ANTHROPOMETRIC MEASURMENTS AND BIOCHEMICAL PARAMETERS IN BLACK WOMEN AT THE UNIT FOR REPRODUCTIVE CARE AT UNIVERSITAS HOSPITAL, BLOEMFONTEIN .

LUCIA MOTSEKI

B. Sc Dietetics

1998316572

Dissertation submitted for the degree
Magister Scientiae (Dietetics) in the Faculty of Health Sciences
University of the Free State.

Study leader: Prof M Slabber

Co-study leader: Dr Nortjé

November 2004

STATEMENT OF DECLARATION

"I Ntsoaki Matumelo Lucia Motseki, declare that the dissertation is hereby submitted by me for the Magister Scientiae (Dietetics) degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I futher more cede copyright of the dissertation in favour of the University of the Free State."

_____

Signature **November 2004**

SUMMARY

The prevalence of infertility in Africa is overshadowed by the high population growth rate in this continent. The number of infertile black African women seeking treatment is on the increase due to the fact that more black women are concentrating on their careers and postponing having children.

The desire to reproduce is a highly motivating factor in most marriages and failure to do so places a lot of stress on the couple. Infertile women in most parts of Africa are treated as outcasts due to their infertile status. In most cases these women are either abused or divorced by their husbands.

In sub-Saharan Africa, sexually transmitted diseases are the most common causes of infertility. Other causes of infertility in women include endometriosis, anovulation, tubal diseases, cervical factors and unexplained infertility.

Anorexia and bulimia nervosa, as well as obesity, produce alterations in the reproductive system of women. Obesity has an effect on ovulation and on the outcomes of in vitro fertilization and assisted reproduction therapy. Anorexia nervosa on the other hand, has also been associated with amenorrhoea and oligoamenorrhoea.

Insulin resistance is another factor that is linked to polycystic ovarian syndrome and infertility. Insulin resistance has also been shown to be prevalent in obese individuals, especially those with android fat distribution. Lowering insulin resistance by weight loss, results in spontaneous ovulation.

The main objective of this study was to determine the anthropometrical and biochemical parameters in infertile black South African women. A total of sixty participants attending the Unit for Reproductive Health, Universitas Hospital, Bloemfontein were included in the study. Anthropometrical data measured included: body mass index; waist-to-hip ratio; waist circumference; neck circumference and body fat percentage. Blood samples were also obtained to determine the levels of fasting insulin, glucose, thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, leptin, prolactin, progesterone, testosterone and C-reactive protein.

The results of this study show that tubal factor infertility was the most prevalent cause of infertility and the second highest cause of infertility was male factors. The median age of the subjects of this study was 32 years.

Sixty percent of the subjects had a gynoid fat distribution. More than a third of the subjects had a body mass index of more than 25 kg/m^2 and none of the subjects in this study had a body mass index of less than 18.5 kg/m^2. Eighty five percent of the subjects had a body fat percentage of more than 32 percent. These results indicate that obesity is a problem among these subjects.

Biochemical parameters indicate that the median concentrations of the reproductive hormones were normal. Only 35 percent of the subjects had hyperinsulinaemia. Almost all of the subjects (83.6%) had leptin concentrations above normal. Median C-reactive protein level was also normal.

No association was found between body mass index and C-reactive protein and insulin. An association was established between leptin concentrations and body mass index and the correlation between these two parameters was very strong. An association was also found between android fat distribution and hyperinsulinaemia.

The high rate of obesity among the subjects of this study, places the subjects of this study at a risk of developing metabolic syndrome and other obesity-related factors. Their obesity status may also be a contributory factor to their infertile status.

There should, be increased awareness of the impact of obesity on infertility and on their general health. Increased physical activity and healthy food choices should be encouraged among black infertile women. Black women should still be made aware of the fact that there are facilities available for treatment of infertility.

OPSOMMING

Die voorkoms van infertiliteit in Afrika word oorskadu deur die hoë populasiegroeitempo in hierdie kontinent. Die aantal swart vroue in Afrika wat aanmeld vir behandeling vir infertiliteit is aan die toeneem as gevolg van die feit dat swart vroue tans meer op hul beroepe konsentreer en later met gesinne begin.

Seksueel oordraagbare siektes is die vernaamste oorsaak van infertiliteit in sub-Sahara Afrika. Ander oorsake van infertiliteit in vroue sluit endometriose, anovulasie, buisfaktore, servikale faktore en ander onverklaarbare faktore in. Vetsug veroorsaak veranderinge in die vroulike reproduktiewe stelsel. Vetsug beïnvloed ovulasie en toon ‘n invloed op die uitkoms van in vitro bevrugtiging en geassisteerde reproduksie terapie. Hiperinsulinemie en insulienweerstandigheid word meer algemeen in vetsugtige persone, veral in diegene met androïde vetsug, aangetref. Insulienweerstandigheid is ‘n faktor wat met polisistiese ovariële sindroom en infertiliteit geassossier word. Die verlaging van insulienweerstandigheid deur middel van massaverlies lei tot spontane ovulasie.

Min navorsing is tot hede oor die kwessie van infertiliteit in die swart gemeenskap gedoen en geen fokus word deur Gesondheidsorgstelsel daarop geplaas. In Suid-Afrika is baie min navorsing oor infertiliteit in die swart bevolking, asook die verband tussen vetsug en infertiliteit in swart vroue gedoen.

Die hoofdoel van hierdie studie was om die antropometriese en biochemiese parameters van infertiele swart Suid-Afrikaanse vroue te bepaal. Die steekproef het bestaan uit sestig swart vroue wat die Reproduktiewesorgeenheid van die Universitas hospitaal, Bloemfontein tussen 1 Maart 2003 en 31 Julie 2004 besoek het. Die volgende antropometriese metings is gedoen: massa, lengte, middel-heup omtrekverhouding, middel en nekomtrek en liggaamsvetpersentasie. Vastende bloedmonsters is ontleed vir insulien, glukose, tiroïedstimuleringshormoon, luteïniseringshormoon, follikelstimuleringshormoon, leptien, prolaktien, progesteroon, testosteroon en C-reaktiewe proteïne.

Die mediaan ouderdome van die vroue was 32 jaar (20.3 – 41.6 jaar). Buisfaktor was die mees algemene oorsaak van infertiliteit terwyl manlike faktore ook ‘n belangrike rol by die fertiliteit van die groep vroue gespeel het.

Meer as een derde van die vroue het ‘n liggaamsmassa indeks (LMI) van meer as 25 kg/m² getoon terwyl geen vroue ondergewig was (LMI< 18.5 kg/m²). Sestig persent van die vroue het ginoïede vetverspreiding getoon. ‘n Liggaamsvetpersentasie groter as 32 persent is by 85 persent van die vroue gevind. Hierdie resultate het bewys dat vetsug ‘n groot probleem onder hierdie vroue is.

Biochemiese parameters het aangedui dat die mediaankonsentrasies van al die voorplantingshormone normaal was. Vyf-en-dertig persent van die vroue het egter hiperinsulinemie getoon. Omtrent al die vroue (83.6%) het verhoogde

leptienkonsentrasies getoon. Die mediaan C-reaktiewe proteïenkonsentrasies was binne die normale reikwydte.

Geen statistiesbetekenisvolle verband is tussen LMI en C-reaktiewe proteïnkonsentrasie asook s-insulienkonsentrasies gevind nie. ‘n Statisties betekenisvolle verband (95% CI: -17.9; -5.3) is tussen leptienkonsentrasies en LMI en ‘n sterk korrelasie (r = 0.77) is ook tussen hierdie twee parameters gevind. Die verband tussen androïede vetsug en hiperinsulinemie was ook statisties betekenisvol (95% CI: -46.7; -0.4) in hierdie vroue.

Vetsug blyk ‘n vername rol te speel in die voorkoms van infertiliteit by hierdie groep vroue in hierdie studie. Die hoë voorkoms van vetsug (46.7%) beklemtoon hul hoë risiko om lewenstyl siektes soos kardiovaskulêre siektes, Tipe 2 diabetes mellitus, hipertensie en polisistiese ovariële sindroom te ontwikkel.

‘n Bewusmakings veldtog moet geloods word waar die publiek bewus gemaak moet word omtrent die impak van vetsug op infertiliteit asook op algemene gesondheid. Verhoogde fisiese aktiwiteit en gesonde voedselkeuses moet aangemoedig word onder swart onvrugbare vroue. Swart vroue moet steeds ook bewus gemaak word van fasiliteite waar infertiliteit behandel kan word.

Verdere navorsing oor die voorkoms van infertiliteit by swart vroue in Suid-Afrika word benodig. Daar word aanbeveel dat sodanige studie(s) ‘n groter steekproef infertiele swart Suid-Afrikaanse vroue insluit, asook ‘n dieetingreep wat sal bepaal wat die invloed van massaverlies by hierdie vroue sal wees. Die verband tussen anovulasie en vetsug moet

ondersoek word sowel as die verband tussen verhoogde leptienkonsentrasies en infertiliteit by swart vroue.

ACKNOWLEDGEMENTS

All my thanks and praise go to God, for all the blessings He has given me, I count it all grace. Through Him I was able to gain strength to complete this dissertation.

I would like to thank the following people and organizations:

- My study leader, Prof M Slabber, for all her assistance, patience and motivation.
- Dr Nortjé, of the Unit for Reproductive Care, Universitas Hospital, Bloemfontein, for all of his advice and expertise.
- Ms R Nel of the Department of Biostatistics at the University of the Free State, for the statistical analysis and her assistance.
- Dr M Meyer and the staff of the Department of Chemical Pathology for their help with the analysis of blood samples.
- Mrs R van der Merwe for her assistance with recruiting subjects.
- Sister Esterhuizen and Sister Nienaber for the blood sampling.
- The Medical Research Council for financial assistance.
- The staff of the Department of Human Nutrition for all their advice and support.
- Ms L Boucher for editing this dissertation.
- All participants for taking part in this study.
- My family and friends for their motivating encouragement and prayers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
AIDS	Acquired immune deficiency syndrome
ART	Assisted reproduction treatment
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
CRH	Corticotrophin-releasing hormone
CRP	C-reactive protein
DHEA	Dehydroxyandrosterone
FSH	Follicle-stimulating hormone
GnRH	Gonadotrophin-releasing hormone
hCH	Human chorionic gonadotrophin
HDL	High-density lipoprotein
HIV	Human immuno-deficiency virus
HPG	Hypothalamus-pituitary-gonadal
HPO	Hypothalamus-pituitary ovarian
IBW	Ideal body weight
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IL	Interleukin
IVF	In vitro fertilization
LDL	Low-density lipoprotein
LH	Luiteinizing hormone
NHANES	National Health and Nutrition Examination Survey
PCOS	Polycystic ovarian syndrome
PID	Pelvic inflammatory disease
PR	Pregnancy rate

RLU	Relative light unit
SHBG	Sex-hormone binding globulin
STD	Sexually transmitted disease
TNF	Tumour necrosis factor
TRH	Thyrotrophic-releasing hormone
TSH	Thyroid-stimulating hormone
TSH-RH	Thyoliberin
URC	Unit for Reproductive Care
USA	United States of America
WHO	World Health Organization
WHR	Waist-hip-ratio

LIST OF APPENDICES

CHAPTER 1
PROBLEM SETTING

1.1 Introduction and problem setting

The desire for reproduction is a basic human instinct. Therefore, infertility causes distress to many couples. The causes of infertility vary from country to country and in different social groups (Willocks & Neilson, 1991, p. 181).

In the United States of America (USA), infertility affects one in six couples seeking a pregnancy (Pilgrim, 2001). In Africa, infertility is a major reproductive health problem with regional prevalence rates of 30 – 40% (Dyer *et al*., 2002b). Prevalence is high, and the underlying pathology frequently affects women's physical health (Dyer *et al*., 2002a).

The causes of infertility among couples are numerous and are distributed evenly among male and female factors (Pilgrim, 2001). The causes of infertility include (Sharara & McClamrock, 2000 & Talbert, 1992, p.3):

i) Tubal factors

ii) Endometriosis

iii) Male factors

iv) Anovulation

v) Unexplained infertility

vi) Luteal-phase defect

vii) Cervical factor

viii) Uterine factor

The main variable of infertility is the incidence of tubal occlusion, caused by either ascending or post-pregnancy infection (Willocks & Neilson, 1991, p. 181). A recent study in the USA, has shown that African American women presented with a longer duration of infertility and a higher incidence of tubal disease than their white counterparts (Sharara & McClamrock, 2000). 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, p. 181).

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 a study undertaken by the World Health Organization (WHO), the cause of infertility in African countries could be attributed to infection- secondary to sexually transmitted diseases and pregnancy complications (Dyer *et al*., 2002a). All of these STD's are associated with the complications of infertility. Another frequent finding in African American women is uterine anomalies such as fibroids. Fifty to seventy five percent of all African American women are affected with fibroid tumors. Some symptoms associated with endometriosis and fibroids are: chronic pelvic pain; back pain; dysmenorrhoea; intermittent bleeding and/or persistent bleeding (Pilgrim, 2001).

African American women also have a higher body mass index (BMI) and require more ovarian stimulation than white women in in vitro fertilization (IVF). They also experience a significantly lower implantation rate and pregnancy rate (PR), and a tendency toward a higher rate of early pregnancy loss. In this study of African American women, black women had a 2.6 fold decreased odds of becoming pregnant as compared with white women. The lower implantation rate and PR could be partly related to the longer duration of infertility and higher BMI (Sharara & McClamrock, 2000).

A relationship between obesity and functional disorders of menstruation and reproductive dysfunction has been implied in the literature for over 40 years (Bates, 1992, p. 192). Overweight and/or obesity have been identified as causes of infertility in women. However, the association between elevated BMI and in vitro fertilization outcome is the subject of much debate (Sharara & McClamrock, 2000).

Women who are obese have a high incidence of amenorrhoea and infertility. It was found that in the USA, 12% of infertility is caused by female body weight disorders. More than 70% of these women conceive spontaneously if their weight is corrected through a weight-reducing diet, as appropriate (Bates, 1992, p. 409). Women in an earlier study in America with presumed anovulation were more than 39 pounds (18kg) heavier than obese women with no menstrual abnormalities (Bates, 1992, p. 190 – 191).

Some investigators have claimed that the high BMI has no adverse effects in black women. Others have documented a significant decrease in both the implantation rate and the pregnancy rate in overweight women (BMI > 25 kg/m²) compared to those with a normal body weight (BMI < 25 kg/m²) (Sharara & McClamrock, 2000). Also, the percentage of women in the USA with menstrual disorders increased as their percentage of weight above ideal body weight (IBW) increased (Bates, 1992, p. 190 – 191).

Infertility is not solely a medical problem. The psycho-social consequences of infertility include stress, anxiety, depression and marital difficulties. Until recently studies have focused predominantly on patients in industrialized countries, while the experience of infertility in the developing world has received comparatively little attention (Dyer *et al.*, 2002a). The effective management of infertility will, therefore, have considerable impact on reproductive health in Africa (Dyer *et al.*, 2002b).

More than many issues in the field of health within the black community, the issue of infertility has been least considered, researched or even discussed among professionals within the health care community (Pilgrim, 2001). Of the many factors affecting IVF outcomes (and therefore causes of infertility), the least studied is ethnicity. Such information, i.e. whether differences exist in IVF outcome among different races, which may allow appropriate alterations in treatment protocols, is very much in need (Sharara & McClamrock, 2000).

1.2 Objectives

The main objective of this study is to describe the anthropometrical and biochemical parameters in infertile, black South African women. The sub-objectives that need to be determined are:

- Body mass index (BMI)
- Waist-hip-ratio (WHR)
- Body fat percentage
- Baseline hormone profiles (i.e. fasting insulin, glucose, thyroid-stimulating hormone, luiteinizing hormone, follicle-stimulating hormone, leptin, prolactin, progesterone, testosterone and C-reactive protein) and
- To determine the association between anthropometric values and hormone levels.

1.3 Study layout

Following this introductory chapter, a review of the literature will be presented in Chapter 2. Here detailed information regarding infertility and all the factors that affect infertility will be discussed. This chapter will present information on how infertility comes about, the role body weight plays in infertility and also the role hormones play in infertility. One will also be able to understand the menstrual cycle and factors that may cause distortion in the normal functioning of the cycle.

In Chapter 3, the methodology of the study will be described. The study design will be discussed here, as well as the method of sampling used; including the inclusion criteria and the sample size. All the anthropometric measurements that were taken will be described. Methods used for blood sampling, preparation of serum, and analyses of all samples are also described. The study procedure, as well as any systematic errors that may have been encountered, will also be discussed.

The results will be presented in Chapter 4. This will be done using tables. The results will give an indication of the median BMI, waist-hip-ratio, and fat percentage. Baseline hormone levels of the subjects will also be presented. This will not be done individually, but the median values and percentiles of the subjects will be given.

Chapter 5 is the discussion chapter where the results that are reported in Chapter 4 will be discussed in detail. In this chapter, it will be possible to describe the baseline characteristics of the subjects in terms of body mass index, body fat percentage, other anthropometrical measurements and all the blood values that have been obtained. The association between the anthropometrical values and hormone levels will also be discussed. The results that have been obtained in this study will then be compared to those found in available literature to see whether there are similarities or differences, especially in terms of body size and the different hormone profiles of the subjects.

Following the discussion of the results, conclusions and recommendations will be made in Chapter 6. The results and discussion given in the previous chapters will be used to draw conclusions regarding the study and, where possible, to make recommendations pertaining to further studies to be conducted, as well as possible modes of treatment for black, infertile women.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The normal reproductive cycle in women consists of a sequence of events that are very well co-ordinated. This chapter gives an overview of the normal reproductive cycle in women and the hormones involved in reproduction. The concept of infertility, as well as those factors that are related to infertility will be discussed. Also, the impact of body weight on infertility and those hormones specifically related to body weight will be mentioned.

2.2 The female reproductive cycle

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, p. 357). 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, p. 3).

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, p. 357).

The reproductive cycle of the female can be divided into three stages (Ferin *et al.*, 1993, p. 3):

1) the follicular phase, the time for follicular growth;

2) the ovulatory period, when final maturation of the oocyte and its release into the reproductive tract occurs; and

3) the luteal phase, when a newly formed corpus luteum secrets hormones in preparation for implantation.

If the egg is not fertilized and implantation does not occur, 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, p. 3).

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, p. 357).

2.2.1 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. 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, p. 19).

The first phase of the cycle is the menses. The onset of menstrual bleeding by convention is termed the first day of the cycle. The follicular phase follows menses. 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. 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). 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. 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, p. 358).

The normal ovulatory menstrual cycle can be summarized as follows (Ferin *et al.*, 1993, pp. 105 – 107):

1. The menstrual cycle is initiated when conditions allow for the preferential release of FSH. After that, a cohort of follicles is recruited and the follicular phase starts.

2. FSH promotes the conversion of androgens to oestrogens. Oestradiol concentrations increase slowly within the follicles and in the peripheral circulation.
3. Within a few days, one of the follicles in the cohort becomes dominant, increasing oestradiol concentrations and decreasing LH and FSH pulse amplitude. Diminished gonadotrophin concentration slows down the growth of all cohort follicles, except for the dominant one. The dominant follicle then continues to grow.
4. The dominant follicle acquires LH receptors. LH stimulates the synthesis of androgens. These androgens are metabolized into oestrogens. Oestradiol is highly mitogenic and potentiates several local growth factors; thus, the selected follicle will grow rapidly and in a few days become a fully mature Graafian follicle.
5. Maturity of the Graafian follicle is marked by high circulating concentrations of oestradiol, the signal to the hypothalamus and pituitary gland that the follicle is ready for the ovulatory signal. The long loop oestradiol positive feedback is activated and as a result, the gonadotrophin surge occurs.
6. The high gonadotrophin concentrations during the surge arrest granulosa cell proliferation and secretory activity in the Graafian follicle. Oestradiol secretion declines rapidly. Granulosa cells begin to luteinize, and as a consequence, a small pre-ovulatory rise of progesterone occurs. Ovulation, the release of the fully grown oocyte, occurs about 18 hours following the gonadotrophin “peak”, or at

least 36 hours after the "initiation" of the surge. At ovulation, the oocyte resumes meiosis.

7. After the release of the ooctye, the granulosa layer becomes vascularized, and the granulosa cell completes the process of luteinization, whereby it acquires de novo steroid synthesis capacity that it previously lacked. LH stimulates progesterone and oestradiol secretion for this newly formed structure, the corpus luteum. Progesterone in combination with oestradiol, in turn, activates the hypothalamic opiate centre, the result of which is to decrease gonadotrophin pulse frequency.
8. The corpus luteum is a transient organ that has an inherent 12 – 15 day life span. Thus, oestradiol and progesterone secretion peaks about seven to nine days after its formation.
9. Luteolysis, the process of regression of the corpus luteum, results in a rapid decline in progesterone and oestradiol concentrations. This leads to menstruation.
10. Through a combination of several factors, which may include the long period of decreased pulse frequency during the luteal phase, a decrease in inhibin secretion and/or of oestradiol and progesterone secretion, FSH concentrations increase relatively to those of LH, and a new cycle is initiated.

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 onset of menstruation delineates the termination of an endometrial cycle and the beginning of a new one (Ferin *et al.*, 1993, p. 4).

2.2.2 The ovulation process

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, pp. 37 – 39).

2.2.3 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, p. 6).

2.3 Reproductive hormones

Fertility in women is tightly regulated by the hypothalamo-pituitary-ovarian (HPO) axis. Any derangement of the HPO axis results in menstrual irregularities and ovulation disorders, with consequent sub- or infertility (Kalro, 2003).

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, p. 4). The hypothalamic-regulating hormones which orchestrate the activities of the anterior pituitary are luteinizing-releasing hormone (LH-RH), corticotrophin-releasing hormone (CRH), growth hormone-releasing hormone (GH-RH), 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, p. 4).

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, p. 5). 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).

2.3.1 Ovarian sex steroid hormone secretion

Ovarian follicles respond to pituitary gonadotrophin secretion by synthesizing the principal ovarian hormones oestradiol and progesterone. Increasing levels of oestradiol feedback to the pituitary gland via a negative – feedback mechanism, resulting 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, p. 358).

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, p. 358).

2.3.2 Hypothalamic GnRH secretion

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. Ovarian function can be restored by the pulsating infusion of GnRH at 70 – 90 minute intervals. (Beckmann *et al.*, 1995, p. 357).

2.3.3 Prolactin

Prolactin is associated with the gonadotrophins, as well as with the growth hormone, and is produced by the mammotropes (lactotropes). Secretion is controlled by dopamine,

which acts as a prolactostatin – inhibits prolactin secretion, serotonin, endorphin and thyroliberin, which stimulates prolactin secretion; and explains why stress (exercise, trauma, myocardial infarction), may promote secretion. Prolactin levels may be elevated in both hyper- and hypothyroidism, because thyroliberin secretion may be increased in both conditions (Meyer *et al.*, 1997, p. 18.17).

Prolactin release is under a tonic inhibitory control by the hypothalamus. The secretion of prolactin is also influenced by (Ferin *et al.*, 1993, pp. 125 – 127):

- *Oestradiol*: The ovarian hormone oestradiol augments prolactin release. Oestradiol 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.
- *Pharmacological agents:* Several drugs increase prolactin, mostly by decreasing dopamine 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.
- *The suckling stimulus*: The amount of prolactin released is proportional to the frequency of suckling; hence, levels of prolactin characterizing the postpartum period are related to the amount of suckling.

- *GnRH*: In some superfused pituitary cell cultures, it was found that GnRH can stimulate prolactin release. This effect is not due to a direct action of the neurohormone on the lactotrope, but rather to the apparent production of a paracrine factor by the gonadotrope, which in turn stimulates the lactotrope to secrete prolactin.

Prolactin is best known for the multiple effects it exerts on the mammary gland. However, it also exerts effects on other targets important to the reproduction of the mammalian species. The varied effects of prolactin on the mammary gland include growth (mammogenesis), synthesis of milk (lactogenesis), and maintenance of milk secretion (galactopoiesis). There are data suggesting that prolactin influences reproductive behaviour. In humans, high prolactin levels are associated with psychosomatic reactions including pseudopregnancy. There are prolactin-receptors in the ventromedial nucleus of the hypothalamus, an area which controls female sexual behaviour (Freeman *et al.*, 2000).

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 steroidogenensis, ovulation, the process of luteinization, and the corpus luteum function (Crosignani *et al.*, 1999).

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, p. 124).

2.3.4 Thyroid-stimulating hormone

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, pp. 255 – 256). 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, p. 18.17). TSH also has these effects on the thyroid: it increases its size, vascularity, iodine uptake, protein synthesis, storage of colloid and the secretion of T3 and T4 (de Swiet *et al.*, 2002, p. 255).

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 hormone-binding 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 long-standing primary hypothyroidism may be responsible for varying degrees of ovulatory dysfunction from luteal-phase insufficiency to oligomenorrhoea or amenorrhoea. (Kalro, 2003).

2.3.5 Luteinizing hormone and follicle-stimulating hormone

The pituitary gonadotrophins FSH and LH are protein hormones secreted by the anterior pituitary gland (Beckmann *et al.*, 1995, p. 357). LH and FSH are glycoproteins from the family which includes TSH and human chorionic gonadotrophin. These hormones consist of a common ∝– subunit and a specific ß – subunit. Both are glycocylated, which determines their bioactivity and half-life (de Swiet *et al.*, 2002, p. 243).

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, p. 18.17).

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, p. 357).

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, p. 244).

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, pp. 4–5).

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, pp. 4–5).

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, p. 5).

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, p. 5).

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, p. 121).

2.3.6 Oestrogen and progesterone

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, p. 244).

Properties of oestrogen (de Swiet *et al.*, 2002, p. 244):

- Structure: Stimulates endometrial growth, maintenance of vessel and skin, reduces bone resorption, increases bone formation, increases uterine growth.
- Protein synthesis: Increases hepatic synthesis of binding proteins.
- Coagulation: Increases circulating levels of factors II, VII, IX, X, anti-thrombin, III and plasminogen, increases platelet adhesiveness.
- Lipid: Increases high-density lipoprotein (HDL) and reduces low-density lipoprotein (LDL), increases triglycerides, reduces ketone formation, increases fat deposition.
- Fluid balance: Salt and water retention.
- Gastro–intestinal: Reduces bowel motility, increases cholesterol in bile.

Although only minute 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, p. 18.39 & p. 19.16).

Functions of oestrogens include the (Meyer *et al.*, 1997, p. 19.16):

1. Promotion of follicle development and ovulation.
2. Stimulation of proliferation of the epithelial cells of the uterine tubes, uterus and vagina.
3. Stimulation of protein synthesis, e.g. contractile proteins in the myometrial muscle fibres of the uterus.
4. Reduction of the membrane potential of the myometrial muscle fibres thus increasing their sensitivity to oxytocin and prostaglandin.
5. Stimulation of duct growth in the mammary glands and involved in lactation.
6. Primary responsibility for the development of the female characteristics.
7. Involvement in skeletal growth and the maintenance of the structural integrity of bones.

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, p. 19.17).

Properties of progesterone (de Swiet *et al.*, 2002, p. 244):

- Structure: Enhances endometrial receptivity, maintains myometrial quiescence, breast development.
- Respiration: Increases respiratory drive.
- Lipid: Reduces HDL and increases LDL.
- Fluid balance: Promotes sodium exertion.
- Bowel: Reduces bowel motility.
- Metabolism: Increases body temperature.

The most important function of progesterone is the regulation of endometrial receptivity (de Swiet *et al.*, p. 244). Other functions of progesterone are that it (Meyer *et al.*, 1997, p. 19.17):

1. Stimulates the secretory activity of the uterine tubes, uterus and vagina.
2. Is 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.
3. 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.
4. Prevents ovulation when present in large amounts.
5. Decreases the number of oestrogen receptors in the endometrial muscle fibres.
6. Promotes protein anabolism.

7. Is responsible for a rise in body temperature at the time of ovulation.
8. Stimulates alveolar formation in the breasts during pregnancy.
9. Stimulates respiration.
10. Antagonizes 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, p. 5).

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, p. 5).

2.3.7 Testosterone

Nearly all circulating testosterone is bound to SHBG and albumin, with free testosterone being the most biologically active form. When elevated insulin levels are present, SHBG levels decrease while free testosterone levels increase (Hunter & Carek, 2003).

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, p. 473). 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, p. 473).

2.4 Infertility

Infertility is defined as one year of attempted conception without success (Smith *et al.*, 2003). Next, infertility and its causes will be discussed with emphasis placed on infertility in black women.

2.4.1 A concept of infertility

The desire to reproduce is an intensely motivating human force. Because of its personal nature, couples may also experience strong religious, cultural and societal pressures to conceive. Therefore, it is understandable that people experiencing infertility often perceive it as a serious life crisis. 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. 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. To some extent, it has led to the rapid evolution of technologic advances in reproductive biology. The physical, psychological and financial challenges of assisted reproductive technology may further impact the couple (Monga *et al.*, 2004; Seibel, 1997, p. 4).

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, p. 181). 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, p. 578). Primary infertility is the term used to describe those couples who have never

achieved a pregnancy, whereas secondary infertility defines patients who have previously achieved a pregnancy of any type and duration (DeCherney, 1990, p. 404).

2.4.2 Causes of infertility

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 STD's 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).

In any series of infertile marriages, the main etiological factor is found in the female more often than in the male (Tindall, 1987, p. 579). In a retrospective study undertaken by Poppe *et al.* (2002), female origin was diagnosed in 45% of the couples with specific causes including endometriosis (11%), tubal disease (30%), and ovarian dysfunction

(59%). Other variables contributing to infertility include age, history of sexually transmitted diseases, education, income level and parity (Green *et al.,* 2001).

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).

Clinical syndromes in females which may be associated with infertility include (Priest, 1985, p. 25):

- Non-consummation
- Tubal spasm and hypogonadism
- Spontaneous abortion
- Hyperemesis and psychological vomiting
- Pre-eclamptic toxaemia
- Amenorrhoea and anovulation
- Anorexia nervosa

2.4.3 Infertility in Africa

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).

2.4.3.1 Infertility in black African women

The problem of infertility in sub-Saharan Africa has received little attention from researchers. It is obscured by the region's high fertility rates, which give rise to a global climate of concern over population growth and high fertility that is not conducive to the perception of infertility as a real problem (Hollos, 2003).

The incidence of infertility is estimated to vary from 10 – 20%, but it appears to be rising. Currently there is an increasing awareness of infertility in Africa as a serious social and public health problem. This increase in public awareness, as well as the availability and scope of infertility services, might be the contributing factor to the apparent increase in prevalence (Chigumadzi *et al.*, 1998; Hollos, 2003).

In a continent where marriage is almost universal, and the purpose of marriage is children, infertility is often viewed as a major tragedy (Dyer, 2002). Loss of self-esteem,

anxiety and depression, hopelessness, guilt and marital difficulties are all recognized consequences of infertility. As the desire to have children has been said to be amongst the strongest emotions that people experience, it is not surprising that infertility has been considered life's worst experience by those who suffer from it (Dyer *et al*., 2002a).

Infertility is recognized as a major cause of divorce and spousal abandonment throughout the continent. (Dyer *et al.,* 2002a). In Nigeria, fertility from the men's point of view means that additional offspring adds to the power and prestige of their sub-lineage or family. Prestige is determined by the number of adult male followers a man can have. Economically in Nigeria, children are important in establishing claims to landholdings in the community, in competition with other sub-lineages, as with many other cultures in Africa (Hollos, 2003).

Women in Africa are particularly affected by their infertile status. Their social status and security usually depends directly on fertility. Those who cannot reproduce are at a substantial risk of divorce, stigmatization, socio-economic deprivation and abuse (Dyer, 2002). For example, infertile women in Mozambique are excluded from important social events and ceremonies. In Gambia, childless women have very few rights to inherit property from their husbands (Dyer *et al.*, 2002a). With children, a woman's prestige and value is assured and increased with each additional child. A woman is considered to be an unfortunate being if she is infertile. Not only is she thought to be disadvantaged economically, but her childlessness also prevents her from attaining full adult womanhood (Hollos, 2003). In a study carried out at a hospital in Durban, South Africa,

women claimed to frequently suffer abuse from their husband's family, and the level of abuse was deemed enough to push a woman to suicide (Dyer *et al.*, 2002b).

It is a fair assumption that the cost of assisted reproduction in most African countries is not covered by public health care or private insurance companies. In this context, cost becomes a critical factor, and any further increase may further limit access to treatment. This is in line with the findings of a study carried out in the United States, where it was found that users of infertility services were more likely than non-users to be white and to have higher incomes. This further complicates the matter of infertile black women seeking treatment, due to the costs which they may not be able to afford (Dyer, 2002; Green *et al.*, 2001).

2.5 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 affected by means of a multitude of factors. The three most common ones are (Seibell, 1997, p. 6):

(1) Excessive weight loss or weight gain;

(2) excessive exercise; and

(3) extreme emotional stress.

Twenty percent above or below ideal body weight may affect ovulation. Also, it must be remembered that obese people may be protein deficient and thin people may be eating quite well. The relationship between excess body fat and ovulatory disturbances appears stronger for early-onset obesity (Seibell, 1997, p. 6).

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, p. 824).

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, p.6).

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, p. 825; Hamilton-Fairley & Taylor, 2003).

2.6 Amenorrhoea

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, p. 115).

Hypothalamic amenorrhoea is characterized by amenorrhoea, hypo-oestrogenism, low or normal serum gonadotrophins, and a broad spectrum of abnormal patterns of hypothalamic GnRH secretion (Perkins *et al.*, 2001). Evidence indicates that psychological, metabolic, and/or nutritional stress could be the basis of hypothalamic amenorrhoea (Andrico *et al.*, 2002).

2.7 Overweight and obesity

An early reference to the influence of obesity on menstruation can be found in the writings of Hippocrates. In an essay on the Scythians, which appears under the heading 'the influence of climate, water supply and situation on health', their reproductive function is described in the following terms: "The girls get amazingly flabby and podgy… People of such constitution cannot be prolific… fatness and flabbiness are to blame. The womb is unable to receive the semen and they menstruate infrequently and little". The 'control subjects' are the serving wenches: "As good proof of the sort of physical characteristics that are favourable to conception, consider the case of serving wenches. No sooner do they have intercourse with a man than they become pregnant, on account of their sturdy physique and their leanness of flesh" (Franks *et al.*, 1996).

Obesity in women is associated with a number of negative health consequences. In the western world, a body mass index (BMI) > 25 kg/m² has been associated with poor education, low income and low socio-economic status. An increased prevalence of metabolic diseases such as diabetes mellitus, gall bladder disease, atherosclerosis, myocardial infarction and stroke, has also been observed in obese individuals, as well as negative psycho-social attitudes from other persons (Wass *et al.*, 1997). Recently, visceral fat accumulation, rather than the total amount of subcutaneous fat, has been shown to be associated with impaired glucose tolerance and lipid metabolism. Upper body obesity (or android obesity), has been identified as an independent risk factor for stroke, CHD and death in both men and women (Kruger, 1999).

2.7.1 Impact of obesity on fertility

Obesity produces a variety of alterations in the reproductive system in humans (Lashen *et al.*, 1999). Obesity affects ovulation, the response to fertility treatment, pregnancy rates and outcome. The fertility of obese women is lower than that of normal weight women in natural cycles and in-vitro fertilization programmes (Clark *et al.*, 1995; Clark *et al.*, 1998).

According to Reid & van Vugt (1987), one of the first attempts to document the relationship between obesity and menstrual dysfunction was presented in 1952. Researchers found a higher incidence of obesity in women with amenorrhoea than they did in the population of normal cycling control subjects with normal cycles. Lashen *et al.*

(1999) reported on a study where obese women with amenorrhoea resumed their menses with weight reduction alone. Most cases of amenorrhoea in women develop shortly after a period of weight gain (Reid & van Vugt, 1987).

Today a BMI of >27 kg/m² is considered to be a factor of decreased fertility. The ovaries of obese women have increased capsular hyalinization, and increased numbers of atypical or atretic follicles (Crosignani, 2002; Glass *et al.*, 1981).

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).

The adverse health implications of obesity are significant. This is evidenced in the many associations with different reproductive hormones, as well as its effect on the menstrual cycle. Therefore, all obese patients, regardless of their menstrual function or fertility, should be encouraged to participate in some form of structured weight control programme (Reid & van Vugt, 1987).

2.7.2 Endocrine abnormalities

As previously stated, obesity affects the results of fertility therapy (Maelli & Grazi, 2002). Obesity 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).

2.7.3 Obesity and spontaneous ovulation

Obesity is associated with three alterations that interfere with normal ovulation, and weight loss improves all three. They are (Speroff *et al.*, 1999, p. 492):

(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.

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 hypothalamo-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).

The mechanisms by which nutritional status influences hypothalamic activity are poorly understood. 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).

2.7.4 Obesity, 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. Insulin is required to facilitate the transport of glucose across cell membranes. Insulin homeostasis is restored by the production of insulin antagonists (e.g. adrenaline and cortisol) within 1 – 2 hours in normal individuals. If the sensitivity of cells to insulin

becomes impaired, the body will respond by producing more insulin and circulation levels may remain high (hyperinsulinaemia). Basal levels of circulating insulin tend to increase with age, and hyperinsulinaemia is strongly associated with adiposity, especially central fatness in men, and with lack of fitness due to inactivity (Garrow *et al.*, 2000, pp. 73 – 74).

The amount of insulin produced in response to a meal is related to a number of factors, including the rate at which sugars are absorbed in the small intestine. Elevated levels of insulin are associated with dyslipidaemia. Rapidly absorbed sugars tend to enter the lipogenic pathway and hence predispose to the obesity that is so common in the modern Western world. The combination of inactivity, easy access to energy-rich foods and consumption of considerable amounts of food often leads to obesity and tissue resistance to insulin (Garrow *et al.*, 2000, pp. 73 – 74).

The insulin resistance results in the inability to cope with the increased blood glucose caused by rapid absorption of the carbohydrates in modern sugary and starchy foods. The highest levels of circulating insulin occur after a meal, with the level depending on (1) the amount of carbohydrates in the meal, (2) their form, (3) the degree of insulin insensitivity, (4) the chemical composition of the starch, (5) the processing method, (6) the presence of viscous fibre, (7) as well as the anti-nutrients in the food or meal consumed. In general, the starch and sugars in modern processed foods are digested and absorbed more rapidly than those in raw or traditional cooked foods (Garrow *et al.*, 2000, pp. 73 – 74; Slabber *et al.*, 1994).

In the 1930s, Himsworth, of the University College Hospital, London, UK, was the first to clearly differentiate between the concepts of insulin secretion and insulin sensitivity. He found that some individuals with diabetes responded rapidly to an injection of insulin, with a fall in blood glucose concentration. Other individuals, typically more obese and with later-onset diabetes, were resistant to the blood glucose-lowering effect of insulin. In the 1960s, this concept was applied to non-diabetic but obese subjects whose metabolism was shown to be resistant to the effects of insulin, and who were found to have high levels of circulating insulin in response to glucose infusion. Since that time it has been recognized that sensitivity to insulin related to body fat content, even within relatively normal ranges of BMI. It has been claimed that at BMI > 30 kg/m^2, the insulin sensitivity index is low (Fryan, 2001).

Obesity has been recognized as an insulin-resistant state for many years, although not all individual obese patients are significantly insulin resistant. 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 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).

Women with abdominal obesity frequently show other signs of hyperandrogenicity, including elevated free testosterone and low sex hormone-binding globulin (SHBG) concentrations (Krotkiewski *et al.*, 1990; Crosignani, 2002). The relationship between abdominal obesity and insulin resistance appears to be closely correlated with the mass of intra-abdominal fat. Insulin has been found to have gonadotrophic effects, stimulating production of ovarian steroids, including androgens (Krotkiewski *et al.*, 1990).

The state of insulin resistance with secondary hyperinsulinaemia is commonly observed in obese, infertile women, especially those women who have PCOS. The exaggerated insulin action on the ovarian tissue may present the pathogenic mechanism leading to the disturbances of the endocrine profile and menstrual cycle, and hence to infertility in some obese women (Hollmann *et al.,* 1996). The disturbances in the menstrual cycle involve a small decrease during the luteal phase. It therefore might be expected that acyclic women are less insulin resistant than are those with intact cycles (Franks *et al.,* 1996).

Insulin is known to stimulate androgen production by ovarian tissue. High fasting and stimulated insulin concentrations are associated with anovulation (Clark *et al.*, 1995). Lowering insulin resistance by weight loss, or administration of an oral hypoglycaemic, results in spontaneous ovulation. Women with reduced weight have lower insulin concentrations (Clark *et al.*, 1998).

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 type 2 diabetes in most patients (Shahid & Schneider, 2000).

2.7.5 Leptin and infertility

Researchers have identified an obesity gene, called *ob,* that is expressed in the fat cells and codes for the protein leptin. Leptin acts as a hormone primarily in the hypothalamus. Leptin suppresses appetite and increases energy expenditure. Mice with a defective *ob* gene do not produce leptin, can weigh up to three times as much as normal mice, and have five times as much body fat. When injected with a synthetic form of leptin, the mice rapidly lose body fat. The fat cells not only lose fat, but they self-destruct, which may explain why weight gains are delayed when the mice are fed again (Whitney & Rolfes, 2002, p. 272).

A genetic deficiency of leptin has been identified in human beings as well. However, 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, p. 272).

2.7.5.1 Leptin's role in reproduction

Leptin research has uncovered some of its other regulatory roles around the body. For example, leptin may inform the female reproductive system about body fat reserves, stimulate growth of new blood vessels, enhance the maturation of bone marrow cells, promote formation of red blood cells, and help support a normal immune response (Whitney & Rolfes, 2002, p. 272). 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).

Among the humoral signals which possibly inform the reproductive cells about nutritional status, leptin is currently emerging as a convincing candidate. Leptin is a recently identified 16kDa proteic hormone synthesized and secreted by mature adipocytes, and serves as an indicator of fat stores to the brain. 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, appear to be dependent factors (Galtier-Dereure *et al.*, 1997).

The role of leptin in reproductive processes has received increasing attention. Leptin, an adipocyte hormone and recently described type-1 cytokine, has angiogenic properties and appears to have a relationship with some reproductive processes. Small 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).

The production of leptin 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 recently suggested. Data 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).

That oestradiol may be involved in the control of leptin production in women is supported by the fact that peri-menopausal women have higher levels of leptin than post-menopausal 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).

The *ob/ob* mice that lack leptin become very obese and infertile and develop insulin resistance. However, infertility is restored in these animals after treatment with recombinant leptin. It has become evident that leptin may act as a link between fat and reproduction. Studies have detected leptin receptor mRNA in the human ovary and specific binding of leptin in ovine granulosa cells (Messinis *et al.*, 1999).

It is possible 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).

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. Five main physiological functions have now been identified for leptin (Garrow *et al.*, 2000, p. 297):

- An inhibitor of food intake (satiety factor)
- A stimulator of energy expenditure
- A signal to the reproductive system
- A role in the production of multiple blood cell types, i.e. haematopoiesis
- A role in the growth of new blood vessels, i.e. angiogenesis

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, p. 297).

Leptin gene expression is regulated by a variety of hormones, growth factors and cytokines. Oestrogens induce, whereas androgens suppress leptin production, providing an explanation for the sexual dimorphism in serum leptin levels. Insulin increases leptin production, and this may contribute to the decrease of plasma leptin levels that occurs during fasting and the hyperleptinaemia that accompanies insulin resistant states. Glucocorticoids increase leptin gene expression independently of their effect on insulin resistance, but may also induce a relative leptin resistance by inhibiting leptin action. Pro-inflammatory cytokines, such as tumour necrosis factor ∝ (TNF-∝) and interleukin 1 (IL-1), may also directly induce leptin gene expression as part of a feedback loop underlying leptin's role in the regulation of local immune response, inflammation, and angiogenesis (Moschos *et al.*, 2002).

There is a variation in leptin levels throughout the menstrual cycle, with higher levels in the mid-luteal rather than the follicular phase. A few studies have shown either small but insignificant trends toward higher serum leptin levels at the end of the cycle, or no fluctuation at all. Although a correlation of leptin levels with changes in serum progesterone and/or oestrogen levels has not been uniform, an association between leptin and soluble receptors of TNF (sTNFR 1 and sTNFR 2), markers of increased TNF-∝ action secreted by pre-ovulatory follicles, has been reported (Moschos *et al.*, 2002).

In girls, leptin increases after puberty. Pre-pubertal levels are correlated with subsequent weight gain. Leptin levels also are high in African-American girls and in patients with precocious puberty. Because a critical threshold in fat mass has to be reached to initiate this process, leptin may be the signal from energy storage to the reproductive axis to elicit sexual development. Oestrogens increase leptin production. In assisted reproductive techniques, superovulation is associated with higher levels. 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).

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).

2.7.5.2 Sexual dimorphism and leptin levels

Even after correcting for body weight and fat mass, women have higher serum leptin levels than men. This sexual dimorphism in serum leptin concentrations has been associated with, or is causally related to a number of factors. Firstly, the pulse amplitude, but not the pulse frequency of leptin secretion from adipose tissue is twofold to threefold higher in females than in males. Secondly, fat mass is increased in females, and there is differential fat distribution with a higher subcutaneous/visceral fat ratio in women than in men. Leptin mRNA expression is known to be higher in subcutaneous than visceral fat depots. Thirdly, women have higher total serum leptin levels, but lower leptin-binding protein levels than men; indicating higher free leptin levels. Finally, female adipose tissue may be more sensitive to hormones (i.e. insulin and glucocorticoids) or other substances that stimulate leptin production. It is known that sex steroids such as oestrogens increase leptin levels, whereas androgens decrease leptin levels (Moschos *et al.*, 2002).

2.7.5.3 Role of leptin in obesity-related reproductive dysfunction

Similar to the *ob/ob* and *db/db* mice, humans who are obese due to mutations in the leptin gene or the leptin receptor gene have also been demonstrated to have reproductive dysfunction. With the exception of these rare cases, human obesity is because of leptin resistance from receptor down-regulation or post-receptor defects rather than leptin deficiency. It has also been shown that increasing obesity is associated with an

increasing frequency of anovulatory cycles and that obese women produced increased numbers of atretic follicles. These findings are consistent with the direct inhibitory action of high leptin levels on ovarian steroidogenesis leading to ineffective follicular maturation. Thus, it can be proposed that the high serum leptin levels of obese women may contribute to reproductive dysfunction at different levels of the hypothalamus-pituitary-gonadal (HPG) axis; that is, a central effect of increasing leptin levels leading to early menarche, which is later followed by resistance to the response of their gonadotropes to GnRH, combined with a peripheral inhibitory effect of the higher leptin levels on ovarian function predisposing to anovulation (Moschos *et al.*, 2002).

2.7.6 C-Reactive Protein and obesity

C-reactive protein (CRP) is an acute-phase reactant produced by hepatocytes in response to a wide range of stimuli. Circulating at low concentrations in healthy individuals, CRP rises dramatically in response to infection, inflammation and injury (Ford, 1999). Elevated serum CRP concentration has been shown to predict future risk of coronary heart disease (CHD). CRP levels below the upper limit of 1mg/dL, have been associated with a 2-to-3 fold increase in risk of myocardial infarction, ischemic stroke, peripheral arterial disease and CHD mortality in healthy men and women (Visser *et al.*, 1999). CRP has been used mostly in clinical settings as part of the diagnostic workup, to monitor disease status, and to monitor treatment results (Ford, 1999).

Elevated CRP levels have been associated with proxy indicators of elevated body fatness (body weight and BMI) independent of age, sex, race and ethnicity (Tchernof *et al.*, 2002; Ford, 1999). The distribution of body fat is associated with CRP concentration independent of BMI (Visser *et al.*, 1999). This is confirmed by a study conducted by Tchernof *et al.* (2002), where it was found that elevated CRP levels were observed primarily in women who were more obese and had more intra-abdominal fat. The best predictor of plasma CRP was body weight (Tchernof *et al.*, 2002). A waist-to-hip ratio (WHR) indicative of a large amount of abdominal visceral fat is associated with low-grade systemic inflammation (Visser *et al.*, 1999). Whether plasma CRP levels are related more closely to abdominal fat distribution than total body fatness, remains unclear (Tchernof *et al.*, 2002).

Among the recently discovered compounds expressed in human adipose tissue is the proinflammatory cytokine interleukin 6 (IL-6). Because of the inflammatory properties of IL-6, including the stimulation of acute-phase protein production in the liver, the release of IL-6 from adipose tissue may induce low-grade systemic inflammation in persons with excess body fat (Visser *et al.*, 1999). It has been proposed that adipose tissue-secreted IL-6 may mediate CRP level increases in obesity (Tchernof *et al.*, 2002). The contribution of adipose tissue in IL-6 secretion has been proposed to be the link between plasma CRP and adiposity, as CRP synthesis in the liver is largely under the control of IL-6. It is possible that this mechanism explains the higher CRP levels in obese patients (Tchernof *et al.*, 2002)

There is increasing evidence that the features of insulin resistance syndrome (namely, abdominal obesity, hyperinsulinaemia, high triglyceride, low HDL cholesterol), are all associated with increased CRP levels (Lemieux *et al*., 2001; Tchernof *et al.*, 2002). Improving insulin resistance with an insulin-sensitizing agent markedly reduces CRP concentrations in the absence of weight loss (McLaughlin *et al.*, 2002).

2.8 Polycystic Ovarian Syndrome (PCOS)

The basic pathophysiologic defect in polycystic ovarian syndrome (PCOS) is not known (Guzick, 2004). The consequences of PCOS include infertility, diabetes, abnormal uterine bleeding, hirsutism, alopecia, acne, increased risk of endometrial cancer and cardiovascular disease.

2.8.1 Definition

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 anovulation 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. In the United States, women are defined to have polycystic

ovarian syndrome if they have chronic anovulation and evidence of androgen excess (Guzick, 2004).

Women with PCOS are seen primarily for menstrual irregularity, androgen excess, and infertility. Women with chronic anovulation and hyperandrogenism have been observed to have an increased prevalence of diabetes and increased risk factors for CHD. Many women with PCOS are similar to those with metabolic cardiovascular syndrome (i.e. Syndrome X), a CHD-associated clustering within the same individual of hyperinsulinaemia, glucose intolerance, dyslipidemia and hypertension. The chronic anovulation of polycystic ovarian syndrome implies unopposed oestrogen and, therefore, an increased risk of endometrial cancer (Guzick, 2004).

2.8.2 Pathophysiology

Although the fundamental pathophysiologic defect in PCOS is unknown, these women have several interrelated characteristics, including insulin resistance, hyperandrogenism, and altered gonadotrophin dynamics. 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).

There is a strong correlation between insulin resistance and hyperandrogenism. Evidence would suggest that the direction of causation is from insulin to androgen, and not the reverse. Weight loss and insulin sensitizers, which also lead to a reduction in insulin, similarly are associated with a reduction in androgens, particularly testosterone and androstenedione (Guzick, 2004).

Another key pathophysiologic feature of PCOS is altered gonadotrophin-releasing hormone dynamics. Both lean and obese women with polycystic ovarian syndrome have increased LH pulse frequency and amplitude. Because androgen production by theca cells is LH dependent, it would seem to follow that the elevated levels of LH seen in women with PCOS are responsible for the excess androgen production (Guzick, 2004).

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).

Absolute concentrations of FSH above a specified threshold are essential for both the initiation of pre-ovulatory follicle development, as well as the selection of a single pre-ovulatory follicle. In the case of PCOS, the concentration of FSH may simply not rise

above levels seen in the mid-follicular range of the normal menstrual cycle, which are insufficient to stimulate pre-ovulatory follicle development (Guzick, 2004).

2.9 Underweight

Underweight is a far less prevalent problem than overweight. Whether the underweight person needs to gain weight is a question of health and, like weight loss, a highly individual matter. People who are healthy at their present weight do not need to loose any weight. Those who are thin because of malnourishment or illness, however, might benefit from a diet that supports weight gain (Whitney & Rolfes, 2002, p. 288).

Changes in the attitudes and practices of western society have led to a striking increase in the prevalence of this problem over the past decade. Widespread promotion of health and fitness has led to the participation in sporting activities by unprecedented numbers of women at all competitive levels (Borini & Asch, 1993, p. 905).

2.9.1 Low body weight

Low body weight can be caused by an intake insufficient in quantity to meet activity needs; excessive activity, such as in the case of compulsive athletes in training; poor absorption and utilization of food consumed; a wasting disease, such as cancer or hyperthyroidism, that increases the metabolic rate and energy needs, and psychological or emotional stress (Laquatra, 2004, p. 587).

Aversion to fatness is not unique to athletes: western society has become preoccupied with the concept that thinness and success go hand-in-hand. Current fashions and television marketing lend the impression that the thin woman is more likely to succeed in all walks of life, ranging from finding a mate to entering the professional or business world. Not surprisingly, there has been a striking increase in the incidence of hypothalamic amenorrhoea and anorexia nervosa in today's women. The factors influencing menstrual function in these circumstances may be complex and are rarely the sole consequence of changes in body weight. Rather, current evidence suggests that a combination of physical, psychological, and nutritional stresses may act additively in the determination of the timing of the onset and rate of pubertal progression, menstrual function, and fertility (Reid & van Vugt, 1987).

The mechanism of the adverse influence of undernutrition on ovarian function is reasonably well understood. This is best illustrated, in women, by the phenomenon of weight loss-related amenorrhoea, in which there is a characteristic disturbance of the hypothalamic control of gonadotrophins by GnRH. In most cases there is a cessation in the release of the gonadotrophins and this is accompanied by decreased pituitary stores of the hormone (Franks *et al.*, 1996; Ferin *et al*., 1993, p. 117).

Marked weight loss with physical or psychological stress will disrupt hypothalamic growth hormone (GnRH) production, leading initially to subtle endocrine abnormalities, such as luteal phase defects, and ultimately, to oligoamenorrhoea or amenorrhoea. The degree of hypothalamic dysfunction in such circumstances appears to be, in part, linked

to the percentage of body fat rather than body weight. Alongside the hypothalamic disturbances, the serum oestradiol levels also fall and there is a reduction in plasma prolactin, together with loss of the nocturnal prolactin rise (Reid & van Vugt, 1987).

2.9.2 Anorexia nervosa and bulimia nervosa

Functional disorders of the hypothalamus are the most frequently encountered hypothalamic disorders in clinical practice. The most extreme weight-related functional disorder of the hypothalamus is anorexia nervosa. This disorder occurs primarily among adolescent white females from middle to upper social class backgrounds, and occurs more frequently in women than in men (Bates, 1992, p. 185).

Anorexia nervosa is a disease characterized by refusal to maintain a minimally normal body weight, intense fear of gaining weight, body image distortion and amenorrhoea in postmenarcheal females. It is a condition characterized by voluntary self-starvation and emaciation. Weight loss is viewed as a sign of extraordinary achievement and self-discipline (Schebendach & Reichert-Anderson, 2004).

Bulimia nervosa, on the other hand, is characterized by repeated episodes of binge eating followed by inappropriate compensatory methods such as purging, including self-induced vomiting or misuse of laxatives, diuretics, or enemas; or nonpurging, including fasting or engaging in excessive exercise (Schebendach & Reichert-Anderson, 2004).

Anorexia nervosa profoundly affects neuroendocrine function. The 24-hour secretion of gonadotrophins reverts to a pubertal pattern in anorectic women, and does not return to an adult pattern until weight is returned to normal. Sex steroid hormone secretion, associated with decreased gonadotrophin secretion, reverts to a pre-pubertal state, thus rendering these women child-like from the viewpoint of reproductive physiology. In a study reported by Bates (1992, pp. 185 - 186), in women weighing between 53% and 64% of ideal body weight (IBW), the LH response was impaired, while the FSH response was equal to that observed in normally menstruating women. In the weight range of 79% to 88% of IBW, the LH response improved but remained less than that in normal women. In women who regained to within 90% – 94% of IBW, the LH response was greater than or equal to that found in normal women (Bates, 1992, p. 185).

Weight loss of approximately 10% of body weight in 1 year can cause amenorrhoea, regardless of premorbid weight. The association of amenorrhoea with anorexia nervosa is well established. Menstrual disturbances often begin prior to significant weight loss and persist after recovery to normal weight (Perkins *et al.*, 2001).

In patients with bulimia nervosa, menstrual irregularities are high. One might predict difficulties achieving pregnancy for women with bulimia nervosa on the basis of lower levels of LH or smaller spikes in LH levels. Abnormal LH secretion may be related to current weight that is less than 85% of the highest lifetime weight, rather than current low body weight (Crow *et al.*, 2002).

2.10 Summary

Infertility is a major problem that involves a number of factors. Not only is it important to look at the biochemical parameters on their own when evaluating infertility, it is also important to take into consideration the nutritional status of the patients. The current chapter has provided a description of the best-known factors related to infertility. The different reproductive hormones have been discussed in detail, as well as possible defects in these hormones that may occur with infertility. Overweight is a growing epidemic world-wide, and Africa, including South Africa, is not immune to this epidemic. It is also well known that obesity is associated with the metabolic syndrome, as well as other factors such as infertility. The relationship between obesity and PCOS has been shown in this chapter, as well as the relationship with insulin resistance. Leptin is emerging as an important hormone in both reproduction and obesity, and the relationship between these factors was also discussed.

Further research still needs to be done on black South African women with regards to infertility and its association with obesity and overweight. There is a growing awareness among the black population concerning the fact that there is treatment available for infertility. The challenge, however, is to make sure that treatment facilities are available to everybody, since one of the factors that prevents people from seeking treatment is the cost of services (Dyer, 2002).

CHAPTER 3
METHODOLOGY

3.1 Introduction

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.

3.2 Operational definitions

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

3.2.1 Black women

For the purpose of this study, black women were defined as those women who have South Sesotho, Setswana or Xhosa as their home languages.

3.2.2 Anthropometrical measurements

Anthropometry is the measurement of body size, weight, and proportions. Anthropometric measures can be used to evaluate nutritional status, whether it be obesity caused by over-nutrition, or emaciation resulting from protein-energy malnutrition (Lee

& Nieman, 2003, p. 164). Anthropometric measurements in this study include the measurement of the body mass index, waist-hip-ratio, neck circumference, as well as body fat percentage.

3.2.2.1 Body mass index

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, p. 139).

BMI can be categorized as follows according to the WHO (Laquatra, 2004, p. 565):

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

3.2.2.2 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, p. 182). 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, p. 10 & Hammond, 2000, p. 372).

3.2.2.3 Neck circumference

Neck circumference refers to the circumference taken around the midway of the neck. A neck circumference of less than 34 indicates normal weight, while neck circumferences of ≥ 34 and ≥ 36 indicate overweight and obesity, respectively (Liubov *et al.*, 2001).

3.2.2.4 Body fat percentage

Most researchers consider bioelectrical impedance analysis (BIA) to be a more accurate method for estimating body fat percentage than are anthropometric measurements (Zhu *et al.*, 2003). Body fat percentage refers to the percentage of fat measured in the body. For the purposes of this study the following cut-off points were used (Lee & Nieman, 1993, p.273):

< 15 ? lean

15 – 22 ? optimal health

23 – 26 ? slightly overweight

27 – 32 ? fat

> 32 ? obese

3.2.3 Biochemical parameters

The following biochemical measurements were taken, and the following cut-off points are given for women (Department of Chemical Pathology, University of the Free State).

- Serum CRP refers to serum C-reactive protein concentration. The optimal CRP concentration value is 3mg/L.

- Serum insulin refers to serum insulin concentration. The optimal fasting range for serum insulin is 5 – 15µU/ml.

- Serum glucose refers to serum glucose concentrations. The glucose fasting reference range is < 6,1mmol/L

- Serum testosterone refers to serum testosterone concentration. The optimal serum testosterone concentration value is between 0.5 and 2.6nmol/L

- 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	10.62 – 81.28nmol/L
Mid-luteal phase	14.12 – 89.14nmol/L

- Serum TSH refers to serum TSH concentration. The optimal TSH reference range is 0.35 – 5.50µIU/ml

- Serum LH refers to the serum LH concentration. During normal menstruation the reference range for LH is as such:

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	1.5 – 9.1mIU/ml

- Serum prolactin refers to the serum prolactin concentration. Serum prolactin concentrations for non-pregnant women: 59 – 619mIU/ml

- Serum leptin refers to the serum leptin concentration. Serum leptin concentrations for women are: 3.7 – 11.1 ng/ml

3.3 Study design

A descriptive study was carried out to investigate the relationship between anthropometrical and biochemical parameters in infertile black women.

3.4 Sample

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

3.4.1. Target population

The study was carried out at the Unit for Reproductive Care (URC) at Universitas Hospital in Bloemfontein. Due to the increasing rate of urbanization, there is an increase in the number of black women attending the URC for treatment. For this study, all new black patients were approached by the researcher to take part in the study.

3.4.2. Sample selection

The total population of black women attending the URC was included in this study.

3.4.3 Sample size

The size of the sample depends on how alike or different its members are with respect to the characteristics of research interest. The more homogeneous the sample, the smaller it will be (Leedy & Ormrod, 2001, p. 221).

The total population of all black women attending the URC for the first time for the period between 01 March 2003 and 31 July 2004 was used. A total of 78 women were recruited, and only 60 women were included in the study. Due to financial constraints and time limits, a larger sample could not be obtained.

3.4.4 Inclusion and exclusion criteria

The inclusion and exclusion criteria used in this study are the same criteria that are used at the URC (Appendix A). They have been modified to suit the objectives of this study. The inclusion criteria are as follows:

i) All patients attending the URC with the following problems:
   - Repeated pregnancy losses
   - Hormonal defects

ii) Hospital as well as private patients are managed at the URC.

iii) Only patients that are psychologically, physically and socially fit to be entered into the assisted reproduction treatment (ART).

iv) Patients must be prepared to have evaluations and special investigations, as prescribed by the URC, carried out on them.

v) Patients must be capable of following the treatment programme fully.

There was only one exclusion criterion for this study. All patients attending the URC are routinely tested for HIV, and those patients who are HIV positive are given further treatment. For this reason, all HIV positive patients were excluded from this study (see Appendix A).

3.5. Study procedure

Figure 1 shows the flow chart for the study procedure.

Patients attending the URC were referred to the clinic either by private practicing medical doctors or by doctors working in hospitals from the Free State, Northern Cape and Kwazulu-Natal provinces, as well as from Lesotho. When patients have received letters of referral, they are required to make appointments for consultations via the secretary at the URC. The patients are told by the secretary to arrive at the clinic in a fasting state for blood sampling, as well as for the Bodystat measurements. According to the precautionary measures for Bodystat measurements, patients were asked not to exercise for the 12 hours prior to the consultation.

Upon arrival at the URC, patients were approached by 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 approved, they were asked to sign the consent form.

Anthropometric measurements were carried out by the researcher using standardized methods and procedures (ISAK, 2001, pp. 7 – 8). After all the measurements were taken, 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. Guidelines for weight loss were given by the researcher to those who needed to lose weight. These guidelines consisted of information on a low-fat, high fibre diet (Appendix B); and those with an ideal body weight were given guidelines regarding eating for good health with the South African Food Based Dietary Guidelines given as a basis for the information (Appendix C).

Fasting blood samples were drawn by the resident registered nurse and taken to the Department of Chemical Pathology for analysis by the staff at that department. The results were then documented by the researcher using Microsoft Excel (MS Windows XP).

The anthropometrical and the biochemical data obtained was then taken to the Department of Biostatistics to be statistically analyzed. After analysis, the data was documented by the researcher in the chapters that follow.

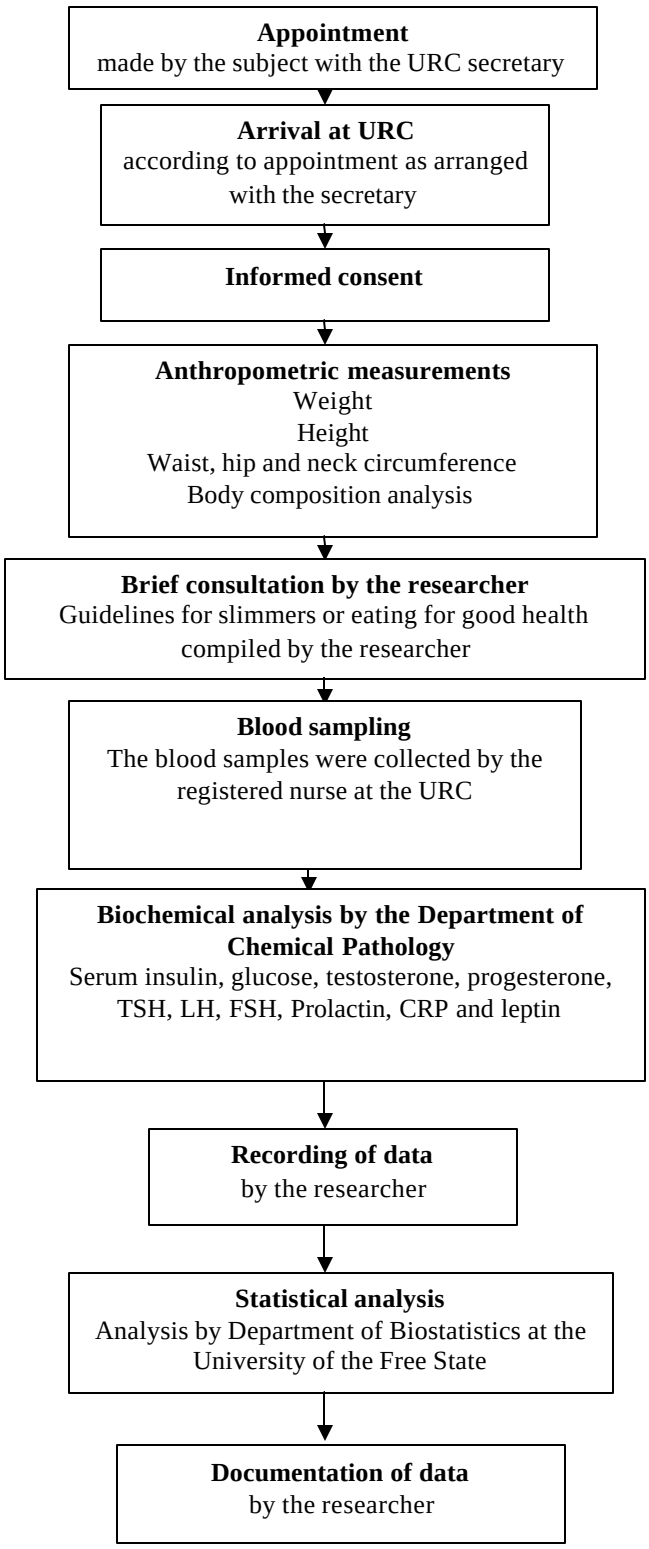

Figure 1: Flow chart of the study procedure

3.6. Choice of apparatus and techniques

3.6.1. Apparatus

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

3.6.1.1. Stadiometer

Height was measured using a stadiometer to the nearest 0.1cm. A stadiometer is a measuring stick attached to a vertical metal board with a movable horizontal headboard. The stadiometer can measure up to two meters (Heymsfield *et al.*, 1999, p. 9).

3.6.1.2. Scale

A platform electronic scale was used to measure the subjects' weight to the nearest 0.1kg (Lee & Nieman, 2003, p. 167). The scale was calibrated before the study.

3.6.1.3. Measuring tape

The measuring tape used was non-extendible, flexible and with a stub of 4cm before the zero line (ISAK, 2001, p. 9).

3.6.1.4. BodystatÒ

A Bodystat®1500 was used to determine body fat percentage. A tiny torch-battery-operated electrical current is sent through the body via electrodes after which an impedance value related to the subject's body fat and lean proportions is registered on the LCD screen. The unit has been precision electronically engineered to the highest quality standard, offering the user a safe and efficient means of measurement. It is able to calibrate itself prior to each measurement (Bodystat® R1500).

3.6.2. Measuring procedures and techniques

Anthropometrical measurements were done according to standardized methods and techniques by the researcher (Lee & Nieman, 2003, pp. 164 – 167).

3.6.2.1. Weight

The scale was placed on a hard surface to ensure an accurate reading. The scale was reading zero before the subject stood on it. Weight was measured 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, p. 19).

3.6.2.2. 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, p.10).

3.6.2.3. Waist and hip circumferences

The waist was measured using a non-stretching tape measure with the measurement taken around the point near the belly button (Sizer & Whitney, 2003, p. 321). 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, p. 372).

3.6.2.4. Neck circumference

A plastic non-stretching measuring tape was used to measure the neck circumference to the nearest 0.1cm. Neck circumference was measured at midway of the neck, between midcervical spine and midanterior neck with the subject standing upright (Liubov *et al.*, 2001).

3.6.2.5. Fat percentage

To measure body fat percentage using the Bodystat®1500, height and weight were first accurately measured and these values, together with the age and activity levels of the subjects were put into the unit. Each subject was asked to remove any socks or stockings and lie flat on a bed with arms and legs spread slightly, without different body parts touching. The self-adhesive disposable electrodes were attached to the right hand and right foot in order to avoid the battery current (low voltage) passing through the side of the body where the heart is situated (Bodystat® R1500).

The Bodystat®1500 has two sets of main leads, which are divided into a red and a black lead. The two sets of main leads are interchangeable. The red lead crocodiles are connected to the electrodes just behind the finger and toe while the black lead crocodile clips are connected to the electrodes on the right wrist and ankle. The enter button is then pressed to send the current through the body and the reading is obtained (Bodystat® R1500).

Upon making the appointment at the URC, the patient was informed to be in a fasting state. This meant that patients were not to eat or drink anything including alcohol and caffeine for 10 – 12 hours before their appointments. Patients were also instructed not to have exercised for the 12 hours prior to their appointments. This was so that reliable values could be obtained with the measurement of fat percentage.

3.6.2.6. Biochemical assays

Blood samples were drawn by registered nurses working at the URC on a full time basis. Blood samples were drawn after an overnight fast of 10 - 12 hours, using standardized laboratory techniques. The blood samples were analyzed at the Department of Chemical Pathology by chemical pathologists.

3.6.2.6.1. Highly sensitive C-reactive protein

Polystyrene particles coated with monoclonal antibodies to CRP are agglutinated when mixed with samples containing CRP. The intensity of the scattered light in the nephelometer depends on the CRP content of the sample. Therefore the CRP concentration can be determined versus dilutions of a standard of a known concentration (Reagent kit package insert).

3.6.2.6.2. Insulin

Serum plasma insulin was determined using the Human Insulin Specific RIA kit by LINCO Research, Inc. This assay does not cross-react with human pro-insulin, and therefore measures 'true' insulin levels (Reagent kit package insert).

In radio-immunoassay, a fixed concentration of labelled tracer antigen is incubated with a constant dilution of antiserum (LINCO Research, Inc. Human Insulin assay utilizes I-

labeled human Insulin and a Human Insulin antiserum) such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by the antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer antigen and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to the antibody will decrease as the concentration of unlabeled antigen increases (Reagent kit package insert).

This can be measured with an instrument that counts radio-activity after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve is set up with increasing concentrations of standard unlabeled antigen. From this curve the amount of antigen in unknown samples can be calculated. The level of insulin in serum, plasma or tissue culture media is determined by the double antibody/PEG technique (Reagent kit package insert).

3.6.2.6.3. Glucose

Serum glucose concentrations were analyzed using the SYNCHRON LX System, which determines glucose concentration by an oxygen rate method employing a Beckman Oxygen electrode (Reagent kit package insert).

A precise volume of sample (10 microlitres) is injected in a reaction cup containing a glucose oxidation solution. The ratio is one part sample to 76 parts reagent. The peak

rate of oxygen consumption is directly proportional to the concentration of glucose in the sample (Reagent kit package insert).

Because oxygen consumption rather than peroxide formation is measured, the only requirement for peroxide is that it must be destroyed by a path not leading back to oxygen. The addition of ethanol to the reagent causes peroxide to be destroyed in the presence of catalase, without yielding oxygen (Reagent kit package insert).

3.6.2.6.4. Testosterone

Androgen assessment is warranted in patients with menstrual cycle abnormalities and hirsutism (Willet, 1996, p. 146).

Method

Testosterone is a C19 steroid hormone. It binds strongly to plasma proteins such as sex-hormone-binding globulin (SHBG) or testosterone-oestradiol-binding globulin. Testosterone also binds with low affinity to CBF (cortisol-binding globulins) and albumin. Less than 2.5% of testosterone circulates unbound to plasma proteins (Reagent kit package insert).

The ACS:180 Testosterone assay is a competitive immunoassay using direct, chemi-luminescent technology. Testosterone in the patient sample competes with acridinium

ester-labelled testosterone in the Lite Reagent for a limited amount of polyclonal rabbit anti-testosterone antibody, which is coupled to paramagnetic particles in the Solid Phase. The assay uses Testosterone Releasing Agent to release bound testosterone from the endogenous binding proteins in the sample. An inverse relationship exists between the amount of testosterone present in the patient sample and the amount of relative light units (RLUs) detected by the system (Reagent kit package insert).

3.6.2.6.5. Progesterone

Progesterone, in conjunction with oestrogens, regulates reproductive tract functions during the menstrual cycle. The major sources of progesterone are the corpus luteum and the placenta in women (Reagent kit package insert). Mid-luteal progesterone determinations are consistent with ovulation (Willet, 1996, p. 278).

Method

The ACS:180 Progesterone assay is a competitive immunoassay using direct, chemi-luminescent technology. Progesterone in the patient sample binds to an acridinium ester-labeled mouse monoclonal anti-progesterone antibody in the Lite Reagent. Unbound antibody binds to a progesterone derivative, covalently coupled to paramagnetic particles in the Solid Phase (Reagent kit package insert).

An inverse relationship exists between the amount of progesterone present in the sample and the amount of RLUs detected by the system (Reagent kit package insert).

3.6.2.6.6. Thyroid-stimulating hormone (TSH)

Low TSH and thyroxine levels signify secondary hypothyroidism and low TSH and elevated thyroxine concentrations are found in persons with primary hyperthyroidism (Willet, 1996, p. 122).

Method

TSH is a glycoprotein with two non-covalently bound subunits. The alpha subunit is similar to those of follicle-stimulating hormone (FSH), human chorionic gonadotrophin (hCH) and luteinizing hormone (LH) (Reagent kit package insert).

The beta subunit of TSH is unique, which results in the specific biochemical and immunological properties of this hormone (Reagent kit package insert).

The ACS:180 TSH assay is a two-site sandwich immunoassay using direct, chemi-luminometric technology, which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is a monoclonal mouse anti-TSH antibody labelled with acridinium ester. The second antibody in the Solid Phase, is a polyclonal sheep anti-TSH

antibody which is covalently coupled to paramagnetic particles (Reagent kit package insert).

A direct relationship exists between the amount of TSH present in the patient sample and the amount of RLUs detected in the system (Reagent kit package insert).

3.6.2.6.7. Luteinizing hormone (LH)

LH is a glycoprotein hormone having two subunits. The alpha subunit is similar to those of FSH, hCG and TSH. The beta subunit is different from those of the other glycoprotein hormones and confers its biochemical specificity (Reagent kit package insert).

Method

The ACS:180 LH assay is a two-site sandwich immunoassay using direct chemi-lumonometric technology, which uses constant amounts of two antibodies that have specificity for the beta subunit of the intact LH molecule. The first antibody, in the Lite Reagent, is a monoclonal mouse anti-LH antibody labelled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-LH antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the amount of LH present in the patient sample and the amount of RLUs detected in the system (Reagent kit package insert).

3.6.2.6.8. Follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) determinations are performed on oligomenorrhoeic and amenorrhoeic women. High levels of FSH are consistent with women who have premature ovarian failure, while basal FSH levels are associated with poor ovarian reserve (Willet, 1996, p. 145).

Method

FSH is a glycoprotein hormone with two subunits. The alpha subunit is similar to those of LH, cCG and TSH. The beta subunit is different from those of the other glycoprotein hormones and confers its biochemical specificity (Reagent kit package insert).

The ACS:180 FSH assay is a two-site sandwich immunoassay using direct, chemi-luminometric technology, which uses constant amounts of two antibodies that have specificity for the intact FSH molecule. The first antibody, in the Lite Reagent, is a polyclonal sheep anti-FSH antibody labelled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-FSH antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the amount of FSH present in the patient sample and the amount of RLUs detected by the system (Reagent kit package insert).

3.6.2.6.9. Prolactin

Prolactin is measured in oligomenorrhoeic and amenorrhoeic women with or without galactorrhoea. Hyperprolactinemia is associated with ovulatory disturbances and luteal phase defects, thus affecting infertility (Willet, 1996, p. 146).

Method

Prolactin is a single chain polypeptide hormone secreted by the anterior pituitary. It is also synthesized by the placenta and is present in amniotic fluid (Reagent kit package insert).

The ACS:180 prolactin assay is a two-site sandwich immunoassay using direct, chemi-luminometric technology, which uses constant amounts of two antibodies. The first antibody, in the Lite Reagent, is a polyclonal goat anti-prolactin antibody labelled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-prolactin antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the amount of prolatin present in the patient sample and the amount of RLUs detected by the system (Reagent kit package insert).

3.7 Statistical analysis

Statistical analysis was carried out by the personnel of the Department of Biostatistics, Faculty of Health Sciences, University of the Free State.

Descriptive statistics, namely medians and percentages for continuous data and frequencies and percentages for categorical data, were calculated by group. The groups were compared by means of 95% confidence intervals.

3.8 Ethical approval

The Ethics Committee of the Faculty of Health Sciences, University of the Free State, gave approval for the study to be carried out.

3.9 Problems encountered during the execution of the study

3.9.1 Subject recruitment

The total number of black women attending the URC at Universitas Hospital was to be included in the study. As previously mentioned, a total of 78 participants were recruited to take part in the study, but only 60 of these patients could be included in the study. The researcher worked together with the unit's secretary when making appointments with the

patients. 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 coming in for appointments and not being 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. None of these patients turned up for their appointments. This affected the total number of subjects recruited.

Another limiting factor in this study was the exclusion of all HIV positive patients from the study. The researcher first saw the patients and carried out the anthropometric measurements, after which the registered nurse took blood samples. Since most of the patients had not had HIV tests carried out on them before coming to the URC, it was not possible to determine their status before the researcher included these patients in the study. This problem delayed the study, because patients who had already been included in the study had to be discarded due to the exclusion criteria when it was discovered that they were HIV positive.

3.9.2 Blood sampling

The progesterone measurement 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. Patients were told of this and asked to come for the blood

sampling on that specific day of their menstrual cycle. Only four of the participants were on day 21 on the day of their consultation. The rest did not keep their appointments. Those patients that did not live in Bloemfontein were given letters to take to their resident doctors and have blood samples drawn by them. It was explained to the subjects that the sampling and courier service costs would be carried by the researcher. None of those patients given letters had their blood samples couriered to the Department of Chemical Pathology, at Universitas Hospital.

3.10 Summary

The methodology of this study is discussed in this chapter. The different measurement techniques, as well as methods, are discussed in detail together with the choice and usage of standardized equipment. These methods were found to be valid and reliable. A list of all the anthropometrical and biochemical measurements was detailed, and all the problems encountered when carrying out the study were discussed.

CHAPTER 4
RESULTS

4.1 Introduction

The collection of data was carried out over a period of seventeen months, i.e. from 01 March 2003 until 31 July 2004). Over this period a total of 78 participants had been recruited. However, only 60 participants could be included in the study. Anthropometrical and biochemical data were collected from these 60 subjects because of the problems discussed in Chapter 3. Due to the skew distribution of the data, the results of this study will be presented by means of medians. The variation round the median value is given as the 25^{th} and 75^{th} percentiles. The results of this study will be presented in this chapter in the following order:

- description of the age and diagnosis of the subjects
- description of the anthropometrical data
- description of the biochemical data
- the association between age and diagnosis
- the association between anthropometric and biochemical parameters
- the correlation between parameters

4.2 Age and diagnosis of the subjects

The median age of the participants in this study was 32.4 years, with the range (20.3 – 41.6) indicating that the women were within the reproductive age.

Table 1 shows the frequencies of the participants classified according to age groups.

Table 1: The age groups of the subjects

	N = 60	
Age (yrs)	**Frequency**	**Percentage**
20 -30	25	41.7
31 – 40	32	53.3
> 40	3	5

More of the women included in this study, fell in the age group of 31 – 40 years while only three women were over forty years old.

The women in this study were diagnosed according to the different infertility related causes, and the number of women with the different diagnoses is set out in Table 2.

Table 2: Diagnosis of the subjects

	N = 60	
Diagnosis	**Frequency**	**Percentage**
Tubal factor	32	56.1
Endometriosis	6	10.7
Male factor	15	26.8
Anovulation	8	14.3
Unexplained infertility	13	22.4

The most prevalent cause of infertility amongst the subjects was tubal factors. Male factor infertility and unexplained infertility attributed to more or less a quarter of the sample's infertility. Endometriosis was the least prevalent diagnosis among the subjects in this study.

4.3 Anthropometrical data of the subjects

Table 3 presents the median, percentiles and the minimum and maximum values of the anthropometrical measurements of the women in this study.

Table 3: Anthropometrical data

	N=60				
	Min	**Max**	**75% (Q3)**	**Med**	**25% (Q1)**
Weight (kg)	45.3	127.8	83.7	75.2	65.3
Height (cm)	151	177	165	161.75	157
BMI (kg/m²)	18.9	48.7	32.6	29.4	25.3
Waist circumference (cm)	64.9	124	96.2	87	78.5
Hip circumference (cm)	55	150	119.4	111.4	106.5
WHR	0.60	1.4	0.83	0.79	0.73
Neck circumference (cm)	29.2	39.4	35.7	33.5	32
Body fat %	22.3	58.1	48.6	42.1	36.5

Median BMI was 29.40 kg/m² while the medians for the neck circumference and body fat percentage were 33.5cm and 42.1%, respectively.

Table 4 shows the anthropometrical measurements presented according to cut-off points as defined for this study (Chapter 3).

Table 4: Frequencies of the anthropometrical data

	N = 60	
Cut off points	**Frequency**	**Percentage**
BMI (kg/m²)		
18.5 – 24.9	14	23.3
25 – 29.9	18	30
≥ 30	28	46.7
Waist circumference (cm)		
> 88	34	56.7
≤ 88	26	43.3
WHR		
< 0.8	36	60
> 0.8	24	40
Body fat percentage (%)		
15 – 22	1	1.7
23 – 26	2	3.3
27 – 32	6	10
> 32	51	85
Neck circumference (cm)		
< 34	32	53.3
≥ 34	14	23.3
≥ 36	14	23.3

According to the BMI, 46.7% of the participants were obese. Most of the women had a waist circumference of more than 88cm and showed gynoid fat distribution.

4.4 Biochemical data

Table 5 shows the values obtained for the different hormones of the subjects, measured for the purposes of this study. Due to problems experienced with blood analysis, leptin could only be determined for 55 subjects in this study.

Table 5: Biochemical data of the subjects

	N = 60				
Normal values	**Min**	**Max**	**75% Q3**	**Med**	**25% Q1**
Insulin (5 – 15 μU/ml)	2.70	37.00	16.10	13.00	8.20
Glucose (≥ 6.1 mmol/L)	1.50	15.30	5.02	4.08	4.55
TSH (≥ 0.35 – 5.5μIU/ml)	0.20	5.50	2.13	1.53	1.03
LH (mIU/ml)	0.50	111	9.05	6.05	4.05
FSH (mIU/ml)	1.10	18.0	6.90	5.25	2.80
LH/FSH ratio	0.21	6.59	2.13	1.35	0.83
Prolactin (≥ 59 - 619mIU/ml)	10.0	738	311.5	242.5	175
Progesterone (nmol/L)	0.340	70.20	17.75	4.41	1.53
Testosterone (nmol/L)	0.40	2.60	1.60	1.20	0.95
Leptin (n = 55) (3.7 – 11.1ng/ml)	5.5	83.0	33.4	23.5	12.4
CRP (< 3mg/L)	0.44	28.70	6.99	3.39	1.01

Median concentrations of biochemical values were mostly within the normal ranges, except for the leptin concentration. Seventy five percent and less subjects had leptin concentrations of 33.4ng/ml. The median leptin concentration was high, with a value of 23.5 ng/ml. Furthermore, the median insulin concentration tended towards the higher value of the normal range for insulin.

Table 6, represents the number of subjects that fell within the categories of low (L), normal (N) and high (H) ranges for the specific hormonal concentrations.

Table 6: Biochemical values set out in categories

	N = 60	
Hormone **Reference ranges**	**Frequency**	**Percentage**
Insulin (µU/ml)		
L - < 5	11	18.3
N - ≥ 5 – 15	28	46.7
H - >15	21	35
Glucose (mmol/L)		
N -<6.1	57	95
H - ≥ 6.1	3	5
TSH (µIU/ml)		
L - < 0.35	2	3.3
N - ≥ 0.35 – 5.5	58	96.7
Prolactin (mIU/ml)		
L - < 59	1	1.7
N - ≥ 59 – 619	57	95
H - > 619	2	3.3
Testosterone (nmol/L)		
L - < 0.5	1	1.7
N - ≥ 0.5 – 2.6	59	98.3
CRP (mg/L)		
N - < 3	28	46.7
H - ≥ 3	32	53.3
Leptin (ng/ml)		
N - 3.7 – 11.1	9	16.4
H - > 11.1	46	83.6

Insulin, CRP and leptin values are the three values that have more subjects within the high categories.

4.5 The association between age and diagnosis

Diagnosis of the subjects according to the different age groups is given in Table 7. Initially, the participants of this study were divided into three age groups. Due to the small number of subjects in the age group of >41 years, this age group was condensed with the 31 – 40 years age group to form the over 31years old age group.

Table 7: The diagnosis of the patients and their age groups

	Age (yrs)		95% confidence interval for the percentage difference
	20 – 30 yrs	[3] **31 yrs**	
Tubal factors	44	65.6	[-43.9% ; 4.0%]
Endometriosis	16.7	6.25	[-6.7% ; 30.1%]
Male factors	50	9.35	[16.8% ; 60.2%]*
Anovulation	16.7	12.5	[-14.3% ; 24.8%]
Unexplained infertility	12.5	29.4	[-35.5% ; 5.5%]

* Statistically significant

In the subjects that were over 31 years old, the most prevalent cause of infertility was tubal factors (65.5%). Statistically significant more younger than older subjects had the male factor as the contributor to infertility. Also, the older subjects had a tendency towards unexplained infertility, but this was not significant.

4.6 The association between anthropometric and biochemical data

The different anthropometrical and biochemical data are shown in relation to the BMI categories in Table 8. The 95% confidence interval (CI) for the percentage differences between BMI and other parameters are given in Table 8. The BMI is divided into three categories where 1 = 18.5 – 24.9 kg/m^2; 2 = 25 – 29.9 kg/m^2 and 3 = ≥ 30 kg/m^2.

Table 8: Anthropometrical and biochemical data in relation to the BMI categories

	BMI (kg/m²)					
	Group 1 18.5 – 24.9 (n=14)	**Group 2 25 – 29.9 (n=18)**	**Group 3 ≥ 30 (n=28)**	**95% Confidence interval for the percentage difference**		
				1 – 2	**1 – 3**	**2 - 3**
	(%)	(%)	(%)			
WHR						
< 0.8 (n = 36)	78.6	77.8	39.3			
> 0.8 (n = 24)	21.4	22.2	60.7	[-26.6%;28.5%]	[-60.2%;-7.4%]*	[-59.0%; -9.1%]*
CRP (mg/L)						
< 3 (n = 28)	57.1	61.1	32.1			
> 3 (n = 32)	42.9	38.9	67.9	[-27.1%3;4.8%]	[-50.8%;5.8%]	[52.4%;0.2%]
Insulin (µU/ml)						
< 5 (n = 11)	28.6	11.1	17.9			
5 – 15 (n = 28)	28.6	72.2	39.3			
> 15 (n = 21)	42.9	16.7	42.9	[-5.0%;53.0%]	[-28.1%;29.5%]	[-7.3%;1.7%]
Leptin (ng/ml)	(n=13)	(n=16)	(n=26)			
3.7 – 11.1 (n = 9)	53.9	12.5	0			
> 11.1 (n = 46)	46.2	87.5	100	[-17.9%;-5.3%]*	[-38.6%;-15.3%]*	[-26.9%;-3%]*

* Statistical significance

The subjects with normal weight and those that were overweight, mostly (78.6% and 77.8% respectively) had a gynoid fat distribution. Most overweight subjects had normal CRP and insulin concentrations, but most of the subjects also had increased leptin concentrations.

The 95% confidence interval showed that there was a significant association between BMI groups and leptin. Other statistically significant differences were observed for the comparison of WHR in relation to the overweight and obese groups.

As with BMI, WHR was also categorized and insulin and leptin were described in terms of the WHR. The 95% confidence intervals for these parameters were also given for the high insulin concentration group.

The association between WHR and insulin is given in Table 9, with the 95% confidence interval being given for the high insulin concentration.

Table 9: WHR and insulin

	WHR		
	< 0.8 n = 36	**> 0.8 n = 24**	**95% Confidence interval for the percentage difference**
Insulin	(%)	(%)	
< 5 (μU/ml)	22.2	12.5	
5 – 15 (μU/ml)	52.8	37.5	
> 15 (μU/ml)	25	50	[-46.7% ; - 0.4%]*

In those subjects with a WHR of < 0.8, most (52.8%) had insulin levels within the normal range, while in subjects with android obesity, 50 of the subjects had high insulin levels.

Table 10 shows the association between WHR and leptin concentrations. The 95% CI was given for the high leptin concentrations.

Table 10: WHR and leptin

	WHR		
	< 0.8 n = 32	**> 0.8 n = 23**	**95% confidence interval for the percentage difference**
Leptin	(%)	(%)	
3.7 – 11.1(ng/ml)	21.9	8.7	
> 11.1 (ng/ml)	78.1	91.3	[-31.2% ; 7.9%]

In subjects with a WHR of < 0.8, 78.1% had leptin levels that were above normal. Most of the android obese women (WHR > 0.8) also had high leptin levels. The association between WHR and leptin was not significant. There was however, a tendency for the android obese subjects to have higher leptin values.

Table 11 describes the CRP levels in relation to insulin and leptin in percentages.

Table 11: CRP levels in relation to insulin and leptin

	CRP (mg/L)		
	< 3	**≥ 3**	**95% confidence interval for the percentage difference**
Insulin (µU/ml)	(%)	(%)	
< 5 (n = 11)	32.1	6.3	
5 – 15 (n = 28)	35.7	56.3	
> 15 (n = 21)	32.1	37.5	[-27.7%; 18.2%]
Leptin (ng/ml)			
3.7 – 11.1 (n = 9)	55.6	43.5	
> 11.1 (n = 46)	44.4	56.5	[-40.9%; 20.1%]

A CRP level of ≥ 3 is considered to be high. With regards to insulin, 57.14% of those subjects with high insulin levels also had high CRP levels. Neither the association between CRP and insulin and leptin was significant.

4.7 The correlation between parameters as determined with the Spearman correlation coefficient

The Spearman correlation coefficients of the various anthropometrical and biochemical data have been set out in Table 12. An r-value of less than 0.5 is considered a poor correlation between the different parameters.

Table 12: Spearman correlation coefficient of anthropometrical and biochemical data

	r-value
BMI vs age	0.21
BMI vs leptin	0.77
BMI vs CRP	0.32
BMI vs insulin	0.08
CRP-C vs insulin	0.24
WHR vs insulin	0.34
Leptin vs CRP	0.32
WHR vs BMI	0.40
WHR vs leptin	0.30

Leptin and BMI showed a strong correlation with each other (r-value = 0.77). On the other hand, the correlation between insulin and BMI was very low, with an r-value of 0.08.

4.8 Summary

The results of this study were discussed in this chapter. The subjects in this study were mostly within their reproductive age. The diagnosis that was more prevalent was that of tubal factor infertility, and endometriosis was the least prevalent cause of infertility. Anthropometrical data indicated a tendency towards overweight and obesity, with most of the subjects having a high BMI. Most of the subjects also had a gynoid fat distribution. Leptin levels increased as BMI increased. The reproductive hormones measured in the subjects of this present study, were within normal ranges.

CHAPTER 5
DISCUSSION

5.1 Introduction

In this chapter the results that were obtained from the anthropometric data and biochemical data of black, infertile women attending the URC at Universitas Hospital, Bloemfontein, will be discussed and interpreted. Few infertility studies have been carried out in South Africa, and especially among black women. Therefore, it was difficult to obtain information regarding the anthropometry of black women to compare with the results of the present study. The results of the present study were thus compared with those of studies conducted in the United States or with data from South African studies.

5.2 Age and diagnosis

The results regarding the ages of the women that were seeking treatment, as well as the relation between the ages and the diagnosis of the participants in this study will be discussed in the following paragraphs.

5.2.1 Age

The reduction in fertility is greatest in women in their late 30s and early 40s (Taylor, 2003). 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 a reproductive age with an age range of 20.3 – 41.6 years, median age of 32.4 and 53.3% of the women were between the ages of 31 – 40 years. Only 5% were over 40 years of age. Chigumadzi *et al.* (1998) found that 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.

The fact that most of the subjects were over 31 years of age, could be attributed to the fact that most black women are not familiar with the availability of infertility treatment facilities, or only found out about them after pursuing other options such as traditional doctors.

5.2.2 Diagnosis

Studies conducted in both South Africa (Chugamudzi *et al.,* 1998) and in the USA (Green *et al.,* 2001) are in agreement with the diagnostic results of the present study which will be discussed in the paragraphs that follow.

5.2.2.1 Tubal factors

Most of the participants in this study, 56.1%, had tubal factors as a cause of 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. The results of this present study correspond well with those of the study conducted in Cape Town, South Africa (on white women), where tubal factors were a cause of infertility in 57% of their subjects (Steward-Smythe & van Iddekinge, 2003).

Tubal factor infertility was the second most prevalent cause of infertility amongst the younger age group of 20 – 30 year olds in the present study. However, tubal factor infertility was the most prevalent cause of infertility among the 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, is indicative of the possibility of pelvic infection. 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.

5.2.2.2 Male factors

Male factor infertility was accounted for in 26.8% of the subjects in the present study, as indicated by the statistically significant association between age and male factor infertility (95% CI: 16.8; 60.2). While it is often the woman who presents initially with difficulty conceiving, infertility is a couple's problem (Case, 2003). This is demonstrated

by the high rate of male factor infertility which was also found in the study by Chigamudzi *et al.* (1998). Male factor infertility was present in 21% of the subjects in their study (Chigamudzi *et.al.*, 1998), while 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.

5.2.2.3 Anovulation

Only 14.3% of the women had anovulation as a cause of infertility. None of the ovulatory factors were abnormal. This could be due to the fact that none of the women in the present study were underweight. Decreased weight disrupts growth hormone production, leading ultimately to oligomenorrhoea or amenorronea (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). 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), for instance, 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).

5.2.2.4 Endometriosis

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, with only 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.

5.2.2.5 Unexplained infertility

Twenty two percent of the subjects in this study had unexplained infertility. This is contradictory to the findings of Chigamudzi *et al.* (1998), who found that in their study only 3.5% of their participants had unexplained infertility. Approximately 30% of infertile couples have unexplained infertility. Unexplained infertility is defined as normal test results in the basic tests for ovulation, sperm production, and Fallopian tube patency. Amongst the younger age group, only 12.5% of the subjects had unexplained infertility as a cause of infertility, while more (29.4%) of the older subjects had unexplained infertility. 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).

5.3 Anthropometrical measurements

The results of the anthropometric measurement of the women in this study will be discussed in the following paragraphs.

5.3.1 Body mass index

The median weight of the subjects in this study was 75.2kg. The height ranged from 151cm – 177cm, with a median height of 161.8cm. The median weight of this study was higher than that found in a study by Wittemer *et al.* (2000), which was only 62.02kg , but the median height of 161.8cm in the present study was similar to the mean height of 164cm found in the study of Wittemer *et al.* (2002).

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 in the developing world. There is an increasing rate of urbanization among the African population in South Africa that has an impact on obesity and its associated consequences in the future (Puoane *et al.*, 2002). Kruger (1999) has found an association between obesity in black women and higher systolic and diastolic blood pressure, lower HDL-cholesterol levels, and higher LDL-cholesterol, triglycerides, fibrinogen, fasting serum glucose and insulin.

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 infertility in the women of this study, where the median BMI was 29.4kg/m². Most women in this study (46.7%) were obese, with only 23.3% having a normal weight, and 30% being overweight (Laquatra, 2004, p. 565). Even though it was not investigated in this study, these women are at a risk for developing PCOS, which has been strongly associated with obesity (Hoeger, 2001).

Most of the normal weight subjects were within the age group 20 – 30 years. However, 55.6% of the overweight subjects also fell in this younger age group. A poor correlation was found between BMI and age ($r = 0.21$). Therefore, it cannot be concluded from this study that a more advanced age contributed to the high prevalence of obesity.

5.3.2 Body fat distribution

Individuals with a 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. 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, p.560).

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 $\geq$ 35 kg/m^2, because these patients will exceed the cut-points (NHLVI, 1998).

The range of the waist circumference in this study population was from 64.9cm – 124cm, with a median of 87cm. More than half of the subjects (56.7%) had a waist circumference that was more than 88cm, which is indicative of increased risk for developing metabolic disorders.

Some people carry their excess fat around and above the waist in the abdominal region; i.e. they have more visceral fat. Others carry their extra fat below the waist in the hips and thighs; i.e. they have more subcutaneous fat. These body types have been dubbed apples and pears, respectively (Grosvenor & Smolin, 2002, p. 255). In the female, body composition parameters and weight status are also clearly associated with reproductive function (Kirchengast *et al.*, 2004). The WHR of the subjects in this study at the 75th percentile was 0.83, while the median WHR was 0.79. Puoane *et al.* (2002) reported a mean WHR ranging between 0.80 – 0.85 in women between the ages of 24 – 54 years in

apparently health women in South Africa. Android obesity patterning affects female reproductive function negatively, in that it is associated with decreased conception rates.

The findings of this current study indicate that the majority (60%) of the women had gynoid obesity, while only 40% had android obesity. These findings are similar to the findings of Slabber *et al.* (1998), who found that in a study population of 69 infertile women (conducted at the same unit as this study), 39.1% of their subjects had android obesity, while 60.9% had gynoid obesity.

In both the normal weight and overweight groups of subjects in this study, most of the women had gynoid obesity, while only 21.4% of the normal weight group and 22.2% of the overweight group had android obesity. This is the opposite to the obese group, where 60.7% of these subjects had android obesity. The correlation between BMI and WHR was also poor ($r = 0.40$). However, a significantly statistical association was found between BMI and the WHR in the overweight and obese groups (95% CI: -52.4; 0.2). Thus, this statistical significance gives an indication that these subjects are at an increased risk of developing anovulation, PCOS and various non-communicable diseases. Slabber *et al.* (1998) suggest that android obesity, and not total body fat, is significantly associated with anovulation, whether PCOS is present or not.

5.3.3 Body fat percentage

Regardless of body weight, 85% of the subjects in this study showed a fat percentage of more than 32, while only 1 subject had a normal fat percentage. The median fat percentage in this study population was 42.1%. In a study undertaken by Zhu *et al.* (2003), in the National Health and Nutrition Examination Survey (NHANES III) in the USA, the mean body fat percentage of healthy black women was 35.9%. These findings were similar to those of the participants in this study. The findings of the present study also agree with the findings of the study by Slabber *et al.* (2001), conducted on 37 obese women at the URC, Bloemfontein. They found that the body fat percentage of their subjects was 48.1%. The high fat percentage in these subjects could possibly be attributed to high energy intakes and lack of physical exercise.

5.3.4 Neck circumference

Cikim *et al.* (2004) state that neck circumference can be used as a simple, easy to perform, quick test that can be used to identify overweight or obese patients. Furthermore Dixon and O' Brien (2002) have found that neck circumference and younger age were independent predictors of higher free androgen index. Neck circumference was also a good clinical predictor of menstrual irregularity, hirsutism, infertility, insulin resistance and PCOS. Liubov *et al.* state that there is a strong association between neck circumference and overweight and obesity indexes and also showed a positive correlation between neck circumference and dyslipidemia.

The results of this study are not in agreement with other research on neck circumference, where a link has been found between neck circumference and other obesity indexes. Only 23.3% of the women in this present study are classified as overweight according to neck circumference, whereas 46.7% were classified as obese according to BMI.

5.4 Biochemical parameters

The results of the biochemical parameters measured in this study will be discussed in the following paragraphs.

5.4.1 Insulin

Fasting insulin levels were determined in all 60 participants, and the median was 13µUI/ml. 46.7% of the subjects had normal insulin levels and over a third had high insulin levels. Hyperinsulinaemia and insulin resistance are well-known features in polycystic ovarian syndrome (PCOS). Therefore, those subjects in this study with increased insulin levels are at a risk of developing PCOS, due to the fact that they 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).

No association was found between insulin and BMI in this present study, and the correlation between these two parameters was very poor ($r = 0.08$). However, when comparing the three BMI groups, the prevalence of increased insulin concentrations was 42.9% in normal weight subjects; 16.7% in overweight subjects; and 42.1% in the obese subjects. 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 insulin concentrations and android obesity (95% CI: -46.7; -0.4). 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.

In a study reported by Lefebvre *et al.* (1997) among PCOS women, 60% of android and only 25% of gynoid women were hyperinsulinaemic. The subjects in this present study with gynoid fat distribution mostly had normal insulin concentrations. However, in the subjects with android obesity, 50% had hyperinsulinaemia, indicating a possible risk for developing PCOS.

5.4.2 Glucose

The median glucose level of the women in this study (4.08mmol/L), showed that most of the women had normal glucose tolerance. Only a few subjects had glucose levels above normal.

An adequate endometrial glucose metabolism is an essential part of endometrial differentiation and decidualization to provide a nutritional and receptive milieu. The relevance of endometrial glucose metabolism is reflected by the storage of glycogen in the endometrial epithelial cells during the proliferative phase, followed by glycogen storage in endometrial stromal cells in the mid-secretory phase, the time of implantation (von Wolff *et al.*, 2003). Therefore, abnormal glucose tolerance does not seem to have an influence in this study population.

5.4.3 Leptin

Leptin levels were determined in 55 subjects, and the median value of leptin in these women was 23.5ng/ml. A study by Iputo *et al.* (2001), conducted on a rural population in the Transkei, showed high leptin concentrations with a mean value of 13.5ng/ml in the women. Slabber *et al.* (2001) found that at baseline, the subjects in their study who were all obese, had leptin levels of 32.5ng/ml. Only 16.4% of the subjects in this present study had normal leptin levels, while the rest had high leptin levels.

Recent observations suggest that leptin may play a role in the regulation of the reproductive axis. In obese subjects with PCOS, a statistically significant hyperleptinemia was found (Orabi *et al.*, 1999). The discovery of leptin receptor mRNA in the brain and ovary, and the observation that leptin can impair the IGF-1 mediated augmentation of oestradiol synthesis by granulosa cells (in rats), suggested that: (1) leptin may act centrally to alter hypothalamic and/or pituitary function and; (2) that leptin may promote ovarian function through direct action on the ovarian follicles. Based on the results of Orabi *et al.* (1999) however, a central mechanism of the effect of leptin on the hypothalamus and/or pituitary was not demonstrated, because no association could be obtained between leptin, prolactin and FSH (Orabi *et al.*, 1999). In this present study, no association was determined between leptin and the reproductive hormones mentioned above.

Serum leptin concentration is primarily a function of the fat content of the human body. Serum concentration of leptin is positively correlated with the two commonly used anthropometric indicators of obesity; namely, the BMI and the total body fat content. It has been suggested that obesity might be a result of tissue insensitivity to leptin - a case of failure in the leptin signalling pathway. However, there is a scarcity of information on serum leptin and its determinants in African communities (Iputo *et al.*, 2001).

The majority of the subjects in this study had leptin levels above the normal range. In these subjects, the leptin levels increased statistically significantly as BMI increased (95% CI: -17.9 ; -5.3). In the group with normal BMI, the median leptin concentration

was 10.3ng/ml. The group that was overweight had a median leptin level of 22.15ng/ml, while the obese subjects' median leptin concentration was 33.55ng/ml. The percentage differences between the normal weight and overweight group was -17.9 ; -5.3. Between the normal weight and obese group it was -38.6 ; -15.3, and between the overweight and obese groups it was -26.9 ; -4.3. These findings of a relationship between BMI and leptin are confirmed by the strong correlation between BMI and leptin, as evidenced by an r-value of 0.77. The findings of this present study are thus similar to the findings of many other researchers who have found a strong correlation between leptin concentration levels and the prevalence of obesity (Iputo *et al.* 2001).

Leptin levels were high in both the gynoid (78.1%) and android (91.3%) obese women in this study. This could be explained by the high number of subjects with high leptin levels. However, no association was found between body fat distribuition and leptin concentrations (95% CI: -31.2; 7.9), and the correlation was also poor (r = 0.3).

5.4.4 C-reactive protein

The results of this study show that the median CRP level was 3.39mg/L, with 53.3% of the subjects having high levels of this hormone. Elevated circulating levels of inflammatory markers have been associated with incidences of cardiovascular disease. CRP concentrations have been shown to be significantly associated with several cardiovascular risk factors, such as age, smoking, hypertension, exercise, plasma lipids, homocysteine, and BMI (Pannacciulli *et al.*, 2001).

Obesity may be one factor linking chronic, sub-clinical inflammation and atherosclerosis. There are several lines of evidence supporting this concept. Dietary fat (Ω-3 polyunsaturated fatty acids) seems to have a direct effect on the synthesis of pro-inflammatory cytokines by peripheral monocytes (TNFα, IL-1). IL-6 and TNFα are expressed in adipose tissue. It has been shown that about 30% of total circulating concentrations of IL-6 originate from adipose tissue in healthy subjects, and that its release is modulated by TNFα (Festa *et al.*, 2001).

Information regarding CRP concentrations in black South African women is scarce. This study, however, shows that in the subjects with normal CRP levels, 55.6% had normal leptin levels, and only 44.4% had high leptin levels. The correlation between CRP and leptin was also very poor (r = 0.32), as with many of the other correlations in this study. There was also no association found between CRP and leptin concentrations (95% CI:

-40.9; 20.1).

Of the obese individuals in this study, 60.7% had high CRP levels, while 61.1% of the overweight subjects had normal concentrations. A poor correlation between BMI and CRP levels in this study was found (r = 0.32). No association was found between BMI and CRP. However, surveys found that the prevalence of elevated CRP levels are higher both in overweight and obese patients than in normal weight subjects. It has been suggested that the association between BMI and CRP might be mediated by the cytokines IL-6 and TNFα, which are both expressed in adipose tissue and referred to as main

regulators of CRC production in the liver (Pannacciulli *et al.*, 2001). Therefore, even though no association was found between BMI and CRP in this study, careful monitoring of CRP levels in obese individuals is necessary to establish the risk of inflammation among obese women.

Pannacciulli *et al.* (2001) found that in their study of 201 infertile women, CRP was positively correlated with fasting insulin. This was not true for this present study where the correlation was poor (r-value = 0.24), and the 95% CI was -27.7 ; 18.2, meaning that there was no association. Of the subjects in this study with high CRP levels, 56.3% had normal insulin levels, while only 37.5% had hyperinsulinaemia. In the women with normal CRP levels, there were no differences in the insulin concentrations.

The findings of Pannacciulli *et al.* (2001) correspond with those of a study by McLaughlin *et al.* (2002), where they found that elevated CRP levels were confined to those obese individuals who were also hyperinsulinaemic, and not to individuals who had normal insulin levels. It has been suggested that heightened inflammatory responses could lead to insulin resistance and compensatory hyperinsulinaemia. This has been attributed in large part to the important role of inflammatory cytokines released from adipocytes; a process presumably made more likely with increased adiposity (McLaughlin *et al.*, 2002).

5.4.5 Reproductive hormones

A problem was encountered in determining the levels of LH, FSH and progesterone in the subjects of this study as explained in Chaper 3. The other hormones will be compared with the findings of similar studies on infertility.

5.4.5.1 Prolactin

Most subjects in this study (95%) showed normal prolactin levels. Only one subject had a low prolactin level, while 3.3% had hyperprolactinaemia. The mean prolactin level was 242.5mIU/ml. Prolactin is involved in the regulation of gonadal function. It stimulates the generation of LH receptors in the gonads. In the female this effect occurs in the ovarian corpus luteum.

Hyperprolactinaemia suppresses gonadal function by short-loop negative feed-back inhibition of LH release. It also reduces GnRH secretion and interferes with ovulation. Therefore, in addition to the galactorrhoea, infertility and amenorrhoea often occur (Laycock & Wise, 1996, p.56 & 80; Cramer *et al.*, 2003). These results and discussion, may partly explain the low incidence of anovulation among the subjects of this study, who mostly had normal prolactin levels.

5.4.5.2 Thyroid-stimulating hormone

TSH was determined in all of the participants in this study and the median was 1.53μIU/ml. All, but two subjects had normal TSH levels. Chigumadzi *et al.* (1998) performed thyroid function tests on 68 infertile black women in their study and, similar to this study, none of the women had abnormal TSH levels.

The prevalence of sub-clinical hypothyroidism has been reported to be 0.7% - 2.3% in a large series of unselected infertile women in a study by Raber *et al.* (2003). Raber *et al.* (2003) found that 34% of the 283 infertile women in their study had hypothyroidism. Cramer *et al.* (2003) found that in their study of 509 women undergoing assisted reproductive technology (ART), 3.1% of the subjects had a TSH value over 5μIU/mL and only one had a value of 55μIU/mL. Therefore, in general, the prevalence of thyroid disorders among infertile women is very low.

With primary thyroid failure, the circulating thyroid hormone level falls, stimulating the pituitary to increase TSH output. Nearly all women with elevated TSH levels have hypothyroidism. It is uncertain whether hypothyroidism can be a cause of recurrent miscarriages. Menstrual irregularities and bleeding problems are common in hypothyroid women (Speroff *et al.*, 1999, pp. 812 – 816).

A suppressed TSH level, on the other hand, with a high T4 or a high T3, confirms the diagnosis of hyperthyroidism. TSH hyper-secretion as a cause of hyperthyroidism is

extremely rare. Menstrual changes associated with hyperthyroidism are unpredictable, ranging from amenorrhoea to oligomenorrhoea, to normal cycles (Speroff *et al.*, 1999, pp. 812 – 816). Most women with hyperthyroidism, however, do not have fertility problems (Cramer *et al.*, 2003).

5.5 Summary

An increasing number of black South Africans are becoming educated and therefore delay having children. The age at which women decide to conceive is increasing, meaning that more couples require assisted reproduction techniques, while others remain subfertile and need more complex treatment such as IVF.

The present study has confirmed the findings of other authors who found tubal factor infertility to be the leading cause of infertility. Africa has seen an increase in the prevalence of sexually transmitted diseases, and with this particular study, it is possible that STDs could be a contributing factor to tubal factor infertility.

It is well known that obesity interferes with certain hormonal and other factors related to reproduction. The nutritional transition that black people find themselves in, means that there is lack of exercise and increased consumption of energy-dense foods. The results of this study show that more than half of the women included were overweight and obese. Therefore, in dealing with infertility, the presence and risk of obesity should be given very serious attention.

Android obesity was not very prevalent in the subjects of this study, as most of the women had a gynoid fat distribution. However, an association between gynoid fat and BMI was not found.

A positive association between leptin and BMI was found. The leptin concentrations of the women in this study increased as BMI increased. This is indicative of leptin resistance.

CRP concentrations have been shown to be associated with increased risk of cardiovascular diseases. Even though the prevalence of subjects with elevated levels of this hormone was not very high, the fact that some of those subjects with elevated concentrations of CRP were also obese, places these individuals at an increased risk of developing metabolic syndrome.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

Despite a high population growth rate in Africa, infertility remains a major reproductive health problem. Prevalence of infertility is high, and the underlying pathology frequently affects women's physical health. Until recently studies have focused predominantly on patients in industrialized countries while the experience of infertility in the developing world has received comparatively little attention (Dyer *et al.*, 2002b).

The main objective of this study was to describe the anthropometrical and biochemical parameters in infertile, black South African women. The biochemical and anthropometrical data was obtained from sixty infertile women, using standardized methods and procedures described in Chapter 3. This study was the first of its kind in determining both the anthropometrical and biochemical parameters and their association in black, infertile women in South Africa.

6.2 Conclusions

The following conclusions could be drawn from this study:

6.2.1 Age and diagnosis

- 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. Endometriosis was the least prevalent cause of infertility. This is in agreement with most studies conducted on infertile women, and especially those studies conducted in Africa.

- No association was found between age and the diagnosis of subjects, except for male factor infertility.

6.2.2 Anthropometry

- Obesity was a cause for concern in the subjects of this study, with almost half of this study population being obese. 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. Although undernutrition is an important hazard in South Africa, none of the women in this study were underweight.

- The high incidence of women with a waist circumference above 88cm is indicative of an increased risk for developing metabolic disorders.

- The finding that over half of the subjects had gynoid fat distribution, suggests that gynoid obesity may pose as much of a threat to the general health status of women as android obesity does. Only a small percentage of the subjects in this study had android obesity, which has been associated, amongst other factors of infertility, with hirsutism. Also, an association was found between BMI and android obesity.

- Most of the subjects had high body fat percentages regardless of body weight. The prevalence of high fat percentages could be due to a lack of physical activity. However, this was not determined in this study.

- For purposes of this study, BMI was a more reliable predictor of obesity than neck circumference.

6.2.3 Biochemistry

- Approximately a third of the participants had high insulin concentrations, while glucose concentrations were normal in most subjects. Even though no association between insulin and BMI could be determined, those subjects with increased insulin concentrations are at an increased risk of developing PCOS and other metabolic disorders.

- There was an association between high insulin levels and android obesity, and most of the subjects with android obesity had hyperinsulinaemia.

- Leptin concentration levels were increased in almost all subjects, which could mean that the women in this study possibly had leptin resistance. A strong correlation was determined between BMI and leptin concentrations. This is in agreement with most studies conducted on leptin and obesity.

- CRP levels were high in more than half of the total population, but no association was found between CRP levels and BMI. Implications of increased levels of CRP, which is associated with inflammation, warrant the increased attention that should be given to this hormone.

- LH, FSH and progesterone levels are difficult to determine in such studies, due to patients being on different days of their menstrual cycles and not during the ovulatory phase of the cycle.

- The reproductive hormones were normal in the black women that participated in this study.

6.3 Recommendations

The recommendations of this study are as follows:

- There should be increased awareness for the black population about the availability of facilities for subjects presenting with infertility. Difficulty in conceiving in the black population has been associated with stigmatization. Therefore, the impact of infertility on the women should not be taken for granted as it does place a great deal of stress on the individual.

- Being overweight has many positive connotations in the African community in South Africa. Research has shown that being obese is perceived as being a reflection of affluence and happiness in many sectors of the African population. The increasing rate of HIV/AIDS also has an effect on the weight status of Africans, because a person that is thin is seen as having the disease. Also overweight is seen as a husband's ability to feed and to support his wife and

family. Therefore, more attention should be given to educating obese individuals about the advantages of achieving and maintaining a healthy weight status. Infertile obese subjects should be made aware of the impact of excess body weight on the outcome of IVF and ART.

- A link between increased CRP concentration levels and the risk of cardiovascular disease has been established in literature; and the increased prevalence of subjects with high CRP levels in this study necessitates that research be carried out to determine the possible association between inflammatory markers and infertility.

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

- Due to the high number of subjects having such high leptin concentrations, research should be carried out to determine the relationship between infertility and hyperleptinaemia in black women.

- A dietary intervention study should also be carried out amongst black, infertile women to determine what the effects of weight loss will be on their infertility.

REFERENCES

Andrico, S., Gambera, A., Specchia, C., Pellegrini, C., Falsetti, L. and Sartori, E. 2002. Leptin in functional hypothalamic amenorrhoea. *Human Reproduction*, vol. 17, no. 8, pp. 2043 – 2048.

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.

Bodystat R1500 - Bodystat®, 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.

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.

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

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.

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.

Cikim, A., Mantar, F., Ozbey, N. and Orhan, Y. 2004. A simple obesity index: neck circumference. *12th International Congress of Endocrinology*.

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 infertile women results in improvement in reproductive outcome for all forms of fertility treatment. *Human Reproduction*, vol. 13, no. 6, pp. 1502 – 1505.

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

Crosignani, P.G., Bianchedi, D., Riccaboni, A. and Vegetti, W. 1999. Management of anovulatory infertility. *Human Reproduction*, vol. 14, suppl. 1, pp. 108 – 119.

Crow, S.J., Thuras, P., Keel, P.K. and Mitchell, J.E. 2002. Long-term menstrual and reproductive function in patients with bulimia nervosa. *American Journal of Psychiatry*, vol. 159, no. 6, pp. 1048 – 1050.

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.

Dixon, J.B. and O'Brien, P.E. 2002. Neck circumference a good predictor of raised insulin and free androgen index in obese premenopausal women: changes with weight loss. *Clinical Endocrinology*, vol. 57, no. , pp. 769 – 778.

Dyer, S.J. 2002. The conflict between effective and affordable health care – a perspective from the developing world. *Human Reproduction*, vol. 17, no. 7, pp. 1680 – 1683.

Dyer, S.J., Abrahams, N., Hoffmann, M. and van der Spuy, Z.M. 2002a. 'Men leave me as I cannot have children': women's experiences with involuntary childlessness. *Human Reproduction*, vol. 17, no. 6, pp. 1663 – 1668.

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 – 1662.

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.

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.

Festa, A., D'Agostino Jr, R., Williams, K., Karter, A.J., Mayer-Davis, E.J., Tracy, R.P. and Haffner, S.M. 2001. The relation of body fat mass and distribution to markers of chronic inflammation. *International Journal of Obesity*, vol. 25, pp. 1407 – 1415.

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

Ford, E.S. 1999. Body mass index, diabetes, and C-reactive protein among US adults. *Diabetes Care*, vol. 22, no. 12, pp. 1971 – 1977.

Franks, S., Robinson, S. and Willis, D.S. 1996. Nutrition, insulin and polycystic ovary syndrome. *Reviews of Reproduction*, vol. 1, no. 1, pp. 47 – 53.

Freeman, M.E., Kanyicska, B., Lerant, A. and Nagy, G. 2000. Prolactin: structure, function, and regulation of secretion. *Physiology Reviews*, vol. 80, no. 4, pp. 1523 – 1631.

Fryan, K.N. 2001. Adipose tissue and the insulin resistance syndrome. *Proceedings of the Nutrition Society*, vol. 60, no.3, pp. 375 – 380.

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.

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.

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.

Grosvenor, M.B. and Smolin, L.A. 2002. *Nutrition: From science to life.* Fort Worth: Harcourt College Publishers.

Guzick, D.S. 2004. Polycystic ovary syndrome. *Obstetrics & Gynecology,* vol. 103, no. 1, pp. 181 -193.

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.

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.

Hoeger, K. 2001. Obesity and weight loss in polycystic ovary syndrome. *Obstetrics and Gynecology Clinics of North America*, vol. 28, no. 1, pp. 85 – 91.

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.

Hollos, M. 2003. Profiles of infertility in Southern Nigeria: Women's voices from Amakiri. *African Journal of Reproductive Health*, vol. 7, no. 2, pp. 46 – 56.

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.

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

Iputo, J.E., Robinson, D. and Mguni, M. 2001. Serum leptin concentration in a rural African population. *East African Medical Journal*, vol. 78, no. 9, pp. 484 – 488.

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

Khalaf, Y. 2003. ABC of subfertility: tubal subfertility. *The British Medical Journal*, vol. 327, no. 7414, pp. 610 – 613.

Kirchengast, S. and Huber, J. 2004. Body composition characteristics and fat distribution patterns in young infertile women. *Fertility and Sterility*, vol. 81, no. 3, pp. 539 – 544.

Krotkiewski, M., Seidell, J.C. and Björntorp, P. 1990. Glucose tolerance and hyperinsulinaemia in obese women: role of adipose tissue distribution, muscle fibre characteristics and androgens. *Journal of Internal Medicine*, vol. 228, no. 3, pp. 385 – 392.

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.

Laschen, H., Ledger, W., Bernal, A.L. and Barlow, D. 1999. Extremeso of body mass do not adversely affect the outcome of superovulation and in-vitro fertilization. *Human Reproduction*, vol. 14, no.3, pp. 712 – 715.

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.

Laycock, J. and Wise, P. 1996. *Essential endocrinology*. 3rd ed. Oxford University Press: Oxford.

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

Leedy, P. and Ormrod, J.E. 2001. *Practical research: planning and design.* 7th ed. New Jersey: Merrill Prentice Hall.

Lefebvre, P., Bringer, J., Renard, E., Boulet, F., Clouet, S. and Jaffiol, C. 1997. Influences of weight, body fat patterning and nutrition on the management of PCOS. *Human Reproduction*, vol. 12, suppl. 1, pp. 72 – 81.

Lemieux, I., Pascot, A., Prud'homme, D., Almeras, N., Bogaty, P., Nadeau, A., Bergeron, J. and Despres, J. 2001. *Arteriosclerosis and Thrombosis and Vascular Biology*, vol. 21, no. 6, pp. 961 – 967.

Liubov, B., Sohar, E. and Laor, A. 2001. Neck circumference as a simple screening measure for identifying overweight and obese patients. *Obesity research,* vol. 9, no. 8, pp. 470 – 477.

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.

McLaughlin, T., Abbasi, F., Lamendola, C., Liang, L., Reaven, G., Schaaf, P. and Reaven, P. 2002. *Circulation*, vol. 106, no. 23, pp. 2908 – 2912.

Messenis, I.E., Milingos, S.D., Alexandris, E., Kariotis, I., Kollios, G. and Seferiadis, K. 1999. Leptin concentrations in normal women following bilateral ovariectomy. *Human Reproduction*, vol. 14, no. 4, pp. 913 - 918.

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.

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

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.

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.

Moschos, S., Chan, J.L. and Mantzoros, C.S. 2002. Leptin and reproduction: a review. *Fertility and Sterility*, vol. 77, no. 3, pp. 433 – 444.

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.

Orabi, H., Ghalia, A. A., Khalifa, A., Mahfouz, H., Shalkani, A. and Shoieb, N. 1999. Serum leptin as an additional possible pathogenic factor in polycystic ovary syndrome. *Clinical Biochemistry*, vol.32, no. 1, pp. 71 – 75.

Ovalle, F. and Azziz, R. 2002. Insulin resistance, polycystic ovary syndrome, and type 2 diabetes mellitus. *Fertility and Sterility*, vol. 77, no. 6, pp. 1095 – 1105.

Pannacciulli, N., Cantatore, F.P., Minenna, A., Bellacicco, M. Giorgino, R. and De Pergola, G. 2001. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. *International Journal of Obesity*, vol. 25, pp. 1416 – 1420.

Perkins, R.B., Hall, J.E. and Martin, K.A. 2001. Aetiology, previous menstrual function and patterns of neuro-endocrine disturbance as prognostic indicators in hypothalamic amenorrhoea. *Human Reproduction*, vol. 16, no. 10, pp. 2198 – 2205.

Pilgrim, T.C. 2001. The hidden problem of infertility in the African American community. Available from http://www.ferre.org/focitext/hiddent.html [Accessed 14 June 2003].

Poppe, K., Glinoer, D., van Steirteghem, A., Tournaye, H., Devroey, P., Schiettecatte, J. and Velkeiners, B. 2002. Thyroid dysfunction and autoimmunity in infertile women. *Thyroid: Official Journal of the American Thyroid Association*, vol. 12, no. 11, pp. 997 – 1001.

Priest, R.G. 1985. *Psychological disorders in obstetrics and gynaecology*. London: Butterworths.

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.

Raber, W., Nowotny, P., Vytiska-Binstorfer, E.V. and Vierhapper, H. 2003. Thyroxine treatment modified in infertile women according to thyroxine-releasing hormone testing: 5 year follow-up of 283 women referred after exclusion of absolute causes of infertility. *Human Reproduction*, vol. 18, no. 4, pp. 707 – 714.

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.

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.

Schebendach, J.E. and Reichert-Anderson, P. 2004. Nutrition in Eating disorders. In *Krause's Food, Nutrition & Diet Therapy*. Ed. by L.K. Mahan & S. Escott-Stump. 11th ed. Philadelphia: W.B. Saunders Company. pp. 594 – 615.

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.

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

Shahid, S.K. and Shneider, H. 2000. Effects of exercise on insulin resistance syndrome. *Coronary Artery Disease,* vol. 11, no. 2, pp. 103 – 109.

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.

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

Slabber, M., Barnard, H.C., Kuyl, J.M., Dannhauser, A. and Schall, R. 1994. Effects of a low-insulin-response, energy-restricted diet on weight loss and plasma insulin concentrations in hyperinsulinaemic obese females. *American Journal of Clinical Nutrition,* vol. 60, no. 1, pp. 48 – 53.

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.

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.

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

Tchernof, A., Nolan, A., Sites, C.K., Ades, P.A. and Poehlman, E.T. 2002. *Circulation*, vol. 105, no. 5, pp. 564 – 569.

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

Visser, M., Bouter, L.M., McQuillan, G.M., Wener, M.H. and Harris, T.B. 1999. *JAMA*, vol. 282, no. 22, pp. 2131 – 2135.

Von Wolff, M., Ursel, S., Hahn, U., Steldinger, R. and Strowitzki, T. 2003. Glucose transporter proteins (GLUT) in human endometrium: expression, regulation, and function throughout the menstrual cycle and in early pregnancy. *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 8, pp. 3885 – 3892.

Wass, P., Waldenstrom, U., Rossner, S. and Hellberg, D. 1997. An android body fat distribution in females impairs the pregnancy rates of in-vitro fertilization-embryo transfer. *Human Reproduction*, vol. 12, no. 9, pp. 2057 – 2060.

Whitney, E.N., Cataldo, C.B., DeBruyne, L.K. and Rolfes, S.R. 2002. *Nutrition for health and health care*. 2nd ed. Australia: Wadsworth Thomson Learning.

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

Willet, G.D. 1996. *Laboratory testing in Ob/Gyn*. Oxford: Blackwell Scientific Publications.

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.

Zhu, S., Wang, Z., Shen, W., Heymsfield, S.B. and Heshka, S. 2003. Percentage body fat ranges associated with metabolic syndrome risk: results based on the third National Health and Nutrition Examination Survey (1988 – 1994). *American Journal of Clinical Nutrition*, vol. 78, no.2, pp. 228 – 235.

APPENDIX A

TOELATINGSKRITERIA

Reproduktiewesorgeenheid

1. Die RSE funksioneer as ‘n tersiêre ondersoek- & behandelingseenheid vir pasiënte met die volgende probleme:
   - Voortplantingsdisfunksie
   - Herhaalde swangerskapsverliese
   - Hormonale defekte
   - Seksuele disfunksie
   - Prementruele sindroom

2. Hospitaalpasiënte sowel as privaatpasiënte word in die RSE hanteer.

3. Geen pasiënt word sonder ‘n amptelike verwysing van ‘n sekondêre hospitaal of ‘n privaatgeseesheer in die RSE gesien nie.

4. Alle pasiënte wat tot die RSE vir ondersoeke en behandeling toegelaat word, moet daartoe bereid wees om hulself aan die kriteria, soos vooraf aan hulle voorgehou, te onderwerp.

5. Slegs pasiënte wat psigies en sosiaal stabile is en wie se algemene geneeskundige toestand van so ‘n aard is, dat swangerskap of die gevolge daarvan, geen beduidende risiko inhou nie, word tot die geassisteerde reproduksieprogram (ART) toegelaat.

6. Pasiënt moet daartoe bereid wees dat die roetine evaluasies & spesiale ondersoeke, soos deur die RSE voorgedkryf, op hulle uitgevoer word.

7. Pasiënte moet in staat wees om die behandelingsprogram nougeset te volg.

8. Hospitaalpasiënte van buite die Vrystaat-Provinsie moet via die voorgeskrewe kanale na die RSE te Universitas Hospitaal verwys word, voordat enige ondersoek of behandeling op hulle uitgevoer sal word.

APPENDIX B

UNIVERSITY OF THE FREE STATE
DEPARTMENT OF HUMAN NUTRITION

GUIDELINES FOR SLIMMERS

HINTS FOR THE PREPARATION OF FOOD

1. Methods for the preparation of food, instead of frying (in oil or fat)
    - ✓ Baking
    - ✓ Oven roasting
    - ✓ Oven broiling
    - ✓ Grilling over coals
    - ✓ Steaming
    - ✓ Stewing
    - ✓ Poaching
    - ✓ Cooking
    - ✓ In microwave oven

2. Use small quantities of cookspray instead of oil or fat in pans. Non-stick pans are also useful.
3. Cut off all visible fat and remove the skin of poultry before it is cooked.
4. Place meat or chicken on a grill if roasted or grilled in the oven.
5. Try to prepare stews, casseroles, soups and sauces in advance so that the dishes can be cooled in the fridge. The solidified fat on the surface can then be scooped off before heating the food.
6. Oil-fried fish must be drained before eating.
7. Eat salads without a salad-dressing or mayonnaise, try low-fat yoghurt.
8. The salt intake can be reduced by using herbs and spices to season food.
9. Try to eat raw vegetables on a regular basis.
10. Always use fresh vegetables and fruit and try to keep them cooled in the fridge until it is used.
11. Limit the addition of sugar, fat/margarine/butter to vegetables.

HINTS FOR FOLLOWING A SLIMMING DIET

1. Eat slowly – to make small amounts more filling:

- Put down the cutlery after each mouthful
- Chew food properly and swallow everything before the next portion of food is taken onto the fork or spoon
- Learn to relax at mealtimes

2. ***Avoid external stimuli when eating***

- Keep serving dishes off the table
- Keep food only in the kitchen, out of sight and as far as possible out of reach
- Store food in non-transparent containers
- Go to the kitchen only at mealtimes
- Eat only at predetermined eating places
- Avoid other activities while eating – remove the television, magazines, newspapers, telephone, etc. from places where eating takes place

3. ***Get involved in alternative activities when you have the urge to eat***

- Start new hobbies
- Get involved in extramural activities
- Do things you enjoy, e.g. phone a friend

4. ***General hints***

- Do not skip meals
- Start meals with a salad

SLIMMING HINTS

1. ***How to eat less***

- Get involved in other activities
- Drink a glass of water or diet cool drink before a meal
- If you feel you have eaten enough, stop eating immediately and keep leftovers for later

2. ***Restricting the excessive intake of sugar***

- Drink diet cool drink instead of sweetened cool drink or fruit juices
- Use artificial sweeteners instead of sugar

3. ***Reducing fat in the diet***

- Use low-fat margarine, low-fat salad dressing or low-fat mayonnaise
- Avoid full-cream milk and full-cream cheese
- Use Spray and Cook or non-stick pans for cooking
- Cut off all visible fat before food preparation, and remove the skin of poultry before cooking
- Use low-fat condensed milk instead of full-cream condensed milk

4. Increase the daily intake of fibre

- Whole-wheat or brown bread instead of white bread
- Porridge rich in fibre such as oats, all bran flakes or wheetbix instead of cornflakes
- Fresh fruit rather than fruit juice
- Fresh, raw vegetables
- Bran added to porridge or sauces

5. Increase daily activity

- Develop a daily exercise programme
- Learn to participate in physical activities such as cycling, tennis, etc.
- Recommended activity: Three times per week for 30 minutes

APPENDIX C

UNIVERSITY OF THE FREE STATE
DEPARTMENT OF HUMAN NUTRITION

EATING FOR GOOD HEALTH

There are no good or bad foods. You do not need to include expensive foods in your diet. Everyone need not eat different foods from the family. Rather, show them how to improve their eating habits.

Try to follow these ideas:

1. **Enjoy a variety of foods**

- Try to eat as many different types of food as you can everyday.
- There are no good or bad foods so you can eat foods which you can afford and which are easy to get.
- Aim to eat at least 3 meals a day.

2. **Make starchy foods the basis of most meals**

- Starchy foods like maize meal, bread, rice, samp, potatoes, porridge, pasta and breakfast cereal are important foods in a healthy, balanced diet.
- These foods should be the central part of each meal.

3. **Eat plenty of fruits and vegetables everyday**

- Vegetables and fruits should be an important part of your daily diet.
- They contain many nutrients that our bodies need.
- You must eat them regularly, if possible at each meal.
- Aim to eat a total of 5 servings a day.

4. **Eat fats sparingly**

- You need some fat for good health.
- But eating too much fat will cause you to become overweight and will increase. your risk of hypertension, heart disease and stroke.
- For good health you should eat as little fat as possible.

5. **Use salt sparingly**

- Foods high in salt and fat can affect blood pressure and increase the risk of stroke and heart disease.
- It is sensible to choose foods low in salt.

6. **Drink lots of clean safe water**

- Drink clean water everyday.
- Water helps your kidneys get rid of the waste products that build up in your body.

7. Eat dry beans, peas, lentils and soya products regularly

- These foods are called legumes.
- You can use them instead of meat to make a balanced meal that costs less.
- Try to eat them at least 3 times a week.
- They are rich in fibre and help to prevent constipation.

8. Chicken, fish, meat, eggs or milk can be eaten everyday

- You can eat small portions of these foods everyday.
- They help build strong muscles.
- Milk products help build strong bones and teeth.

9. If you drink alcohol, drink sensibly

- Limit your alcohol intake to 1 – 2 standard drinks per day.
- A standard drink is a small glass of beer, a tot of spirits or half a glass of wine.

10. Be active

- Good health is linked to activity and nutrition.
- The more active you are, the better your health.
- Increased activity will improve your blood sugar level, your blood pressure will come down and it will be easy to maintain a healthy weight.

APPENDIX D

UNIVERSITY OF THE FREE STATE
DEPARTMENT OF HUMAN NUTRITION

ANTHROPOMETRICAL MEASUREMENTS AND BIOCHEMICAL PARAMETERS IN INFERTILE BLACK WOMEN AT THE UNIT FOR REPRODUCTIVE CARE IN UNIVERSITAS HOSPITAL, BLOEMFONTEIN

1. Respondent number: _____ 1☐☐☐3

2. Date: _____/_____/_____ 4 ☐☐☐☐☐☐☐☐11

3. Name & Surname:_____

4. Date of birth:____/____/____ 12☐☐☐☐☐☐☐☐19

5. Age: _____ 20☐☐ 21

6. Medical diagnosis:

Tubal factor (Y/N)	☐ 22
Endometriosis(Y/N)	☐ 23
Male factor (Y/N)	☐ 24
Anovulation (Y/N)	☐ 25
Unexplained infertility (Y/N)	☐ 26

7. Anthropometrical measurements:

7.1. Weight: _____.___kg 27 ☐☐☐☐☐31

7.2. Height: _____cm 32 ☐☐☐☐☐36
_____cm
_____cm

7.3. Body mass index: _____kg/m² 37 ☐☐☐☐☐ 41

7.4. Waist circumference: _____cm 42☐☐☐☐☐46
_____cm
_____cm

7.5. Hip circumference: _____cm 47☐☐☐☐☐51
_____cm
_____cm

7.6. Neck circumference: _____ 52☐☐☐☐☐56
_____cm
_____cm

7.7. Fat %: _____.___% 57☐☐☐☐☐61
_____.___%

APPENDIX E

UNIVERSITY OF THE ORANGE FREE STATE
DEPARTMENT OF HUMAN NUTRITION

CONSENT LETTER AND FORM

Dear Madam

The Department of Human Nutrition is conducting research to determine the characteristics of infertile black women attending the Unit for Reproductive Care at Universitas hospital in Bloemfontein. This study will help to improve the treatment of black women who are infertile or have other reproductive problems.

You are therefore being asked to partake in this study where blood samples will be drawn and body measurements will be taken. The information obtained from you will remain confidential. If you feel you do not want to participate in the study, please feel free to say so.

Thank you for your co-operation.

Yours sincerely

Ntsoaki Lucia Motseki

I …… willingly agree to participate in the study and give permission for my results to be used in the study confidentially.

Signature ……

Date ……/……/……

YUNIVESITHI YA FOREISITATA
LEFAPHA LA PHEPO E NTLE

LENGOLO LE FOROMO YA TUMELLO

Motswadi

Lefapha la Phepo e Ntle le etsa dipatlisiso tse tla bontshang hore basadi ba batsho ba sa kgoneng ho ba le bana ke ba jwang, ho bo mme ba tsamayang Unit for Reproductive Care, sepetleleng sa Universitas mona Bloemfontein. Dipatlisiso tsena di tla thusa ho phahamisa maemo a phekolo ho bo mme ba batsho ba sa kgoneng ho pepa kapa ba nang le mathata a mang a popelo.

Ka hoo re kopa ha o ka nka karolo dipatlisisong tsena moo teng re tla nkang madi hape re methe botenya kapa bosesane ba mmele wa hao. Ditaba tse tla nkuwa ho wena ha di ka ke tsa phatlalatswa, mme di tla dula e le lekunutu. Ha ka nako enngwe le enngwe o utlwa hore ha o batle ho nka karolo ho dipatlisiso tsena, o ka tlohella ho etsa jwalo.

Re lebohela tshebedisano mmoho ya hao.

Wa lona

Ntsoaki Lucia Motseki

Nna ..... ke dumela ho nka karolo dipatlisisong tsena ntle le ho qobellwa, mme ke fana ka tumello ya hore diphetho tsa ka di sebediswe lekunutung.

APPENDIX F

University of the Free State
P.O. Box 339
Internal Box G24
Bloemfontein
9300
05 February 2003

Dear Sir or Madam

The Department of Human Nutrition is carrying out a study to determine the anthropometric and biochemical characteristics in black women attending the Unit for Reproductive Care at Universitas, Bloemfontein.

The women have to have a number of blood tests carried out on them to determine the levels of certain hormones one of them being progesterone. However, the progesterone levels need to be determined on the 21st day of the menstrual cycle.

We are therefore asking that you help us in obtaining a sample of 10ml of blood 21 days after the last menstrual period and sending it by courier to the Department of Chemical Pathology at Universitas Hospital in Bloemfontein.

Your cooperation will be very much appreciated.

Thanking you in advance

Ntsoaki Lucia Motseki (Miss)