) the response to clomiphene citrate is good. In this study PCO patients of younger age (24.2±0.33 years) who were given six months treatment with glucophage, resulted in conceptions, but older patients (29.5±0.54 years) after three months and (29.3±0.64 years) after six months treatment with glucophage needed ovulation induction with increasing dose of clomiphene citrate for conception. Similarly much older patients (30.2±0.63 years) after three months and (29.7±0.38 years) after six months treatment with glucophage also needed induction with human menopausal gonadotrophins. Rafique (2004, unpublished),concluded that older patients required higher dose of clomiphene citrate i.e 100 mg compared to young patients who conceived with 50 mg clomiphene citrate .

PRIMARY AND SECONDARY INFERTILITY

This study data comprised higher percentage (60.4%) of primary infertile patients compared to secondary infertile patients (39.5%).WHO (2001) also reported 60-80% polycystic patients were of primary infertility in most of the countries.

Maheshwari et al (2008) also observed that polycystic primary infertile patients were in a higher percentage (51.4%) compared to secondary infertile patients.

In primary infertile patients of this study prolonged treatment with glucophage(6 months) resulted in conceptions but not w ith short term treatment(3 months).However glucophage in combination with clomiphene citrate (50 mg and 100 mg dose) and HMG injected in 3 months group successfully conceived. Those who were treated with these combinations for six months showed significantly higher conceptions than in three months treatment particularly when HMG was added to glucophage and clomiphene citrate.

A similar picture was seen with secondary infertile patients but highly significant conceptions were observed when these patients were treated with glucophage in combination with clomphene (50 mg dose) in six months treatment group. This study suggested that both primary infertile and secondary infertile patients would conceive in significantly higher number if treatment be given for a longer period with combination of medicines (glucophage+clomiphene+HMG).

ANTHROPOMETRIC PARAMETERS

Anthropometric parameters of infertile PCOS studied by various scientists and this study are given in table 14.

In this study patients conceived with glucophage for three months treatment showed no significant change in BMI, but significant decrease in BMI was seen in those who were treated for six months. Diamanti-Kandarakis (1999) reported obesity in PCOS patients treated with glucophage for three months and six months. Ng et al (2001) in Chinese PCOS patients and Gennarelli et al (2000) in Italian PCOS patients showed low values for BMI. Fleming et al (2002) s howed significant decrease in BMI in U.K. P COS patients who were treated with metformin for 14 weeks. Pasquali et al (2000) in Italian PCOS patients treated with metformin for six months showed significant decrease in BMI. Similar results with metformin treatment were also reported by Harborne et al (2005). Statistically not significant decrease in BMI was seen by Zafar (2006) with glucophage/metformin treatment and by Palomba et al(2007) in PCOS patients treated with metformin or glucophage.

Significant reduction in waist-hip ratio in conceived PCOS women was observed in this study after three and six months treatment with glucophage.

Table 14: DIfferent studies in which Glucophage was used in PCOS patients.

STUDY	Location/ YEAR	Dose of glucophage	MEAN AGE (YRS)	TYPE OF PATIENTS/ number	DURATION OF TREATMENT	Anthropometric parameters	Biochemical parameters	Endocrine parameters
PRESENT STUDY GP A=who took glucophage for three months Gp B=who took glucophage for six months conceived patients	Pakistan /2008	1500 mg/day	GP A 26.8±0.45 GP B 27.7±0.32	INFERTILE PCOS/142	GP A=three months Gp B= six months	GP A GP B Waist:hip ratio =cm 0.87±0.01 B 0.87±0.01 B 0.82±0.01 P 0.75±0.01P Subscapularis skin fold thickness =mm 53.32±0.44 B 52.8±0.42 B 51.86±0.43 P 50.5±0.45 P BMI=Kg/m2 33.9±0.70 B 33.1±0.59 B 32.9±0.66 P 31.2±0.53 P	GP A GP B Glucose fasting =mg/dl 123.4±1.0 B 120.7±1.7B 112.0±1.1 P 104.6±0.8 P Insulin=ulu/ml 30.9±0.71 B 31.0 ±0.6B 28.4±0.53 P 26.8±0.69 P Leptin=ng/ml 17.8±0.65 B 17.5±0.5B 16.5±0.48 P 15.8 ±0.48P	GP A GP B LH=ulu/ml 12.8±0.30 B 12.6±0.20B 11.9±0.26 P 7.3±0.22 P Estradiol pg/ml 147.0±5.8 B 141.0±4.9B 153.6±5.6 P 170.4±3.5P Testosterone ng/ml 2.11±0.09 B 2.07±0.07B 1.45±0.05 P 0.70±0.06P
Palomba et al	Italy / 2007	1700 mg/day	24.3±3.1	INFERTILE PCOS/14	24 months	Waist hip ratio=cm 0.69±0.1 B BMI= Kg/m 22.24±2.7 B No significant p	Fasting glucose (mg/dl) 84.7 ± 9.0B Fasting insulin (uU/ml) 14.6 ± 5.3B No significant p -	LH=mIU/ml 16.1 ±4.4B E2 (pg/ml) 40.8 ± 4.8B T (ng/ml) 0.7 ± 0.5B 1.5 _ 0.5P
Eisenhardt et al	Germany /2006	1500 mg/day	27.0	PCOS/22	3 MONTHS	BMI 28.9 (23.3–34.1) B 31.1 (22.9–34.2)P	Fasting glucose (mg/dl) 83.0 (78–90) B 83.0 (72–94) P Fasting insulin (uU/ml) 20.0 (14–28) B 20.0 (15–26)P	LH (IU/liter) 7.1 (4.7–9.9) B 8.3 (3.6–16.0)P T (nmol/liter) 1.59 (1.14–2.31)) B 1.59 (1.04–2.07)P Estradiol (pmol/liter) 121.1 (99.5–146.8) B 223.9 (130.3–365.3)P
Fleming et al	UK /2002	1700 mg/day	28.6	INFERTILE PCOS/23	14 weeks	BMI=kg/m2 35.2B 34.6P Waist hip ratio=cm 0.88B 0.88P	Fasting glucose (nmol/liter) 188B 204P Fasting insulin (mIU/liter) 16.8B 16.4P Leptin (ng/ml) 41.1 B 37.3P	E2 (pmol/liter) 142B 226P T (nmol/liter) 3.1 B 3.5P

STUDY	Location/ YEAR	Dose of glucophage	MEAN AGE (YRS)	TYPE OF PATIENTS/number	DURATION OF TREATMENT	Anthropometric parameters	Biochemical parameters	Endocrine parameters
Jakubowicz et al	Virginia /2001	1500 mg/day	27 ±1	PCOS/26	4 weeks	BMI= kg/m2 31.8 ±0.3 B 31.8 ±0.3P Waist Hip Ratio 0.84 ±0.1B 0.84 ±0.2P	Fasting glucose (mmol/L) 4.9 ±0.1B 4.3 ±0.2P Fasting insulin (pmol/L) 206 ±27B 79 ±14P	Testosterone (nmol/L) 339±6.35B 133 ±16P
Pasquali et al	Italy / 2000	1700 mg/day	30.8 ±6 7.4	PCOS/20	7 months	BMI (Kg/m2) 39.8± 7.9B 36.4 ± 7.4P. Waist HipRatio 0.87 ± 0.07B 0.86 ± 0.07P	Glucose, fasting (mg/dL) 99 ± 29B 90 ± 17P Insulin, fasting (mU/mL) 43.0 ± 30.4B 21.6 ± 31.2P	LH (mIU/mL) 8.45 ±3.44 B 7.37 6 3.87 P P (ng/mL) 0.59 ± 0.23B 3.51 6 5.08P T (ng/mL) 0.68 ± 0.35B 0.49 ± 0.25P

B- Baseline values

P-Post treatment values

There is no significant difference between baseline and post treatment values where P is not given

The data of the Present study is of conceived patients (n=142) out of total 288 polycystic infertile patients

In studies by Pasquali et al (2000) PCOS women were given metformin 850 mg for six months duration which caused non significant decrease of waist: hip ratio. No effects on waist: hip ratio was observed by Jakubowies et al (2001) after four weeks treatment of PCOS patients with glucophage and study by Fleming et al (2002) in PCOS patients after 14 wks treatment of metformin. Palomba et al (2007) who studied the effect of Metformin or Glucophage in serial fashion at different time interval after six months, twelve months, then at eighteen and again at twenty four months and found initial decrease but later no significant decrease in levels.

The mean baseline value of subscapularis skin fold thickness in this study was high (50-53 mm) while Gennarelli et al (2000) in Italy in 72 women with PCOS found that mean subscapularis skin fold thickness was lower (27.2 mm). The high value indicated insulin resistance as subcutaneous truncal abdominal fat is highly correlated with insulin resistance (Ross et al, 1996).Subscapularis skin fold thickness >50 mm indicates insulin resistance Gennarelli et al (2000).

In this study after three months treatment with glucophage the values decreased significantly in conceived patients while this decrease was not significant in not conceived patients. After six months treatment subscapularis skin fold thickness decreased highly significantly both in conceived and not conceived patients which indicated that longer duration of treatment is highly effective .

Waist circumference is predictor of visceral fat and indicator of insulin resistance (Hartz et al, 1984).Waist girth and Subscapularis skin fold measure two different types of fats visceral and subcutaneous truncal fat (Bouchard et al, 1993) both types being independently associated with insulin resistance (Ross et al,1996).

FASTING GLUCOSE, INSULIN, GLUCOSE INSULIN RATIO (G: I ratio), QUICKI

In this study the patients who conceived with glucophage treatment for three months and six months there was decrease in mean fasting blood glucose and fasting insulin.The patients who were treated for six months with glucophage showed significant increase in QUICKI and glucose insulin ratio

Jakubowicz et al (2001) after four weeks of treatment of polycystic patients (n=26) with 500mg Glucophage 3 times daily resulted in significant decrease in serum insulin concentrations from 206 $\pm$ 27 pmol/L to 79 $\pm$ 14 pmol/L ($P < 0.001$). Fasting serum glucose concentrations decreased from 4.9 $\pm$ 0.1 mmol/L to 4.3 $\pm$ 0.2 mmol/L ($P = 0.004$).

Zafar (2006) in a study of 22 polycystic infertile women after six months treatment with Glucophage recorded decrease in fasting serum insulin from 23.6 micro U/ ml to 20.2 micro U/ml (P=0.00). On the other hand Eisenhardt et al,(2006) studied women with PCOS (n = 45), aged 21–36 yr, different parameters were checked at baseline, after four weeks, eight weeks and twelve weeks. There was no difference in the levels of fasting Glucose mg/dl from 83.0 baseline levels to 81.5 after twelve weeks. Fasting Insulin levels (uU/ml) also showed no significant difference from baseline levels (20.0) to at twelfth weeks (20.0). Similarly Fasting Glucose/Insulin ratio did not show any significant change from baseline levels (4.0) to after twelve weeks (4.4) of treatment with Metformin. QUICKI showed no significant change from baseline to post treatment values.

Tang et al (2006) in U.K in ninety four PCOS patients used Metformin tablets 850 mg twice daily from the start of downregulation process until the day of oocyte collection underwent 101 consecutive IVF/ICSI cycles. There were no significant changes in Fasting Serum Glucose levels between baseline and at the day of oocyte retrievel. However metformin significantly reduced the fasting insulin levels after four weeks of medication ($p<0.05$)

SERUM FASTING LEPTIN

In this study serum Leptin levels decreased significantly in those conceived patients who were treated for six months with glucophage, but there was no decrease in serum leptin in patients who were treated for three months with glucophage.

Similarly Pasquali et al (2000) in Italy studied a group of 20 women with PCOS who were given metformin 850 mg twice daily) for 6 months. They significantly ($p<0.05$) decreased their leptin concentrations from 42 ng/ml to 30 ng/ml. Fleming et al (2002) also showed that after 14-wk treatment of PCOS patients (n=45), the circulating Leptin concentration declined in the metformin-treated group from 41.1ng/ml to 37.3 ng/ml ($P< 0.05$).

In this study the mean values were less than as given by Fleming et al (2002) (17.5 vs 37.3). Astonishingly in this study we found insulin levels which were high compared to other studies as described previously, however leptin levels were lower. This can be explained as obesity in PCOS is characterized by an increase in visceral fat (Bjorntorp, 1996) i.e. an increase in the type of fat that relatively underscretes leptin compared with subcutaneous fat.

These results may be explained by the presence of a PCOS-specific form of Insulin Resistance in adipocytes, which impairs the simulatory effect of insulin on leptin secretion (Ciaraldi et al.,

1997). This conclusion is consistent with the negative correlation of serum leptin concentrations with insulin sensitivity in both slim and obese women with PCOS reported by Micic et al. (1997). Study by Ng et al, 2001 in Chinese PCOS women also showed that fasting leptin (μg/l) after three months treatment with Glucophage decreased significantly from 10.2 μg/l to 7.9 μg/l ($P<0.01$)

REPRODUCTIVE HORMONES

Endocrine parameters of infertile PCOS studied by various scientists and this study are given in Table 14.

This study showed that patients who conceived after three months treatment with glucophage had highly significantly decreased serum LH and testosterone levels but there was no significant difference in serum FSH, estrogen, progesterone and prolactin.The patients conceived with six months treatment with glucophage also had highly significantly decreased serum LH and testosterone,but showed highly significant increase in estradiol levels, but no effect on level of FSH, estrogen, progesterone and prolactin.

Fleming et al(2002) showed significant decrease in testosterone levels after 14 weeks treatment of PCOS women with metformin .On the other hand Ng et al (2001) in Chinese, Unlühizarci et al (2000) in Turkish,Holte et al (1999) in Sweedish, Tang et al (2006) in British PCOS women treated with metformin for twelve weeks did not show any change in serum FSH and LH levels. Pasquali et al (2000) treated PCOS Italian women with metformin (850 mg dosage twice a day)for six months,but there was no change in serum LH,FSH and progesterone levels. However they observed that significant decrease in testosterone levels, but significant increase in estradiol levels as was seen in this study. Eisenhardt et al,(2006) studied women with PCOS (n = 22), aged 21–36 yr,treated with metformin. their different parameters were checked at baseline, after four weeks, eight weeks and twelve weeks. Testosterone levels (nmol/lite) showed no significant difference ($P>0.05$) from baseline at twelfth week. In contrast, basal LH (IU/liter) slightly increased in the first 2 months of the study from baseline levels and declined again at twelfth week. Basal FSH and stimulated LH and FSH levels were unaffected by metformin. Estradiol levels (pmol/liter) showed highly significant rise at twelfth week ($P = 0.005$).

BLOOD PRESSURE AND CHOLESTROL

PCOS women who conceived after three months and six months treatment with glucophage showed highly significant decrease in systolic and diastolic blood pressure and serum cholesterol levels.

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CONCEPTIONS

In this study the overall conceptions after three months treatment with glucophage were highly significantly low (p=0.0041) compared to those who were given glucophage for six months. This showed that initially these patients who were given 6 months treatment with glucophage responded significantly in terms of conception than those who took glucophage treatment only for three months. This suggests that initial duration of treatment is highly effective for conception. Clomiphene citrate and Human menopausal gonadotrophins in combination with glucophage has proved a better medicine for the treatment of infertility in PCOS. Glucophage/Metformin dosage was 500 mg three times daily. Clomiphene citrate maximal dosage was 100mg daily for 5 days. Combination of clomiphene with glucophage and combination of HMG with glucophage resulted in conceptions of those patients who did not conceive with glucophage alone. This also depends on the age of the patient as younger patients responded to initial (lesser dose combination) medication while older patients required higher dose of combination treatment.

Different authors using glucophage/metformin and clomiphene got different results in their studies Legro et al (2007) in the study concluded that clomiphene (dosage 150 mg/day for 5 days) resulted in significantly greater live birth rates than metformin. The authors concluded that clomiphene is superior to metformin as first-line therapy for infertility in women with PCOS. Ward et al (2003), were of the opinion that metformin is superior to clomiphene to achieve a singleton gestation for all pregnancies but with clomiphene (50 mg/day) they observed multiple pregnancies with significant neonatal and maternal morbidity, and neonatal mortality. Palomba et al (2007) administered metformin at a dose of 850 mg twice daily and clomiphene was initiated at a standard dose of 50 mg daily for 5 d with increases up to 250 mg daily for 5 d if ovulation did not occur. They concluded that n on-obese women with PCOS and infertility treated with metformin had greater live birth rate than those treated with clomiphene (58 vs. 19%) and 6-month clomiphene or metformin treatment resulted in cumulative pregnancy rates of 49 and 63%, respectively (P= 0.2).

Combination treatment of glucophage and clomiphene citrate is also supported in a study by Malkawi and Qublan (2002) in Jordan, where twenty-eight clomiphene citrate-resistant polycystic ovary syndrome women received metformin, 850mg twice daily throughout the cycle along with 50 mg clomiphene citrate for 5 days. They observed statistically significant increase in the rates of ovulation (68.6% versus 25%, $p<0.05$) and pregnancy (56.3% versus 16.6%, $p<0.05$).

The addition of metformin to clomiphene in clomiphene-resistant anovulation is recognised as a valuable treatment option before starting with exogenous gonadotrophins (Nestler et al, 1998 and Lord et al, 2003). Less is known about the addition of metformin during gonadotrophin induction of ovulation. Different studies have described co-administration of metformin with gonadotrophin induction of ovulation in normogonadotrophic anovulatory patients (De Leo et al, 1999 and Yarali et al, 2002). De Leo et al (1999) designed experiments to evaluate whether pretreatment with metformin improves FSH induced ovulation in women with clomiphene resistant polycystic ovary syndrome. They concluded that by reducing hyperinsulinism it favours orderly follicular growth and the androgens and E2 serum concentrations were significantly lower in cycles with metformin co-treatment.

Yarali et al (2002) selected 32 PCOS patients with normal glucose tolerance and clomiphene-resistant anovulation. These patients were treated with metformin for 6-week pretreatment period. All anovulatory patients were treated with FSH using a low-dose step-up protocol as given by (White et al,1996). There was no significant difference in ovarian response but significantly lower serum androgens (free testosterone) were described after metformin treatment.

Van-Santbrink et al (2005) in Rotterdam, Netherland added metformin to gonadotrophin ovulation induction in insulin-resistant, normo-gonadotrophic, anovulatory women, resulting in three pregnancies (one early miscarriage and two ongoing singleton pregnancies). Although metformin treatment did not result in a (significant) decrease of insulin resistance, it did cause a significant decrease in androgen serum concentrations and improved the endogenous gonadotrophin–oestrogen balance, as was demonstrated in the former studies of DeLeo et al (1999) and Yarali et al (2002). In conclusion, these results suggest that metformin may improve the endocrine profile (by decreasing hyperandrogenaemia) and, in that way, facilitates monofollicular development during gonadotrophin ovulation induction.

In this s tudy the group of patients who were given glucophage for three months and those conceived, did not show any significant difference in conception rate compared

to those who continued glucophage during the pregnanacy or those who did not continue. Significantly higher single birth rate was found in patients who continued glucophage during their pregnancy. Abortions,postnatal deaths and preterm delivery rate was significantly higher in patients who did not continue glucophage during the pregnancy duration.

The group of patients who were given glucophage for six months and those who conceived, live birth rate and single births were significantly higher in patients who continued glucophage during pregnancy duration. Abortions, still births and threatened abortions were higher in patients who did not continue gucophage during the pregnancy.

Different authors using metformin reported less pregnancy complications. Jakubowicz et al,(2002) in Venezuella treated 96 nondiabetic women with the polycystic ovary syndrome with metformin, those who became pregnant continued metformin at a dose of 1000–2000 mg daily. In all women who used Metformin the Early Pregnancy Loss rate was less (8.8%)while in control group it was 41.9% ,($P<0.001$).

The findings support the hypothesis that decreasing hyperinsulinemic insulin resistance, with metformin, in women with the polycystic ovary syndrome, decreases the rate of early pregnancy loss. In contrast, the rate of early pregnancy loss of 8.8% in the women treated with metformin is similar to the rate of 10–15% reported for clinically recognized pregnancies in normal women as described by Gray and Wu (2000) and Regan et al (1989). Without Glucophage or Metformin spontaneous abortion is common occurs in 44% pregnancies (Glueck et al, 1999), 55% (Moghetti et al, 2000), 39% (Glueck et al, 2001) and 25% (Wang et al, 2001). Glueck et al (2002) compared 72 women with PCOS who conceived on metformin (2.55 g/day) and 100 women who conceived without taking metformin. 84 fetuses of the women treated with metformin there were 63(75%) normal live births and 14 (17%) first trimester spontaneous abortions. 100 fetuses of women who did not take metformin had 34(34%) live births and 62(62%) spontaneous abortions. L ike the present study they concluded that metformin therapy during pregnancy in women with PCOS was associated with reduction in spontaneous abortions.

CONCLUSION

It was concluded from the study that

1. Comparatively younger PCOS women responded to glucophage treatment in terms of conception more than the older infertile women.
2. Primary infertile women conceived with prolonged treatment (6 months) of glucophage or combination of glucophage with clomiphene and HMG. The same was observed with secondary infertile women.
3. Significant reduction in anthropometric parameters was observed such as BMI, waist hip ratio and subscapularis skin fold thickness.
4. Significant reduction in biochemical parameters was observed such as fasting glucose, insulin, leptin, blood pressure, cholesterol etc.
5. Treatment with glucophage significantly decreased serum LH and testosterone.
6. The overall conception after six months treatment with glucophage were highly significantly more compared to those who were given glucophage for three months.
7. Live birth rate and single births were significantly higher in patients who continued glucophage during pregnancy duration. Abortions, still births and threatened abortions were higher in patients who did not continue gucophage during the pregnancy.

CHAPTER-5

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