THE CORELLATION BETWEEN

ANTHROPOMETRY AND INFERTILITY IN WOMEN

A THESIS SUBMITTED IN
FULFILLMENT OF THE
REQUIREMENT FOR THE DEGREE
OF

MASTER OF SCIENCE (Clinical Biochemistry)

In the

Department of Molecular Medicine,

School of Medical Sciences

College of Health Sciences

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NOVEMBER, 2011

DECLARATION

I hereby declare that this submission is my own work towards the MSC, and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABBREVIATIONS

17 β HSD	-	17 β – hydroxysteriod dehydrogenase
5 α – DHT	-	5α- dihydrotestosterone
AKT	-	Activated Kinase
AMP	-	Adenosine monophosphate
BMI	-	Body Mass Index
CAH	-	Congenital Adrenal hyperplasia
CCK	-	Cholecystokinin
CD4	-	Cluster Differentiation
CRH	-	Corticotrophin – releasing hormone
CVD	-	Cardiovascular disease
DBP	-	Diastolic blood pressure
DHEAS	-	Dehydroepiandrosterone
DHT	-	Dihydrotestosterone
ELFA	-	Enzyme Linked Fluorescent Assay
FBS	-	Fasting Blood Glucose
FFA	-	Free Fatty Acid
FSH	-	Follicle Stimulating Hormone
GHRH	-	Growth hormone – releasing hormone
GLUT 4	-	Glucose transporter 4
GnRH	-	Gonadotropin – releasing hormone
HA	-	Hyperandrogenism
hcG	-	Human Chorionc gonadotrophin

HDL	-	High Density Lipoprotein
HOMA	-	Homeostasis model assessment
HPO	-	Hypothalamic pituitary ovarian – axis
IGF – I	-	Insulin-like growth factor I
IGFBP – I	-	Insulin like growth factor binding protein – I
IGT	-	Impaired glucose tolerance
IL	-	Interleukin
IR	-	Insulin resistance
IRS – 1	-	Insulin Receptor Substrate 1
IVF	-	In vitro Fertilization
LDL	-	Low Density Lipoprotein
LH	-	Luteinizing Hormone
LHRH	-	Luteinizing releasing hormone
MAPK	-	Mitogen Activated Protein Kinase
mRNA	-	messenger Ribonucleic Acid
MSH	-	Melanocyte Stimulating hormone
NHANES Survey	-	National health and Nutrition Examination
P 450 c17α	-	Cytochrome P450 17 α hydroxylase
P I3 – K	-	Phosphatidylinositol 3 – kinase
PAI – I	-	Plasminogen – activator inhibitor activity
PCOS	-	Polycystic Ovarian Syndrome
PID	-	Pelvic Inflammatory Disease
PIF	-	Prolactin Inhibiting Factor
PRG	-	Progesterone

PRL	-	Prolactin
SEM	-	Standard error of mean
SHBG	-	Sex Hormone Binding Globulin
SPR	-	Solid Phase Receptacle
SSBG	-	Sex steroid binding globulin
StAR	-	Steriodogenic acute regulatory protein
STD's	-	Sexually Transmitted Diseases
T – CHO	-	Total Cholesterol
T2DM	-	Type II Diabetes Mellitus
TAG	-	Triacylglycerides
TNF-α	-	Tumuor necrosis factor α
TRH	-	Thyrotrophic hormone – releasing hormone
TSH	-	hyriod Stimulating Hormone
VLCD	-	Very-Low-calorie diet
VLDL	-	Very Low Density Lipoprotein
WC	-	Waist Circumference
WHO	-	World Health Organization
WHR	-	Waist to Hip ratio

ABSTRACT

Infertility owing to obesity and overweight is a serious problem in Ghana now, affecting a large number of women because of the impact of foreign lifestyles on our societies. Excess weight is not only linked to increased risk of chronic disease and life threatening comorbidities such as diabetes, hypertension and dyslipidemia but has also been shown to increase risk of reproductive problems. A descriptive study was carried out to investigate the correlation between anthropometrics and infertility in women. This study was aimed at investigating the correlation between anthropometric indices such as Body mass index (BMI), waist to hip ratio (WHR) and waist circumference (WC) and infertility in women. In all, one hundred and forty four (144) women (32.5 ± 12.5 years, range 20-45yrs) visiting the Department of Obstetrics and Gynaecology at the Komfo Anokye Teaching Hospital for infertility issues were randomly recruited into the study by a single qualified Obstetrician and Gynaecologist. Ethical clearance was received from Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi. All subjects gave informed consent to take part in the study after verbal and written explanation of the methods and risks involved had been given. Anthropometric measurements (BMI, WHR, HC, and WC) were carried out by qualified personnel using standardized methods and procedures. Venous blood samples were taken for the biochemical assays (Blood Glucose, Total Cholesterol, Triglycerides, HDL-Cholesterol, LDL-Cholesterol and VLDL. Other Hormonal assays including, 21-day Progesterone, Prolactin, Follicle Stimulating Hormone and Luteinizing Hormone were analyzed. According to classifications by BMI, the subjects were categorized into four groups; Normal (BMI 18.5-25 kg/m^2), Overweight (BMI 25-30 kg/m^2), Obese I (BMI 30-35 kg/m^2) and Obese II (BMI 35-40 kg/m^2). The mean BMI was 28.92±4.88. With regards to WHR, the subjects were categorized into three groups; Group I (WHR 0.80 or below) were subjects with Low risk of developing cardiovascular disease (CVD), Group II (WHR 0.81-0.85) were subjects with moderate risk of developing CVD and Group III (WHR > 0.85) were subjects with high risk of developing cardiovascular disease. The mean WC measurement for the subjects was 87.92±10.10. There was significant correlation between most of the anthropometric indices. The correlation coefficients noticed between BMI, WHR, WC, HC and Weight was very significant ($p<0.0001$). The

prevalence of overweight and obesity (using BMI) among the infertile women in this study was 34.72 and 45.14% respectively. Again, using WHR, more of the infertile women were obese with prevalence of 54.17%. The most prevalent duration of infertility in the study was between 1 to 5 years with majority of the subjects (about 62.5% of the total population) in the overweight and obese groups. The longest duration of infertility in the study was between 21 to 25 years. Approximately 28.5, 17.36, 14.6, 9.7, 9.7, 7.02, 6.9 and 4.2% of the participants had the following infertility condition(s) respectively: Tubal factors, Male Factors, Ovulation problems, Unexplained Causes, Uterine problems, Hyperprolactinaemia, PCOS and Endometriosis. Overweight and obesity was highest among the Akans (63.89%), least among the Northerners (7.64%), Ewes (4.17%) and the Gas (4.17%). There was significant positive correlation between fasting blood glucose (FBS) ($p<0.0028$), total cholesterol (T-chol) ($p<0.0003$), triglycerides ($p<0.0014$), LDL-cholesterol ($p<0.00001$) and the Obese II group. This is evident of high prevalence of dysmetabolism such as diabetes, cardiovascular risk factors and dyslipidemia with increasing body weight gains among the infertile women. There was no strong correlation between BMI and the hormonal profiles of the infertile women. Altogether the findings of this study have revealed for purposes of this study, that BMI is a more reliable predictor of dysmetabolism such as diabetes, dyslipidemia and cardiovascular risk factors compared to WHR. Tubal factor infertility was the most prevalent cause of infertility, followed by male factors whereas endometriosis was the least prevalent cause of infertility in the study. This is in agreement with other studies conducted on infertile women, and especially those studies conducted in Africa. Women with excess body weight are more likely to have fertility problems and therefore weight reducing therapies such as exercises and dietary interventions must be incorporated into the treatment of infertility.

ACKNOWLEDGEMENT

A wealth of gratitude is given to God Almighty from whom all good things come for His grace and mercy towards me and for seeing me through the execution of this project work. Without Him this project will not have been successful. Thoughtful recognition and profound appreciation to my supervisor, Dr. W.K.B.A Owiredu whose countless pieces of advice, corrections and words of encouragement were much required for this achievment. Daddy, am so grateful, God richly bless you. I wish to express my intense gratitude to my parents Mr. and Mrs. Ofori for the motivational support, encouragement, prayer and financial support and also for their investment in me that has taken me this far in my educational career. My special thanks to the obstetrician and Gynaecologist Dr. Archer Turpin of the Komfo Anokye Teaching Hospital who gave me permission to collect samples at their facilities. My profound thanks go to the entire staff of the Clinical Biochemistry Department of Komfo Anokye Teaching Hospital for helping me in the collection and analysis of the samples. Special recognition to Mr. Simon Bani Bannison of the Tamale Teaching Hospital for helping me analyse my hormonal samples. I am grateful to all the women seeking infertility treatment who availed themselves to participate in this study. I am highly indebted to them. My sincere thanks go to Dr. Christian Obirikorang for helping me with the statistical analysis and all my colleagues at the Department of Molecular Medicine whose constructive criticism helped in the writing of my thesis. Finally, I want to thank all my friends for their unceasing support.

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CHAPTER ONE

INTRODUCTION

With a rising prevalence of infertility among women and men in Ghana, the problem needs recognition as a public health issue. Even though the causes of infertility are numerous, recent studies have shown that obesity is largely linked to infertility. A large proportion of infertile women have polycystic ovarian syndrome (PCOS) which is also linked with increased risk of obesity and other metabolic anomalies (Acien *et al*, 1999). The association between obesity and/or PCOS and hyperinsulinaemia, hyper androgenism and abnormal secretion of other hormones, such as leptin**,** underlies many reproductive disorders observed among infertile women. Thus, in sub fertile/infertile women with PCOS, overweight or obesity usually is more prevalent (Acien *et al*, 1999). In Ghana, the work by Amoah (2003) in urban and rural Accra showed that the overall crude prevalence of overweight and obesity rate was 23.4 and 14% respectively among adults aged 25 years and above. The rates were higher in females than in males. A study to examine the risk associations between indices of obesity, cardiovascular risk factors and morbidity conditions among Penteco-Charistmatic Ghanaians in Kumasi by Owiredu *et al* (2008), revealed the prevalence of overweight and obesity to be 25.07 and 20.37% respectively. This study also found the prevalence of obesity in women to be nearly twelve times that in males. Another study which gave credence to earlier reports of an increase in the prevalence of overweight and obesity among urban Ghanaians by Agyemang *et al* (2008), who showed that urban Ghanaians had a significantly higher combined prevalence of overweight and obesity, thus 22.0% for men and 50.0% for women, than their rural counterparts with 10.3% for men and 19.0% for women. Again, obesity was found to be higher in women than in men. Although obesity is largely linked to infertility, this study will draw on diverse approaches that have been extensively done linking anthropometric measurements to infertility. The study will also investigate the impact of anthropometric indices such as Body Mass Index (BMI), Waist to Hip Ratio (WHR) and Waist Circumference (WC) on female fertility hormone profile, and their correlation in premenopausal women.

1.1 STUDY AIMS AND SPECIFIC OBJECTIVES

This study seeks to explore the extent to which body measurements (anthropometry) correlates with infertility in women. In particular, the study focuses on anthropometric measurements and its effects on infertility in women.

The specific objectives of this study are to:

- Investigate the correlation between anthropometric indices such as Body mass index (BMI), waist to hip ratio (WHR) and waist circumference (WC) and infertility in women.
- To conduct an observational study on the impact of obesity and overweight on female fertility hormone profile in premenopausal women.
- To investigate the correlation between obesity and infertility in women.

1.1.2 SCOPE OF THE STUDY

The details of the study will cover overweight and obesity risk factor assessments that will lead to infertility for early clinical diagnosis and prevention. Patients' current health status after a gynaecological examination and medical history will be used as source of material for the underlying factors leading to the patients' infertility. Relevant questionnaire will be used to gather data for lifestyles such as smoking and alcohol consumption status and behavioural patterns. Body measurements such as Body Mass Index (BMI), Waist to Hip Ratio (WHR) and Waist Circumference (WC) will be taken. Venous blood samples would be drawn for biochemical determinations such as Follicle Stimulating hormone (FSH), Luteinizing Hormone (LH), Prolactin (PRL) and Progesterone (PRG).

Again, lipid profile including triglycerides (TAG), total cholesterol (T-CHO), high density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels will be determined alongside fasting blood sugar (FBS).

1.1.3 JUSTIFICATION

Obesity and overweight are common conditions in the developed countries and they carry many health consequences, including some reproductive disorders. There is a very high prevalence of obese women in the infertile population and many studies have highlighted the link between obesity and infertility (Robert *et al.*, (2004).

Infertility owing to obesity and overweight is a serious problem in Ghana now, affecting large number of women and causing much suffering because of the impact of foreign lifestyles on our societies. A study to examine the risk associations between indices of obesity, cardiovascular risk factors and morbidity conditions among Penteco-Charistmatic Ghanaians in Kumasi by Owiredu *et al* (2008), revealed the prevalence of overweight and obesity to be 25.07 and 20.37% respectively. This study also found the prevalence of obesity in women to be nearly twelve times that in males. Another study on the 'Epidemiology of Obesity in Ghana' by Biritwum *et al* (2006), revealed that the prevalence of obesity is found to be 5.5% and higher among females 7.4% compared to males 2.8%. It was more common among the married than unmarried. Obesity was highest among the employed compared to self-employed or the not working for pay. Obesity was highest in Greater Accra 16.1% and virtually not present in Upper East or Upper West regions. By ethnicity, obesity was highest among Ga Adangbe, Ewes and Akans 14.6%, 6.6% and 6.0% respectively. With an overall crude prevalence of overweight and obesity rate of 23.4% and 14.1% for females and males respectively (Amoah (2003) in Ghana now, and a decreasing fertility rate of 3.78%, (*CIA world fact Book*) there is the need to study the correlation between anthropometry and infertility in women.

LITERATURE REVIEW

1.2.1 Study Background

Infertility is defined as the inability of a couple to conceive after one year of regularly timed (at least 3 times a week) unprotected inter-course (Tietz, 2006). It is estimated that 25% of couples will experience an episode of infertility during their reproductive life. Primary infertility refers to couples or patients who have had no previous recognized pregnancies. Secondary infertility encompasses patients who have previously conceived, but are currently unable to conceive. It is also termed sub-fertility. Infertility problems often arise as a result of hormonal dysfunction of the hypothalamic-pitiutary-gonadal axis. Measurement of peptide and steroid hormones in the serum is therefore an essential aspect in the evaluation of infertility. The single most important factor in determining fertility is the age of the female partner. Fertility declines sharply after the age of 37 and therefore fertility is significantly halved if the female partner is 35 years or more (Tietz, 2006).

Obesity is rapidly increasing worldwide (Ogden *et al.*, 2006). Obesity results from a chronic imbalance between energy intake and energy expenditure. It is well known that an increase in body weight and fat tissue is associated with several abnormalities of sex steroid balance. Obesity alters important homeostatic factors such as pancreatic secretion of insulin. Hyperinsulinemia and insulin resistance are widely accepted to be involved in the underlying mechanisms linking obesity to multiple metabolic abnormalities and to alteration in steroidogenesis. Such alterations involve both androgens and oestrogens and the overall carrier protein, sex-hormone-binding-globulin (SHBG) (Pasquali, 2003). The net decrease in SHBG concentration observed in obesity, leads to alterations in the availability of free circulating androgens and oestrogens, for delivery to target tissues. In insulin resistance syndrome, excess insulin is capable of stimulating steroidogenesis, excessive androgen production from the theca cells and excessive oestrogen production from the granulosa cells of the ovaries. In addition, by directly inhibiting SHBG synthesis, excess insulin may further increase the delivery of free androgens to target tissues.

The excess in local ovarian steroidogenesis induced by excess circulating insulin may cause premature follicular atresia and then favour anovulation (Pasquali, 2003).

Excess weight is not only linked to increased risk of chronic disease (Must *et al.*, 1999), but has also been shown to increase risk of reproductive problems (Catalano, 2007). Several studies have shown that women with excess body weight are more likely to have fertility problems (Pasquali, 2006; Gesink Law *et al.*, 2007). The adverse effects may be reversible with weight loss (Ramlau-Hansen *et al.*, 2007). It is less clear whether men also experience reduced fertility with excess weight. A higher frequency of women diagnosed with disorders of menstruation, infertility, diabetes mellitus in pregnancy and other significant sequelae are either overweight or obese (Sharpe and Franks, 2002). In addition, polycystic ovarian syndrome (PCOS), a condition characterized by hyperandrogenism and menstrual disturbances, further complicates the issue (Norman *et al*., 2002). The relative importance of PCOS status and overweight/obesity in this group of women is yet to be fully understood, although increasing evidence suggests that Body Mass Index (BMI), Waist to Hip Ratio (WHR) and Waist Circumference (WC) contributes significantly towards the severity of many problems, such as the risk of miscarriage (Wang *et al*., 2002). Understanding the dynamics in glucose metabolism and consequently modified androgen secretion in overweight/obese women with PCOS is the key for assessing the link between obesity and the risk of various reproductive disorders (infertility) in this group of women.

The causes of infertility among couples are numerous and are distributed evenly among male and female factors (Pilgrim, 2001). The causes of infertility include tubal factors, endometriosis, male factors, anovulation, unexplained infertility, luteal phase defect, cervical factors and uterine factors (Sharara & McClamrock, 2000). One of the most common causes of infertility in women is pelvic inflammatory disease (PID). Half of the most frequently reported infections are sexually transmitted diseases (STD's), including the most common Chlamydia (Pilgrim, 2001). In general, tubal disease is most common in developing countries and in poor social groups where medical services are not readily available (Willocks & Neilson, 1991). An intensive work by (Willocks & Neilson, 1991) gave the main variable of infertility as the incidence of tubal occlusion, caused by either ascending or post-pregnancy infection. The issue of infertility should not be seen solely as a medical condition but the psychological and social aspect of it requires equal considerations.

Counselling forms an essential part of infertility management. Couples with infertility problems tend to suffer from low self- esteem (Stuart Campbell & Ash Monga, 2005).

Therefore the psycho-social consequences of infertility which includes stress, anxiety, depression and marital difficulties must be duly considered. The effective management of infertility will, therefore, have considerable impact on reproductive health in Africa (Dyer *et al.*, 2002).

1.2.2. Overweight and Obesity

The World Health Organization (WHO) predicts that overweight and obesity may soon replace more traditional public health concerns such as under nutrition and infectious diseases as the most significant cause of poor health (Seidell, 1995). This prediction has become necessary because as of 2005 the WHO estimates that at least 400 million adults (9.8%) are obese, with higher rates among women than men (WHO, 2000). Obesity has become such a serious and prevalent condition constituting economic burden in the developed countries (Kopelman, 2000) and is on a gradual ascendancy in the developing countries. Obesity is a condition in which excess body fat has accumulated to an extent that health may be negatively affected (Seidell, 1995). Obesity is commonly defined as a body mass index (BMI) of 30 kg/m^2 or higher (Seidell, 1995). This definition distinguishes obesity from being pre-obese or overweight, which is classified as a BMI of 25 kg/m^2 but less than 30 kg/m$^{2.}$ Overweight and obesity is associated with various diseases, particularly cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, and osteoarthritis (Yamashita *et al.*, 1996; Bjorntorp, 1991).

In absolute terms, obesity is an increase of body adipose (fat tissue) mass (Seidell, 1995). The most common clinical methods used to estimate obesity are by body mass index (BMI) and in terms of its distribution via the waist–hip ratio (Bjorntorp, 1991). BMI is calculated by dividing the subject's mass by the square of his or her height, typically expressed in metric units. Although BMI is a useful clinical tool that correlates well with adiposity, it does not distinguish between lean and fat mass unlike more precise techniques such as underwater weighing, skinfold thickness, magnetic resonance imaging, dual energy X-ray absorptiometry and infrared

spectroscopy. The location of adipose tissue (peripheral or abdominal) adversely affects health. The abdominal adipose tissue consists of visceral (the mesenteric and the greater and lesser omental depots contained within the body cavity surrounding the internal organs and subcutaneous tissue (under the skin).

Abdominal visceral fat correlates more strongly with insulin resistance, metabolic and reproductive fitness than subcutaneous fat (Folson *et al.*, 1993; Zaadstra *et al.*, 1993) although the subcutaneous depot is likely to also contribute to metabolic abnormalities (Miles, 2005). Waist to hip ratio (WHR) or waist circumferences which is measured midway between the lowest rib and the iliac crest provide a reasonable estimate of abdominal fat. In women a WHR of >0.8 or a waist circumference ≥80cm indicate increased risk of obesity associated metabolic complications and ≥88cm indicates substantially increased risk (Seidell, 1995).

1.2.3. OBESITY, INSULIN RESISTANCE AND HYPERINSULINAEMIA

1.2.3.1 Insulin Resistance and Hyperinsulinaemia

The rise in plasma glucose that follows a carbohydrate-containing meal (the glycaemic response), is accompanied by the production of insulin from the beta cells of the pancreas. In a person with normal metabolism, the elevated blood glucose level causes beta (β) cells in the Islets of Langerhans located in the pancreas to release insulin into the blood. The insulin in turn causes insulin-sensitive tissues in the body (e.g., muscle, adipose) to absorb glucose and thereby lower the blood glucose level. The beta cells reduce insulin output as the blood glucose level falls, with the result that blood glucose is maintained at approximately 5 mmol/L (mM) (90 mg/dL) (McGarry, 2002). In an insulin-resistant person, normal levels of insulin do not have the same effect in controlling blood glucose levels. During the compensated phase on insulin resistance insulin levels are higher, and blood glucose levels are still maintained. If compensatory insulin secretion fails, then either fasting (impaired fasting glucose) or postprandial (impaired glucose tolerance) glucose concentrations increase. Eventually, type 2 diabetes occurs when glucose levels become higher throughout the day as the resistance increases and compensatory insulin secretion fails. The elevated insulin levels have additional effects which cause further abnormal biological effects throughout the body (McGarry, 2002).

Insulin resistance is defined as a decreased biological response to normal concentrations of circulating insulin and is found in both obese, non diabetic individuals and patients with type 2 diabetes (Tietz, 2006). The insulin resistance syndrome (also known as syndrome X or the metabolic syndrome) is a constellation of associated clinical and laboratory findings, consisting of insulin resistance, hyperinsulinaemia, obesity, dyslipidaemia (high triglyceride and low HDL cholesterol), and hypertension. The metabolic syndrome is diagnosed if an individual has three or more of the following criteria;

- Abdominal obesity: waist circumference greater than 35 inches in women or 40 inches in men.
- Triglycerides greater than 150 mg/dL
- HDL cholesterol less than 50 mg/dL (women) or less than 40 mg/dL (men)
- Blood pressure greater than or equal to 130/85 mmHg
- Fasting plasma glucose (FBS) greater than or to 110mg/dL (Tietz, 2006).

This incidence of insulin resistance is observed through impaired insulin stimulated glucose uptake at the muscle, adipose tissue and liver; hepatic glucose overproduction and release; increased lipolysis at the adipose tissue and consequent increased circulating free fatty acids (FFA); reduced lipogenesis from circulating triglycerides and impairment of glycogen synthesis (Reaven, 1995; Goldstein, 2002; Kahn, 2000).

1.2.3.1.1 Mechanisms of Insulin Resistance

Insulin resistance occurs due to abnormalities in any of the downstream facets of the insulin signalling pathway (Kahn, 2000) including down regulation of adipose tissue GLUT4 or impaired translocation of skeletal muscle GLUT4 (Sherperd and Khan, 1999) impaired muscle and adipocyte insulin receptor number binding and activity, reductions in expression and action of downstream proteins including IRS - 1 and P13 – K and upregulation of enzyme activity involved in suppressing insulin signalling or inhibitory molecules in the insulin signalling pathway including phosphotyrosine phosphatise, protein kinase C substrates and Rad (Kahn, 2000). At large, the activity of the mitogen activated protein kinase (MAPK) pathway appears to be preserved (Cusi et al., 2000) although some members of the MAPK family

could regulate both the metabolic and mitogenic actions of insulin are dysregulated in T2DM or hyperinsulinaemia (Koistenen *et al.*, 2003; Lee *et al.*, 2003)

1.2.3.1.2 Obesity and Insulin Resistance

Obesity is a very common cause of insulin resistance although not all individual obese patients are significantly insulin resistant. Obesity, specifically abdominal obesity and visceral obesity are important contributors to insulin resistance (Abate *et al.*, 1995). Defining obesity in terms of a central distribution with increased waist: hip ratio makes it a more-accurate marker of resistance. Excessive release of free fatty acids from adipocytes is a feature of obesity and may be greater in central obesity. Increased free fatty acid (FFA) concentrations may reduce skeletal-muscle glucose metabolism and provide a possible explanation for the insulin resistance of obesity. The hyperinsulinaemia observed in obese subjects could be due to enhanced insulin secretion, reduced insulin clearance, or the combination of the two (Bell, 1997). An increase in adipose tissue mass leads to increased FFA release derived from lipolysis of stored adipocyte triacylglycerol. FFA impair insulin mediated glucose uptake in skeletal muscle, adipocytes and the liver, decrease hepatic insulin sensitivity and increase hepatic glucose output through preferential oxidation of FFA over glucose (Boden *et al.*, 1994; Randle *et al.*, 1963). In visceral fat compared to subcutaneous fat, catecholamine induced lipolysis is increased (Rebuff-Scrive *et al.*, 1990) and insulin-mediated suppression of lipolysis reduced, increasing FFA turnover and release which can increase very low density lipoprotein (VLDL) triglyceride and glucose synthesis (Zierath *et al.*, 1998). Differential expression of adrenergic receptors can also contribute to these differences, with visceral adipocytes expressing elevated lipolytic type 1 and 2 β-adrenergic receptors (Hellmer *et al.*, 1992) and reduced levels of anti-lipolytic α2-receptors and reduced inhibition by α2 receptor agonists (Vikman *et al.*, 1996). Adipocytes-secreted proteins (adipokines such as tumor necrosis factor-α or TNF-α, interleukin-6 or IL-6, IL-8, resistin, leptin and adiponectin) and inflammatory cytokines are circulating factors elevated in obesity that can influence IR (Kahn, 2000).

Some researchers report that TNF-α is elevated in obesity and abdominal obesity (Tsigos *et al.*, 1999). IL-6 and IL-8 secretion is elevated in visceral compared to subcutaneous adipocytes and related to insulin resistance (IR) in some reports (Bruun

et al., 2004). It is not clear whether resistin is associated with obesity and IR (Rea *et al*., 2004) and it may play a greater role in immune and inflammatory processes in humans (Haluzik and Haluzikova 2006). An additional proposed insulin sensitizer is adiponectin. Adiponectin administration enhances hepatic insulin action and reduces circulating glucose (Berg *et al*., 2001), of which levels negatively correlate with insulin sensitivity and are reduced in obesity (Arita *et al*., 1999). The aetiology of insulin resistance is highly variable, and it is not clear that any one cause is responsible for a majority of cases. It appears that a genetically determined difference in the response to hyperinsulinemia and insulin resistance determines the features of the syndrome in a given individual. Racial differences may also be important, since the full syndrome is more likely to be expressed in Caucasians. Early in the development of the syndrome, when beta cell function is adequate, insulin resistance is fully compensated for by increased insulin secretion and the result is the hyperinsulinaemic but normoglycaemic state. Beta cell function gradually deteriorates over time, and varying degrees of impaired glucose tolerance will develop into type2 diabetes in most patients (Shahid & Schneider, 2000).

1.2.4 Impact of Obesity on Fertility

The distribution of body fat is clearly related to infertility. Central obesity measured by an increased waist:hip ratio is associated with a lower probability of conception (Wass *et al.*, 1997). Women with a waist:hip ratio of less than 0.8 have a higher pregnancy rate than women with ratios of more than 0.8. Upper body fatness has been found more often in women with polycystic ovarian syndrome (PCOS), as well as other endocrinological and metabolic changes, such as increased concentrations of free and total testosterone, androstenedione, oestradione, insulin, LDL cholesterol, triglycerides and blood glucose. Little is known regarding whether android body fat distribution, independent of obesity or anovulation, is related to fertility (Wass *et al.*, 1997; Crosignani, 2002).

Fertility processes involve a complex of factors and mechanisms of both ovarian and extra ovarian origin. Obesity may interfere with many neuroendocrine and ovarian functions, thereby reducing both ovulatory and fertility rates in otherwise healthy women. Oligo-ovulation, anovulation and subfertility are present in obese females with a relative risk of anovulatory infertility for women with a BMI >27 compared

with women of BMI 20-25. Many obese women have normal ovulatory menstrual cycles, remain fertile and have no apparent hyperandrogenism. However, currently there is substantial evidence to support the relationship between obesity and anovulatory infertility (Norman *et al*., 2002). Obesity during puberty and early adolescence has a strong association with infertility in the future. The mechanisms via which obesity is linked to anovulation remain unclear, and most likely several hormonal changes are involved (Pasquali, 2003). In fact, body fat distribution has been shown to substantially affect SHBG concentrations. Fat accumulation in the abdominal viscera (visceral fat) has been described as a possible cause of insulin resistance and the resulting metabolic syndrome. Female subjects with central obesity and with higher proportion of visceral fat usually have high insulin resistance leading to lower SHBG concentrations in comparison with matched subjects with peripheral obesity. The net decrease in SHBG concentration observed in obesity, leads to alterations in the availability of free circulating androgens and oestrogens, for delivery to target tissues. Due to the greater reduction of SHBG concentration, the percentage free testosterone fraction tends to be higher in women with central obesity than in those with peripheral obesity leading to a state of 'functional hyperandrogenism'. The pattern of body fat distribution can regulate androgen production and metabolism to a significant extent. In fact, women with central obesity have higher testosterone production rates than those with peripheral obesity (Pasquali, 2003).

Approximately half of all women with PCOS are overweight or obese. Polycystic ovarian syndrome is the most common cause of anovulatory infertility in young women and the history of weight gain frequently precedes the onset of clinical manifestations of the syndrome, suggesting a pathogenetic role of obesity in the development of PCOS and the related infertility. Even though the total BMI in non-obese women with PCOS is normal, the intra-abdominal preperitoneal and visceral fat accumulation may contribute to the hormonal dysregulation leading to anovulation (Norman *et al*., 2002).

1.2.4.1 Obesity and Endocrine Abnormalities

Obesity adversely affects the results of fertility therapy (Maelli & Grazi, 2002), and it is associated with hormonal disturbances, decreased sex hormone-binding globulin, elevated serum oestradiol and elevated levels of androgens (Chong *et al.*, 1986). Obese anovulatory women show higher concentrations of oestrone and/or free oestrone than do either ovulatory obese women or women with normal weight. The fact that adipose tissue can act as a steroid reservoir and a site of peripheral conversion of androgens to oestrogen, could account for the greater oestrogen concentrations in obese women than in women of normal weight. However, this does not explain the differences in circulating oestrogen concentrations between weight-matched anovulatory and ovulatory obese women. Weight loss is not accompanied by a fall in serum oestrone concentrations, as one might expect with a reduction in adipose tissue. Mobilization of steroids from the sizeable fat tissue reservoir could be one explanation (Reid & van Vugt, 1987).

Oestrogen augments the release of LH and inhibits the release of FSH, thus leading to an increased LH/FSH ratio. The elevated LH level in turn stimulates androgen secretion by theca cells of the ovary, providing the precursors for continued oestrogen production in adipose tissue. This vicious cycle results in simultaneous occurrence of hyperandrogenism and hyperoestrogenism. Long term acyclic oestrogen exposure may lead to excessive endrometrial growth, resulting initially in oligomenorrhoea interspersed with episodes of menorrhagia. In some women this ultimately leads to the development of endometrial hyperplasia or adenocarcinoma (Reid & van Vugt, 1987). Basal serum LH and FSH are normal in obesity, but nocturnal LH secretion is decreased. Serum FSH or serum LH might be elevated in obese women. In subjects with gonadal dysgenesis, there is an inverse correlation between 24-hour intergrated serum LH levels and total body water to body weight, a ratio that is inversely related to the percentage of body fat. The pre-ovulatory serum FSH rise is sub-normal in obese pre-pubertal girls than in girls of normal weight. Data suggest that amenorrhoea in obesity is not due to primary ovarian failure, which should be associated with elevated serum LH and FSH, but rather to some hypothalamic-pituitary abnormality (Glass *et al.*, 1981). Hyperandrogenism may be etiologically related to amenorrhoea in obesity. Amenorrhoeic subjects have elevated free androgen levels, while obese eumenorrhoeic subjects do not. Therefore, hyperandrogenism is associated with the amenorrhoea of obesity. The

hyperandrogenism is not secondary to the amenorrhoea, because amenorrhoeic subjects of normal weight do not have elevated free androgen levels. The conversion of androstenedione to oestrone is increased in obese women. This enhanced conversion of androgens to oestrogens may be carried out in the adipose tissue itself, since fat in vitro can convert androstenedione to oestrone and testosterone to oestradiol.

Obese women often have menstrual cycles with inadequate progesterone production during the luteal phase; a change that may account for decreased fertility (Glass *et al.*, 1981).

1.2.4.2 Obesity and Spontaneous Ovulation

Obesity is associated with three alterations that interfere with normal ovulation, and these derangements can be reversed through weight loss. These alterations includes; (1) Increased peripheral aromatization of androgens to oestrogens; (2) decreased levels of SHBG resulting in increased levels of free oestradiol and testosterone ; and (3) increased insulin levels that can stimulate ovarian stromal tissue production of androgens (Speroff *et al.*, 1999).

Excessive visceral body fat is associated with insulin resistance, hyperinsulinaemia and high insulin-like growth factor – I (IGF – I) bioactivity as a result of a decreased concentration of insulin- like growth factor binding protein -1 (IGFBP – 1). IGF – I is a sensitizing factor that enhances the ability of granulosa cells in small antral follicles to respond to FSH facilitating the induction of LH receptors. In the thecal cells, both insulin and IGF-I stimulate ovarian androgen synthesis. Therefore, insulin and IGFs are important intra-ovarian regulators, and systemic or local disturbances may result in alterations of spontaneous ovulation (Galtier-Dereure *et al.*, 1997). In addition to this direct role on ovarian function, body fat appears to be strongly related to the activity of the hypothalamic-pituitary axis. Excessive weight particularly influences the concentration of LH, which is probably the key hormone in the relationship between reproduction and metabolism. Obesity is associated with excessive LH concentrations, and it has been shown that a high concentration of LH results in a lower chance of conception (Galtier-Dereure *et al.*, 1997). In overweight women and/or those with polycystic ovary syndrome (PCOS), an increase in the number of fat cells results in a cascade of changes, involving increased leptin and

insulin levels and a preferential increase in LH, but not FSH, levels. The net effect of these changes is to stimulate the partial development of follicles that secrete supranormal levels of testosterone, but which rarely ovulate (hence low progesterone) (Sharpe and Franks, 2002). The aromatizing function of adipose tissue is possibly a means by which obesity impairs gonadotrophin secretion. As hyperinsulinaemia decreases SHBG concentrations, obesity is associated with high concentrations of unbound androgens. Excessive bioavailability and aromatizing of androgens generates increased oestrone concentrations, which in turn triggers a rise in LH secretion. LH subsequently stimulates the production of ovarian androgens, thus enhancing substrate availability for the aromatizing system (Galtier-Dereure *et al.*, 1997).

1.2.5 Leptin and Obesity

Leptin is a hormone involved in long-term regulation of energy homeostasis that reduces food intake through hypothalamic action (Flier and Maratos, 1998). Leptin is produced in the adipose tissue in proportion to body fat content and in human obesity circulating leptin and adipocyte leptin (messenger ribonucleic acid) mRNA levels are commonly increased (Considine *et al*., 1996). Conversely, leptin is potentially elevated in subcutaneous compared to visceral adipose tissue and is proposed to have insulin sensitizing effects (Sivit *et al*., 1997), an effects on reducing insulin secretion, increasing lipid oxidation and decreasing lipid synthesis in skeletal muscle, the pancreatic β cells and other tissues (Kulkarni *et al*., 1997). All the same, very few obese people have a leptin deficiency. In fact, blood levels of leptin usually correlate directly with body fat: the more fat, the more leptin. Obese people generally have high leptin levels, and when people with low leptin levels gain weight, their leptin concentrations increase. Speculation is that leptin rises in an effort to suppress appetite and inhibit fat storage, but its action is ineffective in obesity. Obesity appears to be associated with an insensitivity or resistance to leptin (Whitney & Rolfes, 2002).

A gene that is expressed only in adipose tissue (*ob*) has been cloned and its protein product, leptin, has been proposed to be a vital signaling factor regulating body weight homeostasis and energy balance. With obese individuals having high leptin levels, there is an assumption that so-called leptin resistance is a major feature of human obesity. Glucocorticoids, and in particular insulin, have been found to

stimulate leptin production, with insulin regarded as a key regulator of this protein. The sympathetic nervous system inhibits leptin production. However, the main physiological functions identified for leptin includes; inhibiting of food intake (satiety factor),stimulating energy expenditure , signaling to the reproductive system, aiding in the production of multiple blood cell types, i.e. haematopoiesis and contributing in the growth of new blood vessels, i.e. angiogenesis(Garrow *et al.*, 2000). Fertility in mammals requires adequate nutrition and reserves of metabolic fuel. If metabolic reserves are low or the system is stressed, reproduction will be inhibited, and leptin seems to be one of the signalling systems for these reproductive changes. The administration of leptin into female *ob/ob* mice corrects their sterility and can result in ovulation, pregnancy, parturition and lactation. Leptin accelerates the onset of puberty in normal female mice, and its effect can occur in the absence of any effect on body weight (Garrow *et al.*, 2000).

1.2.5.1 Role of Leptin in Reproduction

Leptin, an adipocyte hormone and recently described type-1 cytokine, has angiogenic properties and appears to have a relationship with some reproductive processes (Mahutte *et al.*, 2003). Leptin acts on receptors in the hypothalamus of the brain where it inhibits appetite by; counteracting the effects of neuropeptide Y (a potent feeding stimulant secreted by cells in the gut and in the hypothalamus); counteracting the effects of anandamide (another potent feeding stimulant that binds to the same receptor and promoting the synthesis of α-melanocyte-stimulating-hormone (MSH), an appetite suppressant. This inhibition is long-term, in contrast to the rapid inhibition of eating by cholecystokinin (CCK). The absence of a leptin (or its receptor) leads to uncontrolled food intake and resulting obesity. Several studies have shown that fasting or following a very-low-calorie diet (VLCD) lowers leptin levels (Dubuc *et al.*, 1998).

Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin (Considine *et al.*, 1996). These people are said to be resistant to the effects of leptin, in much the same way that people with type2 diabetes are resistant to the effects of insulin. The high sustained concentrations of leptin from the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at

some point so the body doesn't adequately receive the satiety feeling subsequent to eating (Considine *et al*., 1996).

Studies have identified an obesity gene, called *ob that* is expressed in the fat cells and codes for the protein leptin. Several strains of laboratory mice are homozygous for single-gene mutations and that causes them to become grossly obese. These strains were grouped into two classes: (1) ***ob/ob*** = mutations in the gene for the protein hormone leptin. When *ob/ob* mice are treated with injections of leptin they lose their excess fat and return to normal body weight. (2) ***db/db*** = mutations in the gene that encodes the receptor for leptin. Study of these animals has led to an understanding of the action of leptin in humans. Leptin also acts on hypothalamic neurons responsible for the secretion of gonadotropin-releasing hormone (GnRH). Women who are very thin from limited food intake or intense physical training may cease to menstruate because of their lack of leptin-secreting fat cells. Treating them with recombinant human leptin can sometimes restore normal menstruation. Leptin also involved in stimulating the sympathetic nervous system to modulate the balance between the formation and breakdown of bone (Whitney & Rolfes, 2002).

In women, higher leptin concentrations are associated with an earlier menarche, while decreased serum leptin concentrations have been associated with the improved ovarian function induced by serum sensitizing therapy (Crosignani, 2002). In the study, rodents harbouring mutations in the leptin gene (*ob/ob* mice), or in the leptin receptor gene (*db/db* mice and *fa/fa* rats) exhibit both obesity and infertility (Galtier-Dereure *et al*., 1997). In leptin-deficient female *ob/ob* mice, treatment with leptin produces a significant weight loss, increases serum concentrations of LH and ovarian and uterine weight in comparison with pair-fed controls, and restores fertility. In the human, the leptin receptor gene is expressed in the hypothalamus and ovary, raising the possibility of a direct effect of leptin on follicular development. Leptin concentrations are seen mainly to be explained by total fat mass, gender and age, while other parameters such as insulinaemia, insulin sensitivity, and visceral fat, and appear to be dependent factors (Galtier-Dereure *et al.*, 1997). Some studies have already demonstrated elevated leptin in the peritoneal fluid of women with endometriosis. There is evidence that oestradiol and progesterone mediate serum leptin levels. A rise in serum leptin has consistently been documented in the luteal and late follicular phase of both natural and gonadotrophin stimulated cycles. Serum

leptin concentrations are higher in the secretory phase than in the proliferative phase of the cycle (Mahutte *et al.*, 2003).

Leptin production can be affected by various hormonal factors including glucocorticoids and insulin. A possible action of leptin on the ovary has been postulated both from the specific binding of this protein in the granulosa cells, and the fact that insulin-like growth factor-I-mediated enhancement of FSH-stimulated oestradiol synthesis by rat and human granulosa cells in vitro can be inhibited by leptin. A relationship between oestrogen and leptin has been indicated recently. Findings in rodents have shown a reduction in serum leptin concentrations, and decrease in the expression of *ob* gene in adipose tissue of ovariectomized animals; changes which are reversed by oestradiol administration. Higher concentrations of leptin were found in women than in men, as well as in pre-menopausal compared to post-menopausal women. Data in normal women have shown higher values of leptin in the luteal than in the follicular phase of the cycle, as well as in the peri-ovulatory period than in the follicular phase (Messinis *et al.*, 1998).

The involvement of oestradiol in the control of leptin production in women is supported by the fact that pre-menopausal women have higher levels of leptin than postmenopausal women, and in general women have higher levels than men. The fact that leptin levels are higher in the luteal than in the follicular phase is a further support to the involvement of gonadal steroids in the mechanism of leptin production during the normal menstrual cycle. An assumption can be made that progesterone, in addition to oestradiol, stimulated leptin production during the luteal phase. It is possible that the production of leptin by the adipocytes is indirectly affected by FSH through an effect of various ovarian substances (Messinis *et al.*, 1998).

It is conceivable that oestradiol during the follicular phase of the cycle primes the adipocytes to the stimulating effect of progesterone. This could explain the significantly higher values of leptin in the early to mid-luteal phase, compared with the mid- to late follicular phase of the cycle. Recent data have suggested that the pre-ovulatory follicle itself may be an important source of leptin. Oestradiol and progesterone, therefore, may act within the follicle to increase leptin production at that site (Messinis *et al.*, 1999). In PCOS, leptin levels are higher in obese women, and may be used as predictors of PCOS. Leptin pulses are synchronous with those of LH, but this synchronization is dampened in PCOS. Leptin is higher in plasma and peritoneal fluid from patients who have endometriosis. This may be due to its

angiogenic activity and its ability to modulate the immune response through receptors in T-cells (CD4) (Sabogal & Munoz, 2001).

Obesity has been related to reduced ovarian response and to increased leptin levels. In fact, low leptin concentrations are predictive of achieving pregnancy, both in normal and in PCOS patients. Conversely, leptin receptors have been identified in granulosa and theca cells, in which they inhibit oestradiol production. Human chorionic gonadotrophin- induced progesterone production in granulosa cells is also decreased by leptin, antagonizing the effects of insulin. Leptin receptors are present in human endometrium and deciduas, potentially participating in the implantation process. Receptor concentration is cycle-related, being increased during the early luteal phase (Sabogal & Munoz, 2001).

1.2.6 Overview of PCOS

Polycystic ovarian syndrome (PCOS) occurs in about 5% to 10% of pre menopausal women and is thought to be caused by a hypothalamic disorder (Tietz, 2006). PCOS is clinically defined by hyperandrogenism with chronic anovulation in women without underlying disease of the adrenal or pituitary glands. This syndrome is characterized by infertility, hirsutism, obesity (in approximately half of those affected), and various menstrual disturbances ranging from amenorrhea to irregular vaginal bleeding (Tietz, 2006). There is increase in the prevalence of obesity, insulin resistance and hyperandrogenism in PCOS. Women with PCOS are at the risk of developing endometrial hyperplasia and endometrial cancer (cancer of the uterine lining) are possible, due to over accumulation of uterine lining, and also lack of progesterone resulting in prolonged stimulation of uterine cells by estrogen. It is however unclear if this risk is directly due to the syndrome or from the associated obesity, hyperinsulinaemia, and hyperandrogenism (Navaratnarajah *et al*, 2008).

Moreover, PCOS is associated with an adverse cardiovascular risk profile. There is an increase in the traditional risk factors for cardiovascular disease (CVD), including dyslipidaemia (decreased plasma high-density lipoprotein cholesterol (HDL-C), impaired fibrinolysis, increased low-density lipoprotein cholesterol (LDL-C), increased inflammation, endothelial dysfunction and hypertension and an elevated prevalence of subclinical and clinical atherosclerosis (Guzick *et al*., 1996). The signs of PCOS can manifest in adolescence and can include premature adrenarche (early maturation of the adrenal gland cortex and activation of adrenal androgen

production) which may contribute to precocious puberty or premature pubarche (the appearance of pubic hair before the age of 8 years) (Rosenfield *et al*., 2000; Ibanez *et al*., 1993). Delayed menarche (onset of menstruation) has also been documented by some researchers (Shadradch *et al*., 2003). Strikingly, the metabolic complications associated with PCOS are also being documented at this early stage with T2DM, IGT (impaired glucose tolerance) and the metabolic syndrome all are reported in lean and overweight adolescents with PCOS (Palmert *et al*., 2002). With its diverse short and long term presentation, PCOS may adversely affect women at varying stages of their life. This contributes to a significant financial burden of which it is estimated that 30.1% is associated with hormonal treatment of menstrual dysfunction, 12.2% infertility care, 14.2% treatment of hirsutism and 40.5% of T2DM related costs (Azziz *et al*., 2005).

1.2.6.1 The Correlation between PCOS and Obesity

The principal features of PCOS are obesity, annovulation (resulting in irregular menstruation) or amenorrhea, acne, and excessive amounts or effects of androgenic hormones. The symptoms and severity of the syndrome vary greatly among women. While the causes are unknown, insulin resistance, diabetes, and obesity are all strongly correlated with PCOS. Reproductive dysfunction occurs with both positive and negative extremes of body weight (Green *et al*., 1988; Lake *et al*., 1997). In early studies, obesity, assessed as 20% over ideal body weight, was present in 43-48% of women of reproductive age with menstrual disorders (n=160) compared to 13% of age- matched controls (n=201) (Rogers and Mitchell, 1953). There has also been a report on direct relationship between menstrual irregularity and or infertility and degree of obesity in women of reproductive age (Green *et al*., 1988; Hartz *et al*., 1979). It is well known that obesity is associated with insulin resistance. Because women with PCOS are often obese, it is not surprising that they would have some element of insulin resistance. The extent of insulin resistance among women with polycystic ovarian syndrome cannot be explained entirely by obesity (Guzick, 2004). In a study of the National Health and Nurses' Health (NHANES), women with ovulatory disorders (n=2 527) were compared to controls with no history of infertility (n=46 718). Increased BMI at age 18 was significantly associated with ovulatory infertility for a BMI of 28-29.9 kgm^{-2} and a BMI $\geq$ 30 kgm^{-2} (Rich-

Edwards *et al*., 1994). Once conception is achieved, an increased risk of pregnancy complications including gestational diabetes and miscarriage may result with increased weight (Sebire *et al*., 2001). The association with abdominal obesity and menstrual abnormalities and infertility is also evident although it is not known whether it is the visceral or subcutaneous store that is related to reproductive dysfunction. In n=40 980 post menopausal women, WHR was significantly associated with a history of infertility (Kaye *et al*., 1990). A study by Zaadstra et al 1993 documented a 30% decrease in probability of conception for each 0.1 increase in WHR in women of reproductive age of n=500 presenting for assisted reproductive treatment. Android obesity is additionally associated with a low pregnancy rate after in vitro fertilization (IVF) (Wass *et al*., 1997). Reduced fecundity (the probability of achieving at least one pregnancy during treatment) is additionally observed with increased weight. In n= 3 586 women receiving assisted reproductive treatment, there was a significant linear reduction in fecundity from non-obese to obese women (Wang *et al*., 2000).

1.2.6.1.1 Obesity and PCOS

The clear association between obesity and abdominal obesity cannot be overlooked, and this is evident in both adulthood and childhood, on menstrual abnormalities and consequent infertility independent of PCOS. Obesity and abdominal obesity in adolescence and adulthood and weight gain after adolescence are predictors of the development of hirsutism and menstrual disturbances in PCOS (Laitinen *et al*., 2003). Moreover, women with PCOS constitute a significant proportion of the infertile population. Women with PCOS tend to have a BMI outside the acceptable range (19-25 kg/m^2) and 40-60% of women with PCOS are overweight or obese (Kiddy *et al*., 1990; Goldzieher and Axelrod 1963). In a recent study comparing women with PCOS and age-matched controls from the NHANES I study, the women with PCOS demonstrated a lower proportion of BMI<25 kg/m^2 and higher proportion of BMI > 30 kg/m^2 and 40 kg/m^2 (Glueck *et al*., 2005). Women with PCOS also display an increased central distribution of adiposity (Pasquali *et al*., 1993) and WHR measurement > 0.8 have been reported in 63% and 53% of women with PCOS (Pasquali *et al*., 1994). This increase in abdominal fat can be observed even in lean women with PCOS compared to weight-matched controls (Rebuffe-Scrive *et al*., 1989) and lean women with PCOS also display an increased amount of

visceral fat compared to lean controls (Yildirim *et al*., 2003). A number of investigators have shown that obesity and abdominal obesity worsen the clinical features of menstrual irregularity and infertility in PCOS (Kiddy *et al*., 1990).

1.2.6.2 Pathophysiology and Aetiology of PCOS

According to Slowey (2001), the definition of PCOS has three requirements. First is the presence of an ovulatory disorder. This may range from oligo-ovulation to annovulation with amenorrhoea. The second feature of PCOS is evidence of androgen excess, either clinical or on laboratory testing. Finally, other sources of anovulation and hyperandrogenism must be ruled out. These sources include hyperprolactinemia, hypothyroidism, late-onset congenital adrenal hyperplasia, Cushing's syndrome, or an androgen-secreting tumour (Guzick, 2004). Polycystic ovaries develop when the ovaries are stimulated to produce excessive amounts of male hormones (androgens), particularly testosterone, either through the release of excessive luteinizing hormone (LH) by the anterior pituitary gland or through high levels of insulin in the blood (hyperinsulinaemia) in women whose ovaries are sensitive to this stimulus. The physiological process of reproduction occurs through the monthly cycle of follicular maturation, ovulation, fertilisatiion or resorption of the corpus luteum and is tightly regulated through a complex interaction of hormones from the hypothalamus and pituitary (the hypothalamic-pituitary-ovarian axis). Gonadotrophin-releasing hormone (GnRH) is synthesized by the preoptic and arcuate nucleus areas of the hypophyseal portal system and acts on the pituitary to stimulate the synthesis and pulsatile secretion of the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Marshall *et al*., 2001).

The primary role of LH is to regulate androgen production in the theca interna and the primary role of FSH is to regulate growth and maturation of ovarian follicles and to stimulate aromatization of androgens to oestrogens. GnRH and gonadotrophins are differentially regulated by a variety of factors including noradrenalin, serotonin, endogenous opioids, angiotensin II, neuropeptide Y, oxytocin, steroids and ovarian factors (Balen, 2004). Folliculogenesis is the process of maturation of the primordial follicle to a primary, pre-antral or secondary, antral or tertaiary and pre-ovulatory or Graafian follicle (Chabbert *et al*., 1998). In PCOS, anovulation and menstrual

irregularity are characterized by excessive early follicular growth with significantly greater amounts of primary and pre-antral follicles (Webber *et al*., 2003), arrested antral follicle development at the 4-7 mm stage and disturbed dominant follicle selection (Franks, *et al*., 2000). The follicles commonly display hypertrophied and luteinized theca interna layers and a thickened ovarian tunica. The pre-antral follicles express the androgen receptor and androgens augment thecal and granulosa cell growth, inhibit follicular atresia and promote granulose cell gonadotrophin responsiveness and steroidogenesis both directly and indirectly through increasing FSH receptor, IGF-I and IGF-I receptor expression (Wiel *et al*., 1999). The increased pre-antral follicle number is likely due to an increased ratio of intra ovarian androgens to oestrogens. The cause of the arrested antral follicle development is unclear. Increased FSH levels may be required for cyclic follicle recruitment to occur due to the presence of excessive local negative inhibitors of FSH activity. These may include anti-mullerian hormone (AMH) which is potentially increased in PCOS as a direct consequence of the increased number of pre-antral follicles or increased follicle secretion of inhibin and oestradiol due to increased follicle number or premature development (Laven *et al*., 2004). A majority of patients with PCOS have insulin resistance and/or are obese. Their elevated insulin levels contribute to or cause the abnormalities seen in the hypothalamic-pituitary-ovarian axis that lead to PCOS. Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol. The excess of adipose tissue in obese patients creates the paradox of having both excess androgens (which are responsible for hirsutism and virilization) and estrogens (which inhibits FSH via negative feedback) (Tietz, 2006).

Excessive LH production or earlier LH receptor gain could lead to premature action on granulose cell LH receptors in the mid-follicular phase and premature arrest of cell proliferation and follicle growth and consequent anovulation (Jakimiuk *et al*., 2001). Despite a number of candidate genes being indentified, a primary pathological cause of PCOS has not been identified. A polygenic mode of inheritance with a number of predisposing genetic and environmental factors contributing to its presentation is therefore more likely. Candidate genes studied include those involved in steroidogenesis, carbohydrate metabolism and fuel homeostasis, gonadotrophin regulation and action and cardiovascular disease (Goodarzi *et al*.,

2006). The exact cause of PCOS is unclear and numerous theories exist. Three main pathophysiological hypothesis have been proposed to explain the endocrine alternations that occur in PCOS: (a) Hypothalamic-pituitary dysfunction involving a primary neuroendocrine defect leading to disruption of the hypothalamic-pituitary axis and excessive LH synthesis and secretion; (b) Primary hypersecretion of androgens involving primarily a defect in ovarian sex steroid synthesis or metabolism and consequent annovulation and (c) Defects in tissue specific IR and consequent hyperinsulinaemia with resultant effects on androgen secretion and anovulation (Matalliotakis *et al.*, 2006). There are thus a wide range of potential central and peripheral abnormalities in PCOS that may interact to contribute to abnormal regulation of gonadotrophin biosynthesis and secretion.

1.2.6.3 Hypothalamic – Pituitary Dysfunction in PCOS

In adult women, a tightly coordinated feedback system exists between the hypothalamus, anterior pituitary, and the ovaries to orchestrate menstruation. FSH serves to stimulate follicular growth and LH stimulates ovulation and progesterone secretion from the developing corpus luteum (Tietz, 2006). Gonadotrophin abnormalities in PCOS include LH hypersecretion due to increased LH pulse amplitude in 30-90% of women with PCOS (Waldstriecher *et al.*, 1988). FSH levels tend to be normal or reduced compared to regularly ovulating women, resulting in an increased LH/FSH ratio (Yen *et al.*, 1997). Women with PCOS may display increased GnRH pulse frequency which would ultimately favour LH over FSH synthesis (Berga *et al.*, 1993). Consistent with this, some investigators have demonstrated continual rapid LH pulse frequency and an absence of the normal menstrual variation in LH frequency in women with PCOS (Waldstriecher *et al.*, 1988). This may be either due to a primary hypothalamic defect or an inherent abnormal pituitary response to GnRH stimulation or failure of inhibitory signals to suppress GnRH pulsatility in PCOS. However, a primary hypothalamic defect is unlikely in the majority of cases of PCOS and abnormal gonadotrophin secretion is more likely to be induced by peripheral hormonal contributors including insulin, inhibin, progesterone or estrogen (Blank *et a*l., 2006). Also, hyperinsulinemia increases GnRH pulse frequency, LH over FSH dominance, increased ovarian androgen production, decreased follicular maturation, and decreased SHBG binding;

all these steps lead to the development of PCOS. Insulin resistance is a common finding among patients of normal weight as well as those overweight patients. The effects of disordered gonadotrophin secretion are also likely to ultimately provide abnormal negative feedback to the hypothalamic pituitary axis and further impede its normal functioning (Sam and Dunaif, 2003).

1.2.6.4 PCOS, Insulin Resistance, and Hyperinsulinaemia

Insulin can contribute to the presentation of PCOS through effects on steroidogenesis and increasing intra-ovarian or circulating androgens and through affecting normal gonadotrophin feedback and release. It can also directly affect folliculogenesis through actions as a mitogenic factor or through stimulating or augmenting local tissue production of growth factors (Nestler, 2000). Insulin resistant women with PCOS are more severely clinically affected than insulin sensitive women with PCOS (DeUgarte *et al*., 2005) and hyperandrogenism (HA) positively correlates with insulin resistance (IR) in obese and lean women with PCOS (Pasquali et al., 1989). As previously discussed, obesity and abdominal obesity are frequent features of PCOS. Whereas overweight women with PCOS will thus exhibit obesity-associated IR and hyperinsulinaemia. Additionally, even IR has been documented in lean women with PCOS compared to weight-matched controls and IR and compensatory hyperinsulinaemia are documented in approximately 40-70% of lean and obese women with PCOS compared to weight-matched controls (DeUgarte *et al*., 2005). This strongly suggests that in a subset of women with PCOS, the IR and consequent hyperinsulinaemia significantly contribute to the aetiology and presentation of PCOS and that these defects in insulin metabolism are separate and additive to that of obesity (Acien et al., 1999). Not all women with PCOS exhibit hyperinsulinaemia and IR (Dale *et al*., 1992) and ethnic background and the heterogeneity and complex aetiology of the syndrome contribute to this discrepancy.

In PCOS, selective tissue sensitivity to the actions of insulin exists and reduced insulin sensitivity has been identified in fibroblasts, skeletal muscle, adipocytes and hepatocytes. The key insulin homeostasis abnormalities identified in PCOS thus far involve defects in receptor and/or post-receptor signal transduction. In cultured adipocytes, intrinsic decreases in maximal insulin-stimulated rates of glucose utilization or transport and GLUT4 (Dunaif *et al*., 1992), insulin-stimulated

autophosphorylation, insulin-induced lipolysis (Ciaraldi *et al*., 1997) and insulin receptor binding were observed. Although impaired hepatic insulin clearance is observed in lean and obese women with PCOS (Ciampelli *et al*., 1997), hepatic insulin sensitivity appears to be reduced predominantly in obese women with PCOS as a result of the synergistic effect of obesity and PCOS (Dunaif *et al*., 1992). In vivo factors including adipokines likely further modulate insulin signaling (Corbould *et al*., 2005). A variety of additional defects in insulin metabolism have been reported in PCOS. A higher level of β-cell function is present as indicated by insulin hypersecretion (Ciampelli *et al*., 1997) and increased insulin response to metabolic stimuli which are present in the absence of IR (Holte *et al*., 1994). Impaired peripheral tissue degradation of insulin (Buffington and Kitabchi, 1994) has also been observed, indicating a further mechanism for augmentation of hyperinsulinaemia.

In PCOS the ovarian tissue does not appear to display IR, unlike skeletal muscle, fibroblasts and adipose tissue. Insulin stimulates thecal androgen overproduction (Nestler and Jakubowiez, 1996). The exact downstream mediators for this are unknown, although there is evidence that cytochrome P450c17α activity is increased by insulin. This may be mediated through P13-K and AKT (activated kinase) activation with the MAPK (mitogen activated protein kinase) pathway remaining unaffected (Nestler and Jakubowiez, 1996), although other work reports that P13-K is not required for other ovarian specific effects of insulin such as stimulation of progesterone production (Poretsky *et al*., 2001). Insulin also synergistically increases the action of LH in women with and without PCOS, potentially through LH cyclic AMP independent effects that stimulate StAR (steriodogenic acute regulatory protein) and P450c17 expression. Insulin has also been reported to potentially stimulate adrenal steroidogenesis through increasing P450c17 responsivity to adrenocorticotrophic hormone, stimulate oestradiol and progesterone production in human granulose cells and increase granulose cell responsiveness to LH or FSH (Willis et al., 1996).

SHBG is a liver derived plasma steroid-binding protein that additionally acts in androgen and oestrogen steroid signaling mediated through the cell membrane SHBG receptor (Kahn *et al*., 2002). Its production and secretion is primarily inhibited by androgens, insulin, IGF-1 and potentially triglycerides and promoted by

oestrogen, cortisol, iodothyronines and growth hormone (GH). SHBG levels are thus lower in obesity and abdominal obesity (Pasquali *et al*., 1990). A decrease in the hepatic production of SHBG by insulin increases total and free or bioavailable androgens (Nestler *et al*., 1991). Dysregulation of other circulating factors can also modulate the relationship between insulin and steroidogenesis including the IGFs and their binding proteins (IGFBP). Hyperinsulinaemia can activate the ovarian-IGF system through reducing hepatic and ovarian IGFBP production and upregulating IGF-I receptor expression (Poretsky *et al.,* 1988). This sensitizes the ovary to the androgenic effects of IGF-I and IGF-II which include augmenting LH and FSH actions on thecal steroidogenesis, granulose and thecal growth and maturation, ocyte maturation and stimulation of the insulin receptor (Poretsky *et al.,* 1999).

Although increased androgen production in women with PCOS is augmented by increased LH and is associated with anovulation, it can be argued that the proximate cause of the anovulation may be insufficient FSH. Follicles in the ovaries of women with PCOS do not mature fully, and the granulosa cells in these arrested follicles are low in number and in aromataze activity. Therefore, oestradiol production by these follicles is limited (Guzick, 2004). A complex interrelationship thus exists between obesity, abdominal obesity and IR in the aetiology and pathogenesis of PCOS. Clinically, improvements in menstrual function and ovulation and reductions in ovarian volume and follicle number are documented following modest weight loss (Crosignani *et al*., 2003). In overweight women with PCOS, lifestyle modification techniques led to a weight loss of 6.3 kg over 6 months (Clark *et al*., 1995) and 6.8% over 48 weeks (Hoeger *et al*., 2004). There is as yet limited additional data on the effect of weight loss on reducing negative reproductive outcomes or pregnancy complications in PCOS although modest weight loss reduces the risk of developing gestational diabetes and Clark et al reported a reduction in miscarriage rates form 75% pre-treatment to 18% post-treatment in women with PCOS. As both T2DM and the metabolic syndrome are more common in PCOS than the overweight population, lifestyle modification strategies therefore also seem appropriate in regards to their reduction of metabolic risks. It is worth noting that a minimal amount of weight loss (5-10%) over as little as 4 weeks is sufficient to improve the clinical reproductive presentation despite subjects remaining clinically overweight or obese (Wahrenberg *et al*., 1999).

As previously discussed, adiposity, abdominal or visceral adiposity, IR and hyperinsulinaemia are key risk factors for metabolic disease. The reduction in these factors following modest weight loss in PCOS highlights the benefits of weight loss in PCOS on metabolic risk factors. Modest weight loss (5-14%) improved dyslipidaemia (increased HDL-C, reduced LDL-C, total cholesterol and triglycerides) (Andersen *et al*., 1995), diastolic blood pressure (DBP), plasminogen-activator inhibitor activity (PAI-I) (a marker of impaired fibrinolysis) and circulating lipids despite weight losses. One additional issue of interest is thus whether women with and without PCOS would experience similar metabolic improvements with the same degree of weight loss (Andersen *et al*., 1995). There is some evidence that subjects with features of the metabolic syndrome (IR, high liver fat, dyslipidaemia) experience greater improvements in fasting insulin and lipid profiles following equivalent weight losses compared to relatively more metabolically healthy individuals (Reaven *et al*., 1995).

1.2.6.5 Excessive Androgen Production and Secretion in PCOS

A large proportion of women with PCOS demonstrate elevated circulating androgens. These are predominantly of ovarian origin (Asuncion *et al*., 2000); although an adrenal contribution to androgen excess has also been suggested with 20-60% of women with PCOS displaying excessive production and secretion of dehydroepiandrosterone sulfate (DHEAS) and 11-hydroxyandrostenedione (Azziz *et al*., 1998; Kumar *et al*., 2005). Ovarian theca cells from women with PCOS display excessive basal and LH-stimulated synthesis and secretion of androgens (dehydroepiandrosterone (DHEA), Δ4-Androstenendione and testosterone) (Nelson *et al*., 1999). Increased P450c 17 expression, increased P450c17 promoter function and reduced degradation of P450c17 mRNA are demonstrated in cultured ovarian thecal cells from women with PCOS (Wickenheisser *et al*., 2005). Peripheral androgen production occurs through conversion of androstenedione to testosterone by 17β-HSD (17β – hydroxysteroid dehydrogenase) in the liver and adipocytes and conversion of testosterone to dihydrotestosterone (DHT) (the active form of testosterone for most receptors) by type 1 or 2 5α-reductase in the skin, brain, liver, prostate, pituitary, epididymis and adipocytes and in the ovary (thecal and granulosa cells) and adrenal gland (Corbould *et al*., 2002). Increased peripheral

steroidogenesis can also contribute to HA and women with PCOS display elevated conversion of circulating DHEA to 5α-DHT and associated metabolites, indicating enhanced 5α-reductase activity (Fassnact *et al*., 2003).

1.2.6.6 Effects of Weight Loss on PCOS and Fertility

An important point is that a minimal amount of weight loss (5-10%) over as little as 4 weeks is sufficient to improve the clinical reproductive presentation despite subjects remaining clinically overweight or obese (Wahrenberg *et al*., 1999). Large reductions in weight may not be needed to restore reproductive function and, therefore, realistic and achievable weight loss goals can be set for overweight infertile women. Indeed, reductions in circulating insulin and testosterone and increases in SHBG are observed as early as 4 weeks post-energy restriction (Hamilton-Fairley *et al*., 1993), supporting the proposed role of IR and hyperinsulinaemia in HA and clinical reproductive dysfunction in PCOS. This also has important implications for which women with PCOS are likely to experience reproductive benefits from weight loss. Eleven out of 25 women responded to improvements in menstrual cyclicity with weight loss (Moran *et al*., 2003). This is consistent with the proposed aetiological role of IR in reproductive dysfunction in PCOS and IR through obesity treatment as a key initial treatment strategy in PCOS.

As previously discussed, adiposity, abdominal or visceral adiposity, IR and hyperinsulinaemia are key risk factors for metabolic disease. The reduction in these factors following modest weight loss in PCOS highlights the benefits of weight loss in PCOS on metabolic risk factors (Moran *et al*., 2003). Modest weight loss (5-14%) improved dyslipidaemia (increased HDL-C, reduced LDL-C, total cholesterol and triglycerides), diastolic blood pressure (DBP) (298), plasminogen-activator inhibitor activity (PAI-I) (a marker of impaired fibrinolysis) and circulating lipids despite weight losses or 5-15% (Anderson *et al*., 1995). One additional issue of interest is thus whether women with and without PCOS would experience similar metabolic improvements with the same degree of weight loss. There is some evidence that subjects with features of the metabolic syndrome (IR, high liver fat, dyslipidaemia) experience greater improvements in fasting insulin and lipid profiles following equivalent weight losses compared to relatively more metabolically healthy individuals. This is demonstrated by reductions in fasting insulin and HOMA

(Homeostasis model assessment) with weight loss for relatively more IR subjects compared to more insulin sensitive subjects with or without PCOS (Moran *et al*., 2003). Therefore, with PCOS women who demonstrate a significantly worsened metabolic profile, a lesser degree of weight loss may be sufficient to improve risk factors for CVD and T2DM than would be necessary for a less metabolically disturbed population. In isocaloric weight loss studies comparing women with and without PCOS, equivalent weight losses (7.5% reduction in BMI or 5kg reduction in weight) induced similar reductions in fasting insulin for women with or without PCOS (Jakubowiez *et al*., 1997; Pasquali *et al*., 2000).

1.2.7. Overview of the Female Reproductive Cycle

In adult women, a tightly coordinated feedback system exists between the hypothalamus, anterior pituitary, and the ovaries to orchestrate menstruation (Tietz, 2006). Every healthy female neonate possesses approximately 400,000 primordial follicles, each containing an immature ovum. During the reproductive life span of an adult woman, 300 to 400 follicles will reach maturity. A single mature follicle is produced during each normal menstrual cycle at approximately day 14 (Tietz, 2006). In the female reproductive cycle, ovulation is followed by menstrual bleeding in a recurring, predictable sequence, if conception does not occur (Beckmann *et al.*, 1995). The dynamic relationships between the different components of the reproductive axis in the adult female are such that this reproductive process occurs in cyclic fashion, in an orderly sequence of events. This sequence involves a remarkable co-ordination between hormonal secretion and morphological changes in various organs (Ferin *et al.*, 1993). This recurring sequence is established at puberty and continues until the time of menopause at around age 50. A woman, therefore, has approximately 30 years of optimal reproductive function. In healthy women, reproductive cycles occur at about 28-day intervals, and most women ovulate 13 to 14 times per year, unless ovulation is interrupted by pregnancy, lactation or oral contraception (Beckmann *et al.*, 1995). The reproductive cycle of the female can be divided into three stages: ***a***. the follicular phase, the time for follicular growth; ***b***. the ovulatory period, when final maturation of the oocyte and its release into the reproductive tract occurs; and ***c***. the luteal phase, when a newly formed corpus luteum secrets hormones in preparation for implantation (Ferin *et al.*, 1993).

In the absence of fertilization and implantation, a new cycle is initiated as soon as the activity of the corpus luteum wanes. If the fertilized egg implants itself in the uterus, the luteal phase is prolonged and becomes the progestational phase of the pregnancy that follows. Ovulation is induced by the sudden release of large amounts of gonadotrophins from the pituitary gland. Ovarian steroids also promote sexual receptivity. Corpus luteum development then follows ovulation spontaneously (Ferin *et al.*, 1993). The reproductive cycle depends on the cyclic interactions between hypothalamic gonadotrophin-releasing hormone (GnRH), the pituitary gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH), and the ovarian sex steroid hormones oestradiol and progesterone. Through positive – and negative – feedback loops, these hormones stimulate ovulation, facilitate implantation of the fertilized ovum, and bring about menstruation. If any one (or more) of the above hormones becomes elevated or suppressed, the reproductive cycle becomes disrupted and ovulation and menstruation cease. In the case of female reproductive dysfunction, it is essential to identify which hormones are either elevated or reduced (Beckmann *et al.*, 1995).

1.2.7.1 The Menstrual Cycle

The first half of the endometrial cycle, before ovulation, is characterized by epithelial and stromal differentiation. During the first half of the post-ovulatory phase, specific changes occur in the endometrial epithelium. In the second half, the histological changes affect the stroma, leading to a predicidual reaction. Decidualization is an irreversible process, and if implantation does not occur, programmed cell death (apoptosis) ensues. If fertilization fails to occur, the stromal reaction regresses and menstruation starts. If pregnancy occurs, the endometrial changes progress to formation of the deciduas (Borini & Asch, 1993).

Menstruation is the shedding of the dead endometrium and ceases as the endometrium regenerates. Menstruation is initiated by the withdrawal of oestrogen and progesterone. The onset of menstrual bleeding by convention is termed the first day of the cycle. The follicular phase, which is the initiation of follicular growth, actually begins during the last few days of the previous luteal phase and terminates at ovulation (Tietz, 2006). Pulsatile GnRH secretion from the hypothalamus results in release of LH and FSH from the anterior pituitary. Follicles are recruited during this

period and granulosa cells surrounding the developing oocytes produce oestrogens. Eventually a dominant follicle develops. The oestradiol produced by the developing follicles causes an orderly endometrial proliferation within the uterus during this stage of the menstrual cycle (Brugh *et al.*, 2002).

The pre-ovulatory phase is the third phase of the cycle. During this brief phase, an LH surge occurs, following an oestradiol surge. This triggers ovulation, which occurs 24 to 36 hours after the LH surge and the peak occurring 10 to 12hrs before ovulation. Ovulation occurs around day 14 of the menstrual cycle (Tietz, 2006). During ovulation, the dominant follicle ruptures, releasing the mature egg. Following ovulation, the final or luteal phase of the cycle is characterized by secretion of progesterone from the corpus luteum (ruptured follicle) with consequent gradual lowering of LH and FSH concentrations. Progesterone is necessary to maintain the endometrium for the impending implantation of an embryo, which occurs most commonly around 5 days after ovulation if fertilization has occurred. Therefore the progesterone reaches a peak of approximately 8 mg/day at about 8 days post ovulation. If the corpus luteum is not supported by human chorionic gonadotrophin (hCG), produced by an implanted embryo, it will involute (Brugh *et al.*, 2002). Once the corpus luteum is involuted and if pregnancy does not occur, menstruation begins, and the cycle repeats (Brugh *et al.*, 2002; Beckmann *et al.*, 1995). In the majority of women, the menstrual cycle lasts between 25 and 30 days, with the distribution within the range skewed toward cycles with a 28 – 30 day length. The average duration of menstrual flow is 4 to 6 days and average menstrual blood loss is 30mL (Tietz, 2006). The onset of menstruation delineates the termination of an endometrial cycle and the beginning of a new one (Ferin *et al.*, 1993).

1.2.7.2 The Ovulation Process

Late in the follicular phase, FSH induces LH receptors on granulosa cells (Tietz, 2006). Ovulation is the end process of a series of events initiated by the gonadotrophin surge and resulting in the release of a mature fertilizable egg from a Graafian follicle. Unfortunately, the precise sequence of local events within the follicle that lead to rupture of the follicular wall and expulsion of the egg is not known. There is no question that the process of ovulation is initiated by the gonadotrophin surge, which occurs in response to the long loop oestradiol positive

feedback, the signal to the brain and pituitary that the dominant follicle has attained maturity. The gonadotrophin surge terminates oestradiol synthesis; the theca cell now changes from an androgen to a progesterone-secreting tissue. Vascular changes in the pre-ovulatory follicle occur within minutes of the LH surge. The multi- layered capillary plexus within the theca dilates causing hyporemia, a prelude to the ovulatory process. About six hours into the LH surge, there is increasing ovarian blood flow due to decreased vascular resistance, increase in capillary and venule permeability leading to an increase in interstitial fluid volume (Ferin *et al.*, 1993). Once a follicle has been stimulated, oestradiol production causes that specific follicle to be more receptive to effects from FSH.

The high concentration of oestradiol just before midcycle is responsible for triggering the positive feedback in the hypothalamus that leads to the midcycle LH surge. The precise cause of ovulation is not known, but ovulation occur 1 to 24 hrs after the LH peak. After ovulation, LH is suppressed by progesterone and oestradiol, but the effect of LH is increased on the corpus luteum. In the event of successful fertilization and implantation, corpus luteum function is sustained by hCG (human chorionic gonadotrophin) produced by the trophoblastic cells of the developing embryo. hCG has high molecular homology to LH and is capable of binding and stimulating LH receptors (Tietz, 2006).

1.2.7.3 Conception

Menstruation does not take place if pregnancy occurs. The oocyte which is released by the Graafian follicle at the time of ovulation is gently swept into the lumen of the fallopian tube by the finger-like structures at the ends – the fimbrae. Ciliated cells in the tubal lumen (the endosalpinx) waft the egg onwards to the ampulla of the tube, which is where fertilization occurs by the union of egg and sperm (Willocks & Neilson, 1991). The fertilized egg is transported back along the fallopian tubes to the uterine cavity.

1.2.8. The Reproductive Hormones

During a normal menstrual cycle, there is a closely coordinated interplay of feedback effects between the hypothalamus, the anterior lobe of the pituitary gland, and the ovaries. This is tightly regulated by the hypothalamo-pituitary-ovarian (HPO) axis and any derangement of the HPO axis results in menstrual irregularities and ovulation disorders, with consequent sub- or infertility (Kalro, 2003). In addition, there are cyclic hormone changes that lead to functional and structural changes in the ovaries (follicle maturation, ovulation, and corpus luteum development), uterus (preparation of the endometrium for possible implantation of the fertilized ovum), cervix (to permit transport of sperm), and vagina (Tietz, 2006).

There are four major hormonal markers that characterize the menstrual cycle: two are of pituitary origin – LH and FSH – and two are of ovarian origin – oestradiol and progesterone (Ferin *et al.*, 1993). The hypothalamic-regulating hormones which orchestrate the activities of the anterior pituitary are luteinizing-releasing hormone (LHRH), corticotrophin-releasing hormone (CRH), growth hormone-releasing hormone (GHRH), somatostatin, thyrotrophic hormone-releasing hormone (TRH) and prolactin inhibiting factor (PIF or dopamine). LH-RH is a decapeptide, which is released in a pulsatile fashion to stimulate release of the gonadotrophins, FSH and LH, from the anterior pituitary (Willocks & Neilson, 1991).

The circulating levels of most major reproductive hormones have been shown to fluctuate, often quite dramatically. This accounts for the large variations shown by individual cycles, even among successive menstrual cycles in the same woman (Ferin *et al.*, 1993). Several hormonal problems can be identified by a simple history and general inspection of the female patient. For example, the presence of hirsutism may indicate androgen excess. Hirsutism in conjunction with obesity may be associated with polycystic ovarian syndrome or Cushing's syndrome. Irregular cycles in association with a low body weight may indicate hypogonadotrophic ovulation problems, as seen in women with eating disorders. A history of hypo- or hyperthyroidism may suggest an associated ovulatory problem. A history of galactorrhoea may indicate the presence of hyperprolatinaemia and even the presence of a prolactinoma (Brugh *et al.*, 2002). Infertility problems often arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. Measurement of peptide and steroid hormones in the serum is therefore an essential aspect in the evaluation of infertility.

1.2.8.1 Ovarian Sex Steroid Hormone Secretion

The process of follicular development, ovulation and the maintenance of the corpus luteum has been described in terms of ovarian physiology. In reality, the ovary, pituitary and hypothalamus act in concert to ensure the growth and development of one ovarian follicle, and to maintain hormonal support of the endometrium to allow implantation (Stuart Campbell & Ash Monga, 2005). Ovarian follicles respond to pituitary gonadotrophin secretion by synthesizing the principal ovarian hormones oestradiol and progesterone. The output of LH and FSH from the pituitary gland is stimulated by pulses of gonadotrophin-releasing hormone (GnRH) produced by the hypothalamus and transported to the pituitary in the portal circulation. Increasing levels of oestradiol feedback to the pituitary gland through a negative – feedback mechanism, results in decreased secretion of FSH and increased secretion of LH. This results in a marked increase in LH secretion, known as the LH surge, which triggers ovulation. With ovulation, the ovarian follicle is converted into a corpus luteum and begins secreting progesterone. During a full reproductive cycle, one oocyte is brought to maturity before ovulation. In the process of bringing one oocyte to maturation, a number of oocytes are stimulated to partial maturation, but subsequently undergo atresia before reaching ovulation.

During the process of follicular maturation, pre-granulosa cells are stimulated by FSH to become granulosa cells, which begin secreting oestradiol. The binding of FSH to receptors in the granulosa cells causes granulosa cell proliferation, increased binding of FSH and increased production of oestradiol. The follicle with the greatest number of granulosa cells, FSH receptors, and the highest oestradiol production becomes the dominant follicle from which ovulation will occur (Beckmann *et al.*, 1995). As a primordial follicle is stimulated, the pretheca cells surrounding the granulosa cells become theca cells. The theca cells secrete androgens which serve as the precursors for the oestradiol production by the granulosa cells (Beckmann *et al.*, 1995). Within the theca cells, LH stimulates the production of androgens from cholesterol and FSH stimulates the conversion of thecally derived androgens to oestrogens through an aromatization reaction (Stuart Campbell & Ash Monga, 2005).

1.2.8.1.1 Hypothalamic GnRH Secretion

Gonadotrophin-releasing hormone triggers the surge of LH that precedes ovulation. There appear to be two separate feedback centers in the hypothalamus: a tonic negative feedback center in the basal medial hypothalamus and a cyclical positive feedback center in the anterior regions of the hypothalamus (Tietz, 2006). Low concentrations of estradiol, such as those that are present during the follicular phase, affect the negative feedback center, whereas high concentrations of estradiol, such as are seen just before the midcycle LH peak, trigger the positive feedback center.

Hypothalamic GnRH is secreted in a pulsating manner from the arcuate nucleus of the hypothalamus. GnRH secretion is influenced by oestradiol and catecholamine neurotransmitters. The neurotransmitters may help explain psychogenic influences on the reproductive cycle. GnRH reaches the anterior pituitary gland through the hypothalamic– pituitary portal plexus. Pituitary gonadotrophin secretion is stimulated and modulated by the pulsating secretion of GnRH. Surgical ablation of the arcuate nucleus in animals disrupts ovarian function, as does continuous infusion of GnRH agonists (Beckmann *et al.*, 1995). The magnitude of the LH response to GnRH increases steadily through the follicular phase and is greatest at the time of the preovulatory surge of LH, after which it declines again.

1.2.8.2 LH and FSH

The pituitary gonadotrophins FSH and LH are protein hormones secreted by the anterior pituitary gland (Beckmann *et al.*, 1995). LH and FSH are glycoproteins from the family which includes TSH and human chorionic gonadotrophin. These hormones are composed of a two peptide chains, usually ∝– and a specific ß – subunit. Both are glycocylated, which determines their bioactivity and half- life (de Swiet *et al.*, 2002). Secretion of the gonadotrophins, FSH and LH, is controlled by luliberin. This stimulates secretion of LH more effectively than follitropin secretion, the plasma levels of the sex hormones (oestradiol and progesterone in females) through positive and negative feedback. It is also controlled by inhibin, a hormone produced by the Graafian follicles in females. Luliberin also inhibits the release of FSH (Meyer *et al.*, 1997). FSH and LH are also secreted in a pulsating fashion in concert with the pulsating release of GnRH. The magnitude of secretion and the rates of secretion of FSH and/or LH are determined by the levels of ovarian steroid

hormones and other ovarian factors. When a woman is in a state of relative oestrogen deficiency, the principal gonadotrophin secreted is FSH. As the ovary responds to FSH secretion with oestradiol production, there is a negative feedback to the pituitary gland to inhibit FSH secretion and facilitate LH secretion (Beckmann *et al.*, 1995). LH and FSH act on the gonads to stimulate gametogenesis and hormone synthesis. During the follicular phase, FSH and LH stimulate oestrogen synthesis by the developing follicle. This initially feeds back to the level of the hypothalamus and possibly to the pituitary to inhibit FSH and LH release (de Swiet *et al.*, 2002). FSH and LH have important actions on the ovary: the main effect of FSH is to stimulate growth and development of Graafian follicles, while LH acts to cause ovulation. Ovarian steroid hormones are produced through the actions of FSH and LH. As the Graafian follicle enlarges, increasing amounts of the oestrogen, oestradiol, are produced. With the mid-cycle surge of LH, ovulation occurs and the Graafian follicle is converted into a corpus luteum from which mainly progesterone is secreted (Willocks & Neilson, 1991).

A sophisticated system of feedback loops controls the sequence of co-ordination of endocrine events during the menstrual cycle. The increasing amounts of oestradiol produced by the Graafian follicle cause negative feedback to the hypothalamus, inhibiting release of LH-RH, and therefore also of FSH. As the levels of oestradiol continue to rise, however, a positive feedback loop is triggered to the anterior pituitary which produces a surge in FSH and, more importantly, a very large surge in LH to cause ovulation. As the amounts of oestradiol and progesterone produced by the fading corpus luteum decrease, a production of FSH picks up and the next cycle commences (Willocks & Neilson, 1991). In regard to LH secretion, the most striking event is a spectacular and abrupt rise in concentrations at the end of the follicular phase: the pre-ovulatory surge. Mean duration of the gonadotrophin surge is 48 hours. It is estimated that ovulation occurs about 18 hours after the LH peak, or 36 hours after the initiation of the LH surge (Ferin *et al.*, 1993). FSH also rises at the end of the follicular phase as part of the pre-ovulatory gonadotrophin surge, but this increase is more modest than that for LH. Of importance to FSH secretion is the slight but physiologically very significant rise in FSH on the day(s) preceding or on the day of menstruation. Peak FSH values at this time are reached about 24 hours after menstrual flow has started: the early follicular phase FSH rises. This is the only

time in the menstrual cycle at which the FSH: LH ratio favours FSH (Ferin *et al.*, 1993).

Quantitative relationships between ovarian steroids and FSH release determine the amounts of FSH released at the end to the menstrual cycle. Sub-normal FSH release or abnormal FSH: LH ratios during the inter-menstrual period may result in deficient follicular growth, a delay in ovulation, and/or deficiencies in the secretory activity of the corpus luteum (presumably because of decreased amount of tissue available for luteinization), decreased progesterone secretion (the inadequate luteal phase syndrome), and potential adverse effects on the implantation process (Ferin *et al.*, 1993). Follicle-stimulating hormone (FSH) is synthesized in the adenohypophysis, and stimulates the growth and maturation of ovarian follicles, stimulates estrogen secretion, promotes the endometrial changes characteristic of the first portion (proliferative phase) of the mammalian menstrual cycle and stimulates spermatogenesis in the male. Luteinizing hormone (LH) is also synthesized in the adenohypophysis and acts with FSH to promote ovulation and secretion of androgens and progesterone. It instigates and maintains the second portion of the mammalian estrus and menstrual cycle. In females it is concerned with corpus luteum formation, and in males it stimulates the development and functional activity of testicular leydig cells (Tietz, 2006).

1.2.8.3 Thyroid Stimulating Hormone

Thyroid- stimulating hormone also called thyrotropin is a glycoprotein hormone synthesized in the adenohypophysis that promotes the growth of, sustains and stimulates the hormonal secretion of the thyroid gland (Tietz, 2006). TSH is released from the anterior pituitary in response to TRH, a tripeptide synthesised in the supraoptic and supraventricular nuclei (de Swiet *et al.*, 2002). TSH contains 209 amino acids arranged in two polypeptide chains. Secretion is controlled by thyroliberin (TSH-RH) – the release is stimulated by stress, and the plasma level of thyroid hormone through negative feedback on the hypothalamus and particularly on the anterior pituitary. Thyrotropin controls the structure of the thyroid gland as well as each phase of its function (Meyer *et al.*, 1997). TSH also affects the thyroid by increasing its size, vascularity, iodine uptake, protein synthesis, storage of colloid and the secretion of T3 and T4 (de Swiet *et al.*, 2002). Thyroid dysfunction is more

common in women than in men. Clinical manifestations of thyroid disease can be subtle and insidious. Various reproductive disorders ranging from abnormal sexual development to menstrual irregularities and infertility have been associated with thyroid disorders. Hyper- and hypothyroidism can result in menstrual irregularities and compromise fertility (Kalro, 2003). Oligomenorrhoea seems to be the most common menstrual disorder in hyperthyroidism and may progress to amenorrhoea. Amenorrhoea is a feature of severe hyperthyroidism, with elevated LH and FSH levels, loss of midcycle LH peak, and consequent anovulation and low progesterone levels. Excess thyroid hormones typically increase sex hormonebinding globulin (SHBG) production and serum levels, reflecting increased tissue response to these hormones. Circulating total oestrogen and testosterone levels are therefore increased, but active or free fractions are often reduced (Kalro, 2003). Hypothyroidism often causes polymenorrhoea and oligomenorrhoea. It occasionally causes anovulation and rarely amenorrhoea. Occasionally, hypothyroidism may be associated with prolonged periods of amenorrhoea and anovulation. Patients with hypothyroidism have reduced levels of SHBG and consequently reduced levels of circulating oestrogens and testosterone. With anovulatory cycles, LH and FSH may also be reduced (Kalro, 2003).

Hypothyroid states are often associated with increased thyrotropin-releasing hormone levels, which increase both TSH and prolactin levels. Hyperprolactinemia from longstanding primary hypothyroidism may be responsible for varying degrees of ovulatory dysfunction from luteal phase insufficiency to oligomenorrhoea or amenorrhoea (Kalro, 2003). Disorders of thyroid function, either hypothyroidism hyperthyroidism, may lead to menstrual disorders and ovulatory dysfunction. Adrenal conditions such as Cushing's syndrome or congenital hyperplasia also cause anovulation (Stuart Campbell & Ash Monga, 2005).

1.2.8.4 Oestrogen and Progesterone

In normal women most oestrogens are secreted by the ovarian follicles and the corpus luteum, and during pregnancy by the placenta. The major oestrogen secreted by the ovary is estradiol, which is bound specifically and with high affinity to SHBG and nonspecifically to albumin. Following ovulation, the corpus luteum continues to synthesize and release oestrogens and progesterone. Their production peaks 7 days

after ovulation and thereafter declines unless conception and implantation occurs. Here the developing embryo releases human chorionic gonadotrophin (hCG) into the maternal circulation which maintains corpus luteum function (de Swiet *et al.*, 2002). The properties of oestrogen are numerous and they include stimulating endometrial growth, maintenance of vessel and skin, reducing bone resorption, increasing bone formation and increasing uterine growth. Oestrogen also increases hepatic synthesis of binding proteins, increases circulating levels of coagulation factors II, VII, IX, X, antithrombin, III and plasminogen, increases platelet adhesiveness. Oestrogen also has attributes of increasing high-density lipoprotein (HDL) and reducing low-density lipoprotein (LDL), increasing triglycerides, reducing ketone formation and increasing fat deposition (de Swiet *et al.*, 2002). Although only insignificant amounts of oestrogen are secreted by the adrenal cortex, it is responsible for most of the oestrogens formed outside the ovaries. This is because it releases androstenedione and dehydroepiandrosterone (DHEA) which are converted to oestrogens by fat cells, hair follicles, etc. Apart from the small amounts produced by the adrenals, most oestrogens are synthesized by the cells of the corona radiata, theca interna and corpus luteum. Some oestrogen is formed from circulating testosterone. The ovary produces two oestrogens, namely, oestradiol and oestrone; the former is biologically more potent (Meyer *et al.*, 1997). Oestrogens functions to promote follicle development and ovulation, stimulates proliferation of the epithelial cells of the uterine tubes, uterus and vagina; Stimulates protein synthesis, e.g. contractile proteins in the myometrial muscle fibres of the uterus: Reduces the membrane potential of the myometrial muscle fibres thus increasing their sensitivity to oxytocin and prostaglandin: Stimulates the duct growth in the mammary glands and involved in lactation: Primary responsibility for the development of the female characteristics. Progesterone is predominantly produced by the corpus luteum in the non-pregnant female. Small amounts are produced by the developing follicle and adrenals (Meyer *et al.*, 1997).

The most important function of progesterone is the regulation of endometrial receptivity

(de Swiet *et al.*, 2002). Other functions of progesterone are that it stimulates the secretory activity of the uterine tubes, uterus and vagina; Its responsible for the pregestational changes in the endometrium, and together with oestrogen is responsible for the cyclic changes that occur in the cervix and the vagina; Increases

the membrane potential of the myometrial muscle fibres, thus decreasing their sensitivity and excitability to oxytocin and prostaglandin. This explains why progesterone therapy is sometimes so effective in threatening abortion. Other function of progesterone includes decreasing the number of oestrogen receptors in the endometrial muscle fibres, promoting protein anabolism, stimulating alveolar formation in the breasts during pregnancy and antagonizing the action of aldosterone on the kidney.

Oestradiol and progesterone also act on the endometrium – the lining tissue of the uterine cavity. Oestradiol stimulates growth of all elements of the endometrium. Under the influence of progesterone from the corpus luteum during the second half of the cycle, the endometrium is converted from a proliferative pattern to a secretory pattern, as the endometrial glands become tortuous and convoluted. As progesterone and oestradiol levels fall towards the end of the cycle, the endometrium can no longer be sustained, so it breaks up and is cast off in the process of menstruation (Willocks & Neilson, 1991). Oestradiol secretion remains low during the early follicular phase period, but increases 1 week prior to the mid-cycle gonadotrophin surge; first at a slow, then at a very rapid, quasi-exponential rate to reach a peak at the time of the onset of the LH surge: the late follicular phase oestradiol peak. Within a few hours after the initiation of the mid-cycle gonadotrophin surge, oestradiol concentrations fall abruptly. They rise again with the appearance of the corpus luteum.

Progesterone secretion remains insignificant throughout the follicular phase, rises suddenly and modestly about 12 hours prior to the onset of the LH surge, then remains at a plateau for about 12 hours: the pre-ovulatory progesterone rise. Progesterone rises again 36 hours after the onset of the LH surge. During the luteal phase, levels of both progesterone and oestradiol rise, to reach a maximum about six to nine days after the mid-cycle gonadotrophin surge: the luteal phase oestradiol and progesterone secretory curve (Ferin *et al.,* 1993).

1.2.8.5 Testosterone

Testosterone is a hormone that belongs to the discrete chemical classification known as steroids. Other members of this classification are such compounds as cholesterol, bile acids, vitamin D, and hormones of the adrenal glands and ovary. The systematic

chemical name for testosterone is 17-beta-hydroxy-4-androsten-3-one. The majority of the circulating testosterone in men comes from production in the interstitial cells of Leydig at the testicles. Additionally, the zonas reticularis and fasciculata of the adrenal gland produce small amounts. Regulation of testicular production occurs via a negative feedback loop system involving the anterior pituitary, hypothalamus, and testicles (Ferin *et al.*, 1993). Periodically the hypothalamus releases pulses of gonadotrophin-releasing hormone (GnRH) into the hypophyseal circulation, which supplies the hypothalamus and anterior pituitary. The GnRH stimulates the anterior pituitary to produce and release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The pulsatile release of GnRH results in LH and FSH also being released into the systemic circulation in a similar pulsatile manner. For normal, healthy males approximately 2 to 4 LH and FSH pulses are observed over a 6- to 8-hour period; however the amplitudes of the LH pulses are much greater than those observed for FSH. At the testicles, LH and FSH interact with their primary target tissue receptors (LH, Leydig cells; FSH, Sertoli cells) located on the respective cell membranes. Once a hormone-receptor complex is formed, there is an adenyl cyclase-mediated increase of cyclic AMP, which produces a phosphorylation of intracellular proteins by activation of a protein kinase mechanism. In the Leydig cells this protein kinase activation leads to a mobilization of steroid precursors, in particular the activation of pregnenolone synthesis from cholesterol. Pregnenolone serves as the parent compound from which testosterone is derived. Synthesized testosterone diffuses from the Leydig cells into the testicular vascular system and/or into adjacent testicle compartments containing the Sertoli cells. In the Sertoli cells, testosterone plays an essential role in the facilitation of the spermatogenesis process (the FSH receptor-hormone formation at the Sertoli cell results in the initiation of the spermatogenesis process) (Hackney, 1996).

A major reproductive role of testosterone involves the development of the sperm cell. At the Sertoli cells of the testicles, testosterone induces a nuclear activation process which stimulates and catalyses the maturation and development of the spermatozoa (i.e., developing sperm) during the process of spermatogenesis. Maintenance of testosterone levels within the Sertoli cells is essential for the development of adequate numbers of mature, viable sperm that are necessary for a male to be fertile.

The majority of the circulating testosterone is transported bound to various carrier proteins (sometimes referred to as binding proteins). The principal carrier protein is sex steroid-binding globulin (SSBG), however other plasma proteins can also bind and carry testosterone to a lesser degree (e.g., albumin, cortisol-binding globulin). The remaining non-bound circulating testosterone is referred to as free testosterone. This free testosterone is considered the biologically active form of the hormone as this portion of the hormone can interact at the target tissue receptors. Circulating bound and free testosterone are collectively referred to as total testosterone. Testosterone also assists in the development and functioning of the male accessory sex glands (prostate, seminal vesicles and epididymides) which aid in the sperm development and function, as well as in the act of copulation. Also attributed to the influence of testosterone are the secondary sex characteristics of males, such as the typical deeper male voice, increased levels of body hair, penile growth, initiation of the sex drive (libido), and more aggressive behavior patterns (Hackney, 1996). The greater the body mass index, the higher the testosterone levels, and therefore hirsutism is more common in overweight anovulatory women (Speroff *et al.*, 1999). Hirsutism is defined as the presence of excessive terminal hair in androgen dependent areas of a woman's body (Hunter & Carek, 2003). Alopecia and acne are also consequences of hyperandrogenism (Speroff *et al.*, 1999).

1.2.8.6 Prolactin

Prolactin (PRL) is a hormone secreted by special cells of the adenohypophysis. The principal hormone that controls the initiation and maintenance of lactation is PRL. However for an appropriate expression of PRL action, breast tissue requires priming by estrogens, progestins, corticosteroids, thyroid hormone, and insulin. PRL induces ductal growth, development of the breast lobular alveolar system, and the synthesis of specific milk proteins, including casein and γ-lactalbumin. PRL, like other pituitary hormones, binds to a specific receptor on the cell membrane of its target organs (breast, adrenal, ovaries, testes, prostate, kidney and liver). However, the exact intracellular mechanism of PRL action is not known. It has been suggested that PRL regulates the transport of lipoproteins in the adrenal gland, ovary and testis to ensure a constant supply of low-density lipoprotein cholesterol for steroidogenesis. In addition, PRL promotes the synthesis of enzymes of the adrenal androgen

pathway, thus facilitating the conversion of pregnenolone to dehydroepiandrosterone and dehydroepiandrosterone sulfate (Tietz, 2006).

Prolactin release is under a tonic inhibitory control by the hypothalamus. The secretion

of prolactin is also influenced by *Oestradiol* (The ovarian hormone oestradiol augments prolactin release and its action is probably responsible for differences in Prolactin concentrations in the adult versus the pre-pubertal or menopausal woman), *Sleep: (*There is a moderate rise in prolactin concentrations during sleep), *Stress: (*Several types of stress e.g. cold, heat, physical aggression or surgery are all known to increase prolactin release. Similarly, certain types of exercise will also result in increased prolactin levels), and *Pharmacological agents: (*Several drugs increase prolactin, mostly by decreasingdopamine activity through specific mechanisms. Tranquilizers may block dopaminergic receptors, such as the phenothiazine derivatives, or inhibit dopamine re-uptake from the interneuronal cleft, such as the tricyclic depressants) (Ferin *et al.,* 1993). Hyperprolactinaemia is the most common hypothalamic-pituitary disorder encountered in clinical endocrinology (Tietz, 2006). PRL concentrations also may be elevated in women who have only suble alterations of fertility such as anovulation with or without menstrual irregularity, amenorrhea and galactorrhea, or galactorrhea alone. Causes of hyperprolactinaemia include: the growth of a Prolactin-producing adenoma; other tumours of the pituitary region which block the inhibitory influence of the hypothalamus; certain endocrine diseases, e.g. primary hypothyroidism; polycystic ovarian syndrome; antidepressants; antihypertensives and oestrogen. Hyperprolactinaemia can disturb ovarian physiology at several levels, including follicular maturation and steroidogenesis, ovulation, the process of luteinization, and the corpus luteum function (Crosignani *et al.*, 1999).

PRL in men is frequently manifested as oligospermia or impotence or both. Elevated levels of PRL concentrations are observed in as many as 30% of patients with PCOS and patients with clinically silent pituitary adenomas. Basal gonadotrophin concentrations are low in most patients with hyperprolactinaemia; most studies suggest that PRL inhibits the release of GnRH, resulting in a state of functional hypogonadotropism (Tietz, 2006). Increasing prolactin levels are frequently associated with disturbances of the menstrual cycle. Most commonly, these are seen

in patients with a prolactin-producing pituitary adenoma. Thus, in the initial evaluation process of the infertile patient with irregular menses or amenorrhoea, it is always important to measure prolactin concentrations (Ferin *et al.*, 1993).

1.2.9 INFERTILITY

1.2.9.1 Overview of Infertility

Ninety percent of healthy couples practicing unprotected intercourse can expect to conceive within one year, and 95% will be successful within two years. Infertility is defined as the inability to conceive after one year of unprotected intercourse (Tietz, 2006). Infecundity is the inability of a couple to achieve a live birth after 12 months of regular unprotected intercourse. Most couples seeking help are in fact subfertile rather than infertile. Some may have normal fertilizing potential (Stuart Campbell & Ash Monga, 2005). It is estimated that 25% of couples will experience an episode of infertility in their reproductive life. Infertility is either classified as primary or secondary. Primary infertility refers to couples or patients who have had no previous successful pregnancies after regular unprotected intercourse for 12 months. Secondary infertility encompasses patients who have previously conceived, but are currently unable to conceive after regular unprotected intercourse for 12 months (Tietz, 2006).

Infertility problems arise as a result of hormonal dysfunction of the hypothalamic-pituitary-gonadal axis. Measurement of peptide and steroid hormones in the serum is therefore an essential aspect of the evaluation of infertility. Fertility in both men and women is at its maximum in the mid-twenties and, in women, declines after the age of 30 years (Willocks & Neilson, 1991). The fertility of a marriage is a sum of the fertilities of the two partners. Low fertility in one can to some extent be balanced by high fertility in the other, whereas low fertility in both partners may result in infertility. This explains why some couples fail to reproduce, yet when they separate and each takes a new mate, they both proceed to have children (Tindall, 1987). The single most important factor in determining fertility is the age of the female partner. If the female partner is 35 years or above, fertility is halved. Fertility declines sharply after the age of 37 (Stuart Campbell & Ash Monga, 2005).

For many couples it is customary to defer investigation until after one year of unprotected intercourse, but it is essential to start early investigation (i.e. after six months after unprotected intercourse) in women above 35 years of age (Stuart Campbell & Ash Monga, 2005). It is difficult to determine the true cause of infertility because there are numerous factors that bias studies. The cause is established only after investigation and therefore can be affected by referral policies, special interests of the clinic, types of couples seeking investigation, resources available and investigations instituted. Unexplained infertility of a couple in one centre may be explained in another that has facilities for more detailed investigations (Stuart Campbell & Ash Monga, 2005).

The prevalence of infertility across sub-Saharan Africa has received scant attention in population research despite the well-known linkage between infertility, sexually transmitted diseases (STDs) and other reproductive tract infections. There is also mounting epidemiologic evidence that African women have the highest rates of disease induced infertility in the world (Ericksen & Brunette, 1996). The desire to reproduce is an intensely motivating human force and childbearing is an important aspect of most marriages. For most couples, the conception and raising of children are the expected outcomes of their sexual relationship Because of its personal nature, couples may also experience strong religious, cultural and societal pressures to conceive. Societal and parental pressures for propagation of the family name can place a psychological burden on the infertile couple. This central role of reproduction in the human experience has contributed greatly to the desire of couples to overcome infertility. Therefore, it is understandable that people experiencing infertility often perceive it as a serious life crisis. The physical, psychological and financial challenges of assisted reproductive technology may further impact the couple (Monga *et al.*, 2004; Seibel, 1997).

1.2.9.1.1 Causes of Infertility

The main causes of infertility are disorders of oocyte production and ovulation, sperm production and delivery, fallopian tube function and implantation of the embryo. The causes of infertility vary from one geographical area to another with social factors having an influence on the cause. In Africa, most infertile women have

tubal infertility whereas in the Western world, it is either male factor infertility or ovulatory disorders (Stuart Campbell & Ash Monga, 2005).

The number of infertile couples seems to be increasing because many couples postpone the start of a family. Delaying pregnancy decreases the number and quality of available eggs and allows a greater length of time for women to develop unwanted sequelae of conditions such as endometriosis, uterine fibroids, and pelvic inflammatory disease (PID) (Brugh *et al.*, 2002). In sub-Saharan Africa sexually transmitted diseases (STDs) most often implicated in infertility are gonorrhoea, chlamydia and syphilis. These STDs prevent conception by either scarring the fallopian tubes as a result of PID, or in the case of syphilis, by causing foetal loss through spontaneous abortion or stillbirth. Although there are causes of infertility in addition to STDs, epidemiologists agree that it is the transmission of STDs and the lack of treatment for these diseases that explains infertility in sub-Saharan women (Ericksen & Brunette, 1996).

Ovulatory disorders (including ovarian and hormonal causes) account for approximately 30%, pelvic factors (including tubal, cervical, and uterine disease) account for approximately 50%, and immunological factors are implicated in approximately 5% of infertility case. Ovulatory dysfunction manifests itself in the presence or absence of normal menses, making it difficult to diagnose. Metabolic diseases of many kinds can affect ovulatory function, including ones that result in androgen excess. PCOS, which results in androgen excess, is the most common cause of anovulation. In women with hirsutism, CAH should be considered. A 21-hydroxylase deficiency or 3-β-hydroxysteroid deficiency may be present in up to 26% of cases. Ovulatory dysfunction can also be secondary to liver or thyroid disorders as well as obesity. As with male infertility, hypogonadism (hypergonadotropic or hypogonadotropic) also results in female infertility. Causes of hypergonadotropic hypogonadism include, gonadal dysgenesis, resistant ovary syndrome, menopause, and luteal phase deficiency. Causes of hypogonadotropic hypogonadism include pituitary or hypothalamic insufficiency and hyperprolactinemia (Tietz, 2006).

Cigarette smoking has been demonstrated in multiple studies to impair fertility potential in a dose-dependent manner in both men and women. Smoking has adverse effects on tubal function, hormonal secretion and cervical mucus production. An association between high alcohol intake and an increased risk of infertility has been

found. Alcohol ingestion has also been shown to cause a decrease in gonadotrophin levels and irregularities in ovulation (Brugh *et al.*, 2002; Eggert *et al.*, 2004).

1.2.9.2 Anovulation

The causes of anovulation can be divided into two categories. One category occurs as a result of hypothalamic-pituitary dysfunction. Hypothalamic-pituitary dysfunction resulting in hypogonadotrophic hypogonadism is characterized by a selective failure of the pituitary gland to produce LH and FSH. In addition to this, women with a low body mass index (BMI) [weight (kg)/ height (m^2)] (for example < 20kg/m^2), may develop amenorrhoea because of a physiological reduction in the hypothalamic production of gonadotrophin-releasing hormone (Hamilton-Fairley & Taylor, 2003).

The second category of anovulation has ovarian causes. In this category, polycystic ovarian syndrome is the most common cause of anovulatory infertility. The primary abnormality is the excess androgen production within the ovary that leads to the recruitment of large numbers of small pre-ovulatory follicles. The androgen fails to respond to normal concentrations of FSH (Hamilton-Fairley & Taylor, 2003). Ovulation can be seriously affected by excessive weight loss or weight gain, excessive exercise and extreme emotional stress (Seibell, 1997). Twenty percent above or below ideal body weight may affect ovulation. The relationship between excess body fat and ovulatory disturbances appears stronger for early-onset obesity (Seibell, 1997). Obese women develop menstrual disorders which are more often anovulation than amenorrhoea. Obese women have an excess number of fat cells in which extraglandular aromatization of androgens to oestrogens occurs. They also have lower circulating levels of SHBG, which allows a larger proportion of free androgens to be converted to oestrone. The increase in SHBG allows an increase in free androgen levels, which initially are removed by an increased rate of metabolic clearance. This compensatory mechanism diminishes over time, and hirsutism can develop (Scherzer & McClamrock, 1996).

As a woman ages, the ovarian follicles diminish in number and become less sensitive to FSH. The process of ovulation becomes increasingly inefficient, less regular, and less predictable than in earlier years. A woman will begin to notice changes in her reproductive cycle at around age 38 to 42. Initially, she will notice a shortening of the cycle length. With increasing inefficiency of the reproductive cycle, the follicular

phase shortens, but the luteal phase is maintained at normal length. With the passing of time, some cycles become anovulatory, so that the frequency of ovulation decreases (Seibell, 1997). Another common cause of anovulation in women is hyperprolactinaemia. It is caused by a pituitary micro-adenoma. This leads to a reduction in the production of pituitary luiteinizing hormone and FSH (Scherzer & McClamrock, 1996; Hamilton-Fairley & Taylor, 2003). Anovulation tends to occur in women at the extremes of reproductive age and it is typified by an irregular cycle, it is more common in obese women (Stuart Campbell & Ash Monga, 2005).

1.2.9.3 Amenorrhoea

In a normal ovulatory menstrual cycle, menstruation occurs every 28 days on average. Normal women display considerable variation in cycle length that varies from 25 to 30 days. Amenorrhea, the absence of menstrual bleeding, is traditionally categorized as either primary (women who have never menstruated) or secondary (women in whom menstruation is present for a variable time and the ceases). Amenorrhea is a relatively common disorder, with an estimated prevalence of 5% in the general population and as high as 8.5% in an unselected adolescent post pubescent population (Tietz, 2006).

Primary amenorrhea is defined as a failure to establish spontaneous periodic menstruation by the age of 16 regardless of whether secondary sex characteristics have developed. About 40% of phenotypic females who have primary amenorrhea (nearly always associated with absence of development of secondary sex characteristics) have Turner's syndrome (55X karyotype) or pure gonadal dysgenesis (either 46 XX or XY karyotype). Mullerian duct agenesis or dysgenesis with absence of the vagina or uterus is the second most common manifestation, and the third most common is testicular feminization (androgen receptor deficiency and normal or elevated plasma testosterone concentrations if the patient is past puberty and is karyotype XY). A 17α-hydroxylase deficiency is a rare form of CAH that is associated with delayed puberty, primary amenorrhea, and hypertension. These patients have a 46 XX karyotype with elevated gonadotrophins, low sex steroids, hypertension, and hypokalemia. Another rare cause of amenorrhea is the so-called resistant ovary syndrome. This primary hypogonadal condition is associated with increased concentrations of plasma FSH and LH, and ovaries that contain

predominantly primordial follicles. It is thought to arise from a defect in FSH receptors (Tietz, 2006).

Secondary amenorrhea is defined as an absence of periodic menstruation for at least 6 months in women who previously experienced menses, or for 12 months in a woman with prior oligomenorrhea. Oligomenorrhea is infrequent menstruation, occurring less than nine times per year. With a few exceptions, the causes of primary and secondary amenorrhea overlap. Pregnancy is the most common cause of secondary amenorrhea and must be considered first and ruled out. Elevated Prolactin, either iatrogenic or induced by a Prolactin-secreting tumor, can result in oligomenorrhea or amenorrhea. About one third of women with no obvious cause of amenorrhea have elevated Prolactin concentrations. It is thought that hyperprolactinemia inhibits the release of LH and FSH. Both hyperthyroidism and hypothyroidism are associated with a variety of menstrual disorders because of their effect on metabolism and interconversion of androgens and estrogens (Tietz, 2006). Recent observations have clearly demonstrated that, while the hypothalamic pituitary ovarian axis is capable of maintaining ovulatory cyclicity on its own, multiple endogenous or environmental influences may impinge on the normal activity of the pulse generator, usually to decrease GnRH pulse frequency and thereby induce cyclic dysfunction. These conditions are usually diagnosed as hypothalamic amenorrhoea. Frequent causes of hypothalamic amenorrhoea are related to exercise diet or stress (Ferin *et al.*, 1993).

CHAPTER TWO

MATERIALS AND METHODS

In this chapter the methodology used for this study is described. The specific techniques, apparatus, methods, biochemical assays and procedures will be described, as well as the methods for statistical analysis.

2.0 Working Definitions

The following operational definitions are defined according to their uses in this study.

2.1 Anthropometrical measurements

Anthropometry is the measurement of body size, weight, and proportions. Anthropometric measures can be used to evaluate nutritional status, be it obesity caused by over nutrition, or emaciation resulting from protein-energy malnutrition (Lee & Nieman, 2003). Anthropometric measurements in this study include the measurement of the body mass index (BMI) and waist- to-hip-ratio (WHR).

2.2 Body Mass Index

Body mass index or Quetelet index, is a statistical measurement which compares a person's weight and height. Body mass index (BMI) is derived mathematically from the height and weight measures. BMI = weight (kg) / Height (m^2).

BMI values correlate significantly with body fatness and obesity, and experts use them to help evaluate a person's health risks associated with underweight (Whitney *et al.*, 2002).

BMI can be categorized as follows according to the WHO (Laquatra, 2004):

Underweight < 18.5 kg/m^2

Normal 18.5 – 24.9 kg/m^2

Overweight 25.0 – 29.9 kg/m^2

Obese class I 30.0 – 34.9 kg/m^2

Obese class II 35.0 – 39.9 kg/m^2

Obese class III > 40 kg/m^2

2.2.1 Waist-hip-ratio

Waist- hip-ratio (WHR) refers to the relationship between the waist circumference and the hip circumference. WHR is calculated by dividing the waist circumference by the hip (or gluteal) circumference. For the purposes of this study, it is recommended that the WHR of adult women be < 0.8 (Lee & Nieman, 2003). A WHR of more than 0.80 in women indicates central body distribution (android obesity), while a ratio of less than 0.80 indicates gynoid obesity (Brown, 2002 & Hammond, 2000).

2.2.2 Biochemical parameters

The following biochemical measurements were taken and the following cut-off points
are given for women.

- Serum glucose refers to serum glucose concentrations. The fasting glucose reference range is 3.6-6.4 mmol/L.
- Serum cholesterol refers to serum cholesterol concentrations. The cholesterol reference range is 3.9 - 5.2 mmol/L.
- Serum triglyceride refers to serum triglyceride concentrations. The triglyceride reference range is 0.41 – 1.88 mmol/L.
- Serum HDL – cholesterol refers to serum HDL- cholesterol concentrations. The HDL- cholesterol reference range is 0.97 – 4.17 mmol/L.
- Serum LDL cholesterol refers to serum LDL-cholesterol concentrations. The LDL-cholesterol reference range is 0 – 4.2 mmol/L.
- Serum VLDL cholesterol refers to serum VLDL-cholesterol concentrations. The VLDL cholesterol reference range is
- Serum progesterone refers to fasting serum progesterone concentration. Serum progesterone concentration values for a normal female are divided according to the following phases:
  Follicular phase 0.48 – 4.45nmol/L
  Luteal phase 0.01 – 20ng/ml
  Mid- luteal phase 14.12 – 89.14nmol/L

- Serum LH refers to the serum LH concentration. During normal menstruation the reference range for LH is as follows:
  Follicular phase 1.9 – 12.5mIU/ml
  Mid-cycle peak 8.7 – 76.3mIU/ml
  Luteal phase 0.5 – 16.9mIU/ml.

- Serum FSH refers to the concentration of serum FSH. Serum FSH concentrations for normally menstruating women are:
  Follicular phase 2.5 – 10.2mIU/ml
  Mid-cycle peak 3.4 – 33.4mIU/ml
  Luteal phase 0.2 – 9.5mIU/ml.

- Serum prolactin refers to the serum prolactin concentration. Serum Prolactin concentrations for non-pregnant women: 2 – 19ng/ml.

2.3 STUDY DESIGN

A descriptive study was carried out to investigate the correlation between anthropometrics and infertility in women.

2.3.4 Sample

Hereafter, the target population, sample size and inclusion criteria will be discussed.

2.3.4.1 Target population

The study was carried out at the Department of Obstetrics and Gynaecology at the Komfo Anokye Teaching Hospital. For this study, all new patients visiting the department for infertility issues were randomly recruited into the study by a single qualified obstetrician and gynaecologist.

2.3.4.2 Sample selection

Infertile women who satisfied the inclusion criteria attending the unit were included in this study after an investigation by the gynaecologist.

2.3.4.3 Sample size

Infertile women referred to the Department of Obstetrics and Gyaenaecology at the Komfo Anokye Teaching Hospital for infertility issues between September 2009, to March 2011 were included. A total of 178 women were recruited, but only 144 women were included in the study. All subjects gave informed consent to take part in the study after verbal and written explanation of the methods and risks involved.

This study was approved by the Committee on Human Research, Publications and Ethics (CHRPE), School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi. All patients enrolling in the study completed a written informed consent form in accordance with the Helsinki Declaration.

2.4 Inclusion and exclusion criteria

The inclusion criteria are sub fertile or infertile women above 18 years who are psychologically, physically and socially fit to be entered into the study and visiting the facility for treatment. The exclusion criteria for this study were women presenting with infectious conditions such as HIV, Hepatitis B and C, and Tuberculosis. All patients referred to the Department are routinely tested for HIV, Hepatitis B and C and Tuberculosis. Those patients who are HIV, Hepatitis B and C and Tuberculosis positive are given further treatment. For this reason, all HIV, Hepatitis B and C and Tuberculosis positive patients were excluded from this study.

2.5 STUDY PROCEDURE

Women with infertility issues referred to the Gynaecology department of the hospital were referred to the clinic by general practitioners at the polyclinic department of the hospital, private practicing medical doctors or by doctors working in hospitals within the Ashanti region and other parts of the country. The patients are arranged for consultations with the Gynaecologist upon their arrival after which they meet the researcher. The aim of the study was explained to the patients. Patients were given the choice to participate in the study, and when they agree to participate, they are asked to sign the consent form. Anthropometric measurements were carried out by qualified personnel using standardized methods and procedures (ISAK, 2001). The patients were asked to complete a questionnaire with the help of the researcher. A brief consultation was conducted by the researcher informing each patient about her

weight status, as well as explaining the possible associations of weight status with the condition of infertility.

The researcher then made appointment with the patients based on knowledge of their last menstrual periods for blood samples to be taken for fasting blood sugars and 21-day progesterone, LH, FSH and PRL levels as well as Lipid profile. The samples were taken to the Chemical Pathology laboratory of KATH for analysis.

2.6 APPARATUS AND TECHNIQUES

2.6.1 Apparatus

All measurements were taken according to standardized methods and techniques using standardized equipment.

2.6.1.1 Scale

A platform electronic scale was used to measure the subjects' weight to the nearest 0.1kg (Lee & Nieman, 2003). The scale was calibrated before the study.

2.6.1.2 Measuring tape

The measuring tape used was non-extendible, flexible and with a stub of 4cm before the zero line (ISAK, 2001).

2.6.1.3 Standiometer

Height was measured using a standiometer to the nearest 0.1cm. A standiometer is a measuring stick attached to a vertical metal board with a movable horizontal headboard. The standiometer can measure up to two meters (Heymsfield *et al.*, 1999).

2.6.2 Measuring procedures and techniques

Anthropometric measurements were done according to standardized methods and techniques by qualified nurses (Lee & Nieman, 2003).

2.6.2.1. Weight

Weight was measured on a bathroom scale with the subject wearing minimal clothing and no shoes. The subject stood still with weight evenly distributed on both

feet, with no additional support while the reading was taken (Heymsfield *et al.*, 1999).

2.6.2.2 Height

Height was measured with the subject standing erect with weight equally distributed on both feet, with the heels together and flat on the floor. The subject looked straight ahead with the line of vision perpendicular to the body (Frankfort Plane). The subject was asked to inhale and the horizontal headboard was brought down firmly on top of the head (Heymsfield *et al.*, 1999).

2.6.2.3 Waist and Hip circumference

The waist was measured using a non-stretching tape measure with the measurement taken around the point near the belly button (Sizer & Whitney, 2003). The hip circumference was measured at the largest circumference between the waist and the knees. Both measurements were measured to the nearest 0.1cm (Hammond, 2000).

2.7. Biochemical Assays

Blood samples were drawn by qualified phlebotomist working at the Clinical Biochemistry department. Blood samples were drawn after an overnight fast of 12 - 16 hours, using standardized laboratory techniques. Blood samples were collected from the ante cubital vein. Rubber tourniquet was applied for less than one minute and the site to be punctured cleaned with 70% methylated spirit. 8ml to 10ml of blood was taken. 2ml of the blood was placed in a tube containing fluoride – oxalate for blood glucose measurement. The samples were then mixed by hand to prevent clotting by turning the test tube up and down. This sample was placed in a centrifuge and spun at 3000 x g for 3 - 5 minutes to obtain the plasma used for the blood glucose test. The rest of the blood samples were placed in plain tubes and allowed to clot. After the blood had clotted it was placed in a centrifuge and spun at 3000 x g for 10 minutes to obtain the sera. The blood samples were analyzed at the Department of Clinical Biochemistry of KATH by the researcher. Various biochemical assays including, Blood Glucose, Total Cholesterol, Triglycerides, High Density Lipoprotein (HDL)-Cholesterol and Low Density Lipoprotein (LDL)-Cholesterol were analyzed using the BT® 3000 Random Access Chemistry System (Elan Diagnostic Systems, USA). Other Hormonal assays including, Progesterone, Prolactin, Follicle Stimulating Hormone and

Luteinizing Hormone were analyzed using the *mini* VIDAS® Hormonal Analyser (BioMerieux® sa, France).

2.7.1 Glucose

Glucose concentrations in the samples were estimated with the glucose oxidase method according to the following reactions (Barham *et al.*, 1972):

$$\text{Glucose} + O_2 \xrightarrow{glucose\ oxidase} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{peroxidase} \text{Quinoneimine} + 4H_2O$$

2.7.2 Total Cholesterol

This method for the measurement of total cholesterol (Allain *et al.*, 1974) in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the absence of the former the mixture of ethyl-N-propyl-m-anisidine (ADPS) and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample. The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when estimated at 500 nm.

$$Cholesterol\ Esters \xrightarrow{CE} Cholesterol + Fatty\ Acids$$

$$Cholesterol + O_2 \xrightarrow{CO} Cholest-4-en-3-one + H_2O_2$$

$$4-AA + ADPS + H_2O_2 \xrightarrow{POD} Quinoneimine\ (red\ dye) + 4H_2O$$

2.7.3 Triglycerides

The present method uses a modified Trinder (Barham *et al.*, 1972) colour reaction to produce a fast, linear, endpoint reaction(Fossati *et al.*, 1982). Triglycerides in the sample are hydrolyzed by lipase to glycerol and fatty acids. The glycerol is then phosphorylated by ATP to glycerol-3-phosphate (G3P) and ADP in a reaction catalyzed by glycerol kinase. G3P is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide by glycerophosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine (4-AAP) and 3, 5-dichloro-2-hydroxy

benzen (3, 5-DHBS) in a reaction catalyzed by peroxidase to yield a red coloured quinoneimine dye. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

$$Triglycerides + H_2O \xrightarrow{Lipase} Glycerol + Fatty\ Acids$$

$$Glycerol + ATP \xrightarrow{Glycerol\ kinase} G3P + ADP$$

$$G3P + O_2 \xrightarrow{Glycerolphosphate\ oxidase} DAP + H_2O_2$$

$$H_2O_2 + 4AAP + 3,5 - DHBS \xrightarrow{Peroxidase} Quinoneimine\ (red\ dye) + 2H_2O$$

2.7.4 HDL-Cholesterol

The method employed in this study is in a two reagent format. The first reagent contains anti human β-lipoprotein antibody which bind to lipoproteins (LDL, VLDL and chylomicrons) other than HDL. The second reagent contains enzymes which then selectively react with the cholesterol present in the HDL particles. Consequently only HDL cholesterol is subject to cholesterol measurement. The intensity of the red colour produced is directly proportional to the HDL-cholesterol in the sample when read at 500 nm.

2.7.5 LDL-Cholesterol

The LDL-Cholesterol concentration (LDL-C) is calculated from the total cholesterol concentration (TC), HDL-Cholesterol concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald's equation found below (Friedewald *et al.*, 1972).

$$LDL = TC - HDL - \frac{TG}{2.2} \quad mmol/l$$

2.7.6 Progesterone

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection, Enzyme Linked Flourescent Assay (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the

assay. The sample is taken and transferred into the well containing alkaline phosphatase labeled anti-progesterone (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyses the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of progesterone present in the sample.

2.7.7 Prolactin

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The sample is taken and transferred into the well containing alkaline phosphatase labeled anti-prolactin (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyses the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of prolactin present in the sample.

2.7.8 Luteinizing hormone (LH)

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The sample is taken and transferred into the well containing alkaline phosphatase labeled anti-LH (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components are eliminated during the

washing steps. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyses the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of LH present in the sample.

2.7.9 Follicle Stimulating Hormone (FSH)

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The sample is taken and transferred into the well containing alkaline phosphatase labeled anti-FSH (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyses the hydrolysis of the substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of FSH present in the sample.

2.8 STATISTICAL ANALYSIS

The OUTLIERS preliminary test for detection of error values was initially applied for statistical analysis. The results were given as mean ± Standard error of mean (SEM). Correlations were evaluated using the Pearson's correlation test. For all statistical comparisons, the level of significance was set at $p<0.05$. All data analysis in this research was done using GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA) and MedCalc ® for Windows version 9.4.2.0.

2.9 LIMITATIONS TO THE STUDY

The researcher made appointments with the patients before their blood samples were taken. The patients were told specifically to come in a fasting state, as well as not having exercised for 12 hours prior to their consultations. The biggest problem encountered was patients refusing to turn up for the appointments or not coming in a

fasting state. These patients were then asked to make other appointments, so that all the measurements could be taken while they were in a fasting state. This hugely affected the anticipated period for the study and the total number of subjects recruited.

Another problem encountered was the measurement of blood pressure. The unit has a centre where all blood pressure measurements are taken for patients visiting specialists. After their body measurements were taken, the participants were asked to go for their blood pressure measurements which only 58 of them did. This could not be used for statistical purposes as against the 144 total subjects in the study.

The progesterone and other hormonal measurements was another problematic issue in this study. This measurement should be determined on day 21 of the menstrual cycle, to indicate whether a patient is ovulating or not. Upon knowledge of a participant's last menstrual period, appointment is made with the patient. Patients were told of this and asked to come for the blood sampling on that specific day of their menstrual cycle. Most of them did not keep their appointments, and the researcher had to contact them and make new appointments with them. These appointment dates were different for each patient making the study more difficult. Those patients that did not live in Kumasi or its environs had to be excluded from the study. It was explained to the subjects that their transportation costs would be carried by the researcher. This created a huge burden for the researcher, delayed the study and also affected the anticipated period for the study and the total number of subjects recruited.

CHAPTER THREE

RESULTS

3.1 Introduction

One hundred and forty four (144) infertile women (32.5 ± 12.5 years, range 20-45yrs) were included in the study. The collection of data was done over a period of nineteen months thus from September 2009 to March 2011. BMI, WC, HC and WHR were the anthropometric parameters analyzed from the data. According to classifications by BMI, the subjects were categorized into four groups; Normal (BMI 18.5-25 kg/m^2), Overweight (BMI 25-30 kg/m^2), Obese I (BMI 30-35 kg/m^2) and Obese II (BMI 35-40 kg/m^2). The mean BMI was 28.92±4.88. With regards to WHR, the subjects were categorized into three groups; Group I (WHR 0.80 or below) were subjects with low risk of developing cardiovascular disease (CVD), Group II (WHR 0.81-0.85) were subjects with moderate risk of developing CVD and Group III (WHR > 0.85) were subjects with high risk of developing cardiovascular disease. The mean WC measurement for the subjects was 87.92±10.10 cm.

3.2 Demographic Characteristics Based On Body Mass Index

The most prevalent duration of infertility in the study was between 1 to 5 years with majority of the subjects (about 80.82% of the total population) in the overweight and obese groups. The longest duration of infertility in the study was between 21 to 25 years. Primary infertility was more prevalent (about 59.72% of the total population) as against 40.28% of secondary infertility with a significant 79.86% combined primary and secondary infertile subjects in the overweight and obese groups. Infertility was highest among the Akans (about 77.78% of the total population), least among the Northerners (11.81%), Ewes (5.55%) and the Gas (4.86%). The baseline clinical characteristics of the study participants are shown in Table 1. Approximately 28.5, 17.4, 14.6, 9.7, 9.7, 7.02, 6.9 and 4.2% had the following infertility condition(s) respectively: Tubal factors, Male Factors, Ovulation problems, Unexplained Causes, Uterine problems, Hyperprolactinaemia, PCOS and Endometriosis. Majority of the study participants were self employed. The

demographic characteristics based on WHR shown in Table 2 similar to that of Table one.

Duration of condition(yrs)	Total 144 (%)	Normal (%)	Overweight (%)	Obese I (%)	Obese II (%)
1 to 5	110(76.39)	20 (18.18)	41 (37.27)	36 (32.73)	13 (11.82)
6 to 10	21(14.58)	5 (17.2)	6 (11.8)	10 (19.6)	0 (0)
11 to 15	9(6.26)	1 (3.5)	3 (5.9)	5 (9.8)	0 (0)
16 to 20	3(2.08)	3 (10.3)	0 (0)	0 (0)	0 (0)
21 to 25	1(0.69)	0 (0)	1 (1.9)	0 (0)	0 (0)
Type of infertility					
Primary	86(59.72)	21 (24.42)	31 (36.05)	26 (30.23)	8 (9.30)
Secondary	58(40.28)	8 (13.80)	20 (34.48)	25 (43.10)	5 (8.62)
Ethnicity					
Akans	112(77.78)	20 (17.86)	41 (36.61)	41 (36.61)	10 (8.92)
Ewes	8(5.56)	2 (25)	4 (50)	2 (25)	0 (0)
Gas	7(4.86)	1 (14.29)	1 (14.29)	2 (28.57)	3 (42.85)
Others*	17(11.80)	6 (35.30)	5 (29.40)	6 (35.30)	0 (0)
Diagnosis					
Tubal Factors	41(28.47)	8(19.51)	11(26.83)	19(46.34)	3(7.32)
Male Factors	25(17.36)	6(24.00)	11(44.00)	6(24.00)	2(8.00)
Ovulation Problems	21(14.58)	4(19.05)	7(33.33)	9(42.86)	1(4.76)
Uterine Problems	14(9.72)	2(14.29)	5(35.71)	6(42.86)	1(7.14)
Unexplained causes	14(9.72)	2(14.29)	8(57.13)	2(14.29)	2(14.29)
Hyperprolactinaemia	14(9.72)	3(21.43)	4(28.57)	6(42.86)	1(7.14)
PCOS	10(6.94)	3(30.00)	3(30.00)	2(20.00)	2(20.00)
Endometriosis	5(3.49)	1(20.00)	2(40.00)	1(20.00)	1(20.00)
Occupation					
Public servants	37(25.70)	6(16.21)	17(45.95)	11(29.73)	3(8.11)
Self employed	95(65.97)	19(20.00)	30(31.57)	37(38.95)	9(9.48)
Student	7(4.86)	3(42.86)	3(42.86)	0(0)	1(14.28)
Unemployed	5(3.47)	1(20.00)	1(20.00)	3(60.00)	0(0)

Table 1. Demographic Characteristics of the study based on Body Mass Index. The values are expressed as percentages (%)

The data are presented as group Means (± SD), sample percentages are in parenthesis. PCOS; Polycystic Ovarian Syndrome. Others*; Dagaabas, Frafras, Dagombas, Grushies and Gonjas.

3.3 Demographic Characteristics Based On Waist to Hip Ratio (WHR).

Table 2. Demographic Characteristics of the study based on WHR. The values are expressed as percentages (%) in parenthesis.

Duration of condition(yrs)	TOTAL 144(%)	0.80 or below (%)	0.81-0.85 (%)	Greater than 0.85 (%)
1 to 5	108(75.00)	17(15.74)	29(26.85)	62(57.41)
6 to 10	22(15.28)	4(18.18)	11(50.00)	7(31.82)
11 to 15	9(6.25)	1(11.11)	1(11.11)	7(77.78)
16 to 20	4(2.78)	1(25.00)	1(25.00)	2(50.00)
21 to 25	1(0.69)	0(0)	0(0)	1(100)
Type of infertility				
Primary	85(59.03)	13(15.30)	22(25.88)	50(58.82)
Secondary	59(40.93)	10(16.95)	21(35.59)	28(47.46)
Ethnicity				
Akans	112(77.78)	15(13.39)	39(34.81)	58(51.75)
Ewes	8(5.56)	2(25.00)	1(12.50)	5(62.50)
Gas	7(4.86)	2(28.57)	1(14.29)	4(57.14)
Others*	17(11.80)	4(23.53)	2(11.76)	11(64.71)
Diagnosis				
Endometriosis	4(2.78)	1(25.00)	2(50.00)	1(25.00)
Tubal Factors	27(18.75)	4(14.81)	9(33.33)	14(51.86)
PCOS	12(8.33)	4(33.33)	5(41.67)	3(25.00)
Uterine problems	22(15.28)	4(18.18)	6(27.27)	12(54.55)
Male Factors	23(15.97)	4(17.39)	5(21.74)	14(60.87)
Hyperprolactinaemia	15(10.42)	4(26.67)	5(33.33)	6(40.00)
Ovulation Problems	23(15.97)	4(17.39)	6(26.09)	13(56.52)
Unexplained causes	18(12.5)	4(22.22)	4(22.22)	10(55.56)
Occupation				
Public servants	36(25.00)	7(19.44)	11(30.56)	18(50.00)
Self employed	95(65.97)	13(13.68)	29(30.53)	53(55.79)
Student	7(4.86)	3(42.86)	2(28.57)	2(28.57)
Unemployed	6(4.17)	0(0)	1(16.67)	5(83.33)

The data are presented as group means (± SEM), sample percentages are in parenthesis. PCOS; Polycystic Ovarian Syndrome. Others*; Dagaabas, Frafras, Dagombas, Grushies and Gonjas.

3.4 Comparisons between Biochemical Parameters with BMI and WHR

With increasing body weight gains as shown in Table 3, there were increasingly adverse cardiovascular risk profiles as confirmed by raised fasting plasma glucose concentration, high serum total cholesterol, high low density lipoprotein cholesterol, high BMI and raised coronary risk levels. This is evident of high prevalence of dysmetabolism such as diabetes, obesity, cardiovascular risk factors and dyslipidemia. There was no strong correlation between BMI and the hormonal profiles of the infertile women. Table 4 shows no significant correlation between the biochemical and hormonal parameters stratified by WHR except for LH.

Table 3. Comparisons of biochemical and hormonal parameters with BMI. The BMI is divided into different classes as normal, overweight, obese I and obese II.

Parameter	Normal	Overweight	Obese I	Obese II	p value
FBS (mmol/l)	5.127±0.244	6.102±0.399	6.01±0.331	8.161±0.595**	0.0028
TChol(mmol/)	5.112±0.224	5.58±0.263	5.818±0.227	7.827±0.918***	0.0003
TG (mmol/l)	1.311±0.132	1.417±0.143	1.539±0.103	2.535±0.488***	0.0014
HDL (mmol/l)	1.661±0.114	1.708±0.096	1.531±0.090	1.341±0.101	0.2117
LDL(mmol/l)	3.162±0.213	3.585±0.213	3.904±0.206	5.954±0.8856***	<0.0001
VLDL(mmol/)	0.2623±0.026	0.2847±0.028	0.3077±0.021	0.5069±0.098**	0.0015
CR	4.676±0.308	6.063±0.850	6.086±0.440	8.654±1.167*	0.0589
Prog(ng/ml)	9.168±1.254	8.849±0.804	7.297±0.682	7.937±2.228	0.4662
FSH (mIU/ml)	21.92±5.215	10.34±2.188	13.01±2.369	17.18±4.972	0.0718
LH (mIU/ml)	11.82±2.692	10.77±1.718	9.56±1.466	14.18±3.457	0.6324
PRL (ng/ml)	20.68±3.343	22.84±2.895	17.35±1.993	18.7±6.579	0.5032

Data are presented as group means (± SEM). Correlation is significant at the level * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$ by Bonferroni's Multiple Comparison test. FBS; Fasting Blood Sugar, TChol; Total Cholesterol, TG; Triglycerides, HDL; High Density Lipoprotein Cholesterol, LDL; Low Density Lipoprotein Cholesterol, VLDL; Very Low Density Lipoprotein, CR; Coronary Risk, Prog; Progesterone(21 day), FSH; Follicle Stimulating Hormone, LH; Leuitinizing Hormone and PRL; Prolactin.

Table 4. **Comparisons of biochemical and hormonal parameters with WHR. The WHR is divided into different groups as low risk (≤0.8), moderate risk (0.81-0.85) and high risk (>0.85).**

Parameter	≤0.8	0.81-0.85	>0.85	p value
FBS **(mmol/l)**	6.295±0.568	5.671±0.322	6.299±0.304	0.3989
T Chol **(mmol/l)**	5.503±0.5114	5.607±0.2256	5.974±0.232	0.4624
TG **(mmol/l)**	1.302±0.217	1.608±0.165	1.597±0.122	0.4347
HDL-C **(mmol/l)**	1.66±0.166	1.521±0.084	1.628±0.071	0.5986
LDL-C **(mmol/l)**	3.597±0.4122	3.763±0.2141	3.96±0.2157	0.6513
VLDL **(mmol/l)**	0.2604±0.0254	0.3216±0.0329	0.3202±0.0243	0.4288
CR	5.717±0.7089	5.86±0.5403	6.241±0.5723	0.8373
Prog (ng/ml)	9.36±1.464	7.764±0.8884	8.423±0.650	0.5851
FSH (mIU/ml)	12.92±3.785	14.05±3.056	15.00±2.263	0.8982
LH (mIU/ml)	7.158±1.707	8.65±1.497	13.2±1.544*	0.0368
PRL(ng/ml)	19.42±4.028	20.76±2.497	19.81±2.172	0.9494

Data are presented as group means (± SEM). Correlation is significant at the level * $p < 0.05$ by Bonferroni's Multiple Comparison test. FBS; Fasting Blood Sugar, TChol; Total Cholesterol, TG; Triglycerides, HDL; High Density Lipoprotein Cholesterol, LDL; Low Density Lipoprotein Cholesterol, VLDL; Very Low Density Lipoprotein, CR; Coronary Risk, Prog; Progesterone(21 day), FSH; Follicle Stimulating Hormone, LH; Leuitinizing Hormone and PRL; Prolactin.

3.5 Associations between Anthropometric indices and Age.

The correlation between the anthropometric variables, body composition and age is presented for the infertile women in Table 5. There was significant correlation between most of the anthropometric indices. Significant correlations were noticed between BMI, WHR, WC and HC with increase in body weight except for height. Table 6 shows the percentages of individuals with the various parameters.

Table 5. Correlations between anthropometric variables with increase in weight of the subjects.

Variable	Normal	Overweight	Obese I	Obese II	*p* value
Age (yrs)	31.14±1.27	31.45±0.83	32.84±0.87	31.85±0.775	0.5799
Weight (kg)	57.17±0.98	69.65±1.09***	82.86±1.12***	96.46±1.51***	<0.0001
Height (cm)	159.4±0.90	160.6±0.85	160.9±0.72	160.8±1.39	0.6881
WC (cm)	78.14±1.13	86.27±1.17***	92.32±1.22***	98.23±2.46***	<0.0001
HC (cm)	94.38±1.17	98.73±1.45	105.70±1.34***	110.60±1.89***	<0.0001
WHR	0.82±0.01	0.87±0..7**	0.87±0.007**	0.89±0.03***	0.0012

Data are presented as group means (± SEM). Correlation is significant at the level * $p < 0.05$, correlation is significant at the level ** $p < 0.001$ and correlation is significant at the level *** $p < 0.0001$. WC; Waist Circumference, HC; Hip Circumference and WHR; Waist to Hip Ratio.

Table 6. Percentages of individuals with various parameters.

Parameters	Conditions	Total	Primary(Infertility)	Secondary (Infertility)
		100.00(144)	59.72(86)	40.28(58)
Age(yrs)	20-29	39.58(57)	70.18(40)	29.82(17)
	30-39	47.22(68)	55.88(38)	44.12(30)
	40-49	13.19(19)	47.37(9)	52.63(10)
WC(cm)	Normal	43.75(63)	65.08(41)	34.92(22)
	Central obesity	56.25(81)	60.49(49)	39.51(32)
BMI (kg/m^2)	Underweight	1.39(2)	100.00(2)	0.00(0)
	Normal	18.75(27)	51.85(14)	48.15(13)
	Overweight	34.72(50)	68.00(34)	32.00(16)
	Obese	45.14(65)	53.85(35)	46.15(30)
WHR	Normal	45.83(66)	53.03(35)	46.97(31)
	Obese	54.17(78)	64.10(50)	35.90(28)
FBS (mmol/L)	Normal	72.92(105)	54.29(57)	45.71(48)
	Diabetic	27.08(39)	56.41(22)	43.59(17)
Total cholesterol	Normal	44.44(64)	70.31(45)	28.13(18)
(mmol/L)	Hyperlipidaemia	55.56(80)	60.00(48)	40.00(32)
Coronary risk (CR)	Normal	57.64(80)	55.42(46)	44.58(37)
	High risk	42.36(61)	70.49(43)	29.51(18)
Progesterone(ng/ml)	Normal	54.86(79)	58.23(46)	41.77(33)
	Paranormal	45.14(65)	61.54(40)	38.46(25)
	Normal	61.81(89)	66.29(59)	33.71(30)
PRL (ng/ml.)	Paranormal	38.19(55)	54.55(30)	45.45(25)
FSH (mIU/ml.)	Normal	75.69(109)	55.96(61)	44.04(48)
	Paranormal	24.31(35)	54.29(19)	45.71(16)
LH (mIU/ml.)	Normal	61.11(88)	59.09(52)	40.91(36)
	Paranormal	38.89(56)	67.86(38)	32.14(18)
Hormonal profile	Normal	54.86(79)	62.03(49)	37.97(30)
	Deranged	45.14(65)	58.46(38)	41.54(27)

The data are presented as group means (± SEM), sample sizes are in parenthesis. WC; Waist Circumference, BMI; Body Mass Index, WHR; Waist to Hip Ratio, FBS; Fasting Blood Sugar, CR; Coronary Risk, FSH; Follicle Stimulating Hormone, LH; Luteinizing Hormone and PRL; Prolactin.

CHAPTER FOUR

DISCUSSION

4.1 Correlations between anthropometric indices and obesity

Obesity is rapidly increasing worldwide (Ogden *et al.*, 2006), and it results from a chronic imbalance between energy intake and energy expenditure. Excess weight is not only linked to increased risk of chronic disease and life threatening comorbidities such as diabetes, hypertension and dyslipidemia (Must *et al.*, 1999), but has also been shown to increase risk of reproductive problems (Catalano, 2007). Several studies have shown that women with excess body weight are more likely to have fertility problems (Pasquali, 2006; Gesink Law *et al.*, 2007). In this study, the mean BMI of the present study population was 28.92±4.88 kg/m^2 which is similar to values reported for women among a Ghanaian population in Accra (Amoah, 2003) and a Pentecostal population in Kumasi-Ghana (Owiredu *et al.*, 2008). The prevalence of overweight and obesity (using BMI) among the infertile women in this study was 34.72 and 45.14% respectively. Again, using WHR, more of the infertile women were obese with prevalence of 54.17%. This is evident that over nutrition was on the increase among the infertile women. Married Ghanaian women usually want to put on weight and prefer to be plumb in order to appear beautiful, as an indication that their husbands are taking good care of them and they are doing well in their marriages. Ghanaians generally associate fatness with beauty in women and success in both sexes. It is, therefore, not surprising that some women and indeed some men are now going out of their way to put on weight in order to appear beautiful or prosperous (Amoah, 2003). This may presumably contribute to the higher rates of overnutrition among the infertile women. There is an increasing rate of urbanization and Westernization among Ghanaian women in the two major cities in Ghana, which has led to the eating of more fried and fatty foods, sauces and soups than they did several decades before because of the proliferation of fast food outlets in the urban centers of the country (Owiredu *et al.*, 2008).

This study is in agreement with another study on the 'Epidemiology of Obesity in Ghana' by Biritwum *et al* (2006), which revealed that the prevalence of obesity was found to be higher among females. It was more common among the married than

unmarried. Obesity was highest among the employed compared to self-employed or the not working for pay and by ethnicity, obesity was highest among Akans.

According to the World Health Organization (WHO), obesity is becoming a major health problem in many developing countries, particularly in adult women. This presents a significant threat to the emergence of non-communicable diseases such as coronary artery disease, diabetes, hypertension and some forms of cancer in the developing world. The findings of this study is in agreement with other African data (Kruger, 1999) and confirms the association found between obesity in black women and higher coronary risk factors, high cholesterol levels, lower HDL-cholesterol levels, and higher LDL-cholesterol, triglycerides and fasting serum glucose.

The fertility of obese women is lower compared to that of women with normal weight, and ovulation disorders are more frequent (Wittemer *et al.*, 2000). Pregnancy is less likely if the woman is obese (Taylor, 2003). This could be a reason for the infertility in the women of this study with mean BMI of 28.92±4.88 kg/m² and prevalence of obesity (BMI) 45.14%. BMI has been widely used as a measure of adiposity in epidemiological studies. However, BMI variability in the present study has been found to be significant for the entire population of the infertile women. In addition to the high degree of obesity, there were significant correlation between BMI and multiple cardiovascular risk factors and comorbidities as confirmed by raised fasting plasma glucose concentration, high serum total cholesterol, high low density lipoprotein cholesterol, high BMI and raised coronary risk levels (Table 3). This is associated with the high prevalence of diabetes, obesity and dyslipidemia among the entire population.

Most women in this study were obese according to the various anthropometric indices employed (BMI; 45.14%, WHR; 54.17%, and WC; 56.25%) with central obesity being more prevalent as demonstrated by the strong correlation between WHR: overweight ($p < 0.0012$) and obese I ($p < 0.0012$) groups (Table 5). Individuals with similar BMI can vary considerably in their abdominal- fat mass, with premenopausal women typically having half the abdominal-fat mass of men. For this reason, a measure of obesity that takes into account the increased risk of obesity-related illness, due to the accumulation of abdominal fat, is desirable. Assessing the WHR is a simple procedure to identifying obese risk patients. Thus, measuring the WHR facilitates preventive medicine through identification and

rigorous treatment of these risk patients. Waist- hip-ratio (WHR) was previously acknowledged as the clinically accepted method of identifying patients with excess abdominal fat accumulation. However, more recently, waist circumference alone has been suggested as being a more practical measure of intra-abdominal fat mass and total body fat (Dalton *et al.*, 2003), and this present study also reveals appreciable prevalence levels of central obesity (WC) of 56.25% among the infertile women. Waist circumference relates closely to BMI and is the best indicator of changes in intra abdominal fat. Also, waist circumference is more strongly associated with metabolic function than WHR (Lean *et al.*, 1995). However, waist circumference cut-points lose their incremental predictive power in patients with a BMI ≥ 35 kg/m², because these patients will exceed the cut-points (NHLVI, 1998). With more than half of the entire participants (56.25%) in this present study having a waist circumference measuring more than 88cm, is indicative of increased risk for developing metabolic disorders.

4.2 Correlations between anthropometric indices and infertility.

The distribution of body fat is clearly related to infertility (Wass *et al.*, 1997). Central obesity measured by an increased waist to hip ratio is associated with a lower probability of conception (Wass *et al.*, 1997). This study can predict that women with a waist to hip ratio of less than 0.8cm could have a higher pregnancy rate than women with ratios of more than 0.8cm as suggested by Wass *et al.*, (1997) and Crosignani, (2002) as most of the subjects in this study have WHR measurements far above 0.8cm. Fertility processes involve a complex of factors and mechanisms of both ovarian and extra ovarian origin. Obesity may interfere with many neuroendocrine and ovarian functions, thereby reducing both ovulatory and fertility rates in otherwise healthy women. In agreement with other data (Norman *et al.*, 2002), this study suggests that oligo-ovulation, annovulation and subfertility are present in obese females with a relative risk of annovulatory infertility for women with a BMI >27 kg/m² compared with women of BMI 20-25 kg/m². Although many obese women have normal ovulatory menstrual cycles, remain fertile and have no apparent hyperandrogenism, the current study has substantial evidence to support the relationship between obesity and anovulatory infertility with 14.58% (Table 1) of the study population having ovulatory problems.

The mechanisms via which obesity is linked to anovulation remain unclear, and most likely several hormonal changes are involved (Pasquali, 2003). In fact, body fat distribution has been shown to substantially affect Sex Hormone Binding Globulin (SHBG) concentrations. Fat accumulation in the abdominal viscera (visceral fat) has been described as a possible cause of insulin resistance and the resulting metabolic syndrome. Female subjects with central obesity and a higher proportion of visceral fat usually have high insulin resistance leading to lower SHBG concentrations in comparison with matched subjects with peripheral obesity. The net decrease in SHBG concentration observed in obesity, leads to alterations in the availability of free circulating androgens and oestrogens, for delivery to target tissues. Due to the greater reduction of SHBG concentration, the percentage of free testosterone fraction tends to be higher in women with central obesity than in those with peripheral obesity leading to a state of 'functional hyperandrogenism'. The pattern of body fat distribution can regulate androgen production and metabolism to a significant extent. In fact, women with central obesity have higher testosterone production rates than those with peripheral obesity (Pasquali, 2003).

In this study, the prevalence of PCOS among the infertile population was 13.9% with majority of them (79.2%) being overweight or obese. Polycystic ovarian syndrome is the most common cause of anovulatory infertility in young women and the history of weight gain frequently precedes the onset of clinical manifestations of the syndrome, suggesting a pathophysiological role of obesity in the development of PCOS and the related infertility. Even though the total BMI in non-obese women with PCOS is normal, the intra-abdominal preperitoneal and visceral fat accumulation may contribute to the hormonal dysregulation leading to anovulation (Norman *et al*., 2002). Moreover, women with PCOS constitute a significant proportion of the infertile population (Kiddy *et al*., 1990). Women with PCOS tend to have a BMI outside the acceptable range (19-25 kg/m^2) and 40-60% of women with PCOS are overweight or obese (Kiddy *et al*., 1990; Goldzieher and Axelrod 1963). This present study is in agreement with other data comparing women with PCOS and age-matched controls from the NHANES I study, the women with PCOS demonstrated a lower proportion of BMI<25 kg/m^2 and higher proportion of BMI > 30 kg/m^2 and 40 kg/m^2 (Glueck *et al*., 2005). Women with PCOS also display an increased central distribution of adiposity (Pasquali *et al*., 1993) and WHR measurement > 0.8 have

been reported in 63% and 53% of women with PCOS (Pasquali *et al*., 1994). This increase in abdominal fat can be observed even in lean women with PCOS compared to weight-matched controls (Rebuffe-Scrive *et al*., 1989) and lean women with PCOS also display an increased amount of visceral fat compared to lean controls (Yildirim *et al*., 2003). A number of investigators have shown that obesity and abdominal obesity worsen the clinical features of menstrual irregularity and infertility in PCOS (Kiddy *et al*., 1990; Pasquali *et al*., 1994). Upper body fatness has been found more often in women with polycystic ovarian syndrome (PCOS), as well as other endocrinological and metabolic changes, such as increased concentrations of free and total testosterone, androstenedione, oestradione, insulin, LDL cholesterol, triglycerides and blood glucose (Wass *et al*., 1997; Crosignani, 2002). Hyperinsulinaemia and insulin resistance are well-known features in polycystic ovarian syndrome (PCOS). Therefore, those study participants with increased plasma glucose levels were at a risk of developing PCOS, due to the fact that they could have hyperinsulinaemia. Whether hyperinsulinaemia in PCOS is primarily due to a defect in insulin action, to increased insulin secretion, to decreased hepatic clearance of insulin, or to an interaction between all these disorders, is however, not clear (Morin-Papunen *et al*., 2000).

A majority of patients with PCOS have insulin resistance and/or are obese. Their elevated insulin levels contribute to or cause the abnormalities seen in the hypothalamic-pituitary-ovarian axis that lead to PCOS. Adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol. The excess of adipose tissue in obese patients creates the paradox of having both excess androgens (which are responsible for hirsutism and virilization) and estrogens (which inhibits FSH via negative feedback) (Tietz, 2006). Insulin resistance can be associated with various other features, including central obesity (Ovalle & Azziz, 2001) which is confirmed by the results of the present study, where an association was found between high glucose concentrations and android obesity II ($p < 0.0028$) as shown in table 3. Moreover, there was a positive significant correlation ($p < 0.0001$; Table 5) between central obesity, overweight and obesity among the infertile women. Morin-Papunen *et al.* (2000) found that serum insulin concentration was significantly correlated to WHR in lean polycystic women. This is

could be evident of an association between abdominal obesity and hyperinsulinaemia in these study participants.

4.3 Associations between Age and diagnosis of infertility

4.3.1 Age and infertility

The single most important factor in determining fertility is the age of the female partner. Fertility declines sharply after the age of 37 and therefore fertility is significantly halved if the female partner is 35 years or more (Tietz, 2006). The reduction in fertility is greatest in women in their late 30s and early 40s (Taylor, 2003). As a woman ages, the ovarian follicles diminish in number and become less sensitive to FSH. The process of ovulation becomes increasingly inefficient, less regular, and less predictable than in earlier years. A woman will begin to notice changes in her reproductive cycle at around age 38 to 42. Initially, she will notice a shortening of the cycle length. With increasing inefficiency of the reproductive cycle, the follicular phase shortens, but the luteal phase is maintained at normal length. With the passing of time, some cycles become anovulatory, so that the frequency of ovulation decreases (Seibell, 1997). For women aged 35 – 39 years, the chance of conceiving spontaneously is about half that of women aged 19 – 26 years. The age related decline in female fecundity is caused by a steadily reducing pool of competent oocytes in the ovaries (Taylor, 2003). For a variety of social, professional, financial, or psychological reasons, many women delay pregnancy until well into their 30s. Most women are unaware of the fact that after age 35 fertility declines and the success of assisted reproductive technologies (ART) also declines dramatically with increasing maternal age (Case, 2003).

As expected, most of the women in the present study were of the reproductive age with an age range of 20 – 45 years, (median age of 32.5) and 47.22% of the women were between the ages of 31 – 40 years. Only 13.19% were over 40 years of age with 39.58% in the 20 – 29 age range. Chigumadzi *et al.* (1998) reported in their study population at King Edward VIII Hospital, Durban, the mean age of black, infertile women was 31 years; while in a study by Wittemer *et al.* (2000), on women referred for IVF in the USA; the women's mean age was 33.07 years. Again, the present study is in agreement with an African study by Motseki (2004) on anthropometric measurement and biochemical parameters in black women at the unit for reproductive care at Universitas Hospital Bloemfontein; the women's mean age was

32.4 years. The fact that most of the infertile women in the present study were over 31 years of age, with 55.88% primary infertility conditions could be attributed to the fact that most of the infertile women were not familiar with the availability of infertility treatment facilities, or only found out about them after pursuing other options such as traditional medicines, herbal preparations and spiritual therapies.

4.3.2 Diagnosis and Causes of Infertility

4.3.2.1 Tubal Factors

Most of the participants, in this study, 28.47% (Table 1), had tubal factors as a cause of their infertility. Dyer, (2002) states that the most common cause of infertility in Africa is tubal disease, secondary to pelvic sepsis. Tubal factor infertility was found to be within the range of 14% - 22% in developed countries (Chigamudzi *et al.*, 1998). Chigamudzi *et al.* (1998) conducted a study at King Edward VIII Hospital in Durban on a total population of 100 black women from an underprivileged society. They found that among these subjects, tubal factors were identifiable in 77% of the women. Green *et al.* (2001) found that of the black women in their study, 41% had tubal factor infertility. Another study conducted in Cape Town, South Africa (on white women), also revealed tubal factors as the lead cause of infertility in 57% of their subjects (Steward-Smythe & van Iddekinge, 2003).

Moreover, a study by Fiander, (1990) at the Bawku Hospital on the causes of infertility among 1000 patients in Ghana revealed that the commonest causes of infertility were male factor (45%) and tubal factors (23%) of those diagnosed. Tubal factor infertility was the most prevalent cause of infertility amongst the overweight and obese groups in the present study and it was the most prevalent cause of infertility among the younger and older age group of the subjects, with endometriosis being the least prevalent cause. Patent Fallopian tubes are a prerequisite for normal human fertility. However, patency alone is not enough; normal function is crucial. They have a critical role in picking up eggs and transporting eggs, sperm, and the embryo. The Fallopian tubes are also needed for sperm capacitation and egg fertilization. Because the egg is fertilized in the Fallopian tubes, and the first stages of development of the embryo occur during its four day journey to the uterine cavity, the tubes are also important in nutrition and development. Pelvic infection is a major cause of tubal sub- fertility. Infective tubal damage can be caused by sexually transmitted diseases, or can occur after miscarriage, termination of pregnancy,

puerperal sepsis, or insertion of an intrauterine contraceptive device (Khalaf, 2003). Other causes of tubal infertility include pelvic inflammatory disease, previous pelvic surgery (especially ruptured appendix) and endometriosis (Case, 2003). The high rate of tubal factor infertility among the subjects of this study, and especially among the older age group from tables 1 and 2, is indicative of the possibility of pelvic infections. Because tubal factors have been associated with STDs, it is also possible that some of these women in this study could have had a history of STDs. In sub-Saharan Africa sexually transmitted diseases (STDs) most often implicated in infertility are gonorrhoea, chlamydia and syphilis. These STDs does either prevent conception by scarring the fallopian tubes as a result of PID, or in the case of syphilis, by causing foetal loss through spontaneous abortion or stillbirth. Although there are causes of infertility in addition to STDs, epidemiologists agree that it is the transmission of STDs and the lack of treatment for these diseases that explains infertility in sub-Saharan women (Ericksen & Brunette, 1996).

4.3.2.2 Male Factors

Male factor infertility was accounted for in 17.36% of the subjects when BMI is used (Table 1), and 15.97% when WHR is used (Table 2) in the present study, and the second most prevalent cause of infertility among the infertile women. While it is often the woman who presents initially with difficulty conceiving, infertility is a couple's problem (Case, 2003). The fertility of a marriage is a sum of the fertilities of the two partners. Low fertility in one can to some extent be balanced by high fertility in the other, whereas low fertility in both partners may result in infertility. This explains why some couples fail to reproduce, yet when they separate and each takes a new mate, they both proceed to have children (Tindall, 1987). Other African studies have also demonstrated high rate of male factor infertility as one of the commonest causes of infertility. A study by Motseki (2004) on anthropometric measurement and biochemical parameters in black women at the unit for reproductive care at Universitas Hospital Bloemfontein found 26.8% of male factor as the second prevalent cause of infertility. This was also found in the study by Chigamudzi *et al.* (1998), where Male factor infertility was present in 21% of the subjects in their study. Green et al. (2001) found that with the black women in their study; only 11.5% had male factors. These findings indicate that among the subjects of this

present study, male factor infertility was a great cause for concern. In a society where the burden is always placed on the female, the implications of this study suggest a burden that should be shared by the couple and not by the woman alone because the fertility of a marriage is a sum of the fertilities of the two partners.

4.3.2.3 Ovulation Factors

Exclusively 14.6% (Table 1) of the women had ovulation problems as a cause of infertility. Decreased weight disrupts growth hormone production, leading ultimately to oligomenorrhoea or amenorrhea (Reid & van Vugt, 1987). Disorders of ovulation account for about 30% of infertility and often presents with irregular periods (oligomenorrhoea) or an absence of periods (amenorrhoea) (Hamilton-Fairley & Taylor, 2003). Ovulation can be seriously affected by excessive weight loss or weight gain, excessive exercise and extreme emotional stress (Seibell, 1997). Twenty percent above or below ideal body weight may affect ovulation. The relationship between excess body fat and ovulatory disturbances appears stronger for early-onset obesity (Seibell, 1997). Obese women develop menstrual disorders which are more often anovulation than amenorrhoea. Obese women have an excess number of fat cells in which extraglandular aromatization of androgens to oestrogens occurs.

The findings of this present study were therefore lower with regards to anovulation as a cause of infertility, as compared to those of other studies. Chigamudzi *et al.* (1998), found that 21% of the subjects in their study had anovulatory factors, while 29% of the subjects of a study conducted in Cape Town had anovulatory factors as a cause of infertility (Steward Smythe & van Iddekinge, 2003). A study by Motseki (2004) on anthropometric measurement and biochemical parameters in black women at the unit for reproductive care at Universitas Hospital Bloemfontein found that 14.3% had anovulatory factors.

4.3.2.4 Endometriosis

The condition of Endometriosis is characterized by the presence and growth of endometrial tissue outside the uterus, and is often associated with symptoms of dysmenorrhoea and dyspareunia. It is also associated with reduced fertility and no causal factor has been proven (Smith *et al.*, 2003; Hart, 2003). The present study showed that endometriosis was not a major cause of infertility, but the least prevalent with only 3.49% (table 1) of the subjects having endometriosis. Motseki (2004),

found 10.7% of the subjects having endometriosis. According to Smith *et al.* (2003) endometriosis is generally found in 5% to 10% of infertile female partners.

4.3.2.5 Unexplained infertility

Apparently, 9.72% (Table 1) of the subjects in this study had unexplained infertility. This is contradictory to the findings of Chigamudzi *et al.* (1998), who found in their study only 3.5% of their participants had unexplained infertility. Unexplained infertility is infertility that is idiopathic in the sense that its cause remains unknown even after an infertility assessment, usually including semen analysis in the man and assessment of ovulation and fallopian tubes in the woman. Approximately 30% of infertile couples have unexplained infertility (Smith *et al.*, 2003). Unexplained infertility is defined as normal test results in the basic tests for ovulation, sperm production, and Fallopian tube patency. The female partner's age is one factor that contributes to the unexplained category. The mean probability of conception with unexplained and short-term infertility is higher than 35%, even without treatment. When the duration of infertility is more than 3 years, conception is less likely (Smith *et al.*, 2003).

4.3.2.6 Polycystic Ovarian Syndrome (PCOS) and Hyperprolactinaemia

The condition of polycystic ovarian syndrome was accounted for in 6.94% (table 1) of the participants in the current study. Polycystic ovarian syndrome (PCOS) occurs in about 5% to 10% of pre menopausal women and is thought to be caused by a hypothalamic disorder (Tietz, 2006). PCOS is clinically defined by hyperandrogenism with chronic annovulation in women without underlying disease of the adrenal or pituitary glands. This syndrome is characterized by infertility, hirsutism, obesity (in approximately half of those affected), and various menstrual disturbances ranging from amenorrhea to irregular vaginal bleeding (Tietz, 2006). The prevalence of this condition was highest among the overweight (30%) and obese groups (40%) of the subjects (Table 1). The principal features of PCOS are obesity, annovulation (resulting in irregular menstruation) or amenorrhea, acne, and excessive amounts or effects of androgenic hormones either clinical or biochemical. The symptoms and severity of the syndrome vary greatly among women. While the

causes are unknown, insulin resistance, diabetes, and obesity are all strongly correlated with PCOS. In all, twelve (12) of the participants had confirmed polycystic ovarian syndrome defined as having menstrual cycles less than 26 days or more than 35 days in length or annovulation demonstrated by a mid- luteal progesterone level less than 4 ng/mL if the cycles were between 26 and 35 days, polycystic ovaries in ultrasound together with hyperandrogenemia and/or hirsutism after related disorders were ruled out. About seven (7) more participants were estimated to develop the syndrome based on their annovulation history, their mid-luteal progesterone levels and possible hyperandrogenism but had no ultrasound confirmation. The prevalence of Hyperprolactinaemia among the population was 9.72% (Table 1) and this was assessed by raised levels of prolactin in the blood. This is a common problem and is more common in women. The prevalence of Hyperprolactinaemia ranges from 0.4% in an unselected normal adult population to as high as 9-17% in women with reproductive disorders (Crosignani, 2002).

4.3.2.7 Uterine Problems

Uterine problems identified among the infertile population were abnormal uterine bleeding largely caused by polyps or fibroids (small and large growths) representing 9.7% (Table 1) of the causes of infertility among the participants. A study by Fiander, (1990) at the Bawku Hospital on the causes of infertility among 1000 patients in Ghana found that 37 patients had uterine factors with 33 of them having fibroids. In the present study, most of the infertile women with uterine problems were in the overweight and obese groups.

4.4. Biochemical parameters and infertility

4.4.1 Fasting Blood Glucose

The prevalence of diabetes mellitus in the present study was 27.08%, with high values among the obese II group (8.161±0.595, $p < 0.0028$) of the infertile population. Most of the participants (72.92%) in the present study had normal glucose levels of 3.6-6.4 mmol/L with 27.08% of the subjects having high fasting blood glucose levels above 7.0mmol/L. Clearly elevated plasma glucose after a meal, a high value two hours after a glucose load, or fasting hyperglycaemia will establish the diagnosis of diabetes mellitus without any need for further diagnostic testing

particularly in a person with classic symptoms of diabetes mellitus. The glucose tolerance test is not necessary if the fasting plasma glucose is greater than 7.0mmol/L and the random plasma glucose is 11.0mmol/L or more in children or in adults. There was strong correlation between FBS and the Obese II group ($p< 0.0028$), table 3. The adverse effects of obesity on infertility are specifically evident in polycystic ovary syndrome. The main factors implicated in the association may be insulin excess and insulin resistance (Pasquali *et al*., 2007), which may be the possible cause of hyperinsulinaemia in PCOS. Whether hyperinsulinaemia in PCOS is primarily due to a defect in insulin action, to increased insulin secretion, to decreased hepatic clearance of insulin, or to an interaction between all these disorders, is however, not clear (Morin-Papunen *et al.*, 2000).

In a study by Morin-Papunen *et al.* (2000) on women with PCOS, the impairment in insulin sensitivity was profound in obese women with PCOS, suggesting that obesity in PCOS contributes to hyperinsulinaemia in a synergetic manner. Insulin resistance can be associated with various other features, including central obesity (Ovalle & Azziz, 2001) which is confirmed by the results of the present study, where an association was found between high FBS and the Obese II group ($p< 0.0028$), table 3. Morin-Papunen *et al.* (2000) found that the serum insulin concentration was significantly correlated to WHR in lean polycystic women. This is confirmed by the hypothesis of an association between abdominal obesity and hyperinsulinaemia in these subjects. Excess body weight, especially when located in the abdominal region, has a strong association with blood glucose levels, insulin resistance and the development of diabetes. This has been a consistent finding across a range of prospective studies (Despres et al. 2001; Boyko et al. 2000; Njolstad et al. 1998). Most studies support that central adiposity is the dominant risk factor for the development of Type 2 diabetes, although there are some exceptions. Perry et al. (1995) and Skarfors et al. (1991) found BMI was the dominant risk factor over other measures of central adiposity for risk of developing Type 2 diabetes. A review by Hodge et al. (2001) concluded that both overall adiposity and central fat distribution were important independent risk factors for Type 2 diabetes. This study has shown significant correlations between BMI and the development of Type 2 diabetes. According to Tietz, 2006, excess weight; obesity and lack of physical activity can contribute to decreased insulin sensitivity. Hereditary factors and autoimmune

conditions may also make it more likely that individuals will develop insulin resistance and conditions such as PCOS that lead to infertility. Other conditions such as excess weight around the waist, high blood pressure or hypertension and high levels of cholesterol also increase the risk of insulin resistance, high blood glucose, type 2 diabetes mellitus and infertility.

4.4.2 Lipid profile and cardiovascular risk.

More than half of the participants (55.56%) in the present study had high cholesterol levels with 42.36% of the entire population with high coronary risk factors and estimated to develop cardiovascular risks. The lipid profile parameters showed higher mean values of serum total cholesterol, LDL-C, VLDL-C and triglycerides while, mean serum cardioprotective HDL-C was low among the subjects of this study. There were significant correlations between serum total cholesterol, triglycerides, and LDL-C, VLDL-C and coronary risk with the obesity II group of the population (table 3), which goes to buttress the point that there is high risk for the development of cardiovascular diseases among the study population. It has been estimated that risk of myocardial infarction is 35% to 55% less in adults and normal weight as compared to obese adults (Manson *et* al, 1992). However, the influence of obesity on cardiovascular risk begins before adulthood and overweight during adolescence is associated with an increased risk of coronary heart disease in male and female subjects (DiPietro *et* al., 1994). As 34.72% (Table 6) participants of the total study population are overweight, so number of at-risk individuals is much higher. Therefore, strategies designed to limit cardiovascular risk should address weight reduction procedures. The findings of this study are consistent with other studies in Ghana by Owiredu *et al.*, 2008 and Eghan and Acheampong, 2003; who found out that total cholesterol levels were higher among their subjects. The current study also found dyslipidemia to be a problem in Ghana, in agreement with Owiredu *et al.,* 2008 but contrary to previous studies by Asibey-Berko and Avorkliyah, 1999; who established that dyslipidemia was not a problem in Ghana. The current study has also established that high total serum cholesterol is not higher among hypertensive patients only and LDL-cholesterol is not only higher in those with hypertension and diabetes as proposed by Eghan and Acheampong, 2003. Consistent with other studies (Ferrara et al. 1997; Stamler et al. 1997; Kuller et al. 1995; Ernst & Obarzanek

1994;), significant correlations have been found between LDL cholesterol and people with excess body weight.

In the light of the present analysis, it is suggested that metabolic variables such as total cholesterol, triglycerides, LDL-cholesterol and HDL-Cho ratio are significant predictors of CVD risk in the groups studied. High levels of plasma cholesterol and triglycerides are well established risk factors for CVD (Bass et al., 1993; Austin et al., 2000). In agreement with other studies, (WHO 2000; Ferrara et al. 1997; Stamler et al. 1997; Ernst & Obarzanek 1994) the present study has established that excess body weight is associated with lower levels of HDL cholesterol. The superiority of HDL-Cho ratio in CVD risk assessment has been established by numerous studies (Stampfer et al., 1991; Shai et al., 2004). Evidence has suggested that HDL is important for prediction of CVD (Meagher, 2004; Mosca et al., 2004). The association of HDL and triglycerides as predictors for coronary events is independent of the total cholesterol level and has a greater predictive potential in females (Castelli, 1992; Castelli et al., 1992). The significance of triglycerides as a predictor of CVD is also demonstrated by the present study. However, HDL does not show any significant associations between the overweight and obese groups. Again, low levels of HDL cholesterol among the population means the tendency for the occurrences of cardiovascular diseases among the infertile women in this study. This study is also in agreement with several studies that have shown consistent positive independent associations between excess body weight and total cholesterol levels (Owen et al. 2003; WHO 2000; Ferrara et al. 1997; Stamler et al. 1997; Ernst & Obarzanek 1994).

4.5 Hormonal parameters and infertility

Majority of the subjects (54.86%) (Table 6) had normal female fertility hormonal profile levels measured on day 21 of the participant's menstrual cycle whilst 45.14% (Table 6) had paranormal or deranged levels of hormonal levels. Serum progesterone levels measured on day 21 were deranged in 45.14% of the participants. Prolactin levels were deranged in 38.19% of the infertile women with paranormal levels in FSH (24.31%) and LH (38.89%) measurements also. Obesity is associated with menstrual irregularity, chronic anovulation, and the PCO-syndrome (Zhang *et* al, 1984 and Hartz *et* al, 1979). Especially women with android obesity seem to be prone to develop menstrual disorders (Barbieri *et* al, 1988 and Nester *et* al, 1989).

The possible role of insulin as the mediating factor has aroused a great deal of interest in recent years. Receptors for insulin have been demonstrated in human ovaries. In vivo and in vitro studies showed a direct stimulatory effect of insulin on ovarian androgen secretion acting in synergism with LH. The increased androgen concentrations serve as substrates for active aromatization into oestrogens in the body fat compartment which is an important source of oestrogens in obese women. The resulting hyperoestrogenaemia is thought to modulate pituitary gonadotrophin release by increasing LH and decreasing FSH secretion. Increased LH levels result in further stimulation of the androgen production. Ovarian follicular maturation deteriorates due to the lack of FSH and the increased androgen concentrations in the ovary. After a variable period of time, these hormonal changes lead to the polycystic morphology of the ovaries due to chronic annovulation (Barbieri *et* al, 1988 and Nester *et* al, 1989). This partly explains why annovulation was very prevalent among the participants. Some significant levels of correlation ($p < 0.03$) was found between LH and central obesity for WHR greater than 0.85. Aside this, FSH, prolactin and 21- day progesterone did not show any significant correlations with WHR, WC or BMI.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Obesity is a cause for concern among the subjects of this study, with almost half of this study population being obese when BMI is used and more than half being obese with WHR. The high prevalence of obesity places the subjects of this study at a risk for developing infertility related disorders such as anovulation, amenorrhoea and PCOS.

The high incidence of women with a waist circumference above 88cm is indicative of central obesity being prevalent in this study, which is suggestive of increased risk for developing metabolic disorders.

For purposes of this study, BMI is a more reliable predictor of dysmetabolism such as diabetes, dyslipidemia and cardiovascular risk factors compared to WHR.

The results from this study present with findings that suggest the development of metabolic disorders due to significant levels of cholesterol, blood glucose and the prevalence of obesity among the subjects.

The women presenting with infertility were within their reproductive years, with the majority of subjects being between the ages of 31 and 40 years. Only a few of the subjects were over 40 years old.

Tubal factor infertility was the most prevalent cause of infertility, followed by male factors whereas endometriosis is the least prevalent cause of infertility in the study. This is in agreement with other studies conducted on infertile women, and especially those studies conducted in Africa

The profile of reproductive hormones progesterone, LH: FSH and prolactin were deranged in about half of the infertile women that participated in this study.

5.2 RECOMMENDATIONS

There should be increased awareness for the infertile population about the availability of facilities for subjects presenting with infertility. Difficulty in conceiving in the infertile population has been associated with stigmatization.

A study consisting of a larger population of infertile women and controls should be carried out. The association of obesity and annovulation could be determined by looking at the association between BMI and progesterone, as well as the LH: FSH ratio extensively. The prevalence of PCOS and its association with obesity among infertile women should also be investigated.

Weight reducing therapies such as exercises and dietary interventions study should also be carried out amongst; infertile Ghanaian women to determine what the effects of weight loss will be on their infertility.

The correlations between central obesity and general adiposity with infertility should be extensively investigated.

REFERENCES

Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest 1995; 96:88-98.

Acien P, Quereda F, Matallin P, Villarroya E, Lopez-Fernandez JA, Acien M, Mauri M and Alfayate R (199) Insulin androgens and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. Fertil Steril 72, 32-40.

Agyemang Charles, Ellis Owusu-Dabo, Ank de Jonge, David Martins, Gbenga Ogedegbe and Karien Stronks. Overweight and Obesity among Ghanaian residents in The Netherlands; how do they weigh against their urban and rural counterparts in Ghana. Cambridge University Press, Cambridge Journals.

Allain, CC, Poon, LS, Chan, CS, Richmond, W, Fu, PC (1974) Enzymatic determination of total serum cholesterol. *Clin Chem* **20**(4)**:** 470-475.

Amoah AG. Obesity in adult residents of Accra, Ghana. *Ethn Dis.* 2003 13(2 Suppl 2):S29-S101.Summer.

Andersen P, Seljeflot I, Abdelnoor M, Arnesen H, Dale PO, Lovik A, Birkeland K. Increased insulin sensitivyand fibrinolytic capacity after dietary intervention in obese women with polycystic ovary syndrome. Metabolism 1995; 44:611-6.

Arita Y, Kihara S, Ouchi N, Takahasi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T,Matsuzawa Y, Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Bios Res Commun 1999; 257:79-83.

Asibey-Berko, E. and W.A. Avorkliyah, 1999. Serum cholesterol levels of male blood donors at korle-bu teaching hospital. Ghana Med. J., 33: 104-107.

Asunction M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Causcasian women from Spain. J Clin Endocrinol Metab 2000; 85:2434-8.

Austin MA, Rodriguez BL, McKnight B, McNeely MJ, Edwards KL, 2000. Low density lipoprotein particle size, triglycerides and high density lipoprotein cholesterol as risk factors for coronary heart disease in old Japanese-American men. American Journal of Cardiology. 86: 412-416.

Australian Institute of Health and Welfare. Australia's health 2006: The tenth biannual health report of theAIHW. Canberra: Australian Institute of Health and Welfare, 2006.

Azziz R, Black V, Hines GA, Fox LM, Boots LR. Adrenal androgen excess in the polycystic ovary syndrom: sensitivity and responsitivity of the hypothalamic-pituitary-adrenal axis. J Clin Endocrinol Metab 1998; 83:2 17-23.

Azziz R, Marin C, Hog L, Badamgarav E, Song P. Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. J Clin Endocrinol Metab 2005; 90:4650-8.

Balen A. The pathophysiology of polycystic ovary syndrome: trying to understand PCOS and its endocrinology. Best Pract Res Clin Obstet Gynaecol 2004; 18:685-706.

Barbieri RL, Smith S, Ryan KJ. The role of hyperinsulinemia in the pathogenesis of ovarian hyperandrogenism. Fertil Steril 1988; 50: 197-212.

Barham, D, Trinder, P (1972) An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst* **97**(151)**:** 142-145.

Bass KM, Newschaffer CJ, Klag MJ, Bush TL, 1993. Plasma lipoprotein levels as predictors of cardiovascular death in women. Archives of Internal Medicine. 153: 2096-2216.

Bates, W. 1992. Nutritional aspects of infertility. In *Infertility: A practical guide for the physician*. Ed. by M.G. Hammond & L.M. Talbert. 3rd ed. Oxford: Blackwell Scientific Publications.

Beckmann, C.R.B., Ling, F.W., Barzansky, B.M., Bates, G.W., Herbert, W.N.P., Laube, D. W. and Smith, R.P. 1995. *Obstetrics and Gynaecology*. 2nd ed. Baltimore: Williams & Wilkins.

Bell, P.M. 1997. Dietary and lifestyle factors contribution to insulin resistance. *Proceedings of the Nutrition Society*, vol. 56, no.1B, pp. 263 - 272.

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001; 7:947-53.

Berga SL, Guzick DS, Winters SJ. Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. J Clin Endocrinol Metab 1993; 77:895-901.

BioMerieux® sa, France.

Biritwum RB, J Gyapong and G Mensah, (2006), Ghana Medical Journal, (The Epidiomiology of Obesity in Ghana).Vol.39 (3), pp 82-85.

Bjorntorp P. Metabolic implications of body fat distribution. Diabetes Care 1991; 14:1132-43.

Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update 2006; 12:351-61.

Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake J Clin Invest 1994; 93:2438-46.

Bodystat R1500 - Bodystatâ, Isle of Man Limited

Borini, A. and Asch, R.H. 1993. Factors that affect implantation in the human. In *Annual Progress in Reproductive Medicine*, Ed. By R.H. Asch & J.W.W. Studd. New York: The Patherton Publishing Group Inc. pp. 19 -25.

Boyko E, Leonetti D, Fujimoto W et al. 2000. Visceral adiposity and risk of Type 2 diabetes: a prospective study among Japanese Americans. Diabetes Care 23(4):465-71.

Brown, J.E. 2002. *Nutrition Now*. 3rd ed. Australia: Wadsworth Thomson Learning

Brugh, V.M, Nudell, D.M and Lipschultz, L.I. 2002. What the urologist should know about the female infertility evaluation. *Urologic Clinics of North America*, vol. 29, no. 4, pp. 983 - 992.

Bruun JM, Lihn AS, Madan AK, Pedersen SB, Schiott KM, Fain JN, Richelsen B. Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue. Am J Physiol Endocrinol Metab 2004; 286:E8-13.

Buffington CK, Kitabchi AE. Evidence for a defect in insulin metabolism in hyperandrogenic women with polycystic ovarian syndrome. Metabolism 1994; 43:1367-72.

CIA WORLD FACT BOOK ,Air Ninja.com

Case, A.M. 2003. Infertility evaluation and management: strategies for family physicians. *Canadian Family Physician*, vol. 49, pp. 1465 - 1472.

Castelli WP, 1992. Epidemiology of Triglycerides: a view from Framingham. American Journal of Cardiology. 70: 3H-9H.

Castelli WP, Anderson K, Wilson PWF, Levy D, 1992. Lipids and risk of coronary heart disease: the Framingham study. Annals of Epidemiology. 2: 23-28.

Catalano P. Management of obesity in pregnancy. Obstet Gynecol (2007) 109:419-433.

Chabbert Buffet N, Djakoure C, Maitre SC, Bouchard P. Regulation of the human menstrual cycle. Front Neuroendocrinol 1998; 19:151-86.

Chigumadzi, P.T., Moodley, M.D. and Bagratee, J. 1998. Infertility profile at King Edward VIII Hospital, Durban, South Africa. *Tropical Doctor*, vol. 28, no. 3, pp. 168-172.

Chong, A.P., Rafael, R.W. and Forte, C.C. 1986. Influence of weight in the induction of ovulation with human menopausal gonadotropin and human chorionic gonadotropin. *Fertility and Sterility*, vol. 46, no. 4, pp. 599 - 603.

Ciampelli M, Fulghesu AM, Cuccineli F, Pavone V, Caruso A, Mancuso S, Lanzone A. Heterogeneity in beta cell activity, hepatic insulin clearance and peripheral insulin sensitivity in women with polycystic ovary syndrome. Hum Reprod 1997; 12:1897-901.

Ciaraldi TP, Morales AJ, Hickman MG, Odom-Ford R, Olefsky JM, Yen SS. Cellular insulin resistance in adipocytes from obese polycystic ovary syndrome subjects involves adenosine modulation of insulin sensitivity. J Clin Endocrinol Metab 1997; 82:1421-5.

Clark, A.M., Ledger, W., Galletly, C., Tanlinson, L., Blaney, F., Wang, X. and Norman, R. J. 1995. Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Human Reproduction*, vol. 10, no. 10, pp. 2705 - 2712.

Clark, A.M., Thornley, B., Tomlinson, L., Galletley, C. and Norman, R.J. 1998. Weight loss in obese infertilewomen results in improvement in reproductive outcome for all forms of fertility treatment. *Human Reproduction*, vol. 13, no. 6, pp. 1502 - 1505.

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC,McKee LJ, Bauer TL, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334:292-5.

Corbould A, Kim YB, Youngren JF, Pender C, Kahn BB, Lee A, Dunaif A. Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling. Am J Physiol Endocrinol Metab 2005; 288:E1047-54.

Corbould AM, Bawden MJ, Lavranos TC, Rodgers RJ, Judd SJ. The effects of obesity on the ratio of type 317beta-hydroxysteroid dehydrogenase mRNA to cytochrome P450 aromatase mRNA in subcutaneous abdominal and intra-abdominal adipose tissue of women. Int J Obes Relat Metab Disord 2002; 26:165-75.

Cramer, D.W., Sluss, P.M., Powers, R.D., McShane, P., Ginsburg, E.S., Hornstein, M.D., Vitonis, A.F. and Barbieri, R.L. 2003. Serum Prolactin and TSH in an in vitro fertilization population: is there a link between fertilization and thyroid function. *Journal of Assisted Reproduction and Genetics*, vol. 20, no. 6, pp. 210-215.

Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. Hum Reprod 2003; 18:1928-32.

Crosignani, P.G. 2002. Resumption of fertility with diet in overweight women. *Reproductive Biomedicine Online,* vol. 5, no. 1, pp. 60 - 64.

Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000; 105:311-20.

D. W. and Smith, R.P. 1995. *Obstetrics and Gynaecology*. 2nd ed. Baltimore: Williams & Wilkins.

Dale PO, Tanbo T, Vaaler S, Abyholm T. Body weight, hyperinsulinemia, and gonadotropin level in the polycystic ovarian syndrome: evidence of two distinct populations. Fertil Steril 1992; 58:487-91.

Dalton, M., Cameron, A.J., Zimmet, P.Z., Shaw, J.E., Jolley, D., Dunstan, D.W. and Welborn, T.A. 2003. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *Journal of Internal Medicine*, vol. 254, no. 6, pp. 555 - 563.

De Swiet, M., Chamberlain, G. and Bennet, P. 2002. *Basic science in obstetrics and gynaecology*, 3rd ed. Edinburgh: Churchill Livingstone.

DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertile Steril 2005; 83:1454-60.

Despres J-P, Lemieuz I & Prud'homme D 2001. Treatment of obesity: need to focus on high risk abdominally obese patients. British Medical Journal 322:716-20.

DiPietro L, Mossberg HO, Stunkard AJ. A 40 year history of overweight children in Stockholm: life-time overweight, morbidity, and mortality. Int J Obes 1994; 18: 585-90.

Dubuc G, Phinney S, Stern J, Havel P (1998). "Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women". *Metab. Clin. Exp.* 47 (4): 429-34. PMID-9550541.

Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T. Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. Diabetes 1992; 41:1257-66.

Dyer, S.J., Abrahams, N., Hoffmann, M. and van der Spuy. 2002b. Infertility in South Africa: women's reproductive health knowledge and treatment-seeking behaviour for involuntary childlessness. *Human Reproduction*, vol. 17, no. 6, pp. 1657-16.

Eggert, J., Theobald, H. and Engfeldt, P. 2004. Effects of alcohol consumption on female fertility during an 18-year period. *Fertility and Sterility*, vol. 81, no. 2, pp. 379-383.

Eghan, Jr., B.A., and J.W. Acheampong, 2003. Dyslipidemia in outpatient at general hospital in Kumasi, Ghana: Cross-sectional study. Croat. Med. J., 44: 576-578.

Ericksen, K. and Brunette, T. 1996. Patterns and predictors of infertility among African women: a cross-national survey of twenty-seven nations. *Social Science and Medicine*, vol. 42, no. 2, pp. 209-220.

Ernst N & Obarzanek E 1994. Child health and nutrition: obesity and high blood cholesterol. Preventive Medicine 12:427-36.

Fassnacht M, Schlenz N, Schneider SB, Wudy SA, Allolio B, Arlt W. Beyond adrenal and ovarian androgen generation: increased peripheral 5alpha-reductase activity in women with polycystic ovary syndrome. J ClinEndocrinol Metab 2003; 88:2760-6.

Ferin, M., Jewelewicz, R. and Warren, M. 1993. *The menstrual cycle: physiology, reproductive disorders and infertility*. New York: Oxford University Press.

Ferrara A, Barrett-Connor E & Shan J 1997. Total, LDL, and HDL cholesterol decrease with age in older men and women. The Rancho Bernardo Study 1984–1994. Circulation 96:37-43.

Fiander A., (1990), Journal of Tropical Doctors, (Causes of infertility among 1000 patients in Ghana), vol 336, pp.137-138.

Flier JS, Maratos-Flier E. Obesity and the hypothalamus: novel peptides for new pathways. Cell 1998; 92:437-40

Folson AR, Kaye SA, Sellers TA, Hong CP, Cerhan JR, Potter JD, Princas RJ. Body fat distribution and 5-year risk of death in older women. Jama 1993; 269:483-7.

Fossati, P, Prencipe, L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* **28**(10)**:** 2077-2080.

Franks S, Mason H, Willis D. Follicular dynamics in the polycystic ovary syndrome. Molecular and Cellular Endocrinology 2000; 163:49-52.

Friedewald, WT, Levy, RI, Fredrickson, DS (1972) Estimation of the concentration of low-density lipoproteincholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**(6)**:** 499-502.

Galtier-Dereure, F., Piyol, P., Dewailly, D and Bringer, J. 1997. Choice of stimulation in polycystic ovarian syndrome: the influence of obesity, *Human Reproduction,* vol. 12, suppl. 1, pp. 88 - 96.

Garrow, J.S., James, W.P.T. and Ralph, A. 2000. *Human Nutrition and Dietetics*, 10th ed. Edinburgh: Churchill Livingstone.

Geelhoed D, International Journal of Gynecology & Obstetrics, Volume 79, Issue 2, Page 137.

Gesink Law D, Maclehose R, Longnecker M. Obesity and time to pregnancy. Hum Reprod (2007) 22:414 420.

Glass, A.R., Burman, K.D., Dahms, W.T. and Boehm, T.M. 1981. Endocrine function in human obesity. *Metabolism*, vol. 30, no. 1, pp. 89 - 104.

Glueek CJ, Dharashivkar S, Wang P, Zhu B, Gartside PS, Tracy T, Sieve L. Obesity and extreme obesity, manifest by ages 20-24 years, continuing through 32-41 years in women, should alert physicians to the dignostic likelihood of polycystic ovary syndrome as a reversible underlying endocrinopathy. Eur J Obstet Gynecol Reprod Biol 2005; 122:206-12.

Goldstein BJ. Insulin resistance as the core defect in type 2 diabetes mellitus. Am J Cardiol 2002; 90:3G-10G.

Goldzieher JW, Axelrod LR. Clinical and Biochemical Features of Polycystic Ovarian Disease. Fertile Steril1963; 14:631-53.

Goodarzi MO, Azziz R. Diagnosis, epidemiology, and genetics of the polycystic ovary syndrome. Best Pract Res Clin Endocrinol Metab 2006; 20:193-205.

Green BB, Weiss NS, Daling JR. Risk of ovulatory infertility in relation to body weight. Fertile Steril 1988; 50:721-6

Green, J.A., Robins, J.C., Scheiber, M., Awadalla, S. and Thomas, M.A. 2001. Racial and economic demographics of couples seeking infertility treatment. *The American Journal of Obstetrics and Gynaecology*, vol. 184, no. 6, pp. 1080-1082.

Guzick DS, Talbott EO, Sutton-Tyrrell K, Herzog HC, Kuller LH, Wolfson SK, Jr. Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. Am J Obstet Gynecol 1996; 174:1224-9; discussion 1229-32.

Guzick, D.S. 2004. Polycystic ovary syndrome. *Obstetrics & Gynecology,* vol. 103, no. 1, pp. 181 -193. Hackney, A.C. (1996). The male reproductive system and endurance exercise. Medicine and Science in Sports and exercise, 28, 180-189.

Hackney, A.C. (1996). The male reproductive system and endurance exercise. Medicine and Science in Sports and exercise, 28, 180-189.

Haluzik M, Haluzikova D. The role of resistin in obesity-induced insulin resistance. Curr Opin Investing Drugs 2006;7: 306-11

Hamilton-Fairley D, Kiddy D, Anyaoku V, Koistinen R, Seppala M, Franks S. Response of sex hormone binding globulin and insulin-like growth factor binding protein-1 to an oral glucose tolerance test in obese women with polycystic ovary syndrome before and after caloric restriction. Clin Endocrinol (Oxf) 1993; 39:363-7.

Hamilton-Fairley, D. and Taylor, A. 2003. ABC of subfertility: anovulation. *The British Medical Journal*, vol. 327, no. 7414, pp. 546-549.

Hammond, K.A. 2000. Dietary and clinical assessment. In *Krause's Food, Nutrition & Diet Therapy*. Ed. by L.K. Mahan & S Escott-Stump. 10th ed. Philadelphia: W.B. Saunders Company. pp. 353 – 379.

Hart, R. 2003. ABC of subfertility: unexplained infertility, endometriosis, and fibroids. *The British Medical Journal*, vol. 327, no. 7414, pp. 721 - 724.

Hartz AJ, Barboriak PN, Wong A, Katayama KP, Rimm AA. The association of obesity with infertility and related menstrual abnormalities in women. Int J Obes 1979;3:57-73.

Haslam DW, James WP (2005). "Obesity". Lancet 366 (9492): 1197–209. di:10.1016/S0140 6736(05)67483-1 PMID 16198769

Hellmer J, Marcus C, Sonnenfeld T, Arner P. Mechanisms for differences in lipolysis between human subcutaneous and omental fat cells. J Clin Endocrinol Metab 1992; 75:15-20.

Heymsfield, S.B., Baumgartner, R.N. and Pan, S. 1999. Nutritional assessment of malnutrition by anthropometric methods. In *Modern Nutrition in Health and Disease*. Ed. by M.E. Shils, J.A. Olson, M. Shike & A.C. Ross. 9th ed. Philadelphia: Lippincott Williams & Wilkins

Hodge AM, Collins VR, Zimmet P et al. 2001. Type 2 diabetes and obesity. In: Ekoe J-M, Zimmet P & Williams R. The epidemiology of diabetes mellitus: an international perspective. West Sussex: Wiley & Sons.

Hoeger KM, Kochman L, Wixom N, Craig K, Miller RK, Guzick DS. A randomized, 48-weeks, placebo-controlled trial of intensive lifestyle modification and/or metformin therapy in overweight women with polycystic ovary syndrome: a pilot study. Fertil Steril 2004; 82:421-9.

Hollmann, M., Runnerbaum, B. and Gerhard, I. 1996. Effects of weight loss on the hormonal profile in obese, infertile women. *Human Reproduction*, vol. 11, no. 9, pp. 1884 - 1891.

Holte J, Bergh T, Berne C, Berglund L, Lithell H. Enhanced early insulin response to glucose in relation to insulin resistance in women with polycystic ovary syndrome and normal glucose tolerance. J Clin EndocrinolMetab 1994; 78:1052-8.

Hunter, M.H. and Carek, P.J. 2003. Evaluation and treatment of women with hirsutism. *American Family Physician*, vol. 67, no. 12, pp. 2565-2572.

Ibanez L, Potau N, Virdis R, Zampolli M, Terzi C, Gussinye M, Carrascosa A, Vicens-Calvet E. Postpubertal outcome in girls diagnosed of premature pubarche during childhood: increased frequency of functional ovarian hyperandrogenism. J Chin Endocrinol Metab 1993;76:1599-603.

International Society for the Advancement of Kinanthropometry (ISAK). 2001. International Standards for Anthropometric Assessment.

Jakimiuk AJ, Weitsman SR, Navab A, Magoffin DA. Luteinizing hormone receptor, steroidogenesis acute regulatory protein, and steroidgenic enzyme messenger ribonucleic acids are over expressed in thecal and granulose cells from polycystic ovaries. J Clin Endocrinol Metab 2001; 86:1318-23.

Jakubowiez DJ, Nestler JE. 17 alpha-Hydroxyprogesterone responses to leuprolide and serum androgens in obese women with and without polycystic ovary syndrome offer dietary weight loss. J Clin Endocrinol Metab 1997; 82:556-60.

Kahn BB, Flier JS. Obesity and Insulin resistance. J Clin Invest 2000; 106:473-81.

Kahn SM, Hryb DJ, Nakhla AM, Romas NA, Rosner W. Sex hormone-binding globulin is synthesized in target cells. J Endocrinol 2002; 175:113-20.

Kalro, B.N. 2003. Impaired fertility caused by endocrine dysfunction in women. *Endocrinology Clinics of North America*, vol. 32, no. 3, pp. 573- 592.

Kaye SA, Folsom AR, Prineas RJ, Potter JD, Gapstur SM. The association of body fat distribution with lifestyle and reproductive factors in a population study of postmenopausal women. Int J Obes 1990; 14:583-91.

Kiddy DS, Sharp PS, White DM, Scanlon MF, Mason HD, Bray CS, Polson DW, Reed MJ, Franks S. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol (oxf) 1990;32:213-20.

Koistinen HA, Chibalin A V, Zierath JR. Aberrant p38 mitogen-activated protein kinase signaling in skeletal muscle from Type 2 diabetic patients. Diabetologia 2003; 46:1324-8

Kopelman PG. Obesity as a medical problem. Nature 2000; 404:635-43.

Kruger, H.S. 1999. The puzzle of obesity in African women: contributing factors and associated risk factors.PhD-thesis, Potchefstroomse Universiteit vir Christilike Hoër Onderwys, Potchefstroom.

Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghates MA, Withers DJ, Gardiner TV, Baitey CJ, Bloom SR. leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and in vitro in mice. J clin Invest 1997:100:2729-36.

Kuller L 1999. The epidemiology of obesity in adults in relationship to cardiovascular disease. In: Fletcher GF, Grundy SM & Hayman LL (eds). Obesity: impact on cardiovascular disease. Armonk, NY: Futura Publishing Co., Inc.

Kuller L, Meilahn E, Bunker C et al. 1995. Development of risk factors for cardiovascular disease among women from adolescence to older ages. American Journal of the Medical Sciences 310 (Suppl 1):S91-100.

Kumar A, Woods KS, Bartolucci AA, Azziz R. Prevalence of adrenal androgen ecess in patients with the polycystic ovary syndrome (PCOS). J Clin Endocrinol (Oxf) 2005; 62:644-9.

Laitinen J, Taponen S, Martikainen H, Pouta A, Millwood I, Hartikainen AL, Ruokonen A, Sovio U, McCarthy MI, Franks A, Jarvelin MR. Body size from birth to adulthood as a predictor of self-reported polycystic ovary syndrome symptoms. Int J Obes Relat Metab Disord 2003; 27:710-5.

Lake JK, Power C, Cole TJ. Women's reproductive health: the role of body mass index in early and adult life. Int J Obes Relat Metab Disord 1997; 21:432-8.

Laquatra, I. 2004. Nutrition for weight management. In *Krause's Food, Nutrition & Diet Therapy*. Ed. by L.K.Mahan & S. Escott-Stump. Philadelphia: W.B. Saunders Company. pp. 558 593.

Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC. Anti-Mullerian hormone serum concerntrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrimol Metab 2004; 89:318-23.

Lean, M.E., Han, T.S. and Morrison, C.E. 1995. Waist circumference as a measure for indicating need for weight management. *British Medical Journal*, vol. 311, no. , pp. 158 - 161.

Lee YH, Giraud J, Davies RJ White MF.c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem 2003; 278:2896-902.

Lee, R.D. and Nieman, D.C. 2003. *Nutritional assessment*. 3rd ed. Boston: McGraw Hill.

Loscalzo, Joseph; Fauci, Anthony S.; Braunwald, Eugene; Dennis L. Kasper; Hauser, Stephen L; Longo, Dan L. (2008). Harrison's principles of internal medicine. McGraw-Hill Medical. ISBN 0-07-146633-9.

Maelli, E.M. and Grazi, R.V. 2002. Metformin and BMI? *Fertility and Sterility*, vol. 78, no. 5, p. 890.

Mahutte, N.G., Matalliotakis, I. M., Goumenou, A. G., Vassiliadis, S., Koumantakis, G.E. and Arici, A. 2003. Inverse correlation between peritoneal fluid leptin concentrations and the extent of endometriosis. *Human Reproduction*, vol. 18, no. 6, pp. 1205-1209.

Manson JE, Tosteson H, Ridker PM, Satterfield S, Hebert P, O'Connor GT, et al. The primary prevention of myocardial infarction. N Engl J Med 1992; 326:1406-16.

Marshall JC, Eagleson CA, McCartney CR. Hypothalamic dysfunction. Mol Cell Endocrinol 2001; 183:29-32

McGarry J (2002). "Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes". *Diabetes* 51 (1): 7-18. doi:10.2337/diabetes.51.1.7. PMID 1175-6317.

Meagher EA, 2004. Addressing cardiovascular disease in women: Focus on dyslipidemia. Journal of the American Board of Family Practice. 17: 424-437.

Messenis, I.E., Milingos, S.D., Zikopoulos, K., Kollios, G., Seferiadis, and Lolis, D. 1998. Leptin concentrations in the follicular phase of spontaneous cycles and cycles superovulated with follicle stimulating hormone. *Human Reproduction*, vol. 13, no. 5, pp. 1152 - 1156.

Metalliotakis I, Kourtis A, Koukoura O, Panidis D. polycystic ovary syndrome: etiology and pathogenesis. Areh Gynecol Obstet 2006; 274:187-97.

Meyer, B. J., Meij, H. S. and Meyer, A. C. 1997. *Human Physiology*. 2nd ed. Cape Town: Juta & Co, LTD.

Miles JM, Jensen MD. Counterpoint: visceral adiposity is not causall related to insulin resistance. Diabetes Care 2005; 28:2326-8.

Monga, M., Alexandrescu, B., Katz, S.E., Stein, M. and Ganiats, T. 2004. Impact of infertility on quality of life, marital adjustment, and sexual function. *Urology*, vol. 63, no. 1, pp. 126 – 130.

Moran LJ, Noakes M, Clifton PM, Tomlinson L, Norman RJ. Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab 2003; 88:812-9.

Morin-Papune, L.C., Vauhkonen, I., Koivunen, R.M., Ruokonen, A. and Tapanainen, J.S. 2000. Insulin sensitivity, insulin secretion, and metabolic and hormonal

parameters in healthy women and women with polycystic ovarian syndrome. *Human Reproduction*, vol. 15, no. 6, pp. 1266 - 1274. Multiple Risk Factor Intervention Trial. American Journal of Clinical Nutrition 65(Suppl.):S272 88.

Mosca L, Appel LJ, Benjamin EJ, 2004. Evidence based guidelines for cardiovascular disease prevention inwomen. Circulation. 109: 672-693.

Must A, Spadano J, Coakley E, Field A, Colditz G, Dietz W. The disease burden associated with overweight and obesity. JAMA (1999) 282:1523-1559.

NHLVI Clinical Guidelines Executive Summary. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. *American Journal of Clinical Nutrition*, vol. 68, no. 4, pp. 899 - 917.

National Heart, Lung, and Blood Institute (1998) (PDF). Clinical Guidelines on thIdentification, Evaluation, and Treatment of Overweight and Obesity in Adults. International Medical Publishing,Inc. ISBN 1-58808-002-1.http://www.nhlbi.nih.gov/guidelines/obesity/ob_gdlns.pdf.

Navaratnarajah R, Pillay OC, Hardiman P (2008). "Polycystic ovary syndrome and endometrial cancer". Semin. Reprod. Med. ***26*** *(1): 62-71.* doi:10.1055/s-2007-992926. PMID 18181084.

Nelson VL, Legro RS, Strauss JF, McAllister JM. Augmented androgen production is a stable steroidogenicphenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 1999; 13:946-57.

Nester JE, Clore JN, Blackard WG. The central role of obesity (hyperinsulinema) in the pathogenesis of thepolycystic ovary syndrome. Am J Obstet Gynecol 1989; 161: 1095 -1097.

Nestler JE, Jakubowiez DJ. Decreases in ovarian cytochrome P450c 17alpha activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engle J Med 1996; 335:617-23.

Nestler JE, Powers LP, Matt DW, Steringold KA, Plymate SR, Rettmaster RS, Clore JN, Blackard WG. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab 1991; 72:83-9.

Nestler JE. Obesity, insulin, sex steroids and ovulation. Int J obes Relat Metab Disord 2000; 24 Suppl 2:S71-3.

Njolstad I, Arnessen E & Lund-Larsen P 1998. Sex differences in risk factors for clinical diabetes in a general population: 12-year follow-up of the Finnmark study. American Journal of Epidemiology 147(1):49-58.

Norman RJ, Davies MJ, Lord J and Moran LJ (2002) the role of lifestyle modification in polycystic ovary syndrome. Trends Endocrinol Metab 13,251-257.

Obarzanek E 1999. Obesity in children, adolescents and families. In: Fletcher GF, Grundy SM & Hayman LL (eds). Obesity: Impact on cardiovascular disease. Armonk, NY: Futura Publishing Co., Inc.

Ogden C, Carroll M, Curtin L, McDowell M, Tabak C, Flegal K. Prevalence of overweight and obesity in the United States, 1999–2004. JAMA (2006) 295:1549-1555.

Ovalle, F. and Azziz, R. 2001. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. *Fertility and Sterility*, vol. 77, no. 6, pp. 1095 - 1105.

Owen C, Whincup P, Odoki K et al. 2003. Birth weight and blood cholesterol level: a study in adolescents and systematic review. Pediatrics 111(5):1081-9.

Owiredu W.K.B.A, M.S. Adamu, N. Amidu, E.Woode, V. Barn, J. Plange-Rhule and C. Opoku - Okrah (2008). Obesity and Cardiovascular Risk Factors in a Pentecostal Poplation in Kumasi-Ghana, Journal of Medical Science, 8 (8); 682-690.

Palmert MR, Gordon CM, Kartashov AI, Legro RS, Emans SJ, Dunaif A. Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome. J Clin Endocrinol Metab 2002; 87:1017-23.

Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Balestra V, Melchionda N, Barbara L. Clinicaland hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab 1989; 68:173-9.

Pasquali R, Casimirri F, Cantobelli S, Labate AM, Venturoli S, Paradisi R, Zannarini L. Insulin and androgen relationships with abdominal body fat distribution in women with and without hyperandrogenism. Horm Res 1993; 39:179-87.

Pasquali R, Casimirri F, Plate L, Capelli M. Characterization of obese women with reduced sex hormone-binding globulin concentrations. Horm Metab Res 1990; 22:303-6.

Pasquali R, Casimirri F, Venturoli S, Antonio M, Morselli L, Reho S, Pezzoli A, Paradisi R. Body fat distribution has weight-independent effects on clinical, hormonal, and metabolic features of women with polycystic ovary syndrome. Metabolism 1994; 43:706-13.

Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, FilicoriM, Morselli-Labate AM. Effect of long-term treatment with metrormin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the

polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism 2000; 85:2767-74.

Pasquali R, Pelusi C, Genghini S, Cacciari M, Gambineri A. Obesity and reproductive disorders in women. *Hum Reprod Update* 2003; **9:**359-72.

Pasquali R. Obesity, fat distribution and infertility. Maturitas (2006) 54:357–371 *physician*. Ed. by M.G. Hammond & L.M. Talbert. 3rd ed. Oxford: Blackwell Scientific Publications.

Perry I, Wannamethee S, Walker M et al. 1995. Prospective study of risk factors for development of non-insulin dependent diabetes in middle aged British men. British Medical Journal 310:5604.

Pilgrim, T.C. 2001. The hidden problem of infertility in the African American community. Available from http://www.ferre.org/focitext/hiddent.html.

Poretsky L, Glover B, Laumas V, Kalin M, Dunaif A. The effects of experimental hyperinsulinemia on steroid secretion, ovarian [125] insulin binding, and ovarian [125] insulin-like growth-factor I binding in the rat. Endocinology 1988;122:581-5.

Poretsky L, Seto-Young D, Shrestha A, Dhillon S, Mirjany M, Liu HC, Yih MC, Rosenwaks Z. Phosphatidyl-inositol-3 kinase-independent insulin action pathway(s) in the human ovary. J Clin Endocrimol Metab 2001; 86:3115-9.

Puoane, T., Steyn, K., Bradshaw, D., Laubscher, R., Fourie, J., Lambert, V and Mbaganga, N. 2002. Obesity in South Africa: the South African Demographic and Health Survey. *Obesity Research*, vol. 10, no. 10, pp.1038 - 1048.

Ramlau-Hansen C, Thulstrup A, Nohr E, Bonde J, Sorensen T, Olsen J. Subfecundity in overweight and obese couples. Hum Reprod (2007) (In press).

Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycles. Its role in insulin sensitivity and the metabolic disturbance of diabetes mellitus. Lancet 1963; i:785-9.

Rea R, Donnelly R. Resistin: an adipocyte-derived hormone.Has it a role in diabetes and obesity?Diabetes Obes Metab 2004; 6:163-70.

Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995; 75:473-86.

Rebuffe-Scrive M, Andreson B, Olbe L, Bjorntorp P. Metabolism of adipose tissue in intra abdominal depotsin severely obese men and women. Metabolism 1990; 39:102-5.

Rebuffe-Serive M, Cullberg G, Lundberg PA, Lindstedt G, Bjorntorp P. Anthropometric variables and metabolism in polycystic ovarian disease. Horm Metab Res 1989; 21:391-7.

Reid, R.L. and van Vugt, D.A. 1987. Weight-related changes in reproductive function. *Fertility and Sterility*, vol. 48, no. 6, pp. 905-913

Rich-Edwards JW, Goldman MB, Willett WC, Hunter DJ, Stampfer MJ, Colditz GA, Manson JE. Adolescentbody mass index and infertility caused by ovulatory disorder. Am J Obstet Gynecol 1994; 171:171-7.

Robert J.Norman, Manny Noakes Rugin Wu, Michael J. Davies, Lisa Moran and Jim X Wang (2004), OxfodJournals of Human Reproduction, Vol.10(3)pp267-280.

Rogers J, Mitchell GW. The relation of obesity to menstrual disturbances. N Engle J Med 1953; 247:53-55

Rosenfield RL, Ghai K, Ehrmann DA, Barnes RB. Diagnosis of the polydystic ovary syndrome in adolescence: comparison of adolescent and adult hyperandrogenism. J Pediatr Endocrinol Metab 2000:13 Suppl 5:1285-9

Sabogal, J.C. and Munoz, L. 2001. Leptin in obstetrics and gynaecology: a review. *Obstetrical and Gynaecology Survey*, vol. 56, no. 4, pp. 225 - 228.

Sadrzadch S, Klip WA, Brockmans FJ, Schats R, Willemsen WN, Burger CW, Van Leeuwen FE, Lambalk CB. Birth weight and age at menarche in patients with polycystic ovary syndrome or diminished ovarian reserve, in a retrospective cohort. Hum Reprod 2003; 18:2225-30.

Sam S, Dunaif A. Polycystic ovary syndrome: syndrome XX? Trends Endocrinol Metab 2003; 14:365-70.

Scherzer, W.J. and McClamrock, H. 1996. Amenorrhoea. In *Novak's Gynecology.* Ed. by Berek *et.al.*, 12th ed. Baltimore: Williams & Wilkins. p. 809 - 832.

Scidell JC. The impact of obesity on health status: some implications for health care costs. Int. J Obes Relat Metab Disord 1995; 19 Suppl 6:S13-6.

Sebire NJ, Jolly M, Harris JP, Wadsworth J. Joffe M, Beard RW, Regan L, Robinson S. Maternal obesity and pregnancy outcome: a study of 287 213 pregnancies in London. Int J Obes Relat Metab Disorb 2001; 25:1175-82.

Seibell, M. M. 1997. Diagnostic evaluation of an infertile couple. In *Infertility: A Comprehensive Text.* Ed. byM.M. Seibel. 2nd ed. Stamford: Appleton & Lange. pp 1 - 78.

Shai I, Rimm EB, Hankinson SE, Curhan G, Manson JE, Rifai N, Stampfer MJ, Ma J, 2004. Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. Circulation. 110: 2824-2830.

Shahid, S.K. and Shneider, H. 2000. Effects of exercise on insulin resistance syndrome.

Coronary Artery Disease, vol. 11, no. 2, pp. 103-109.

Sharpe RM and Franks S (2002) Environment, lifestyle and infertility an inter-generati**on**al issue. Nature Cell Biol 4(Suppl),s33-40

Sharara, F.I. and McClamrock, H.D. 2000. Differences in in vitro fertilization (IVF) outcome between white and black women in an inner city, university-based IVF program. *Fertility and sterility*, vol. 73, no. 6, pp. 1170 - 1173.

Shepherd PR, Kahn BB. Glucose transporters and insulin action implications for insulin resistance and disbetes mellitus. N Engl J Med 1999; 341:248-57.

Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normalrats. Endocrinology 1997; 138:3395-401.

Sizer, F. and Whitney, E. 2003. *Nutrition concepts and controversies*. 9th ed. Australia: Wardswoth Thomson Learning.

Skarfors E, Selinus K & Lithell H 1991. Risk factors for developing non-insulin dependent diabetes: a 10 year followup of men in Uppsala. British Medical Journal 303:755-60.

Slabber, M., Lusardi, L-M., Meyer, M. and Joubert, G. 2001. Effects of dietary manipulation on weight loss and endocrinological parameters in obese women. *Internation Journal of Obesity and related metabolic disorders*, vol. 25, Suppl. 2, p.S145.

Slabber, M., Walsh, C.M., Oosthuizen, A.P., Nortje, J.D., Wessels, P.H. and Nel, M. 1998. The association between fat distribution and ovulation. *SAMJ*, vol., no., p. 885.

Slowey, M.J. 2001. Polycystic ovary syndrome: new perspective on an old problem. *Southern Medical Journal*, vol. 94, no. 2, pp. 190 -195.

Smith, S., Pfeifer, S.M. and Collins, J.A. 2003. Diagnosis and management of female infertility. *Journal of the American Medical Association*, vol. 290, no. 13, pp. 1767 - 1770.

Speroff, L., Glass, R. H. and Kase, N. G. 1999. *Clinical Gynaecologic Endocrinology & Infertility*. 3rd ed. Baltimore: Williams & Wilkins.

Stamler J 1991. Epidemiologic findings on body mass and blood pressure in adults. Annals of Epidemiology 1(4):347-62.

Stamler J, Briefel R, Milas C et al. 1997. Relation of changes in dietary lipids and weight, trial years 1–6, to changes in blood lipids in the special intervention and usual care groups in the Multiple Risk Factor Intervention Trial. American Journal of Clinical Nutrition 65(Suppl.):S272–88.

Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH, 1991. A prospective study of cholesterol, apolipoproteins and the risk of myocardial infarction. New England Journal of Medicine. 325: 373-381.

Steward-Smythe, G.W. and van Iddekinge, B. 2003. Lessons learned from infertility investigations in the public sector. *SAMJ*, vol. 93, no. 2, pp. 141 - 143.

Stuart Campbell and Ash Monga (2000). Gynaecology by Ten Teachers, 17th Edition, BookPower Publications, pp83- 97.

Taylor, A. 2003. ABC of subfertility: extent of the problem. *British Medical Journal*, vol. 327, no. 7412 , pp. 434 -436.

Tietz (2006), Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition, Elsevier Saunders Publishers, pp2021-2027.

Tindall, V. R. 1987. *Jeffcoate's principles of gynaecology*. 5th ed. Butterworths: London.

Tsigos C, Kyrou I, Chala E, Tsapogas P, Stavridis JC, Raptis SA, Katsilambros N. Circulating tumor necrosis factor alpha concentrations are higher in abdominal versus peripheral obesity. Metabolism 1999; 48:133-5.

Vikman HL, Savola JM, Rassmaja A, Ohisalo JJ. Alpha 2A-adrenergic regulation of cyclic AMP accumulation and lipolysis in human omental and subcutaneous adipocytes. Int J Obes Relat Metab Disord 1996; 20:185-9.

WHO 2000. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. WHO Technical Report Series 894. Geneva: WHO.

WHO 2003. Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series 916. Geneva: WHO.

Wahrenberg H, EK I, Reynisdottir S, Carlstrom K, Bergqvist A, Arner P. Divergent effects of weight reduction and oral anticonception treatment on adrenergic lipolysis regulation in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab 1999;84:2182-7.

Walstreicher J, Santoro NF, Hall JE, Filicori M, Crowley WF, Jr. Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovarian disease: indirect evidence for partial gonadotroph desensitization. J Clin Endocrimol Metab 1988; 66:165-72.

Wang JX, Davies M, Norman RJ. Body mass and probability of pregnancy during assisted reproduction treatment: retrospective study. Bmj 2000;321:1320-1.

Wang JX, Davies MJ and Norman RJ (2002) Obesity increases the risk of spontaneous abortion during infertility treatment. Obes Res 10,551-554.

Wass P, Waldenstrom U, Rossner S, Hellberg D. An android body fat distribution in females impairs the pregnancy rate of in-vitro fertilization-embryo transfer. Hum Reprod 1997; 12:2057-60.

Webber LJ, Stubbs S, Stark J, Trew GH, Margara R, Hardy K, Franks S. Foundation and early development of follicles in the polycystic ovary. Lancet 2003; 362:1017-21.

Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. J Clin Endocrinol Metab 1999; 84:2951-6.

Whitney, E. N. and Rolfes, S. R. 2002. *Understanding Nutrition*. 9th ed. Australia: Wadsworth.

Wickenheisser JK, Nelson-Degrave VL, McAllister JM. Dysregulation of cystochrome P450 17 alpha-hydroxylase messenger ribonucleic acid stability in theca cells isolated from women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90:1720-7.

Willis D, Mason H, Grilling-Smith C, Franks S. Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulose cells of normal and polycystic ovaries. J Clin Endocrinol Metab 1996; 81:302-9.

Willocks, J. and Neilson, J.P. 1991. *Obstetrics and Gynaecology*. 4th ed. Edinburgh: Churchill Livingstone.

Wittemer, C, Ohl, J., Bailly, M., Betthar-Lebugle, K. and Nisand, I. 2000. Does body mass index of infertile women have an impact on IVF procedure and outcome? *Journal of Assisted Reproduction and Genetics*, vol. 17, no. 10, pp. 547 - 552.

World Health Organisation. Obesity: preventing and managing the global epidemic. Report of WHO consultation. WHO Technical Report Series 894. Ed. Geneva: \World Health Organisation, 2000.

World Health Organization (2000) (PDF). Technical report series 894: Obesity: Preventing and managing theglobalepidemic Geneva: World Health Organization.

Yamashita S, Nakamura T, Shimomura I, Nishida M, Yoshida S, Kotani K, Kameda-Takemuara K, Tokunaga K, Matsuzawa Y. Insulin resistance and body fat distribution. Diabetes Care 1996; 19:287-91.

Yen SS, Vela P, Rankin J. Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol Metab 1997; 30:435-42.

Yildirim B, Sabir N, Kaleli B. Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. Fertile Steril 2003; 79:1358-64.

Zaadstra BM, Seidell JC, Van Noord PA, te Velde ER, Habbema JD, Vrieswijk B, Karbaat J. Fat and femalefecundity: prospective study of effect of body fat distribution on conception rates. Bmj 1993; 306:484-7.

Zhang YW, Stern B, Rebar RW. Endocrine comparison of obese menstruating and amenorrheic women. J Clin Endocrinol Metab 1984; 58: 1077-1083.

Zierath JR, Livingston JN, Thorne A, Bolinder J, Reynisodottir S, Lonnqvist F, Arner P. Regional difference in insulin inhibition of non-esterified fatty acid release frok human adipocytes: relation to insulin receptor phosphorylation and intracellular signaling through the insulin receptor substrate-1 pathway. Diabetologia 1998; 41:1343-54.