A STUDY OF DIFFERENT CLINICAL AND BIOCHEMICAL PARAMETERS IN POLYCYSTIC OVARY SYNDROME AFFECTING OVULATION INDUCTION OUTCOME AND FERTILITY POTENTIAL

Dr TI Siebert

Promotor: Prof TF Kruger

December 2008

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2008

This work is dedicated to my beloved son Enrico

INDEX

Acknowledgements

I wish to express my appreciation and gratitude to:

The Cape Provincial Administration and **Tygerberg Academic Hospital** for their financial assistance and the use of their facilities.

Professor TF Kruger, Chairman: Department of Obstetrics and Gynaecology, Stellenbosch University and Tygerberg Academic Hospital, for his interest in the work, and for the opportunity of further study.
As my promotor, for his guidance, enthusiasm and unfailing confidence in my abilities.

Dr CJ Lombard and the **Institute of Biostatistics, Medical Research Council**, for statistical analysis of results.

The **fellows in Reproductive Medicine**, current and past, and the **registrars** working at the endocrinology clinic for their help with the study.

The personnel and colleagues of the Institute of Reproductive Medicine at Vincent Pallotti and the Andrology Laboratories (Tygerberg Academic Hospital), for their assistance and friendliness.

Mrs Madaleine Schultz-du Toit for assistance in the preparation of the manuscript.

My father, Johnny Siebert for the constant support and love that he gave me.

My family and friends, especially Mirna my wife, Ezann my daughter and Ruan and Enrico my sons, for their love and understanding, and for their patience and constant inspiration.

i

PROTOCOL

TITLE

A study of different clinical and biochemical parameters in polycystic ovary syndrome (PCOS) affecting ovulation induction outcome and fertility potential.

LITERATURE REVIEW

The polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. In 1935 Steyn and Leventhal[1] described the association of amenorrhoea, obesity, infertility, hirsutism and bilateral enlarged ovaries. Till today the diagnostic tools in use remain topical and controversial. There are two definite schools of thought regarding the diagnosis of PCOS. In the UK the classical ultrasound features[2] are the cornerstone of the diagnosis which includes the clinical and biochemical presentation. On the contrary, in the USA, PCOS is diagnosed on the clinical and biochemical evidence with the exclusion of CAH, hyperprolactinaemia and hypothyroidism.[3]

Fortunately in 2003 the Rotterdam consensus statement[4] was made to give clinicians guidance in the diagnosis of PCOS. This statement concluded that the diagnosis of PCOS could be made if two of the following features are present: chronic anovulation; polycystic ovaries on ultrasound; hyperandrogenism and exclusion of other endocrinopathies.

Familial clustering of cases suggests that genetic factors play an important role in the diagnosis of PCOS. Using a candidate gene approach, Franks et al[5] found evidence for the involvement of two key genes in the aetiology of PCOS. They suggest that the steroid synthesis gene CYP 11a and the insulin VNTR regulatory polymorphism are important factors in the genetic case of PCOS. It is, however, unlikely that these two are the only genes involved in the aetiology of PCOS.

On the basis of the theory that hyperinsulinaemia negatively effects ovulation and that it is an important role-player in the pathophysiology of PCOS, it is postulated and has been proven that insulin sensitisers may improve the endocrine imbalances and result in normal menses, ovulation and normalisation of hyperandrogenism.[3] It is also known that obesity on its own, and in association with hyperinsulinaemia, is associated with relative gonadotropin resistance.[6] By using a simple formula we can isolate the hyperinsulinaemia/insulin resistant patient and commence with a combination of weight loss and insulin sensitisers. *At this stage the HOMA (homeostasis model assessment) has been proven to be of great success in identifying the scenario.*[7]

$$HOMA = \frac{\text{fasting insulin x fasting glucose}}{22,5}$$

The value of more than 2,5 is generally accepted as insulin resistant, the same is true for a fasting insulin level of more than 17 IU/ml.

The HOMA is not the only method to use for the diagnosis of insulin resistance(IR). A more scientific method is the euglycemic clamp test. This test is unfortunately very expensive and time consuming. This is one of the main reasons why the HOMA remains the most frequently used diagnostic test for Insulin resistance in PCOS patients in the gynaecological clinic. Very recently an article published concluded that the HOMA is not very sensitive to diagnose IR in lean type 2 diabetic patients.[8] Other tests also available as markers of IR is fasting insulin/glucose levels[9] and hypertrigliseridemia.[10] For the reasons mentioned, we will use the HOMA in combination with fasting insulin levels to diagnose IR.

Numerous articles have been published regarding the optimal protocol for ovulation induction in the PCOS patient. Obesity is defined as a BMI of greater then 30kg/m2 and is found in 30 – 50% of women with PCOS.[11] As mentioned, obesity on its own is associated with ovulation resistance. Even a minor weight loss of 5% often result in normalisation of cycles and ovulation.[12]

Clomiphene citrate (CC), an anti-oestrogen, is the drug most regularly used for ovulation induction. The primary site of action is the hypothalamus where it binds to estrogen receptors and blocks the negative feedback effect of circulating estrogens and ultimately results in an increase in gonadotrophin releasing hormone secretion.[13] As previously mentioned, insulin sensitisers is more frequently apply to induce ovulation induction in the PCO patient.

In financially restricted clinics ovarian drilling remain an effective alternative in CC-resistant anovulatory women with PCOS.[14] On the other hand, in private non-financial restricted clinics, the debate regarding the optimal ovulation inducting protocol is far from settled. In an article published,[15] the author concluded that a low dose of purified FSH is a very effective mode of induction, whether if it is the best, remains to be confirmed. They also found a minimal incidence of hyperstimulation with FSH. We are still awaiting results of good randomised trials of recombinant FSH. In a Cochrane Review, ovarian drilling for OI was critically assessed. The conclusion was that ovarian drilling was not better, but also not less effective than gonadotropin therapy as a secondary treatment for CC-resistant women. In a recent article[14] an insulated needle was used for the ovarian drilling. They concluded that ovarian drilling is an effective alternative treatment in CC-resistant women and that an insulated needle is associated with a minimal amount of adhesion formation.

Very recently aromatase inhibitors proved to be very successful to achieve ovulation induction.[16] Aromatase is a sytochrome P450 hemoprotein-containing complex that catalyses the rate limiting steps in the production of estrogens, that is, the conversion of androstenedione and testosterone to estrone + estradiol.[17] The hypothesis of ovulation induction with aromatase inhibitors is based on the fact that these drugs may act locally in the ovary to increase follicular sensitivity to FSH.[18] Ovulation induction can also be achieved by releasing the hypothalamus or pituitary from estrogen negative feedback on GnRH and gonadotropin secretion, resulting in an increase gonadotropin production which could stimulate ovarian follicular development.[18]

When and if the PCOS individual falls pregnant, the belief is that the LH hypersecretor is associated with an increase of miscarriages. In a recent article this finding was challenged. The author concluded that LH hypersecretion was not associated with an increased miscarriage rate.[19] Whether LH hypersecretion is associated with poorer OI response remains controversial.

It is well known that PCOS has long-term metabolic effects. To screen for insulin resistance may identify these patients. In a very recent article published in Diabetes Care, they concluded that a combination of fasting insulin and triglyserides is a very simple and accurate method to screen for insulin resistance.[20]

AIMS OF THE STUDY

Chapter 1 presents a literature study on the diagnostic debate of PCOS. The literature study includes a discussion of the recent Rotterdam consensus statement regarding the diagnosis of PCOS. This is followed by a discussion on the essential work-up of the patient presenting with PCOS. Finally, chapter 1 presents a discussion on the complexity of the different variations in women presenting with PCOS.

Chapter 2 is a literature review on ovulation induction methods in patients who present with PCOS. This literature study puts special emphasis on the different available methods used for ovulation induction in women with PCOS and the profounding effect weight loss will have in managing these patients. This chapter also addresses the use of newer agents, like aromatase inhibitors (Letrozole), and the current role of each of these agents in ovulation induction protocols.

Chapter 3 is a literature overview on the effect of Metformin in Clomiphene-resistant PCOS women. The inclusion criteria of this review was all prospective randomized trials where Metformin was added for ovulation in the Clomiphene-resistant PCOS patient. The data is presented as a meta-analysis.

Chapter 4 is a prospective randomise control trial to evaluate the benefit of metformin if added to Clomiphene in a primary ovulation induction protocol in comparison to Clomiphene alone. This chapter also evaluates all factors influencing ovulation outcome. Finally in the discussion section all the recent studies published addressing this topic were reviewed.

Chapter 5 is a *literature review* to evaluate the classification systems for semen parameters and the in vivo fertility potential. This data is also used to establish fertility/subfertility thresholds for semen parameters.
This chapter also presents the results of a *prospective* and *retrospective study* of the semen analysis of the partners of women with PCOS. We believe that this population presents the best reference group to study the semen profile of the general male population.

Chapter 6 is a summary of the results of these studies and serves as an evidence based approach for ovulation induction in women with PCOS.

MATERIALS AND METHODS

1. Literature review

A literature review, using MEDLINE, will be performed to assess the biochemical and clinical profile of the patient presenting with PCOS. The long-term complications of the syndrome will be discussed to highlight the utmost importance of lifestyle changes as the primary step in the management of the patient with PCOS. This review will also include all different options of ovulation induction regimens available in patients with PCOS, who desire a pregnancy.

A structured literature review will also be performed to assess the efficacy of metformin in the CC-resistant patient. In this review we will only use prospective randomised trials available and aim to present the data in the form of a meta-analysis. We will also focus on other management options for the CC-resistant patient.

2. The study

This is a prospective study at the clinics mentioned. All patients will be diagnosed as having PCOS according to the Rotterdam statement. The patients diagnosed with PCOS will be motivated to loose at least 5% of their body weight. Patients will be encouraged to follow a fat free diet and motivated to participate in exercises for at least 40 minutes per day for 3 days per week. Base line bloods will consists of FSH, LH, fasting insulin and glucose, lipid profile, TSH, prolactin, 17OH Progesterone, DHEAS, SHBG and testosterone (four tubes). A gynaecological ultrasound will also be performed at presentation. All patients diagnosed with PCOS will be motivated to loose at least 5% of their body weight. The BMI of all these patients will be calculated and monitored at the follow-up visits.

3. Inclusion criteria

All patients diagnosed with PCOS will be included in the study. If they have not lost weight, they will also be included.

4. Exclusion criteria

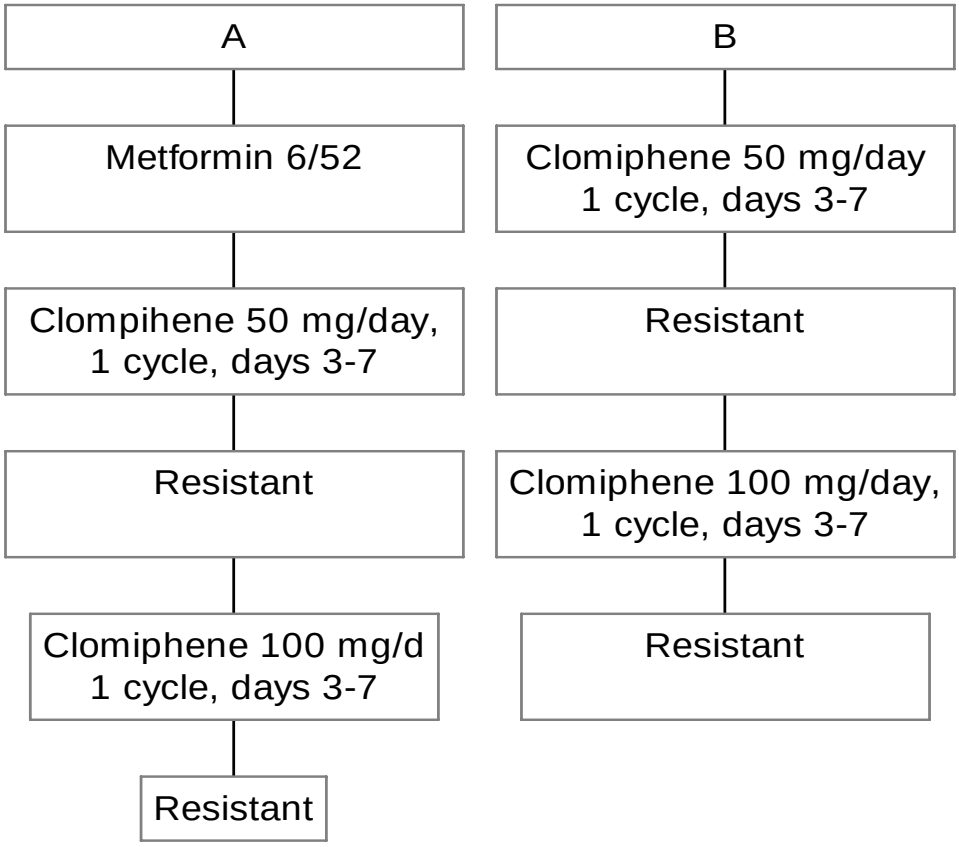
A patient presenting with any other reason of anovulation or hirsutism.

The partners of all the patients diagnosed with PCOS will be asked to give a semen sample. All semen samples will be investigated according to the Tygerberg Strict Criteria. If the morphology is in the P-pattern (poor pattern) group, all slides will be evaluated by one observer, TFK.

The available data will give a profile of the semen analysis of the partners of PCOS-patients. This profile of the semen analysis of the partners of the PCOS patient will provide a possible prediction of the semen profile of the general male population.

Patients diagnosed with PCOS and motivated to loose 5% of their body weight will be randomised on different ovulation management protocols as outlined in the following algorithm.

OVULATION INDUCTION PROTOCOLS



If leg A is selected, the patient will receive metformin 850 mg b.d. for 6/52. Ovulation will be monitored with ultrasound of follicles and confirmed with day 21 progesterone. If the patient did not ovulate on metformin alone, clomiphene citrate 50mg/day, days 3-7 will be added. Ovulation will be monitored as above. If still anovulatory, clomiphene citrate will be increased to a maximum of 150 mg/d, days 3-7. If leg B is selected, clomiphene citrate 50mg/day day 3-7 will be used, and ovulation monitored as mentioned. If still anovulatory clomiphene citrate will be increased to a maximum of 150 mg/d, days 3-7.

Regression analysis of the available data will be conducted to establish the biochemical and clinical profile of the patient resistant to clomiphene and metformin. By using the regression analysis, we will attempt to identify which of these factors influence ovulation outcome.

With the available data of the semen profiles of the partners of these patients, we will also attempt to use this database as a possible reflection of the semen analysis of the normal population.

STATISTICS

A power calculation was performed to assess the number of patients needed to do the regression analysis. The statistician, Dr C Lombaard did a two group test to calculate the numbers to randomise. A two group test with a 0.050 one-sided significance level will have a 90% power to detect the difference between a Group1 proportion of 0,500 and a Group 2 proportion of 0,800 (odds ratio of 4,00) when the sample size in each group is 42.

SETTING

1. Tygerberg Fertility Clinic.
2. Reproductive Institute at Vincent Pallotti.

ETHICAL APPROVAL

Was obtained: 2003/013.

REFERENCES

1. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935;29:181-191.

2. JA Eden, Polycystic ovary syndrome: diagnostic and clinical aspects. Gynaecology Forum 1997:2.

3. Lena H Kim, Anne E Taylor, Robert L Barbieri. Insulin sensitizers and polycystic ovary syndrome: can a diabetes medication treat infertility? Fertil Steril 2000;73:1097-1098.

4. The Rotterdam ESHRE/ASRM sponsored PCOS consensus group. Revised 2003 consensus on diagnostic criteria and long-term health risk related to polycystic ovary syndrome (PCOS). Human Reproduction 2004;19(1):41-47.

5. Stephen Franks. The genetic basis of polycystic ovary syndrome. Hum Reprod 1997;12:2641-2648.

6. Peter Fedorcsak, Per Olav Dale, Ritsa Storeng, Tom Tanbo, Thomas Abyholm. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovary syndrome. Hum Reprod 2001;16(6):1086-1091.

7. Steven M Haffner, Clicerio Gonzalez, Heikki Miettinen, Esmarie Kennedy, Michael P Stern. A Prospective Analysis of the Homa Model. Diabetes Care 1996;19:1138-1141.

8. Eun Seok Kang et al. Limitation of the validity of the homeostasis model assessment as an index of insulin resistance in Korea. Metabolism 2005;54:206-211

9. Carmina E, Lobo RA. Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. Fertility and Sterility 2005;83:521-2

10. Sang Ah Chang et al. Body mass index is the most important determining factor for the degree of insulin resistance in non-obese type 2 diabetic patients in Korea. Metabolism 2004;53:142-146

11. Franks S. Polycystic ovarian syndrome: a changing perspective. Clin Endocrinol 1989;31:87-120.

12. Huber-Buchholz MM, Carey DGP, Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab 1999;84:1470-1474.

13. Emrr Seli, Antony J Duleba. Optimizing ovulation induction in women with polycystic ovary syndrome. Obstet Gynaecol 2002;14(3):245-254.

14. Afaf Felemban, Seang Lin Tan, Togas Tulandi. Laparoscopic treatment of polycystic ovaries with insulated needle cautery: a reappraisal. Fertil Steril 2000;73:266-269.

15. Roberto Marci, Alfred Senn, Salvatore Dessole, Alain Chanson, Ernest Loumaye, Pierre De Grandi, Marc Germond. A low-dose stimulation protocol using highly purified follicle-stimulating hormone can lead to high pregnancy rates in in vitro fertilization patients with polycystic ovaries who are at risk of a high ovarian response to gonadotropins. Fertil Steril 2001;75:1131-1137.

16. Mitwally MFM, Casper RT. The use of an aromatase inhibitor for induction of ovulation in cases of clomiphene citrate failure (abstract). In: Program and abstract of the 16th Annual Meeting of the European Society for Human Reproduction and Embryology (ESHRE); Bologna, Italy, June 2000;15:71.

17. Cole PA, Robinson CH. Mechanism and inhibition of cytochrome P-450 aromatase. J Med Chem 1990;33:2933-2944.

18. Mohamed F Mitwally AB, Roberts F, Casper A. Aromatase inhibition for ovarian stimulation: future avenues for infertility management. Current Opinion in Obstet Gynaecol 2002;14:255-263.

19. Raj Rai, May Backos, Grances Rushworth, Lesley Regan. Polycystic ovaries and recurrent miscarriage – a reappraisal. Hum Reprod 2000;15:612-615.

20. McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, Duncan AW. Diagnosing insulin resistance in the general population. Diabetes Care 2001;24:460-464.

CHAPTER 1

DIAGNOSTIC FEATURES OF POLYCYSTIC OVARY SYNDROME

1.1 INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women.[1] It was first described by Stein and Leventhal in 1935[2], by the association of infertility, obesity, hirsutism and bilateral enlarged polycystic ovaries. As a syndrome, PCOS has consequently over the years followed an interesting history, with much debate and often poor consensus regarding its diagnostic criteria. A variety of histological, biochemical and sonographic features have been described, but until recently no general agreement on definition has been reached.

1.2 DEFINITION: THE DIAGNOSTIC DEBATE

The National Institute of Health (NIH) in Bethesda, USA. held its first international consensus conference on PCOS in April 1990 – which ironically made obvious that there was no true consensus.[3] Nonetheless, a clinical and working definition emerged from the United States following the NIH conference. This suggested that diagnosis of PCOS consisted of chronic anovulation with biochemical evidence of hyperandrogenism and the exclusion of other causes, such as hyperprolactinaemia and non-classical congenital adrenal hyperplasia (NCAH).[3,4] Ovarian morphology on sonar was not regarded as part of the criteria. In other words, diagnosis is made on clinical and biochemical criteria alone.

On the other hand, the predominantly European working definition of PCOS[5] comprises sonographically diagnosed polycystic ovary morphology – usually using the ultrasound criteria associated with oligomenorrhoea or amenorrhoea and/or signs of hyperandrogenaemia.[6]

1.3 ROTTERDAM ESHRE/ASRM-SPONSORED PCOS CONSENSUS WORKSHOP

May 2003 brought the Rotterdam consensus workshop on polycystic ovary syndrome, sponsored by European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). This workshop was attended prominently by well-published authors from both sides of the Atlantic. A ‘consensus statement’[7] was released on the revised 2003 diagnostic criteria, and proves to be detailed and inclusive. The report was based on clinical evidence rather than majority opinion.

In essence, there are three major criteria, with two out of three required for diagnosis:

- Oligo- or anovulation;
- Clinical and/or biochemical signs of hyperandrogenism (with the exclusion of congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumours, thyroid abnormalities and hyperprolactinemia);
- Polycystic ovaries on ultrasound.

The report further acknowledged the problems with this criteria with regards to trial protocol and data. For example, where pregnancy is the trial outcome, of course the inclusion criteria of anovulation is clearly of significance. However, where clinical improvement of hirsutism is the outcome, less emphasis need be placed on ovulatory function.

The statement entails a detailed discussion on the terms 'hyperandrogenism' – both clinically and biochemically, with specific reference made to the limitations of laboratory measurement of circulating androgens and comment that such evidence is not required as proof of clinical hyperandrogenism.

Whether this carefully researched and constructed document will be used as a general reference in its scientific research field, will only become known in hindsight. Interestingly, Adam Balen from the United Kingdom, who presented at the consensus workshop the report on the revised definitions of ultrasound assessment, co-authored an article on the clinical overview on PCOS[8]. In this paper, he defined PCOS as a sonographic finding of PCOS plus either oligo- or amenorrhoea, obesity or hyperandrogenism.

Another prominent figure on the scientific committee of the workshop, Ricardo Azziz of the US, also published a prevalence study in June 2004.[9] In this study he used the NIH inclusion criteria for his definition. Both these examples emphasises the problems with adopting a new definition in a scientific field.

1.4 ORIGINS AND POTENTIAL GENETIC DETERMINANTS

The first signs of PCOS may be an early adrenarche with an early appearance of pubic hair.[10] It is increasingly being recognised that oligomenorrhea in adolescence may be one of the first manifestations of PCOS.[11-13] Although PCOS is not diagnosed until two to three years after menarche, it is believed its origins lie in childhood or fetal life, since excess androgen exposure to animals *in utero* produces PCOS-like features.[14-17] The severity of hyperinsulinaemia manifest in

adulthood in over 50% of even normal weight women with PCOS is influenced by both genetic and environmental factors, particularly obesity.[18] Consequently, although a woman may have the predisposition to PCOS, whether genetic or environmental, it is the development of insulin resistance due to the deposition of adipose tissue that leads to the manifestation of the phenotype of PCOS. Hence it is then also possible that with weight loss she may loose some of the features of PCOS.[19-23]

There appears to be a genetic basis for PCOS as evidenced by this familial concordance, with 24% of mothers and 32% of sisters being affected.[24] The syndrome appears to have an autosomal dominant mode of inheritance, with premature balding in men as the putative male phenotype.[24] Genetic linkage with insulin resistance and obesity has been reported via the common allelic variation at the VNTR locus in the promoter region of the insulin gene. Anovulatory hyperinsulinaemic women are more likely to have inherited this class III/III allele, particularly from their fathers.[24]

The ovarian androgen production in women with PCOS is accelerated due to the increased ovarian theca cell androgenic enzymatic activity of 3 beta hydroxysteroid dehydrogenase (HSD) 17 alpha hydroxilase/C17,20 lyase, a product of CYP 17.[25] The commonly found associated metabolic derangement of insulin resistance in PCOS is believed to be due to impairment of the ovarian insulin signal transduction augmenting[4] cytochrome P450scc, the rate-limiting step in ovarian steroidgenesis, and cytochrome **P450c17A,** the androgenic enzyme 17 alpha hydroxylase/C17,20 lyase.[25,26]

Genetic abnormalities that produce these altered enzyme activities have been difficult to determine. Possible mutations linked to these alterations are associated with the CYP21 gene[27] and the insulin receptor.[28] Elevated plasminogen activator inhibitor-1 (PAI-1) has been implicated in the increase propensity towards miscarriage and cardiovascular risk factors amongst women with PCOS.[29] The presence of an increase in PAI-1 results in a higher incidence of thrombosis. In this study, it has been suggested that there is a higher level of PAI-1 among women with PCOS, which among other risk factors, will lead to an increase in cardiovascular complications.[29]

It would appear that there are many genetic polymorphisms in women with PCOS and, hence, the influence of an adverse environment (whether antenatal, due to excess androgen exposure during childhood, or in adulthood due to obesity), on the genetic predisposition leads to the appearance of the PCOS phenotype.[30]

1.5 PREVALENCE

The assessment of the prevalence of PCOS is fraught with problems. Data are often difficult to compare from one study to another due to the inconsistency in standardisation of diagnostic criteria, making meta analyses difficult to perform. The inadequacies of the NIH and European systems of classification has become obvious, both in the interpretation of data and also in the diagnosis of PCOS.

We know that the finding of polycystic ovaries (PCO) alone does not necessarily indicate the presence of the syndrome.[31] Prevalence studies for these sonographic ovarian findings place the incidence in the order of 17-22%, figures that seem remarkably constant worldwide.[32-35] Only 7% of the eumenorrhoeic women in Polson's 1988 study of 257 women had polycystic ovaries.[32] In contrast, 86% of women with irregular cycles had PCO. Transvaginal ultrasound places this figure somewhat higher, at 21-28%, and it appears that younger women have a higher incidence of PCO than women over 35 years.[34] Many of the subjects recruited in the Polson study did in fact have clinical problems, although they had not sought medical attention for them, demonstrating the difficulty with performing such studies in a "normal" population group.[32]

A 3-11% prevalence of the syndrome is reported, depending on the criteria used for definition.[34] A recently published USA prevalence study[9], on 347 women seeking a pre-employment medical, found the prevalence of PCOS at 6,6% using modified NIH criteria of oligo-ovulation rather than amenorrhoea. It also emerged that 86% of women presenting with both menstrual dysfunction and hirsutism had PCOS, whereas only 8% with menstrual dysfunction alone (no hirsutism) had PCOS. In this study, prevalence rates between black and white subjects were not significantly different.

A problem with the NIH definition arises in cases where clinically the patient must have the syndrome although she does not comply with the criteria. For example, a woman with polycystic ovaries and hyperandrogenism who is ovulatory would, by NIH criteria, not be diagnosed as PCOS. However, an anovulatory woman with hyperandrogenism but sonographically normal ovaries will benefit from the diagnosis by the European criteria.

1.6 CLINICAL PRESENTATION

As the most common of endocrinopathies and reproductive disorders in women, it is essential that we be aware of PCOS and detect the obvious signs to enable timely diagnosis. It is presented

clinically primarily by menstrual irregularity, androgen excess (hirsutism), acne, androgen-dependent alopecia and infertility.[4]

The first of these clinical features, menstrual irregularity, is subsequent to ovulatory dysfunction. This may be defined by a history of eight or fewer menstrual cycles in a year, or menstrual cycles that are shorter than 26 days or longer than 35. Alternately, it is indicated where cycle length is 26-35 days and a day 22-24 (mid-luteal) progesterone of less than 4ng/ml confirms anovulation.[9]

Over the last decade we have become more aware of the higher prevalence of metabolic problems associated with PCOS, the so-called metabolic syndrome.[36] Women with this syndrome are frequently obese, with increased risk of hyperinsulinemia, impaired glucose tolerance (IGT) and even frank diabetes. An association with hypertension and dyslipidemia is also well described in the literature.[7] The consequent cardiovascular risk implications make clinical detection of polycystic ovary syndrome and further identification of its metabolic sequelae a very relevant health issue. In fact, the ESHRE/ASRM 2003 statement includes a consensus guideline regarding indications for screening for metabolic disorders in PCOS (Table 1).[7] Chronic anovulation also implies unopposed oestrogen and a consequent increased risk of endometrial carcinoma.

Azziz discusses an approach to screening hirsute woman in clinical practice from a cost-effective perspective.[37] In his guideline, he suggests that all hirsute women first be screened for ovulation, even those claiming to be eumenorrhoeic, because in fact 40% of these are oligo-ovulatory. He further recommends that oligo-ovulatory hirsute women be screened via TSH (thyroid stimulating hormone) (for coincidental thyroid dysfunction) and via 17-hydroxyprogesterone (to exclude NCAH). He recommends that routine gonadotrophin testing not be done, since only 50-60% of PCOS subjects have an elevated LH/FSH ratio. This may at best confirm what is suspected, but is often erroneously used to exclude the diagnosis. Screening must be done for diabetes, as 30% of PCOS subjects have IGT and 8% frank type II diabetes. Routine sonogram of the hirsute patient is not considered necessary, although it stands to reason that where there are other suggestive symptoms of PCOS, ultrasound should form part of the diagnostic analysis.[38]

Obesity is an important association with PCOS. We know that response to treatment is reduced with increased BMI. Weight loss itself may be associated with attenuation of symptoms and reduction of circulating androgens and insulin, and even spontaneous ovulation. Weight loss has no effect on gonadotrophin secretion though.[31]

Obese patients may reveal the presence of a cutaneous indicator of hyperinsulinaemia called acanthosis nigricans, an association described in 1980 by Barbieri and Ryan as the "HAIR-AN" syndrome (hyperandrogenism, insulin resistance and acanthosis nigricans).[4]

An interesting study assessing the effectiveness of interviewing as a means of predicting PCOS as a less cost-limiting and time-saving approach was also done.[24] Instead of costly biochemical testing, the questionnaire centred on androgenic symptoms and was given to patients, their mothers and sisters. The questionnaire consisted of the history of possible androgenic symptoms of PCOS and was presented to patients and their first degree female relatives, who were also evaluated by physical and laboratory investigations. The sensitivity, specificity and positive predictive value (PPV) and negative predictive value (NPV) for the detection of PCOS by interview, were calculated. The NPV of the proband interview was significantly lower for sister than for mothers (82% vs 100%, respectively; p-value < 0.5). When the family member completed the written questionnaire directly, the specificity and NPV of self-reporting were equally high (> 90%), for both mothers and sisters. Thus direct interviewing of PCOS patients, or their mothers and sisters, reliably predicts reliable status, but patient interview alone, will not predict PCOS in almost 50% of the affected sisters.

1.7 DIAGNOSIS

1.7.1 Ultrasonography/imaging

The most widely accepted sonographic criteria of PCO for almost 20 years was described in 1985.[6] The PCO was defined as the presence, in one plane, of multiple cysts, 2-18 mm in diameter, distributed evenly around the ovarian periphery, with an increased ovarian stroma. The Adams criteria[6] have been adopted by many subsequent studies following this seminal paper on polycystic ovaries.

Adams had only transabdominal sonar at her disposal in 1985[6]. The advent of transvaginal ultrasound with its greater resolution has today largely superseded the transabdominal approach, although the latter still has a very definite place.[38] The transvaginal approach, with modern high frequency (>6 MHz). probes provide a more accurate view, and especially in obese patients avoids the homogenous appearance of ovaries that may be erroneously found on a transabdominal scan.

A paper[38], first presented at the ESHRE/ASRM workshop in 2003, provides a comprehensive view on the current approach to polycystic ovary imaging. It provides a critical discussion on the

methods available today, and enumerates the criteria for definition in women on oral contraceptives and in the menopause.

The revised sonographic criteria[38] define PCO in the finding of either of the following:

- 12 or more follicles measuring 2-9 mm diameter
- Increased ovarian volume (>10 cm^3)

The presence of a single PCO is sufficient for diagnosis. Distribution of follicles and quantification of ovarian stroma is no longer essential to diagnosis.

The recent and innovative techniques of 3-D ultrasound and magnetic resonance imaging (MRI) may provide even more sensitive means of detection of the PCO. The 3-D sonar is limited by the greater cost, training and data analysis it requires. However, excellent correlation between 2-D and 3-D measurements for ovarian volume and morphology were reported at the ESHRE/ASRM workshop.[38]

MRI as a diagnostic tool provides superb ovarian imaging, and as such would likely increase the detection rates of abnormal ovarian morphology dramatically, but has cost and practicality limitations. However, it has a place in other related areas of study.[39] Transvaginal colour Doppler has demonstrated that polycystic ovaries have an increased ovarian blood flow and blood vessels of greater diameter than normal ovaries, in keeping with the well-described feature of ovarian enlargement.[39] A study using dynamic contrast-enhanced (DCE) MRI has also shown the enhancement behaviour of the ovaries of PCOS women corresponding with these findings[40], which may broaden diagnostic and treatment parameters. DCE-MR imaging as a method has thus far been used primarily in the field of breast cancer research, focusing on the assessment of angiogenesis. Increased concentrations of biochemical factors associated with this process, such as vascular endothelial growth factor (VEGF), have been reported as expressed in human ovaries[40]. Coupled with the finding of increased follicular fluid VEGF levels found in ovarian hyperstimulation syndrome patients (OHSS, the most serious iatrogenic complication of ovulation induction), DCE-MR imaging may in the future be utilised in predicting OHSS.

1.7.2 Biochemical diagnosis

The pathogenesis and pathophysiology of PCOS is still incompletely understood. What we do recognise as inter-related characteristics are insulin resistance (IR), hyperandrogenism and altered gonadotrophin dynamics.[4] This association between PCOS and disordered carbohydrate metabolism

was historically first noted by Achart and Thiers in 1921, as the “diabetes of bearded women”. By 1980, this PCOS association were demonstrated with hyperinsulinaemia.[3,41]

Insulin resistance may be defined as a subnormal biological response to a given level of insulin. Dunaif published a now classic study in 1989 on the association of insulin resistance in PCOS, which indicates that the extent of IR cannot be explained by obesity alone.[3] IR in obese PCOS was greater than in obese normal subjects. Among non-obese women, those with PCOS had higher IR than the controls (Fig.1).[4]

Dunaif subsequently sought to demonstrate a causality of relationship between insulin resistance and hyperandrogenaemia.[3] Ovarian tissue sensitivity to hyperinsulinaemia appears to drive ovarian and adrenal androgen production, stimulating proliferation of the pilosebaceous unit and suppression of sex hormone binding globulin (SBHG), thereby further increasing the bioavailability of free testosterone. The directionality of this relationship is now accepted as probable[42], though not certain.[8]

We are aware that early detection and treatment of IR and its metabolic sequelae is likely to have far-reaching health benefits, but testing does not necessarily identify women who will respond to insulin sensitisers, nor does treatment usually normalise their endocrine picture.[42] The assessment, moreover, of insulin resistance and a clear diagnostic strategy to define its parameters is at present still an area of debate.

The gold standard for testing IR is the euglycaemic insulinaemic clamp test, in which insulin is administered intravenously at a fixed dose while glucose is simultaneously infused at the rate required to maintain the glucose at a predetermined level. It is a method that is expensive, time-consuming and labour-intensive[8]. Therefore, it is inappropriate for an office setting.

Homeostatic measurements of fasting glucose/insulin ratios, such as the homeostatic model assessment (HOMA), and the quantitative insulin sensitivity check (QUICKI), are the most frequently used techniques[8]. These tests are simple and easy to apply. The HOMA index is probably the most commonly utilised formula in our clinical setting, simply calculated by the product of fasting insulin (I_o) and fasting glucose (G_o), divided by constant 22.5. A level above 2,5 generally being accepted as consistent with IR.

$$HOMA = [I_o\,(uIU/ml)\ X\ G_o\,(mmol/L)] / 22.5$$

Both these tests are widely considered to have a good correlation with the clamp technique, and may be used in normo- and hyperglycaemic patients.[8] A recent study in Greece[43] specifically on PCOS women failed to demonstrate this correlation. They concluded that metabolic or hormonal factors particular to PCOS might have influenced this lack of correlation between their findings and those of other insulin resistant groups. Putative markers of insulin resistance[42] that are current areas of research are homocysteine,[44] plasminogen activator inhibitor-1, adiponectin, endothelin-1, SHBG and insulin-like growth factor binding protein-1 (IGF-1).[44,11] The value of obtaining relatively non-invasive, sensitive and specific serological markers for insulin resistance holds much appeal. This area of research is consequently one of much current interest.

1.7.3 Endocrine diagnosis

The endocrine hallmarks of polycystic ovary syndrome are hyperandrogenaemia and, to a lesser extent, elevated secretion of the gonadotrophin, luteinising hormone (LH).[35] Both obese and lean women have an increased, 24-hour, mean concentration of LH, with an increased pulse frequency and amplitude.[4,45] This may suggest the presence of a hypothalamic defect in PCOS[3], but it is more widely accepted that these abnormalities of gonadotrophin release are in fact secondary to ovarian pathology and chronic anovulation, with the polycystic ovary itself central to the pathogenesis of the syndrome.[31]

Androgen production by the ovarian theca cells is LH-dependent. It would thus appear that the excess androgen production is subsequent to elevated LH levels, supported by the finding that suppression of LH by gonadotrophin releasing hormone analogues or the oral contraceptive suppresses androgen levels.[4]

Follicle stimulating hormone (FSH) concentrations are usually in the midfollicular range of eumenorrhoeic women, but lower than those in the early follicular phase.[4,35] Whether this relative insufficiency plays a more direct causative role in anovulation is contentious as it has been postulated that threshold levels for the initiation of ovulation may be inadequate. The finding that most women with PCOS respond to clomiphene citrate, which itself works by stimulating pituitary release of FSH, provides supporting evidence for this hypothesis.[4]

A characteristic finding is the increase of LH relative to FSH. Some 50-60% of subjects have an elevated LH/FSH ratio, with a ratio[37] greater than 2:1 being commonly accepted as consistent with PCOS[35]. Because of the pulsatile nature of gonadotrophin release, however, a single blood assay may fail to detect this.[3] Assessment of serum concentrations of gonadotrophins, and LH in

particular, is limited by data that reflect divergent results with different assay kits on the same serum sample. Assay-related reference ranges may largely attenuate this problem[44], which appears to be improving from what was experienced a decade ago.

Serum levels of testosterone (T), in particular the free T index, are increased in PCOS averaging at 50-150% higher than normal.[35] The clinical expression of this hyperandrogenism shows a wide spectrum, with well-documented racial differences in expression.[46] Recently, a study conducted in America, was published and the aim of this study was to determine the prevalence of diagnosed PCOS. This study took place in Northern California with a very heterogenous set of patients. The files of 11035 women were studied. The authors observed a definite difference in clinical presentation and associated risk factors among different racial groups.[46] Anovulatory but non-hirsute women with PCOS have similar levels to hirsute women.[35] Testosterone is bound to SBHG, the expression of which appears to be linked to BMI via the insulin mechanism. In women with PCOS, low SHBG levels have been found to correlate with insulin resistance[42], thereby increasing the unbound testosterone fraction with its ensuing effects.

Androstenedione (A4) has also been reported as elevated in the PCOS,[4,31] but the ESHRE/ASRM guidelines exclude it from routine testing in the assessment of hyperandrogenaemia. A small percentage of PCOS patients may exhibit elevated levels of dehydroepiandrosterone sulphate (DHEAS), though again here evidence for routine testing was lacking, according to the consensus statement. Nevertheless, DHEAS and A4 have thus far been accepted widely as additional androgens that, like testosterone, may typically be elevated in PCOS, as reported by many investigators.[9]

Oestrogen levels in PCOS follow an acyclical pattern as a consequence of anovulatory cycles.[31] Early and midfollicular levels are normal, but there is no preovulatory or mid-luteal increase in oestrogen levels.[35] With progesterone deficiency and increased peripheral conversion of androgens to oestrogen by adipose tissue, unopposed oestrogen results in menstrual dysfunction and irregular bleeding, with a long-term increased risk of endometrial carcinoma.[4]

1.8 PCOS IN ADOLESCENCE

Another early manifestation of PCOS is often the presence of menstrual irregularity in adolescence. Most adolescents with menstrual irregularity[47] or persistent acne[48] will have PCOS, particularly if associated with a raised body mass index (BMI). Menstrual irregularity that does not resolve within the first two years of menarche will be associated with the clinical and metabolic features of PCOS

in up to 70% of girls.[48,49] There is also evidence that the occurrence of precocious puberty is often followed by the development of PCOS in adolescence.[47,50,51]

The therapeutic management of the features of PCOS in this sensitive group of young women, beyond simple measures to control excessive weight gain, is essentially limited to control of the menstrual cycle using the combined oral contraceptive pill in conjunction with an anti-androgen. A more controversial approach has been to treat the underlying hyperinsulinaemia on a long-term basis with an insulin sensitiser with or without additional anti-androgenic treatment.[30] The third-generation oral contraceptive pill, either alone or in a combination pill with cyproterone acetate, has demonstrable and equal benefit to girls with PCOS with regard to cycle regulation, improvement in the Ferriman–Gallway (FG) score, serum androgen profile and lipid profile.[52,53]

In women with PCOS, the addition of metformin to a traditional third-generation combined oral contraceptive pill, either with or without cyproterone acetate, leads to an improvement in insulin sensitivity, androgen profile, sex hormone-binding globulin and waist–hip ratio in obese subjects, with no significant effects on lipid metabolism, although more favourable changes were noted in the serum-free androgen levels.[53-56]

The introduction of an oral contraceptive containing drosperinone with anti-mineralocorticoid and anti-androgenic properties, the so-called fourth-generation combined oral contraceptive pill, has increased the therapeutic options for these young women. Since drosperinone is an analogue of spironolactone it antagonises the oestrogen-induced activation of the renin-aldosterone system to reduce sodium and water retention. In addition to the beneficial effects with regard to a reduction in weight and improvement in androgenic symptoms it also has a more favourable effect on the lipid profile than traditional third-generation combined contraceptives.[57]

Many studies[58-63] have provided evidence for the hypothesis that size at birth is related to the risk of developing disease in later life. In particular, links are well established between reduced birthweight and increased risk of coronary heart disease, diabetes, hypertension and stroke in adulthood. These relationships are modified by patterns of postnatal growth. The most widely accepted mechanisms thought to underlie these relationships are those of fetal programming by nutritional stimuli or excess fetal glucocorticoid exposure. It is suggested that the fetus makes physiological adaptations in response to chages in its environment to prepare itself for postnatal life. These changes may include epigenetic modification of gene expression. Less clear at this time are

the relevance of fetal programming phenomena to twins and preterm babies, and whether any of these effects can be reversed after birth.[64]

1.9 PCOS AND LATER LIFE

The diagnosis of PCOS has serious implications not only for a woman's reproductive potential but also for her future long-term health.

In a population of women with PCOS, approximately 30% will have impaired glucose tolerance (IGT) and up to 10% will have diabetes[65,66], while in women with a BMI < 27 kg/m^2 the prevalence of IGT and diabetes is 10.3% and 1.3%, respectively.[65]

It is a recommendation of the Rotterdam ESHRE/ASRM consensus meeting that women with PCOS and a BMI in excess of 27 kg/m^2, should undergo a glucose tolerance test and a metabolic screen.[7]

Women with PCOS are at an increased risk of an adverse cardiovascular profile. In women with PCOS, elevated androgen and insulin levels (Figure 1) are associated with an unfavourable lipid profile with an increase in LDL, a decrease in HDL and increases in total cholesterol and triglyceride levels.[67,68] Women with PCOS are at a 2.5-fold increased risk of coronary atherosclerosis[67], carotid artery atherosclerosis[69] and arterial stiffness compared to controls.[70]

In women with PCOS, unopposed oestrogen arising from chronic anovulation may constitute a risk factor for endometrial hyperplasia and cancer, although epidemiological evidence of links between PCOS and endometrial cancer is limited.[71]

Despite some reports that the incidence of benign breast disease is increased in women with PCOS[65], this has not been confirmed and the evidence for an increased risk of breast cancer in women with PCOS is lacking.[72]

1.10 CONCLUDING REMARKS

It is unclear whether PCOS represents a single disorder or a conglomeration of different disorders with similar clinical presentation.A clinical presentation or Phenotype of PCOS may also reflect different etiology or pathophysiological differences. According to the 1990 NICHD definition, PCOS may present as three phenotypes.[74]

In a recent article[75], it was hypothesised that the three clinical phenotypes of PCOS represent different forms of the same metabolic disorder. Three hundred and sixteen women diagnosed as having PCOS were evaluated.

The oligo (oligo-ovulation) + HA (hyperandrogenism) + hirsutism phenotype represented 48% of subjects, oligo + HA represented 29% of the subjects and oligo + hirsutism represented 23% of subjects. These three phenotypes did not differ in mean BMI, waist-to-hip ratio, racial composites, degree of oligo-ovulation, prevalence of acne or family history of hyperandrogenic symptomatology. However, subjects demonstrating the oligo + HA + hirsutism phenotype were the youngest and had the greatest degrees of hyperandrogenemia, hyperinsulinemia and ß -cell dysfunction. Patients with the oligo + hirsutism phenotype were the oldest and had the mildest degrees of hyperandrogenemia, hyperinsulinemia and ß-cell dysfunction. Subjects with the oligo + HA phenotype demonstrated intermediate degrees of hyperandrogenemia and metabolic dysfunction.[75] This set of data suggested that it is the degree to which the ß-cell is able to compensate for the degree of insulin resistance, and not the degree of insulin resistance per se, that determines the severity of the phenotype.

They also concluded that the lower levels of hyperinsulinemia are related to lower androgen levels and slightly less severe hirsutism, whereas the greater degrees of hyperinsulinemia favour the development of hirsutism and frank hyperandrogenism.[75]

Finally, it remains unclear whether the three clinical phenotypes of PCOS described represent a continuum within a single population or are the result of differences in underlying pathophysiologic mechanisms, and whether the clinical phenotype predicts differences in the long-term risks of these patients for developing type 2 diabetes mellitus or cardiovascular disease.

The above study clearly confirms the controversy regarding the possible aetiology and diagnostic criteria for PCOS.[75]

The diagnosis and the debate of what encompasses this syndrome are hopefully becoming clearer. With the revised 2003 guidelines[7], more accurate prevalence statistics ought to become available, thereby increasing awareness of a common problem that deserves a high index of suspicion in any clinical practice including women of reproductive age.

The health impact of PCOS is enormous, and with the increasing prevalence of obesity and diabetes worldwide, is likely to increase.

It is therefore of utmost importance to adhere to current diagnostic guidelines. This will help us to gain valuable information and conduct non-biased research seeking the answers for this poorly understood disease.

1.11 REFERENCES

1. Adams J, Polson D W, Franks S. 'Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism'. *B Med J* 1986;293:355-359.
2. Stein IF, Leventhal M. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935;29:181-191.
3. Dunaif A. Insulin resistance and the polycystic ovary syndrome: Mechanism and implications for pathogenesis. Endocrine Reviews 1997;18(6):774-800
4. Guzick DS. Polycystic ovary syndrome. Am Coll Obstet Gynecol 2004;103(1):181-193.
5. Homberg R. What is polycystic ovarian syndrome? Hum Reprod 2002;17(10):2495-99.
6. Adams J, Polson DW, Abdulwahid N, et al. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotrophin releasing hormone. Lancet 1985;ii:1375-1399.
7. Fauser B, Chang J, Azziz R et al. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.
8. Balen A, Rajkhowha M. Polycystic ovary syndrome. Best Pract Res Clin Obstet Gynecol 2003;17:263-274.
9. Azziz R, Woods KS, Reyna et al. The prevalence and features of polycystic ovary syndrome in an unselected population. J Clin Endrin Metab 2004;89:2745-2749.
10. Lucky AW, Rosenfield RL, McGuire J et al. Adrenal androgen hyperresponsiveness to adrenocorticotropin in women with acne and/or hirsutism: adrenal enzyme defects and exaggerated adrenarche. Journal of Clinical Endocrinology and Metabolism 1986;62:840-848.
11. Van Hooff M, Voorhorst FJ, Kaptein MB et al. Predictive value of menstrual cycle pattern, body mass index, hormone levels and polycystic ovaries at age 15 years for oligo-amenorrhoea at 18 years. Hum Reprod 2004;19:383-392.
12. Van Hooff MH, Voorhorst FJ, Kaptein MB et al. Endocrine features of polycystic ovary syndrome in a random population sample of 14-16 year old adolescents. Hum Reprod 1999;14:2223-2229.

13. Ibanez L, Potau N, Marcos MV & De Zegher F. Adrenal hyperandrogenism in adolescent girls with a history of low birthweight and precocious pubarche. Clin Endocrinol (Oxford) 2000;53:523-527.
14. Abbott DH, Dumesic DA & Franks S. Developmental origin of polycystic ovary syndrome – a hypothesis. J Endocrinol 2002;174:1-5.
15. Abbott DH, Barnett DK, Bruns CM & Dumesic DA. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? Hum Reprod Update 2005;11:357-374.
16. Robinson JE, Birch RA, Taylor JA et al. In utero programming of sexually differentiated gonadotrophin releasing hormone (GnRH) secretion. Domestic Animal Endocrinology 2002;23:43-52.
17. Short RV. Sexual differentiation of the brain of the sheep: effects of prenatal implantation of androgen. Film: general discussion. Ciba Foundation Symposium 1978;62:257-269.
18. Chang RJ, Nakamura RM, Judd HL & Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metabol 1983;57:356-359.
19. Kiddy DS, Hamilton-Fairley D, Bush A et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxford) 1992;36:105-111.
20. Norman RJ, Noakes M, Wu R et al. Improving reproductive performance in overweight/obese women with effective weight management. Hum Reprod Update 2004;10:267-280.
21. Crosignani PG, Colombo M, Vegetti W et al. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. Hum Reprod 2003;18:1928-1932.
22. Clark AM, Thornley B, Tomlinson L et al. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod 1998;13:1502-1505.
23. Hoeger KM, Kochman L, Wixom N et al. A randomised, 48-week, placebo-controlled trial of intensive lifestyle modification and/or metformin therapy in overweight women with polycystic ovary syndrome: a pilot study. Fertil Steril 2004;82:421-429.
24. Kahsar-Millar MD, Azziz R. The effectiveness of interview for predicting the presence of polycystic ovary syndrome. Gynecol Endocrin 2003;17:449-454.
25. Nelson VL, Qin Kn KN, Rosenfield RL et al. The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. J Clin Endocrinol Metabol 2001;86:5925-5933.

26. Wickenheisser JK, Nelson-Degrave VL & McAllister JM. Dysregulation of cytochrome P450 17alpha-hydroxylase messenger ribonucleic acid stability in theca cells isolated from women with polycystic ovary syndrome. J Clin Endocrinol Metabol 2005;90:1720-1727.
27. Witchel SF & Aston CE. The role of heterozygosity for CYP21 in the polycystic ovary syndrome. J Ped Endocrinol Metabol 2000;13(supplement 5):1315-1317.
28. Ukkola O, Rankinen T, Gagnon J et al. A genome-wide linkage scan for steroids and SHBG levels in black and white families: the HERITAGE Family Study. J Clin Endocrinol Metabol 2002;87:3708-3720.
29. Diamanti-Kandarakis E, Palioniko G, Alexandraki K et al. The prevalence of 4G5G polymorphism of plasminogen activator inhibitor-1 (PAI-1) gene in polycystic ovarian syndrome and its association with plasma PAI-1 levels. Eur J Endocrinol 2004;150:793-798.
30. Hart R, Norman R. Polycystic ovarian syndrome – prognosis and outcomes. Best Prac Research Clin Obstet Gynaecol 2006;20(5):751-778.
31. Van der Spuy Z. The polycystic ovary syndrome. JEMDSA 2000; 5: 122-128.
32. Polson DW, Adam J, Wadsworth J, Franks S. Polycystic ovaries – a common finding in normal women. Lancet 1988;i:870-872.
33. Clayton RN, Ogden V, Hodgkinson J et al. How common are polycystic ovaries in normal women and what is the significance for the fertility of the population? Clin Endorin 1992;37:127-134.
34. Vanky E, Kjotrod S, Salvesen KA et al. Acta Obstet Gynecol Scand 2004;83:482-486.
35. Franks S. Polycystic ovary syndrome. N Eng J Med 1995;333:853-861.
36. Van der Spuy. Polycystic ovary syndrome – reproductive disturbance of metabolic disorder? S Afr Med J 1998;88:1509-1511.
37. Azziz R. The time has come to simplify the evaluation of the hirsute patient. Fertil Steril 2000;74:870-872.
38. Balen AH, Lave JSE, Tan S et al. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update 2003;9:505-514.
39. Carmina E. Longo A, Lobo RA. Does ovarian blood flow distinguish between ovulatory and anovulatory patients with polycystic ovary syndrome? Am J Obstet Gynecol 2003;189:1283-1286.
40. Erdem CZ, Bayar U, Erdem LO et al. Polycystic ovarian syndrome: dynamic contrast-enhanced ovary MR imaging. Eur J Radiol 2004;51:48-53.
41. Burghen GA, Givens JR. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50(1):113-116.

42. Legro R, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovarian syndrome: purposes and pitfalls. Obstet Gynecol Surv 2004;59:141-154.
43. Diamanti-Kandarakis E, Kouli C, Alexandraki K et al. Failure of mathematical indices to accurately assess insulin resistance in lean, overweight and obese women with polycystic ovary syndrome. J Clin Endocrin Metab 2004;89:1273-1276.
44. Schachter M, Razial A, Friedler S et al. Insulin resistance in patients with polycystic ovarian syndrome associated with elevated levels of plasma homocysteine. Hum Reprod 2003;18:721-727.
45. Fielding AM. Serum LH analysis: solutions and problems. Br J Obstet Gynaecol 2004;111:92.
46. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91(4):1357-1363.
47. Lazar L, Kauli R, Bruchis C et al. Early polycystic ovary-like syndrome in girls with central precocious puberty and exaggerated adrenal response. Eur J Endocrinol 1995;133:403-406.
48. Homburg R & Lambalk CB. Polycystic ovary syndrome in adolescence e a therapeutic conundrum. Hum Reprod 2004;19:1039-1042.
49. Venturoli S, Porcu E, Fabbri R et al. Longitudinal change of sonographic ovarian aspects and endocrine parameters in irregular cycles of adolescence. Ped Research 1995;38:974-980.
50. Ibanez L, Valls C, Potau N et al. Polycystic ovary syndrome after precocious pubarche: ontogeny of the low-birthweight effect. Clin Endocrinol 2001;55:667-672.
51. Ibanez L, Ferrer A, Ong K et al. Insulin sensitization early after menarche prevents progression from precocious pubarche to polycystic ovary syndrome. J Ped 2004;144:23-29.
52. Mastorakos G, Koliopoulos C & Creatsas G. Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. Fertil Steril 2002;77:919-927.
53. Cibula D, Fanta M, Vrbikova J et al. The effect of combination therapy with metformin and combined oral contraceptives (COC) versus COC alone on insulin sensitivity, hyperandrogenaemia, SHBG and lipids in PCOS patients. Hum Reprod 2005;20:180-184.
54. Morin-Papunen L, Vauhkonen I, Koivunen R et al. Metformin versus ethinyl estradiol-cyproterone acetate in the treatment of nonobese women with polycystic ovary syndrome: a randomised study. J Clin Endocrinol Metab 2003;88:148-156.
55. Morin-Papunen LC, Vauhkonen I, Koivunen RM et al. Endocrine and metabolic effects of metformin versus ethinyl estradiol-cyproterone acetate in obese women with polycystic ovary syndrome: a randomised study. J Clin Endocrinol Metab 2000;85:3161-3168.

56. Elter K, Imir G & Durmusoglu F. Clinical, endocrine and metabolic effects of metformin added to ethinyl estradiol-cyproterone acetate in non-obese women with polycystic ovarian syndrome: a randomised controlled study. Hum Reprod 2002;17:1729-1737.

57. Gaspard U, Endrikat J, Desager JP et al. A randomised study on the influence of oral contraceptives containing ethinylestradiol combined with drospirenone or desogestrel on lipid and lipoprotein metabolism over a period of 13 cycles. Contraception 2004; 69:271-278.

58. Barker DJ, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. Br Med J 1993;306:422-426.

59. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577-580.

60. Rich-Edwards JW, Stampfer MJ, Manson JE et al. Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. Br Med J 1997;315:396-400.

61. Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD. Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 1996;348:1478-1480.

62. Stein CE, Fall CH, Kumaran K, Osmond C, Cox V, Barker DJ. Foetal growth and coronary heart disease in south India. Lancet 1996;348:1269-1273.

63. Osmond C, Barker DJ, Winter PD, Fall CH, Simmonds SJ. Early growth and death from cardiovascular disease in women. Br Med J 1993:307:1519-1524.

64. De Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. Australian and New Zealand Journal of Obstetrics and Gynaecology 2006;46:4-14.

65. Legro RS, Kunselman AR, Dodson WC & Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metabol 1999;84:165-169.

66. Ehrmann DA, Barnes RB, Rosenfield RL et al. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999;22:141-146.

67. Christian RC, Dumesic DA, Behrenbeck T et al. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metabol 2003;88:2562-2568.

68. Legro RS, Kunselman AR & Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Amer J Med 2001;111:607-613.

69. Lakhani K, Hardiman P & Seifalian AM. Intimaemedia thickness of elastic and muscular arteries of young women with polycystic ovaries. Atherosclerosis 2004;175:353-359.

70. Meyer C, McGrath BP & Teede HJ. Overweight women with polycystic ovary syndrome have evidence of subclinical cardiovascular disease. J Clin Endocrinol Metabol 2005;90:5711-5716.

71. Hardiman P, Pillay OC & Atiomo W. Polycystic ovary syndrome and endometrial carcinoma. Lancet 2003;361:1810-1812.

72. Anderson KE, Sellers TA, Chen PL et al. Association of Stein-Leventhal syndrome with the incidence of postmenopausal breast carcinoma in a large prospective study of women in Iowa. Cancer 1997;79:494-499.

73. Soran A, Talbott EO, Zborowski JV & Wilson JW. The prevalence of benign breast disease in women with polycystic ovary syndrome: a review of a 12-year follow-up. Int J Clin Pract 2005;59:795-797.

74. Zawadki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome; towards a rationale approach. In: Dunaif A, Givens JR, Haseltine F, Merriam G, Polycystic ovary syndrome. Boston: Blackwell Scientific 1992:377-84.

75. Chang WY, Knochenhauer ES, Barolucci AA, et al. Phenotypic spectrum of polycystic ovary syndrome: Clinical and biochemical characterization of the three major clinical subgroups. Fertil Steril 2005;83(6):1717-1723.

TABLE 1. Criteria for the metabolic syndrome in women with PCOS (three of five qualify for the syndrome)[7]

Risk factor	Cut off
1. Abdominal obesity (waist circumference)	>88cm
2. Triglycerides	≥150mg/dL / ≥1.8mmol/L
3. HDL cholesterol	<50mg/dL / <1.3mmol/L
4. Blood pressure	≥130/≥85mmHg
5. Fasting and 2-h glucose from oral GTT	Fasting glucose 110-126mg/dL or 6-7mmol/L 2-h glucose 140-199mg/dL or 7.8-11.1mmol/L

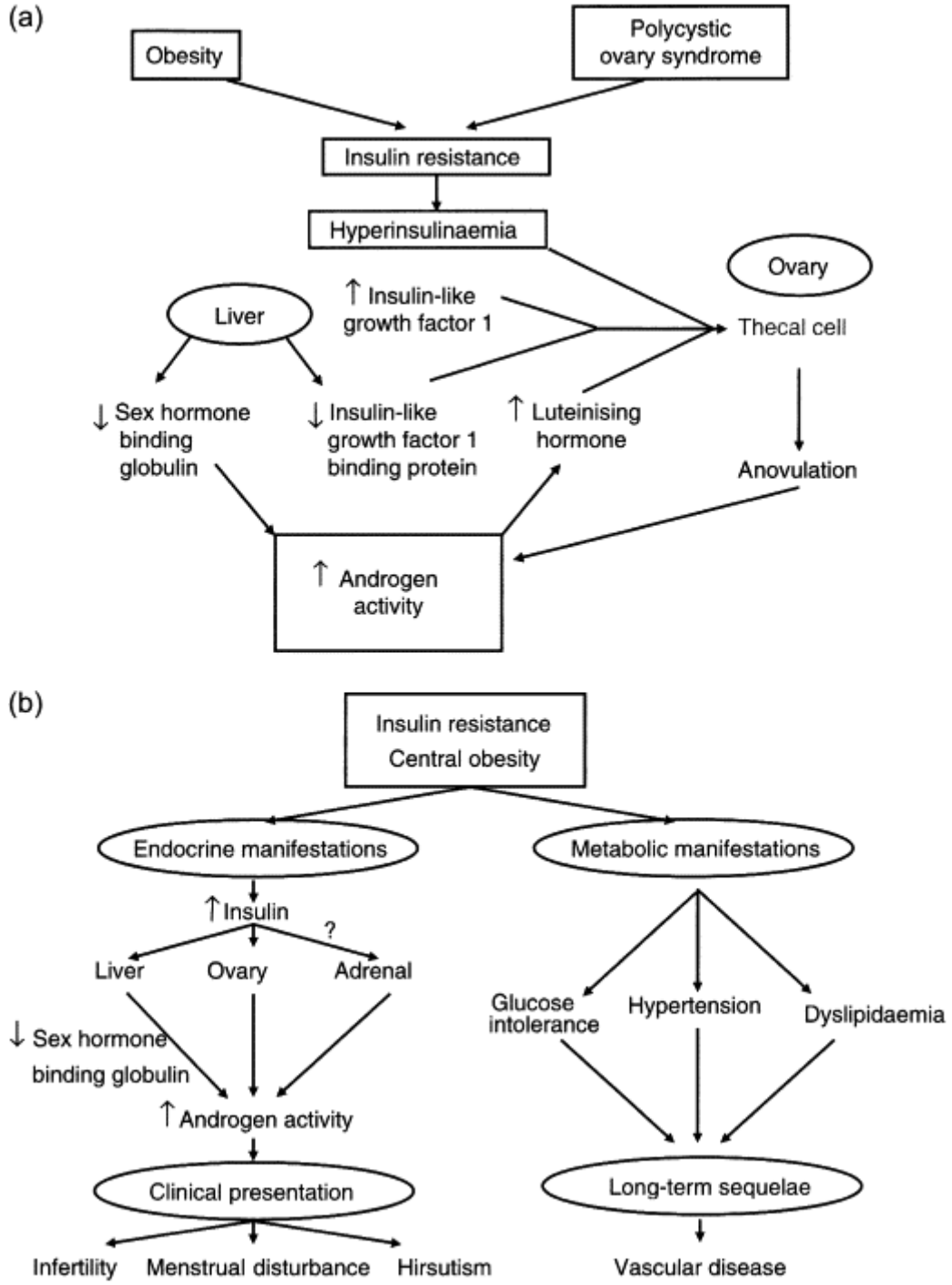


FIGURE 1(a) Potential mechanisms by which defects in insulin metabolism promote increased androgen activity at the level of the ovary. (b) Central role of insulin resistance in both the clinical presenting features and the long-term sequelae of polycystic ovary syndrome. (Reproduced with permission from Moran & Norman Understanding and managing disturbances in insulin metabolism and body weight in women with polycystic ovary syndrome.[30]

CHAPTER 2

OVULATION INDUCTION IN WOMEN WITH PCOS

2.1 INTRODUCTION

Women with PCOS have an increased incidence of World Health Organisation (WHO) group II anovulatory infertility.[1]

The aetiology of the association of anovulation with PCOS is believed to be hyperinsulinaemia and is accentuated by obesity.[2,3] Approximately 50% of women with PCOS are overweight[4] and indeed there is evidence that even normal weight women with PCOS have increased intra-abdominal fat.[5] More than 50% of lean women with PCOS are insulin resistant.

Hyperinsulinaemia and elevated leptin production from adipose tissue lead to increased ovarian androgen production by increasing ovarian theca cell cytochrome P450-scc and "cytochrome P450c-17" enzyme activity[6], as well as by increasing the frequency of luteinising hormone (LH) pulses, thus augmenting ovarian androgen production.[7] This is in addition to the increase in serum free androgen levels, due to the inhibition of hepatic sex hormone binding globulin. The result is that serum and ovarian androgen levels are raised in association with impaired folliculogenesis. Methods employed to induce ovulation consist of weight loss, anti-estrogens, insulin sensitisers, gonadotrophins, laparoscopic ovarian drilling and letrozole.[1]

2.2 WEIGHT LOSS

As described, obesity is very common in women with PCOS. It is also very important to distinguish between different localisations of fat deposits. Despite not distinguishing between lean and fat mass, BMI (body mass index, weight in kg per height in m^2) is a useful clinical tool that correlates reasonably well with adiposity. It is also apparent that body fat distribution has a crucial impact on metabolic and reproductive fitness.[8,9] Different abdominal fat regions may additionally confer differing risks with evidence suggesting abdominal visceral fat correlates more strongly with insulin resistance and markers of the metabolic syndrome than subcutaneous fat.[10] Waist hip ratios (WHR) or waist circumferences provide reasonable estimate of abdominal fat without distinguishing between abdominal and visceral fat. Generally, a WHR > 0,9 for men and > 0.8 for women defines an increased risk of CVD.[11]

2.2.1 Obesity and reproductive processes

Reproductive processes are influenced by body weight, and reproductive dysfunction is present with both positive and negative extremes of body weight .[12] Menstrual disturbances including

oligomenorrhoea, amonorrhoea, and anovulation have been consistently related to obesity in women.[13] This relationship was also observed for infertility. In a subset of the Nurses' Health Study, women with ovulatory disorders were compared to controls with no history of infertility. Increased BMI at age 18 was significantly associated with ovulatory infertility.[14] Furthermore once conception is achieved, an increased risk of pregnancy complications (including gestational diabetes) and miscarriage may result with increased weight.[15] There is thus a clear association between obesity, both in adulthood and childhood, on menstrual abnormalities and consequent infertility.

2.2.2 Weight loss and subsequent reproductive improvement

Resumption of ovulation occurred with weight losses of 5,6 to 6,5 kg in anovulatory women.[16] This amount of weight loss is generally sufficient to reduce abdominal fat and improve insulin sensitivity. A reduction in body weight of 2 to 5% was associated with restoration of ovulation, an 11% reduction in abdominal fat, a 4cm reduction in waist circumference and a 71% increase in insulin sensitivity.[17] Large changes in weight may not be needed to restore reproductive function, and realistic and achievable target weight loss goals can be set for women to improve their reproductive fitness.

2.2.3 How is this weight loss best achieved?

The NIH document "Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults" recommends a multifaceted approach to treating obesity.[18] (Table 1)

2.2.4 Dietetic treatment and lifestyle changes

Dietary management aims for gradual weight loss (0,5 to 1 kg per week) through energy intake reduction and increasing physical activity.[19] A low fat (30% of energy and saturated fat 10% of energy), moderate protein (15%) and high carbohydrate intake (55%) and increased consumption of fibre, wholegrain breads and cereals and fruit and vegetables in conjunction with moderate regular exercise (30 to 60 minutes per day) is proposed to aid in weight loss and maintenance both in general population and in obese infertile women PCOS.[19]

Smoking is a major risk factor for female sub-fertility, expressed time to pregnancy, for pre-term birth and for low birth weight in babies.[20] High levels of alcohol intake have been associated with reduced fertility and increased risk of spontaneous abortion.[21] Cognitive behaviour therapy and

reduction of psychosocial stressors can aid in both weight loss and maintenance of the reduced weight.[22]

Weight loss should therefore be the first choice of action in obese, infertile women. This goal may be difficult to achieve and maintain and it is therefore crucial to identify means to increase the ease of achieving and maintaining weight loss.
Principles identified in the general population and in obese infertile women include adoption of healthy eating habits and moderate amounts of low-intensity exercise that can be sustained as lifestyle changes.[19] (Table2)

2.3 CLOMIPHENE CITRATE

Ovulatory dysfunction is one of the most common causes of reproductive failure in sub-fertile and infertile couples.[23] Women with PCOS have an increased incidence of World Health Health Organisation (WHO) group II anovulatory infertility.[1] Clomiphene citrate (C/C) is the most common initial treatment used in anovulatory infertile women. The first clinical trial of C/C therapy demonstrated successful ovulation in 80% of women, half of whom achieved pregnancy during treatment.[24]

2.3.1 Pharmacology

C/C is a nonsteroidal triphenylethylene derivate that exhibits both estrogen agonist and antagonist properties.[25] In general C/C acts solely as competitive estrogen antagonist. About 85% of an administered dose is eliminated after approximately 6 days, although traces may remain in the circulation for much longer.[26] C/C is a mixture of two distinct steroisomers, enclomiphene and zuclomiphene. Available data indicate that enclomiphene is responsible for the ovulation inducing action of C/C.[25,27] The levels of enclomiphene rise rapidly after administration and is cleared from the circulation soon thereafter. Zuclomiphene is cleared more slowly and the levels of this less active isomer remain detectable in the circulation for more than a month after treatment and may accumulate over consecutive cycles of treatment.[28]

The structural similarity to estrogen allows C/C to bind to estrogen receptors (ER). In contrast to estrogen C/C binds ER for an extended period of time and eventually depletes ER concentrations.[25] Depletion of the hypothalamic ER prevents correct interpretation of circulating estrogen levels. Reduced levels of estrogen block the negative feedback effect of estrogen on the anterior pituitary, stimulating an increased secretion of gonadotrophins thus augmenting follicular selection and stimulation.

2.3.2 Indications

2.3.2.1 Anovulation

The causes of anovulation are many and varied. Correct diagnosis may suggest specific treatment and many associated conditions may have longer-term health consequences. Thyroid disease, pituitary tumors, eating disorders, extreme of weight loss and exercise, hyperprolactinemia, PCOS and obesity may be identified. C/C is the initial treatment of choice. However, given its hypothalamic site of action, C/C is often ineffective in hypogonadotrophic hypogonadism. Associated endocrinopathies should always first be treated appropriately.[23]

2.3.2.2 Luteal phase deficiency

The corpus luteum is derived from the follicle that ovulates, therefore its functional capacity is in part dependant on the quality of the preovulatory follicle development. In this context C/C is one logical treatment option for luteal phase deficiency.[29] Progesterone levels are typically higher after C/C treatment than in spontaneous cycles.[30]

2.3.2.3 Unexplained infertility

In couples whose infertility remains unexplained after thorough investigation, empiric treatment with C/C may be justified. This is particularly true for young couples with a short duration of infertility.[31] The efficacy of empiric C/C treatment may be attributed to correction of subtle and unrecognised ovulatory dysfunction.[32]

2.3.2.4 Standard therapy

C/C is administered orally, typically starting on the third to the fifth day after the onset of menses. The ovulation rates, conception rates and pregnancy outcome are similar regardless whether treatment begins on cycle day2, 3, 4 or 5.[33]

Treatment normally begins with a single 50-mg tablet daily for 5 consecutive days, increasing by 50-mg increments in subsequent cycles until ovulation is induced. Most women ovulate in response to treatment with 50 mg (52%) or 100 mg (22%). Higher doses have also been used but less successful (150 mg, 12%, 200 mg, 7%).[34]

Lower doses (e.g., 25 mg/day) need to be further investigated in women who demonstrate sensitivity to C/C or constantly develop large ovarian cysts.

C/C treatment will successfully induce ovulation in approximately 80% of cases. Likelihood of response declines with increasing age, body mass index (BMI), and free androgen index.[35] Approximately 70% to 75% of anovulatory women who respond to C/C may be expected conceive within six to nine cycles of treatment.[36]

2.3.2.5 Side effects

C/C is generally very well tolerated. Some side effects are relatively common but they are typically modest and manageable.

Vasomotor flushes (hot flashes) occur in approximately 10% of C/C-treated women, typically disappear soon after treatment stops.[23] Mood swings are also common. Visual disturbances, including blurred or double vision, scotomata, and light sensitivity are generally uncommon (<2% prevalence) and reversible. There are isolated reports of persisting symptoms and more severe complications such as optic neuropathy.[37] Whenever visual disturbances are identified it is very important to stop treatment and consider alternatives. Less specific side effects include breast tenderness, pelvic discomfort, and nausea all observed in 2% to 5% of C/C-treated women.

In addition to the successful ovulation induction action of C/C, C/C also exerts undesirable and unavoidable adverse anti-estrogenic effects in the periphery (endocervix, endometrium, ovary, ovum and embryo) that may explain the discrepancy between the ovulation and conception rates observed in C/C-treated women. However, there is very little or no compelling evidence to support these notions. The quality and quantity of cervical mucus production in C/C treatment cycles may sometimes be reduced, but rarely to the extent which may interfere with sperm transport or sperm survival.[38] Limited endometrium proliferation has been observed in some C/C-treated patients,[24] but the effect is minor or not at all evident in the large majority of women. If endometrium proliferation is a problem in a specific patient it would be advisable to use an alternative like letrozole.[39-41] Adverse effects of C/C on mouse ovum fertilization and embryo development have been demonstrated in vitro,[42] but circulating levels of C/C never reach the concentrations required to produce these effects, even after several treatment cycles.[28]

2.3.2.6 Complications

2.3.2.6.1 *Multiple gestation*

Multifollicular development is relatively common during C/C treatment and the risk of multiple gestation is clearly increased to approximately 8% overall.[43] The overwhelming majority of

multiple pregnancies that result from C/C are twin gestations, triplet and higher order pregnancies are rare but may occur.

2.3.2.6.2 *Congenital anomalies*

There is no evidence that C/C treatment increases the overall risk of birth defects or of any one anomaly in particular.[44,45]

2.3.2.6.3 *Spontaneous abortion*

A number of studies have described abortion rates that are not different from those observed in spontaneous pregnancies (10% to 15%).[46,47]

2.3.2.6.4 *Ovarian hyperstimulation syndrome*

The incidence of ovarian hyperstimulation syndrome (OHSS) in C/C-treated women is difficult to determine, as definitions of the syndrome vary widely among studies. Whereas mild OHSS (moderate ovarian enlargement) is relatively common, severe OHSS (massive ovarian enlargement, progressive weight gain, severe abdominal pain, nausea and vomiting, hypovolemia, ascites and oliguria) is rarely observed.[23]

2.3.2.6.5 *Ovarian cancer*

Two epidemiologic studies suggested that the risk of ovarian cancer might be significantly increased in women exposed to ovulation induction drugs.[48,49] In contrast to these results, subsequent studies have failed to confirm those findings.[50-53] A recent pooled analysis of eight case-control studies concluded that neither fertility drug use for more than 12 months was associated with invasive ovarian cancer.[54]

Taken together, available data suggest that any adverse anti-estrogenic effects of C/C present no significant obstacle in the majority of treated women.

2.4 INSULIN SENSITIZERS

Hyperinsulinemia and insulin resistance play an important role in the pathogenesis of PCOS.[55,56] Hyperinsulinemia enhances ovarian androgen production and decreases serum concentrations of sex hormone binding globulin (SHBG), resulting in an increased amount of unbound serum androgens.[57] Hyperinsulinemia may also increase ovarian E2 production by granulosa cells.[58]

The use of insulin sensitizers may restore the endocrine milieu and promote a normal menstrual cycle and ovulation by normalizing serum insulin and androgen levels.[59,60,61]

2.4.1 Metformin

2.4.1.1 Pharmacology

Of the insulin sensitising drugs, metformin has been the one studied most widely and has the most reassuring safety profile.[62] Metformin is a biguanide, it enhances insulin sensitivity in both the liver, where it inhibits hepatic glucose production, and the peripheral tissue, where it increases glucose uptake and utilization into muscle tissue. By increasing insulin sensitivity, metformin reduces insulin resistance, insulin secretion and hyperinsulineamia.[63] The most common side effects of metformin is nausea, vomiting and other gastro intestinal symptoms.[64] Metformin is contraindicated in the presence of even mild renal impairment because of a danger of lactic acidosis and it is associated with a decrease absorption of vitamin B12.[65] There is also no literature about the safety of long term use of metformin in young women.

2.4.1.2 Dose

Most studies or case reports of metformin[66-77] but not all,[78-80] have demonstrated that metformin administered at a dose of 500 mg three times daily (1,5 gr daily) increases menstrual cyclicity, improves spontaneous ovulation and promotes fertility.

It is interesting to speculate whether the response rate might have been higher had a dose of metformin of 1,000 mg twice daily been administered. In a dose response study of type II diabetic patients, the 2,000 mg daily dose of metformin was found to be optimal in improving glucose homeostasis,[81] and it is reasonable to assume that the higher dose might prove more beneficial in women with PCOS as well**.**

2.4.1.3 Clinical effects

In a recent systematic review and meta-analysis,[65] the authors commented on the proven effects of metformin when administered in PCOS patients.

2.4.1.3.1 *Ovulation rate*

A statistical significant effect of metformin when compared to placebo was observed ($P<0.0001$).

2.4.1.3.2 *Weight*

No evidence of effect was found from metformin on body weight or body mass index.

2.4.1.3.3 *Blood pressure*

The analysis showed a significant reduction for metformin in both systolic blood pressure and diastolic blood pressure.

2.4.1.3.4 *Insulin*

Metformin had a significant effect in reducing fasting insulin (P= 0.0001).

2.4.1.3.5 *Lipids*

Total cholesterol, high density lipoprotein cholesterol and triglycerides showed no evidence of a significant treatment effect with metformin, but low density lipoprotein cholesterol was significantly reduced in the metformin group.

2.4.1.4 Metformin and ovulation

As documented in the meta-analysis,[65] metformin showed a significant effect compared to placebo on ovulation (P<0,0001).

A recent structured literature review published, reported on the effect of metformin when added to clomiphene- resistant PCOS patients. In this review the authors documented a significant effect when metformin was added to clomiphene in the clomiphene-resistant PCOS patient.[82] (See chapter 3)

At the time of the meta-analysis[65], the question to be answered was, should metformin replace clomiphene as primary ovulation induction agent in women with PCOS? Recently 4 prospective randomized control trials were published, trying to answer the above question.[83-86] (In the discussion of chapter 5 the outcome of these trials is discussed). In the study by Legro et al,[85] they studied 626 patients with PCOS. This is by far the biggest trial and they concluded that C/C was superior to Metformin in achieving live birth rates and equal to the combination of Metformin and C/C in achieving pregnancies.

2.4.2 Trioglitazone

Trioglitazone, pioglitazone and rosiglitazone are part of a newer group of insulin sensitizers, the thiazolidinediones.

Five studies have reported on the use of trioglitazone in PCOS.[87-91] Each of these studies demonstrated an improvement in ovulation in the women treated with trioglitazone. The most recent trial was a multicenter, one-year study of over 400 women with PCOS.[87] This study demonstrated a dose-responsive improvement in ovulation with trioglitazone, lending substantial weight to the idea that insulin sensitivity influences ovulation. In conjunction with an increase insulin sensitivity , trioglitazone therapy consistently reduced circulating free testosterone, dehydroepiandrosterone sulfate, estrone, and LH levels and increased levels of sex hormone binding globulin.[92]

Because of the reported cases of hepatotoxicity associated with trioglitazone therapy, it has been withdrawn from the market in the UK until the issue of hepatotoxicity risk is settled.

Rosiglitazone and pioglitazone related to the same pharmacological group have been reported to be safer but clinical experience is still limited.[93-95]

2.5 LAPAROSCOPIC OVARIAN DRILLING

Laparoscopic ovarian drilling (LOD) has been widely used to induce ovulation in PCOS women after failure of treatment with C/C. It was first described in 1984 as a laparoscopic alternative to ovarian wedge resection by laparotomy.[96] Many authors have reported high ovulation (80%) and pregnancy rates (60%) following LOD.[96-104] The mechanism of action of LOD is not fully understood. It is therefore not exactly clear why some PCOS patients will not respond to LOD. A possible explanation is that the amount of ovarian tissue destroyed during LOD is not sufficient to produce an effect in some patients.[105] It is also believed that ovarian diathermy works by increasing the sensitivity of the ovaries to endogenous FSH, and that only a minimal amount of thermal injury is required. Another possible explanation of failure to respond may be an inherent resistance of the ovary to the effects of drilling.[105]

A retrospective study has determined that three punctures per ovary are sufficient to produce the beneficial effect of ovarian drilling.[106] A significant side effect of ovarian drilling is the occurrence of pelvic adhesions and to minimise this significant risk a fine electrodiathermy needle should be employed.[107]

In a recent Cochrane review the authors concluded that there is no evidence of a difference between laparoscopic ovarian drilling (with or without medical ovulation induction) compared to ovulation induction with gonadotrophins for women with PCOS and C/C-resistance for the outcomes of

pregnancy and ovulation after 12 months follow up.[108] They also stated that multiple pregnancy rates are increased with gonadotrophins and are almost nonexistent with ovarian drilling. With regard to adhesion formation, there is currently insufficient evidence to favour any one surgical technique over another.[108]

In a recent study,[105] the authors studied 200 PCOS patients and evaluated the influence of the various pre operative characteristics on the ovulation and pregnancy rates after LOD. Women with body mass index > 35kg/m2, serum testosterone concentration >4,5nmol/l, free androgen index >15 and with duration of infertility > 3 years seem to be poor responders to LOD. The authors recommended alternative methods of treatment for this group of patients such as weight reduction, metformin, gonadotrophin therapy or IVF. In the LOD responders, Serum LH levels > 10IU/l appeared to be associated with higher pregnancy rates.

In another study,[109] the authors studied 83 women with C/C-resistant PCOS. These women had LOD and were followed up post operatively to evaluate factors influencing ovulation outcome. They concluded that women who were younger than 13 at menarche, had a LH/FSH ratio below 2 and a glucose level below 4,5mmol/l were more likely to have persistent anovulation.

LOD may be an alternative choice for C/C-resistant women with PCOS.[107,110]

2.6 CLOMIPHENE AND DEXAMETHASONE

The use of dexamethasone (0,5 - 2mg from days 2 – 6) as an adjunct to C/C treatment, when compared to C/C alone, based on two studies[111,112]**,** demonstrated a major benefit with regard to ovulation and pregnancy, with the number to treat for each additional pregnancy being only 2,7.[113] The mechanism of action is potentially by suppressing adrenal androgen secretion, facilitation of folliculogenesis by augmenting follicle-stimulating hormone (FSH) secretion, or by suppression of the large amplitude LH secretion.[113] The addition of dexamethasone to C/C may, therefore, may be considered in women with a high LH level or with an elevated adrenal androgen, dehydroepiandosterone (DHEA) level, although it is recognised that these two studies were not entirely comparable and that further research is required.[113]

2.7 GONADOTROPHIN THERAPY

Gonadotrophin therapy is often used as a second line therapy in anovulatory women with PCOS if they were either resistant to ovulation induction with anti-oestrogen treatment or failed to conceive. However, women with PCOS are particularly sensitive to gonadotrophin therapy and have a

significant chance of multiple follicular development and cycle cancellation.[114] In addition, the frequent development of multiple follicles leads to the risk of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). To overcome this risk, a "low-dose step-up" protocol is well established in fertility practices.[115] Treatment with metformin concurrently with low-dose gonadotrophin stimulation may improve the mono-follicular ovulation rate.[116] In an attempt to predict treatment response an article was published assessing initial patient characteristics and the subsequent risk of OHSS.[117] Initial characteristics predicting multifollicular development were hyperandrogenism, increased LH, and increased antral follicle count, and those for better chances of ongoing pregnancy in FSH ovulation induction include younger age, lower androgens and lower insulin growth factor I.[117]

Gonadotrophin therapy remains a successful option for ovulation induction in C/C-resistant PCOS women.

2.8 AROMATASE INHIBITOR TREATMENT

2.8.1 Introduction

Clomiphene citrate (C/C) is frequently used for ovulation induction and is highly effective in initiating ovulation in patients with PCOS[118]. However, despite a 75% -80% ovulation rate with C/C use, the cumulative pregnancy rate after 6 months of treatment is only 40% - 45%[119].

In patients who do not respond to treatment with C/C, metformin can be added (see Chapter 3). Gonadotrophins can also be used in the C/C-resistant patient with an increased risk of hyperstimulation syndrome and multifetal pregnancies (see discussion on gonadotrophins).

2.8.2 Pharmacology

Aromatase inhibitors were originally developed for the treatment of breast cancer. Aromatase is a cytochrome P-450 hemoprotein that catalyses the rate-limiting step in estrogen synthesis, that is, the 3-hydroxylation step in the conversion of androstenedione and testosterone to estrone and E2, respectively[120]. The most widely used aromatase inhibitor is letrozole. It has been suggested that letrozole increases endogenous gonadotrophin secretion, as seen with C/C. However, unlike C/C, letrozole does not cause a decrease in estrogen receptors[121].

2.8.3 Treatment regimens

In a recent study, 179 patients were prospectively randomised. This study aimed to compare the three most common used doses: 2,5, 5 and 7,5mg from day 4 to day 8[122]. This study reported a

significantly higher (P<0.05) number of follicles on the day of administration of human chorionic gonadotrophin (hCG) in the 7.5mg group. However, the pregnancy and miscarriage rates were similar in the three groups. The authors concluded that it seems that the use of higher doses of letrozole offers no advantage in terms of pregnancy rates over the lower (2.5mg) dose.

In another study by Bayar et al[123], they compared the use of letrozole with the use of C/C. This was a prospective randomised study of 74 patients. In this study the median endometrial thickness on the day of hCG administration did not differ between the two groups. The ovulation rates and pregnancy rates did also not differ significantly.

2.8.4 Current issues

In a study by Biljan et al[124], the authors evaluated the outcome of 150 babies conceived after the use of letrozole and compared this data to a large control group of spontaneous conceptions. The outcome of this study suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac and bone malformations in the newborns. As a result of this study, on November 17th, 2005, Novartis Pharmaceuticals issued a statement to physicians in Canada and worldwide advising that letrozole use in premenopausal women, specifically its use for ovulation induction, is contraindicated[125].

This study was followed by a retrospective study on 911 newborns from women conceived following C/C and letrozole treatment[126]. Overall, congenital malformations and chromosomal abnormalities were found in 14 of 514 newborns in the letrozole group (2.4%) and in 19 of 397 newborns in the C/C group (4.8%). The major malformation rate in the letrozole group was 1.2% (6/514) and in the C/C group was 3% (12/397). In addition, the rate of all congenital cardiac anomalies was significantly higher (P:0.02) in the C/C group (1.8%) compared to the letrozole group(0.2%).

The authors concluded that congenital cardiac anomaly is less frequent in the letrozole group and that there was no difference in the overall rates of major and minor congenital malformations among newborns from mothers who conceived after letrozole or C/C treatments.

Based on current data letrozole may be an acceptable alternative to C/C as an ovulation induction drug in patients with PCOS.

2.9 REFERENCES

1. Hart R, Norman R. Polycystic ovarian syndrome – prognosis and outcomes. Best Prac Research Clin Obstet Gynaecol 2006;20(5):751-778.
2. Guzick DS. Polycystic ovary syndrome. Am Coll Obstet Gynecol 2004;103(1):181-193.
3. Chang RJ, Nakamura RM, Judd HL & Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metabol 1983;57:356-359.
4. Gambineri A, Pelusi C, Vicennati V et al. Obesity and the polycystic ovary syndrome. International Journal of Obesity & Related Metabolic Disorders: J Int Ass Stud Obesity 2002;26:883-896.
5. Yildirim B, Sabir N & Kaleli B. Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. Fertil Steril 2003; 79: 1358-1364.
6. Nestler JE. Insulin regulation of human ovarian androgens. Hum Reprod 1997; 12(supplement 1):53-62.
7. Franks S, Mason H & Willis D. Follicular dynamics in the polycystic ovary syndrome. Mol Cell Endocrinol 2000;163:49-52.
8. Clark AM, Thornley B, Tomlinson L, Galletley C & Norman RJ. Weight loss results in in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod 1995;10:2705-2712.
9. Zaadstra BM, Seidell JC, Van Noord PA et al. Fat and female fecundity: Prospective study of effect of body fat distribution on conception rates. Br Med J 1993;306:484-487.
10. Yamashita S, Nakamura T, Shimomura I et al. Insulin resistance and body fat distribution. Diabetes Care 1996;19:287-291.
11. Dobbelsteyn CJ, Joffres MR, MacLean DR & Flowerdew G. A comparative evaluation of waist circumference, waist-to-hip ratio and body mass index as indicators of cardiovascular risk factors: The Canadian Heart Health Surveys. Int J Obes Relat Metab Disord 2001;25:652-661.
12. Lake JK, Power C & Cole TJ. Women's reproductive health: The role of body mass index in early and adult life. Int J Obes Relat Matab Disord 1997;21:432-438.
13. Hartz AJ, Barboriak PN, Wong A, Katayama KP & Rimm AA. The association of obesity with infertility and related menstrual abnormalities in women. Int J Obes 1979;3:57-73.
14. Rich-Edwards JW, Goldman MB & Willett WC. Adolescent body mass index and infertility caused by ovulatory disorder. Am J Obstet Gynecol 1994;171:171-177.
15. Sebire NJ, Jolly M, Harris JP et al. Maternal obesity and pregnancy outcome: A study of 287 213 pregnancies in London. Int J Obes Relat Metab Disor 2001;25:1175-1182.

16. Clark AM, Thornley B, Tomlinson L, Galletley C & Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod 1998;13:1502-1505.

17. Huber-Buchholz MM, Carey DG & Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: Role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab 1999;84:1470-1474.

18. National Institute of Health. Clinical guidelines on the identification, evaluation and treatment of overweight and obesity in adults: Evidence report. Journal of Obesity Research 1998;6 (Suppl 2).

19. Norman RJ and Moran LJ. Weight, fertility and management approaches. In: Advances in fertility studies and reproductive medicine IFFS 2007. Eds: TF Kruger, Z van der Spuy, Kempers RD. Juta 2007;4:24-35.

20. Satcher D. Women and smoking: A report of the surgeon general. Atlanta, GA: Centres for Diesease Control 2001.

21. Grodstein F, Goldman MB & Cramer DW. Infertility in women and moderate alcohol use. Am J Public Health 1994;84:1429-1432.

22. Skender ML, Goodrick GK, Del Junco DJ et al. Comparison of 2-year weight loss trends in behavioral treatments of obesity: Diet, exercise and combination interventions. J Am Dietetic Assoc 1996;96:342-346.

23. The Practice Committee of the American Society for Reproductive Medicine. Use of clomiphene citrate in women. Fertil Steril 2006;86(4):187-193.

24. Greenblatt RB. Chemical induction of ovulation. Fertil Steril 1961;12:402-404.

25. Clark JH, Markaverich BM. The agonistic-Antagonistic properties of chomiphene: a review. Pharmacol Ther 1982;15:469-519.

26. Mikkelson TJ, Kroboth PD, Cameron WJ, Dittert Lw, Chungi V, Manberg PJ. Single-dose pharmacokinetics of clomiphene citrate in normal volunteers. Fertil Steril 1986;46:392-396.

27. Van Campenhout J, Borreman E, Wyman H, Antaki A. induction of ovulation with cisclomiphene. Am J Obstet Gynecol 1973;115:321-327.

28. Young Sl, Opsahl MS, Fritz Ma. Serum concentrations of enclomiphene and zuclomiphene across consecutive cycles of clomiphene citrate therapy in anovulatory infertile women. Fertil Steril 1999;71:639-644.

29. Quagliarello J, Weiss G. Clomiphene citrate in the management of infertility associated with shortened luteal phases. Fertil Steril 1979;31:373-377.

30. Guzick DS, Zeleznik A. Efficacy of clomiphene citrate in the treatment of luteal phase deficiency: quantity versus quality of preovulatory follicles. Fertil Steril 1990;54:206-210.

31. Fisch P, Casper RF, Brown SE, Wrixon W, Collins JA, Reid RL, et al. Unexplained infertility: evaluation of treatment with clomiphene citrate and human chorionic gonadotropin. Fertil Steril 1989;51:828-833.

32. Glazener CM, Couson C, Lambert PA, Watt EM, Hinton RA, Kelly NG, et al. Clomiphene treatment for women with unexplained infertility: placebo-controlled study of hormonal responses and conception rates. Gynecol Endocrinol 1990;4:75-83.

33. Wu CH, Winkel CA. The effect of therapy initiation day on clomiphene citrate therapy. Fertil Steril 1989;52:564-568.

34. Gysler M, March CM, Mishell DR Jr, Bailey EJ. A decade's experience with an individualized clomiphene treatment regime including its effect on the postcoital test. Fertil Steril 1982;37:161-167.

35. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. Predictors of patients remaining anovulatory during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. J Clin Endocrinol Metab 1998;83:2361-2465.

36. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. Fertil Steril 2002;77:91-97.

37. Purvin V. Visual disturbance secondary to clomiphene citrate. Arch Ophthalmol 1995;113:482-484.

38. Maxson WS, Pittaway DE, Herbert CM, Garner CH, Wentz AC. Antiestrogenic effect of clomiphene citrate: correlation with serum estradiol concentrations. Fertil Steril 1984;42:356-359.

39. Dickey RP, Olar TT, Taylor SN, Curole DN, Matulich EM. Relationship of endometrial thickness and pattern of fecundity in ovulation cycles: effect of clomiphene citrate alone and with human menopausal gonadotropin. Fertil Steril 1993;59:756-760.

40. Eden JA, Place J, Carter GD, Jones J, Alaghband-Zedeh J, Pawson Me. The effect of clomiphene citrate on follicular phase increase in endometrial thickness and uterine volume. Obstet Gynecol 1989;73:187-190.

41. Randall JM, Templeton A. Transvaginal sonographic assessment of follicular and endometrial growth in spontaneous and clomiphene citrate cycles. Fertil Steril 1991;56:208-212.

42. Schmidt GE, Kim MH, Mansour R, Torello L, Friedman CI. The effects of enclomiphene and zuclomiphene citrates on mouse embryos fertilized in vitro and in vivo. Am J Obstet Gynecol 1986;154:727-736.

43. Schenker JG, Jarkoni S, Granat M. Multiple pregnancies following induction of ovulation. Fertil Steril 1981;35:105-123.

44. Ahlgren M, Kallen B, Rannevick G. Outcome of pregnancy reuslting from clomiphene therapy. Acta Obstet Gynecol Scand 1976;55:371-375.

45. Correy JF, Marsden DE, Schokman FC. The outcome of pregnancy resulting from clomiphene induced ovulation. Aust NZ J Obstet Gynaecol 1982;22:18-21.

46. Gysler M, March CM, Mishell DR Jr, Bailey EJ. A decade's experience with an individualized clomiphene treatment regime including its effect on the postcoital test. Fertil Steril 1982;37:161-167.

47. Dickey RP, Taylor SN, Curole DN, Rye PH, Pyrzak R. Incidence of spontaneous abortion in clomiphene pregnancies. Hum Reprod 1996;11:2623-2628.

48. Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. Am J Epidemiol 1992;136:1184-1203.

49. Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG. Ovarian tumors in a cohort of infertile women. N Engl J Med 1994;331:771-776.

50. Venn A, Watson L, Lumley J, Giles G, King C, Healy D. Breast and ovarian cancer incidence after infertility and in vitro fertilization. Lancet 1995;346:995-1000.

51. Modan B, Ron E, Lerner –Geva L, Blumstein T, Menczer J, Rabinovici J, et al. Cancer incidence in a cohort of infertile women. Am J Epidemiol 1998;147:1038-1042.

52. Mosgaard BJ, Lidegaard O, Kjaer SK, Schou G, Andersen AN. Infertility, fertility drugs and invasive ovarian cancer: a case-control study. Fertil Steril 1997;67:1005-1012.

53. Potashnik G, Lerner-Geva L, Genkin L, Chetrit A, Lunenfeld E, Porath A. Fertility drugs and the risk of breast and ovarian cancers: results of a long-term follow-up study. Fertil Steril 1999;71:853-859.

54. Ness RB, Cramer DW, Goodman MT, Kjaer SK, Mallin K, Mosgaard BJ, et al. Infertility, fertility drugs and ovarian cancer: a pooled analysis of case-control studies. Am J Epidemiol 2002;155:217-224.

55. PCOS consensus. The Rotterdam ESHRE/ASRM-Spnsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-47.

56. PCOS consensus. The Rotterdam ESHRE/ASRM-Spnsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Fertil Steril 2004;81:19-25.

57. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanisms and implications for pathogenesis. Endocrine Reviews 1997;18:774-800.

58. Coffler MS, Patel K, Dahan MH et al. Enhanced granulosa cell responsiveness to FSH during insulin infusion in women with PCOS treated with Pioglitazone. J Clin Endocrinol Metab 2003;88:5624-5631.

59. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. New Engl J Med 1998;338:1876-1880.

60. Lord JM, Flight IHK, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003;327:951-953.

61. Kashyap S, Wells GA, Rosenwaks Z. Insulin-sensitizing agents as primary therapy for patients with polycystic ovary syndrome. Hum Reprod 2004;19:2474-2483.

62. Nestler JE, Stovall D, Akhter N, Iuorno MJ, Jakubowicz DJ. Strategies for the use of insulin-sensitizing drugs to treat infertility in women with polycystic ovary syndrome. Fertil Steril 2002;77(2):209-215.

63. Fauser B, Tarlatzis B, Chang J, Azziz R, Legro R, Dwailly D, Franks S, Balen AH, Bouchard P, Dahlgren E et al. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-47.

64. Fleming R, Hopkinson ZE, Wallace AM, Greer IA, Sattar N. Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomised double blind placebo-cntrolled trial. J Clin Endocrinol Metab 2002;87:569-574.

65. Lord JM, Flight IHK, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003;327:951-956.

66. Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia and systolic bloo pressure, while facilitating normal menses and pregnancy. Metabolism 1994;43:647-654.

67. Velazquez E, Acosta A, Mendoza SG. Menstrual cyclicity after metformin therapy in polycystic ovary syndrome. Obstet Gynecol 1997;90:392-395.

68. Morin-Papunen LC, Koivunen RM, Ruokonen A, Martikainen HK. Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. Fertil Steril 1998;69:691-696.

69. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 1998:1876-1880.

70. Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L. Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. Metabolism 1999;48:511-519.

71. Sarlis NJ, Weil SJ, Nelson LM. Administration of metformin to a diabetic woman with extreme hyperandrogenemia of nontumoral origin: management of infertility and prevention of inadvertent masculinization of a female fetus. J Clin Endocrinol Metab 1999;84:1510-1512.

72. Ibanez L, Valls C, Potau N, Marcos MV, de Zegher F. Senstization to insulin in adolescent girls to normalize hirsutism, hyperandrogenism, oligomenorrhea, dyslipidemia and hyperinsulinism after precocious pubarche. J Clin Endocrinol Metab 2000;85:3526-3530.

73. Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. Fertil Steril 2000;73:1149-1154.

74. Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, et al. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomised, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab 2000;85:139-146.

75. Paquali R, Gabmineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, et al . Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab 2000;85:2767-2774.

76. Seale FG, Robinson RD, Neal GS. Association of metformin and pregnancy in the polycystic ovary syndrome. A report of three cases. J Reprod Med 2000;45:507-510.

77. Vandermolen DT, Ratts VS, Evans WS, Stovall DW, Kauma SW, Nestler JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. Fertil Steril 2001;75:310-315.

78. Crave JC, Fimbel S, Lejeune H, Cugnardey N, Déchaud H, Pugeat M. Effects of diet and metformin administration on sex hormone-binding globulin, androgens, and insulin in hirsute and obese women. J Clin Endocrinol Metab 1995;80:2057-2062.

79. Acbay O, Gundogdu S. Can metformin reduce insulin resistance in polycystic ovary syndrome? Fertil Steril 1996;65:946-949.

80. Ehrmann DA, Cvaghan MK, Imperial J, Sturis J, Rosenfield RL, Polonsky KS. Effects of metformin on insulin secretion, insulin action, and ovarian steroidogenesis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1997;82:524-530.

81. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial.

82. Siebert TI Kruger, TF, Steyn DW, Nosarka S. Is the addition of metformin efficacious in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome? A structured literature review. Fertil Steril 2006;86(5):1432-1437

83. Palomba S, Orio F, Falbo A, Manguso F, Russo T, Cascella T, et al. Prospective parallel randomised, double-blind, double-dummy controlled clinical trial comparing Clomiphene Citrate and Metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with Polycystic Ovary Syndrome. The Journal of Clinical Endocrinology & Metabolism 2005;90(7):4068-4074.

84. Moll M, Bossuyt PMM, Korevaar JC, Lambalk CB, Van der Veen F. Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double-blind clinical trial. BMJ 2006;332:1485-1489.

85. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, Metformin, or both for Infertility in the Polycystic Ovary Syndrome. N Engl J Med 2007:356(6)551-566.

86. Neveu N, Granger L, St-Michel P, Lavoie HB. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction and achievement of pregnancy in 154 women with polycystic ovary syndrome. Fertil Steril 2007:87(1):113-120.

87. Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, et al . Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 2001;86:1626-1632.

88. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R. The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 1996;81:3299-3306.

89. Ehrmann DA, Schneider DJ, Sobel BE, Cavaghan MK, Imperial J, Rosenfield RL, Polonsky KS. Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1997;82:2108-2116.

90. Hasegawa I, Murakawa H, Suzuki M, Yamamoto Y, Kurabayashi T, Tanaka K. Effect of troglitazone and ovulatory performance in women with insulin resistance-related polycystic ovary syndrome. Fertil Steril 1999;71:323-327.

91. Mitwally MF, Kuscu NK, Yalcinkaya TM. High ovulatory rates with use of troglitazone in clomiphene-resistant women with polycystic ovary syndrome. Hum Reprod 1999;14:2700-2703.

92. Mitwally MF, Casper RF. Insulin resistance in polycystic ovary syndrome and the role of oral hypoglycemic agents. Mid East Fertil Soc J 2000;5(1):2-12.

93. Miller JL. FDA approves pioglitazone for diabetes. Am J Health Syst Pharm 1999;56(17):1698.

94. Samraj GP, Kuritzky L, Quillen DM. Improving management of type 2 diabetes mellitus: 5 Thiazolidinediones. Hosp Pract (Off Ed) 2000;35(1):123-124,129-132.

95. Peraldi P, Xu M, Spiegelman BM. Thiazolidinediones block tumor necrosis factor-alpha-induced inhibition of insulin signaling. J Clin Invest 1997;100:1863-1869.

96. Gjonnaess H. Polycystic ovarian syndrome treated by ovarian electrocautery through the laparoscope. Fertil Steril 1984;41:20-25.

97. Gjonnaess H. Ovarian electrocautery in the treatment of women with polycystic ovary syndrome (PCOS). Factors afferting the results. Acta Obstet Gynecol Scand 1994;73:407-412.

98. Abdel-Gadir A, Mowafi RS, Alnaser HMI, Alrashid AH, Alonezi OM, Shaw RW. Ovarian electrocautery versus human gonadotrophins and pure follicle stimulating hormone therapy in the treatment of patients with polycystic ovarian disease. Clin Endocrinol 1990;33:585-592.

99. Kovacs G, Buckler H, Bangah M, Outch K, Burger H, Healy D, Baker G, Phillips S. Treatment of anovulation due to polycystic ovarian syndrome by laparoscopic ovarian electrocautery. Br J Obstet Gynaecol 1991;98:30-35.

100. Armar NA, Lachelin GC. Laparoscopic ovarian diathermy: an effective treatment for anti-oestrogen resistant anovulatory infertility in women with the polycystic ovary syndrome. Br J Obstet Gynaecol 1993;100:161-164.

101. Naether OGJ, Baukloh V, Fischer R, Kowalczyk T. Long-term follow-up in 206 infertility patients with polycystic ovarian syndrome after laparoscopic electrocautery of the ovarian surface. Hum Reprod 1994;9:2342-2349.

102. Li TC, Saravelos H, Chow MS, Chisabingo R, Cooke ID. Factors affecting the outcome of laparoscopic ovarian drilling for polycystic ovarian syndrome in women with anovulatory infertility. Br J Obstet Gynaecol 1998;105:338-344.

103. Felemban A, Tan SL, Tulandi T. Laparoscopic treatment of polycystic ovaries with insulated needle cautery: a reappraisal. Fertil Steril 2000;73:266-269.

104. Amer SAK, Gopalan V, Li TC, Ledger WL, Cooke ID. Long-term follow up of patients with polycystic ovarian syndrome after laparoscopic ovarian drilling: clinical outcome. Hum Reprod 2002a;17:2035-2042.

105. Amer SAK, Li TC, Ledger WL. Ovulation induction using laparoscopic ovarian drilling in women with polycystic ovarian syndrome: predictors of success. Hum Reprod 2004;19(8):1719-1724.

106. Amer SA, Li TC & Cooke ID. Laparoscopic ovarian diathermy in women with polycystic ovarian syndrome: a retrospective study on the influence of the amount of energy used on the outcome. Hum Reprod 2002;17:1046-1051.

107. NICE. National Institute of Clinical Excellence Report on Fertility Assessment and Treatment for People with Fertility Problems. London: Department of Health UK, 2004.

108. Farquhar C, Lilford RJ, Marjoribanks J, Vandekerckhove P. Laparoscopic "drilling" by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. (Review) The Cochrane Library 2007;3:1-11.

109. Van Wely M, Bayram N, van der Veen F, Bossuyt PMM. Predictors for treatment failure after laparoscopic electrocautery of the ovaries in women with clomiphene citrate resistant polycystic ovary syndrome. Hum Reprod 2005;20(4):900-905.

110. Dutch Health Council guideline. Available at: http://www.cvz.nl/resources/ rSBA97-007_tcm13-2650.pdf

111. Daly DC, Walters CA, Soto-Albors CE et al. A randomised study of dexamethasone in ovulation induction with clomiphene citrate. Fertil Steril 1984;41:844-848.

112. Parsanezhad ME, Alborzi S, Motazedian S & Omrani G. Use of dexamethasone and clomiphene citrate in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome and normal dehydroepiandrosterone sulfate levels: a prospective, double-blind, placebo-controlled trial. Fertil Steril 2002;78:1001-1004.

113. Beck JI, Boothroyd C, Proctor M et al. Oral anti-oestrogens and medical adjuncts for subfertility associated with anovulation. Coch Database of Systematic Reviews 2005;1:CD002249.

114. Nugent D, Vandekerckhove P, Hughes E et al. Gonadotrophin therapy for ovulation induction in sub-fertility associated with polycystic ovary syndrome. Coch Database of Systematic Reviews 2000;4:CD000410.

115. Messinis IE. Ovulation induction: a mini review. Hum Reprod 2005;20:2688-2697.

116. De Leo V, la Marca A, Ditto A et al. Effects of metformin on gonadotropin-induced ovulation in women with polycystic ovary syndrome. Fertil Steril 1999;72:282-285.

117. Van Santbrink EJ, Eijkemans MJ, Laven SJ, Fauser BC. Patient-tailored conventional ovulation induction algorithms in anovulatory infertility. TEM 2005;16:381-389.

118. Hammond MG. Monitoring techniques for improved pregnancy rates during clomiphene ovulation induction. Fertil Steril 1984;42:499-509.

119. European Society for Human Reproduction and Embryology. Female infertility: treatment options for complicated cases. The ESHRE Capri Workshop. Hum Reprod 1997;12:1191-1196.

120. Brodie A, Lu Q, Liu Y, Long B. Aromatase inhibitors and their antitumor effects in model systems. Endocr Relat Cancer 1999;79:109-114.

121. Mitwally MF, Casper RF. Aromatase inhibition reduces the dose of gonadotropin required for controlled ovarian hyperstimulation. J Soc Gynecol Investig 2004;11:406-415.

122. Badawy A, Metwally M, Fawzy M. Randomised controlled trial of three doses of letrozole for ovulation induction in patients with unexplained infertility. RBM Online 2007;14(5):559-562.

123. Bayar U, Basaran M, Kiran S, Coskun A, Gezer S. Use of an aromatase inhibitor in patients with polycystic ovary syndrome: a prospective randomised trial. Fertil Steril 2006;86(5):1447-1451.

124. Biljan MM, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. Fertil Steril 2005;84(supp 1); O-231, Abstract 1033.

125. Fontana PG, Leclerc JM. Contraindication of Femara® (letrozole) in premenopausal women. http:www.ca.novartis.com/downloads/en/letters/ femara_hcp_e_17_11_05.pdf

126. Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, Librach C, Greenblatt E, Casper RF. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril 2006;85(6):1761-1765.

TABLE 1. National Institute of Health Clinical Guidelines for long-term treatment of overweight and obesity

Effective weight loss and long-term results – National Institute of Health Guidelines
1. Sensible diet and changes eating habits for long term
2. Effective physical activity programme sustainable long term
3. Behaviour modification, reduction of stress, wellbeing
4. Combination of dietary and behaviour therapy and increased physical activity
5. Social support by physician, family, spouse, peers
6. Smoking cessation and reduction in alcohol consumption
7. Avoidance of "crash diets" and short-term weight loss
8. Minor roles for drugs involved in weight loss
9. Avoidance of aggressive surgical approaches for majority
10. Adaptation of weight-loss programmes to meet individual needs
11. Long-term observation, monitoring and encouraging of patients who have successfully lost weight

Adapted from [17]

TABLE 2. Principles for treatment of infertility in obese women

Principles for treatment of infertility in obese women
Assessment of BMI and waist circumference / WHR
Assessment of metabolic risk profiles (lipid profile, glucose intolerance), particularly in women with PCOS
Encouraging weight loss through diet/exercise/lifestyle modification a) Energy deficit of ~ 500 – 600 kcal/day b) Moderate exercise/ lifestyle modification c) Diet composition: Fat ≤ 30 % of energy (saturated ≤ 10% of energy, reduce trans fatty acids, increase mono-unsaturated and polyunsaturated fatty acids). Carbohydrate ~ 55% of energy, protein ~ 15% of energy
Reduction of alcohol intake and cessasion of smoking
Reduction of psychosocial stressors
Use of a group environment in providing support, aiding weight loss and maintainance of weight loss
Tailoring intervention to a individual's weight and current dietary and exercise patterns (with use of dietitian of appropriate)

Adapted from [19]

CHAPTER 3

IS THE ADDITION OF METFORMIN EFFICACIOUS IN THE CLOMIPHENE-RESISTANT PCOS PATIENT? (A STRUCTURED LITERATURE REVIEW)

3.1 INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a very common endocrinopathy among infertile female individuals and affects approximately 6% of the general female population[1]. The most prominent presenting characteristics are anovulation and hyperandrogenism.

The diagnosis of PCOS was recently debated and suggestions followed in the Rotterdam consensus statement[2]. This statement concluded that the diagnosis of PCOS could be made if two of the following are present: chronic anovulation, polycystic ovaries on ultrasound, and hyperandrogenism[2].

Insulin resistance and concomitant hyperinsulinemia are frequently found in obese PCOS women (65%)[3,4]. The incidence of insulin resistance among lean PCOS women is nearly 20%[3]. This results in hyperinsulinemia and enhances the LH driven production of androgens from ovarian theca cells[4]. Hyperinsulinemia, insulin resistance and an increase in androgen production are all linked together in PCOS patient[4,5]. It is also known that patients with PCOS and insulin resistance are often resistant to ovulation induction. Is the answer in the management of infertile PCOS women then the use of insulin sensitizers? Previous articles have been published where insulin sensitizers such as biguanides (metformin)[6] and thiazolidinediones (troglitazone), have been used and proven to improve metabolic abnormalities in PCOS patients[7]. Unfortunately, nearly all of these studies were observational studies. (See chapter 2, Discussion Metformin)

Metformin, a biguanide, is normally used in non-insulin dependent diabetes and the mechanism of action includes inhibition of gluconeogenesis in the liver and increasing the peripheral uptake of glucose. Metformin reduces levels of LH, hyperinsulinemia and also decrease ovarian production of androgens[8].

Infertility secondary to chronic anovulation is one of the most common clinical presenting features[1]. Clomiphene citrate (C/C) is the standard drug used for ovulation induction in women with PCOS[9,10,11]. PCOS patients are frequently resistant to C/C and these results in numerous cycles where C/C is unsuccessfully used for ovulation induction. The continuous use of C/C has also been linked to possible higher ovarian cancer risk[12]. (See chapter 2, Discussion Clomiphene) The possible solution for an optimal protocol in ovulation induction is for the clinician to know the

optimal time when to introduce insulin sensitizers to improve ovulation induction among PCOS patients.

The aim of this literature search is to establish if metformin is efficacious when given to the C/C-resistant PCOS patient.

3.2 MATERIALS AND METHODS

This study was reviewed by the Stellenbosch University IRB (2003/013) and approval was given to proceed.

Studies were identified using several search strategies. The National Library of Medicine's MEDLINE database was searched form 01 January 1980-2005. The following medical subject headings (MESH) were used: metformin, ovulation induction, C/C-resistance. The MEDLINE search was performed on titles, abstracts and key words of the listed articles.

Clinical trials comparing two groups of patients were selected only if they met the inclusion criteria and if the outcome data were provided to enable statistical pooling of the data.

Our inclusion criteria were prospective randomised control trials where metformin was randomised either with placebo or C/C to induce ovulation induction in the C/C-resistant patient. The dosage of Metformin used in all articles was 850mg twice a day or 500mg three times a day. Most authors defined C/C-resistance as no response in three consecutive cycles to a maximum dosage of C/C 100-150mg administered day four to eight of the cycle. The primary outcome of interest was ovulation.

3.3 VALIDITY ASSESSMENT AND DATA EXTRACTION

A score was given to each trial using the same scoring system by Soliman *et al*[13]. Six methodological variables namely, randomization, group demographics, placebo use, follow-up, co-intervention and patient cycle differentiation were chosen (Table I). Each trial was assessed and ranked for its methodological rigor and its potential to introduce bias. The methodological strength of each trial was evaluated in a systematic fashion (Table II). Trials were given scores that were divided by the maximum possible score and a percentage performance was given to each trial. Performance scores ranged from 50% to 92% for the studies analyzed.

3.3.1 Statistical analysis

The data on the outcomes of each include trial were summarized in two-by-two tables. The odds ratio (OR) was calculated for the use of metformin in the C/C-resistant patient. The overall combined OR, together with its 95% confidence interval (CI) was calculated using the Mantel-Haentszel method. This statistic is also presented as the overall effect. Statistical significance was inferred with a two-tailed p value of 0.05 or less.

The weight of each study in each analysis was calculated as inversely proportional to the variance. The degree of heterogeneity of studies was calculated using the chi-square test. Where the p-value was < 0.05, the OR and 95% CL are still reported, but the applicable studies were re-analyzed to find an explanation for any differences. We used a fixed effect analysis because we assumed that the intervention was similar in each study. We also applied a random effect analysis to each data set.

3.4 RESULTS

Twenty trials were evaluated. Eight trials compared the efficacy of metformin in the C/C-resistant patient regarding ovulation induction. Six trials met the inclusion criteria and were selected for analysis. Three groups were identified regarding the study structure.

There were no significant differences when the fixed effect analysis model was used in comparison with the random effect analysis model. We therefore report only the results obtained with the fixed effect analysis.

3.4.1 Group 1

Four trials were prospective double-blind placebo controlled[14,15,16,17]. Each of these trials randomised metformin with placebo in the C/C-resistant patient. In one trial[16] there was no difference in outcome. The other three trials[14,15,17] had a statistical significant improvement when metformin was added to C/C in the C/C-resistant patient (Figure 1). When the data of the four trials were pooled the test for the overall effect was p=0.0006 with an OR of 4 and 95%CI of 1.81-8.84.

3.4.2 Group 2

In two of the trials the randomization was only prospective and not double blind[18,19]. Each of these trials prospectively randomised and compared the addition of metformin with placebo in the C/C-resistant patient. In both trials there was a statistical improvement when Metformin was added

(Figure 2). When the data of the two trials were pooled the overall effect was p<0.00001 with an OR of 20.94 and 95% CI of 6.24-70.27.

3.4.3 Combined analysis of groups 1 and 2

The data of these two groups were combined to increase the numbers and to give the meta-analysis more weight (Figure 3). This combined data show an overall effect, p<0.00001 and an OR of 6.82 with a 95% CI of 3.59-12.96.

3.4.4 Group 3

The third group consisted of two trials[20,21]. In these two trials the investigator looked prospectively at a cohort of C/C-resistant patients when metformin was added without randomization.

Batukan and Baysal[20] added metformin to 29 C/C-resistant patients; 65.2% of these patients became pregnant when metformin was added. In the second study by Parsanezhad *et* al.[21], metformin was added to 41 C/C-resistant patients. None of these patients were ovulating before the addition of metformin and 13 (39.39%) ovulated after treatment.

3.5 DISCUSSION

The fertility specialist cannot consider any medical treatment in PCOS patients with anovulation if lifestyle intervention is not practiced. In a study by Norman *et al*.[22], they demonstrated that lifestyle modification led to increased insulin sensitivity and also resulted in improved ovulation and fertility in obese women with PCOS. This approach of lifestyle modification, which includes weight-reducing diet and exercise, should be the first step in the management of the obese patient with PCOS[23].

Two excellent review articles were published recently[24,25]. In the one review[25], the studies by Nestler[18], Malkawi[19] and Sturrock[17] were not included in their analysis. In the other review[24], the study by Nestler[18] was not included. In this review[24] the authors included a study by Yarali[26] where FSH was added, which made the set of data very heterogeneous. Based on the above-mentioned facts and the fact that C/C-resistance is a major problem in the handling of the PCOS patient, we performed another meta-analysis with more articles to our disposal and according to the selection criteria as outlined. For the meta-analysis, we obtained data from four prospective randomised double blind trials and two prospective randomised (not double blind) trials. The data on the first four articles[14,15,16,17] clearly showed a statistical significant effect in favor of ovulation with addition

of metformin. When the data of the two prospective randomised articles[8,19] were pooled with the first mentioned data set it further confirmed the positive effect on ovulation with the addition of metformin in the C/C-resistant patient (Figure 3). Although the prospective randomised studies used in the meta-analysis are strong pieces of evidence in favor of the use of metformin in C/C-resistant patients, we must emphasize the small number of patients in the studies as well as the heterogenous set of data. Future randomised control studies should address this defect. It is interesting to note that the positive effect with the addition of Metformin in the C/C-resistant patient is further strengthened by two cohort studies[20,21].

In contrast with the above-mentioned studies where metformin was added only after C/C- resistance was observed, Fleming *et al.*[27] performed the only prospective double-blind placebo controlled trial where metformin was primarily randomised with placebo in women with oligo-amenorrhoea and PCOS. In this study 45 women used metformin and 47 used placebo. Twenty three percent of the metformin treated group ovulated and only thirteen percent in the placebo group ovulated. This difference was modest, but statistical significant. It is, however, important to note that the dropout rate in the metformin group was 30% due to side effects. The main side effects were nausea and gastrointestinal complications. (See chapter 2, Discussion Metformin)

In a review article by Nestler *et al.*[28] the opinion was expressed that for practical purposes all patients should be regarded as insulin resistant. However, if we compare on the one hand the significant benefit of the addition of metformin in the C/C-resistant patient with on the other hand the results of Fleming *et al.*[27], it will be difficult to conclude that all PCOS women should receive metformin to achieve ovulation. It is our opinion that the side effects must be taken in consideration before prescribing the drug. A percentage of patients will definitely benefit by simple lifestyle measures as well as C/C alone as primary ovulation induction method.

Based on our study it can be concluded that metformin is highly effective in achieving ovulation in the C/C-resistant patient. We also recommend that all obese PCOS patients seeking fertility help should be guided using a lifestyle modification program that should include weight-reducing diet and exercise[22,23]. When this goal is achieved the patient can be started on C/C and if C/C-resistance is present, metformin can be added to achieve ovulation.

3.6 REFERENCES

1. Polson DW, Adams J, Wadsworth J and Franks S. Polycystic ovaries - a common finding in normal women. Lancet 1998;1:870-872.

2. The Rotterdam ESHRE/ASRM sponsored PCOS consensus group. Revised 2003 consensus on diagnostic criteria and long-term health risk related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41-47.
3. Chang RJ, Nakamura RM, Judd HL and Kaplan SA. Insulin resistance in no obese patients with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:356-359.
4. Dunaif A. Insulin resistance and the polycystic ovarian syndrome: mechanism and implications for pathogenesis. Endocrinol Rev 1997;18:774-800.
5. Burghen GA, Givens JR and Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinemia in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113-115.
6. Velazquez EM, Mendoza SG, Hamer T, Sosa F and Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenaemia and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 1994;43:647-654.
7. Erhmann D, Schneider DJ, Sobel BE, Cavaghan MK, Imperial J, Sturis J *et al*. Troglitazone improves defects in insulin action, insulin secretion, ovarian steroid genesis, and fibrinolysis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 1997;82:2108-2116.
8. Nestler JE and Jakubowicz D. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab 1997;82:4075-4079.
9. Shepard MK, Balmaceda JP and Leija CG. Relationship of weight to successful induction of ovulation with Clomiphene citrate. Fertil Steril 1979;32:641-645.
10. O'Herlihy C, Pepperell RJ, Brown JB, Smith MA, Sandri L and McBain JC. Incremental Clomiphene therapy: a new method of treating persistent anovulation. Obstet Gynaecol 1981;58:535-542.
11. Lobo RA, Gysler M, March CM, Goebelsmann U and Mishell DR Jr. Clinical and laboratory predictors or Clomiphene response. Fertil Steril 1982;37:168-174.
12. Rossing MA, Daling JR, Weiss NS, Moore DE and Self SG. Ovarian tumours in a cohort of infertile women. Br J Pharmacol 1994;331:771-776.
13. Soliman S, Daya S, Collind J *et al*. The role of luteal phase support in infertility treatment: a meta-analysis of randomised trials. Fertil Steril 1994;61:1068-1076.
14. Kocak M, Caliskan E, Simsir C and Haberal A. Metformin therapy improves ovulatory rates, cervical scores, and pregnancy rates in Clomiphene citrate-resistant women with polycystic ovary syndrome. Fertil Steril 2002;77:101-106.
15. Vandermolen DT, Ratts V, Evans WS, Stovall DW, Kauma SW and Nester JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patient with

polycystic ovary syndrome who is resistant to clomiphene citrate alone. Fertil Steril 2001;75:310-315.

16. Hung Yu Ng E, Ming Sun Wat N and Chung Ho P. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomised, double-blinded placebo-controlled trial. Hum Reprod 2001;16:1625-1631.
17. Sturrock NDC, Lannon B and Fay TN. Metformin do not enhance ovulation induction in clomiphene resistant polycystic ovary syndrome in clinical practice. Br J Pharmacol 2002;53:469-473.
18. Nestler J, Jakubowich DJ, Evans WS and Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Eng J Med 1980;25:1876-1880.
19. Malkawi HY and Qublan HS. The effect of metformin plus clomiphene citrate on ovulation and pregnancy rates in clomiphene-resistant women with polycystic ovary syndrome. Saudi Med J 2002;23:663-666.
20. Batukan C and Baysal B. Metformin improve ovulation and pregnancy rates in patient with polycystic ovary syndrome. Arch Gynecol Obstet 2001;265:124-127.
21. Parsanezhad ME, Alborzi S, Zarei A, Dehbashi S and Omrani GH. Insulin resistance in Clomiphene responders and non-responders with polycystic ovarian disease and therapeutic effects of metformin. Internat J Obstet Gynecol 2001;75:43-50.
22. Norman RJ, Davies MJ, Lord J and Moran IJ. The role of lifestyle modification in polycystic ovary syndrome. Trends Endocrinol Metab 2002;13:251-257.
23. Clark AM, Thornley B, Tomlinson L, Galletley C and Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod 1998;13:1502-1505.
24. Lord JM, Flight IHK and Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. Brit Med J 2003;327:1-6.
25. Costello MF and Eden JA. A systematic review of the reproductive system effects of metformin in patient with polycystic ovary syndrome. Fertil Steril 2003;79:1-13.
26. Yarali H, Yildiz BO, Demirol A, Zeyneloglu HB, Yigit N, Bukulmez O, Koray Z. Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomised trial. Hum Reprod 2002;17:289-294.
27. Flemming R, Hopkinson ZE, Wallace AM, Greer IA and Sattar N. Ovarian Function and metabolic factors in women with oligomenorhea treated with metformin in a randomised double blind placebo-controlled trial. J Clin Endocrinol Metab 2002;87:569-574.

28. Nestler JE, Stovall D, Akhter N, Luorno MJ and Jakubowicz DJ. Strategies for the use of insuline-sensitizing drugs to treat infertility in women with polycystic ovary syndrome. Fertil Steril 2002;77:209-215.

TABLE 1. Validity criteria and scoring for methodology assessment of studies

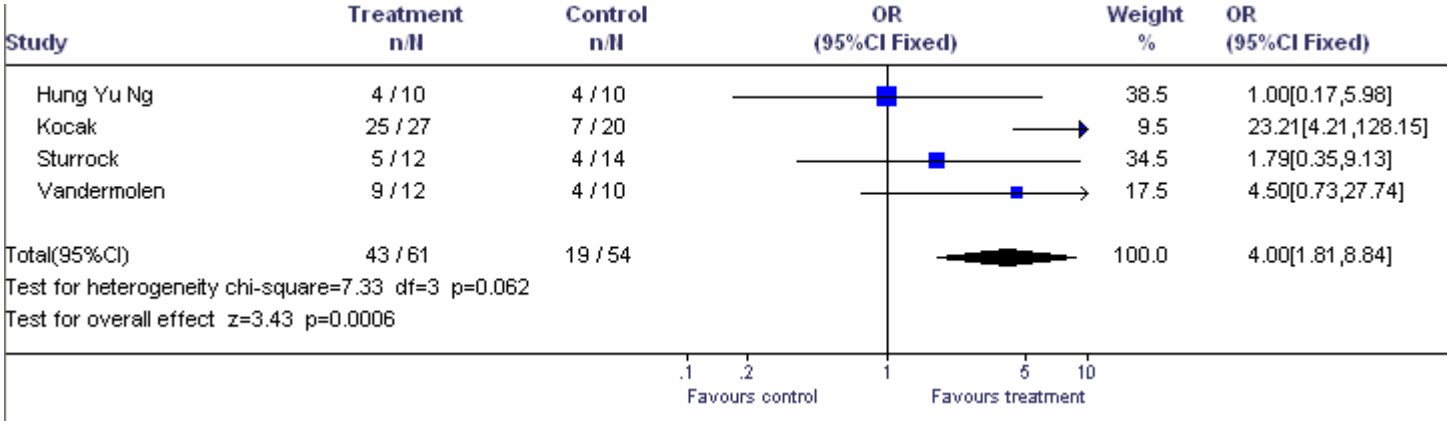
Category	Score	Method
A. Randomization	3	Randomised by central means (telephone and pharmacy) or sealed accounted envelopes.
	2	Alternating numbers.
	1	Methods not described.
B. Group Demographics	2	Demographics comparable.
	1	Demographics not described.
C. Placebo use	2	Placebo or other treatment used in control group.
	1	No placebo or other treatment.
D. Follow-up	2	Outcome data for primary analysis complete.
	1	Outcome data incomplete.
E. Co-intervention	2	Other than for use of treatment versus control, protocol involved same drugs.
	1	Difference in protocols that may lead to contaminated results.
F. Patient and cycle differentiation	3	Only first treatment cycle included.
	2	Patients included for more than 1 cycle.
	1	Cycles and patients not differentiated.

TABLE 2. Validity criteria score

Study	Score	Randomization	Demo-graphics	Placebo/ Other	Follow-up	Co-intervention	Cycles	Total
Batukan	50%	0	1	1	2	1	2	7
Parsenezhad	57%	0	2	1	2	1	2	8
Nestler	85%	2	2	2	2	2	2	12
Malkawi	78%	2	1	2	2	2	2	11
Hung Yu Ng	92%	3 computer/ sealed envelopes	2	2	2	2	2	13
Sturrock	85%	2	2	2	2	2	2	12
Kocak	92%	3 sealed envelopes	2	2	2	2	2	13
Vandermolen	92%	3 computer generated	2	2	2	2	2	13

FIGURE 1.

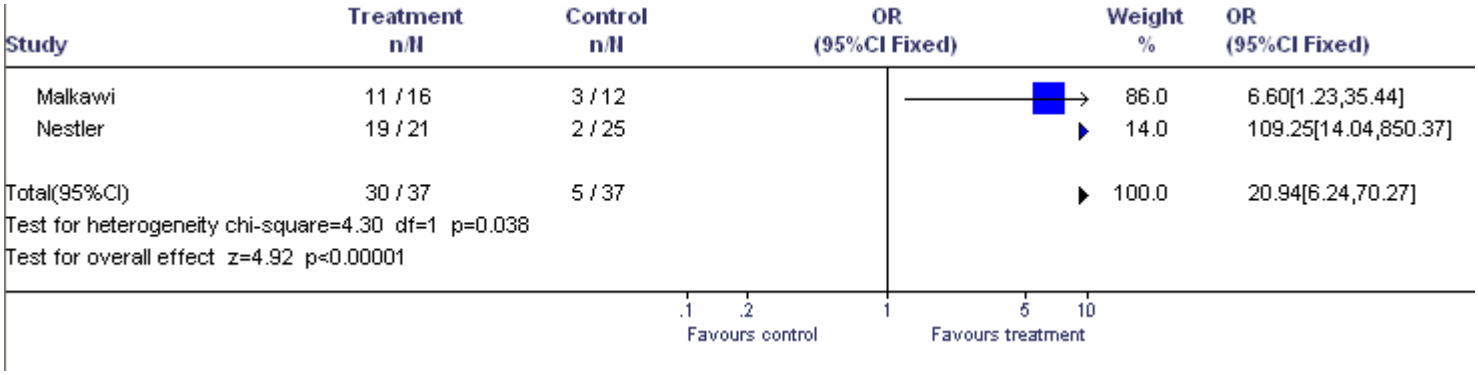
Group 1: Four trials where the addition of metformin was randomised in a prospective double-blind placebo controlled fashion in the C/C resistant patient



n/N = the number of women where ovulation induction was achieved / the total number of women in the group

FIGURE 2.

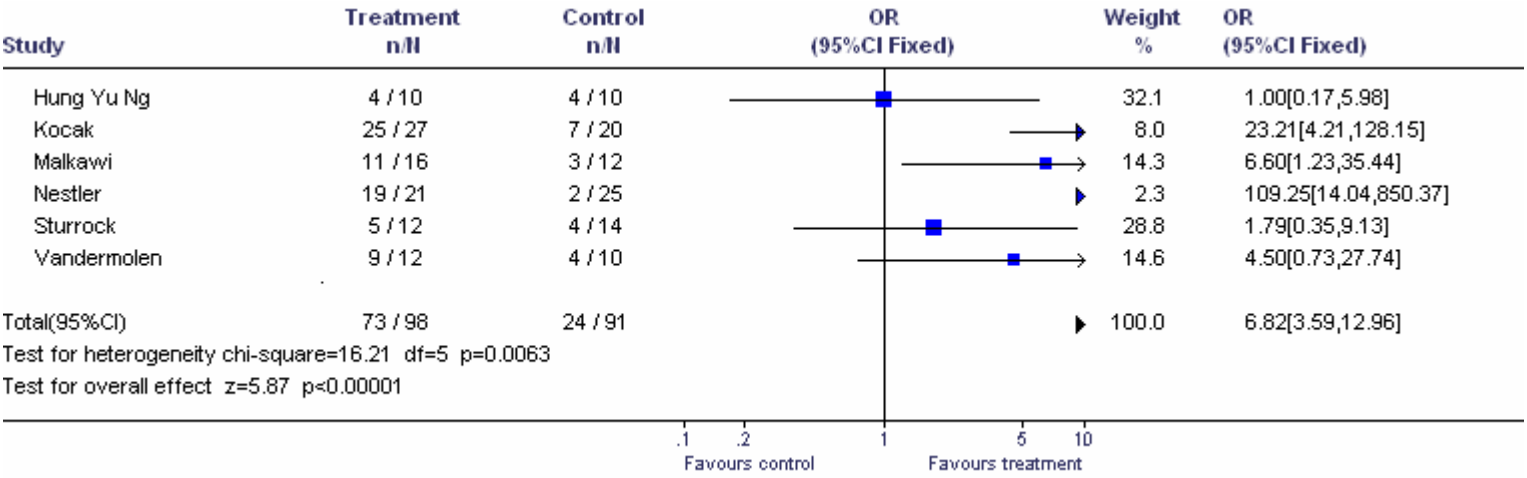
Group 2: Two trials where the addition of metformin was prospectively randomised in the C/C resistant patient



n/N = the number of women where ovulation induction was achieved / the total number of women in the group

FIGURE 3.

Group 3: Pooled data of group 1 and 2.



n/N = the number of women where ovulation induction was achieved / the total number of women in the group

CHAPTER 4

EVALUATING THE EQUIVALENCE OF CLOMIPHENE CITRATE WITH AND WITHOUT METFORMIN IN OVULATION INDUCTION IN PCOS PATIENTS: A RANDOMISED CONTROLLED TRIAL

4.1 INTRODUCTION

Polycystic ovary syndrome is one of the most common endocrinopathies, affecting 5-10% of women of reproductive age[1]. Various criteria have been proposed for the diagnosis of PCOS which hampered research into this common disorder[2,3]. Fortunately, in 2003 a joint consensus meeting between the American Society of Reproductive Medicine and the European Society of Human Reproduction and Embryology proposed a unifying definition[4]. Oligo-anovulation due to ovarian dysfunction continues to be the pivotal feature that makes this syndrome the major cause of anovulatory infertility in developed countries[5].

Clomiphene citrate(C/C) was the first agent used in experiments for ovulation induction in oligomenorrheic women[6]. For many years it was and may still be the first therapeutic option managing anovulatory infertility. The treatment with C/C in anovulatory PCOS women is related to an ovulation rate of 60-85% and a pregnancy rate of 30-40%[7]. Reasons for this discrepancy may be due to the anti-oestrogenic effect of C/C acting at both an endometrial and ovarian level, in addition to the development of a hostile cervical mucus[8].

The addition of metformin to C/C in C/C-resistant women significantly improves the ovulation rate. The meta analysis in a Cochrane review reported a significant benefit for metformin compared to placebo for ovulation in anovulatory women with PCOS[9]. Another metanalysis showed a significant positive effect of metformin when added to C/C in the C/C-resistant PCOS patient[10]. The first head to head study comparing C/C with metformin has recently been published[11]. It demonstrated that both medications are highly effective for ovulation induction, but that metformin use results in higher cumulative pregnancy rates. However, this study was restricted to young, lean PCOS women without glucose tolerance problems or tubal or male factors.

From the available data it remain unclear whether the addition of metformin to C/C is superior to C/C alone as a primary induction agent and where metformin should be introduced in ovulation induction protocols in the PCOS patient.

The aim of this study was to evaluate the benefit of metformin if added to C/C in a primary ovulation induction protocol in comparison to C/C alone.

4.2 MATERIALS AND METHODS

4.2.1 Patients

This study was approved by the Ethical Committee of Stellenbosch University at Tygerberg Academic Hospital (*2003/013*). Informed consent was obtained from each patient involved. A total number of 107 patients diagnosed with PCOS were enrolled for ovulation induction in a treatment period of 15 months. The inclusion criteria required that all couples needed to present with a history of infertility for at least 18 months. The diagnosis of PCOS was based on the recent Rotterdam consensus statement. All patients had a complete infertility and PCOS work up consisting of weight and body mass index (BMI), hysterosalpingogram(HSG), basal hormonal tests (FSH, LH, TSH, Prolactin, 17-OH Progesterone, DHEAS, SHBG, Testosterone, fasting insulin, fasting glucose and fasting lipid profile.), semen analysis on the husband and where indicated a diagnostic hysteroscopy and laparoscopy was performed. Patients with known tubal factors, azoospermia or severe oligoteratozoospermia were excluded from this study. The Tygerberg strict criteria was used to evaluate the sperm morphology and the rest of the semen parameters according to the WHO manual 1999[12].

All obese patients (BMI >25) were informed to loose at least 5% of their weight and to participate in exercise for at least 40 minutes per day 3 days per week. They were motivated regarding short-term positive impact of weight loss regarding ovulation induction and long term benefits on development of Diabetes Mellitus, ischaemic heart disease and lipid abnormalities.

4.2.2 Study

This was a prospective randomised controlled trial of 107 consecutive PCOS patients. *Inclusion criteria*: all patients diagnosed with PCOS were included. The diagnosis of PCOS was according to the recent Rotterdam consensus statement. *Exclusion criteria*: patients known with tubal factors, azoospermia, severe oligoterato-zoospermia or any other reason for anovulation or hirsutism were excluded.

The randomization was computer generated and patients were randomised into two groups. Group A received pre treatment with metformin 850mg twice a day for at least 6 weeks before C/C was added and the metformin was used throughout the study period. Group B received C/C without pre treatment of metformin. In both groups C/C was given at a starting dose of 50mg day 4-8 and increase with increments of 50mg to a maximum of 150mg if no response was achieved. We did

not pre-treat patients in group B with placebo. This is a definite shortcoming of the study. However, the drop-out rates in the 2 groups were similar.

The patients were followed up with transvaginal ultrasound to record follicular growth and endometrial response. Day 21 progesterone was drawn to confirm ovulation. (Figure 1)

4.2.3 Statistical Analysis

An intention to treat analysis was performed for the primary outcome ovulation success.

For a full intention to treat analysis, we have to include all randomised women, those who were lost to follow-up too.

A secondary analysis of the patient factors associated with ovulation was also performed. The Mann-Whitney test was used for the comparison of the ovulation and non-ovulation groups with respect to characteristics such as 17OH Progesterone, Testosterone, SHBG and fasting insulin. For the significant factors identified in this analysis a further logistic regression analysis was done of ovulation success on the specific factor with adjustment for a treatment effect.

4.3 RESULTS

The results are presented in different sections. The first part is the primary analysis to test the equivalence in ovulation between the two treatment arms. The second part is a secondary analysis to assess the possible association between the different factors and ovulation.

4.3.1 Intention to treat analysis (Table 1)

The intention to treat analysis, which include the patients who were lost to follow up too, was performed. (Table 1)

In the M+C/C arm 34/52 (65.4%) achieved ovulation compared to 36/55 (65.5%) in the C/C alone arm. The estimated mean different is 0% with 90% confidence intervals -16% to 18%. Since this interval does not fit within the equivalence interval (-10% to 10%) we cannot conclude equivalence.

The intention to treat analysis strengthens the conclusion that metformin should not be added to C/C since the ovulation rates achieved, as intended when randomizing the women in the trial, were identical.

4.3.2 Primary outcome (Table 2)

The ovulation rate achieved in women in the M+C/C arm was 34/42 (81%) compared to 36/48 (75%) in the C/C arm. (Table 2) The treatment effect ((M+C/C) –C/C) is 6% with 90% confidence interval of -9% to 20%. Since this interval does not fit within the equivalence interval we cannot conclude equivalence. Using the confidence interval we can also not conclude superiority of metformin and C/C versus C/C alone since the interval spans 0%, the reference value of no difference between the arms. In this analysis, the patients who were lost to follow-up were excluded.

4.3.3 Patient Characteristics

In the metformin + C/C group, 42/52 women had a positive outcome (81%) compared to 48/55 in C/C group (87%). The total loss to follow-up was 17 patients (16,3%). 10 patients were lost to follow-up in group A, and 7 patients were lost to follow-up in group B.

Since the duration of the treatment is different with M+C/C being much longer one would expect this arm to have a higher dropout. The follow-up achieved was similar in both groups. The baseline characteristics in the two arms of the study were similar. There were 2 women diagnosed with pregnancy before follow-up. These two women received C/C 50mg and did not attend their first follow-up. They were regarded as having had a successful ovulation at 50mg.

4.3.4 Comparison of the dosage level of ovulation success or failure (Table 3)

The estimated treatment effect by C/C dosage show an increased effect by dose. However the sample size within each dose is small and a test for a dose by treatment effect is not significant, p=.414. The 90% confidence intervals for the estimated treatment effect is also given for completeness. (Table 3)

4.3.5 Determinants of ovulation

The descriptive characteristics of the factors considered as possible determinants for ovulation is mentioned in the Materials and Methods section. These factors were weight and body mass index (BMI), hysterosalpingogram(HSG), basal hormonal tests (FSH, LH, TSH, Prolactin, 17-OH Progesterone, DHEAS, SHBG, Testosterone, fasting insulin, fasting glucose and fasting lipid profile.), and a semen analysis.

The Mann Whitney test was used to do a non-parametric comparison of ovulating versus non-ovulating women, for each of the factors, to assess if any of these factors were associated with ovulation outcome.

From this analysis, weight (p=.021), DHEAS (p=.05), 17OH-progesterone (p=.027), SHBG (p=.036) and BMI (p=.009) were significant factors. Marginal risk factors for ovulation outcome were height (p=.097) and fasting glucose (p=.085).

To further evaluate the factors affecting ovulation, a logistic regression model was used where the factors found above were evaluated with an adjustment for a treatment effect. The variable SHBG is a significant factor after adjustment for treatment with odds ratio (OR) 1.04; 95% CI :1.0 to 1.07; p=.049. It is positively associated with ovulation.

The variables 17OH-progresterone (OR=.82; 95%CI: .67 to .99; p=.043), BMI (OR=.90; 95%CI: .82 to .98; p=.0.018) and weight (OR=.97; 95%CI: .94 to 1.0; p=.049) were also significant factors after adjustment for treatment. These factors were negatively associated with ovulation. In this study all women with a BMI below 27 kg/m^2 achieved ovulation irrespective of treatment received.

The variables DHEAS and fasting glucose were no longer significant factors after adjustment for treatment.

4.4 DISCUSSION

In the treatment of women with PCOS who wants to get pregnant our study could not establish equivalence or find any benefit of adding metformin to C/C comparing to the standard treatment with C/C alone in women receiving these options as primary induction choice. We found no significant differences in outcome of ovulation induction in the two different groups studied. We also observed no difference in the discontinuation rate between the two groups.

In addition to the results of our study, four prospective randomised controlled trials were recently published[11,13,14,15]. The primary aim of these studies was to compare C/C with metformin alone or in combination when studied as primary ovulation induction agents in women with PCOS. In the first study by Moll et al[13], they prospectively randomised 228 women. The primary aim of this study was the ovulation rate. The ovulation rate in the metformin and C/C group was 64% compared with 72% in the placebo and C/C group which was not statistical significant. There was

no difference in the pregnancy rates or the abortion rates of the 2 groups and the mean BMI was 28 in both groups.

In the second study by Legro et al[14], 626 PCOS patients were randomised. The primary outcome of their study was live birth rates. They concluded that C/C (22,5%) is superior to metformin (7,2%) but similar to the combination group (26,8%) in achieving live birth rates. As a secondary outcome ovulation was addressed, again metformin alone performed significant worse than C/C alone or the combination of C/C and metformin. They did not observe any difference in the abortion rates between the 3 groups and observed a significant better live birth rate if the BMI is less than 30 regardless the treatment option used.

In the third study by Neveu et al[15], they prospectively randomised 154 patients with PCOS. In this study they observed a significant better ovulation rate when on metformin alone (75,4%) compare to C/C alone(50%). In the combination group of C/C and metformin the ovulation rate (63,4%) was not significantly different to the metformin alone but significant better than the C/C alone. However, pregnancy rates were equivalent in the three groups. They also observed a better ovulatory response in the women with a lower BMI in the C/C group and patients with a BMI of 27-35 responded better to metformin for ovulation induction. The mean BMI of the study was 31. This study had a better ovulation rate in the metformin and the combination group, but no difference in pregnancy rates between the three groups. These three authors concluded that it is not beneficial to add metformin to C/C in primary ovulation induction protocols.

In the fourth study by Palombo et al[11], they included 100 PCOS women. In this study, they too did not observe any difference between the metformin group and the C/C group regarding the ovulation rates. However, when analyzing the data regarding cumulative pregnancy rates there were a significant better rate 15,1% in the metformin group versus a 7,2% in the C/C group. It is however important to note that women with a BMI>30 were excluded from this study and they concluded that metformin was superior to C/C in achieving a live birth.

In one of the first studies to address this topic, Nestler et al[16] conducted a multicenter study. In this study they studied 61 obese PCOS women. They concluded that spontaneous ovulation induced by C/C may be increased in obese women with PCOS by decreasing serum insulin concentrations with metformin. This was not a prospective randomised control trial and it was also a very small study.

In our study, we prospectively randomised 107 patients and 17 (16,3%) patients were lost to follow up. In the study by Moll et al[13], they lost 63 (27,6%) patients to follow up. In their study more patients were lost in the metformin group, which might have been due to the side effects. In contrast, in our study a similar number of women were lost to follow up in the two groups studied.

If we look at the primary characteristics of the two groups in our study, no significant differences were noted. The combination of metformin and C/C had a 6% better ovulation rate as C/C alone. This trend may only be a chance effect or may be significant if the numbers were more.

In the study by Moll et al[13] a similar outcome to our study was observed, however Legro et al[14] observed a poorer ovulation rate in the metformin group versus C/C alone or the combination of the two drugs. In contrast, Neveu et al[15] observed a poorer ovulation rate in the C/C alone group versus the metformin or metformin and C/C combination. However, when these authors commented on live birth rates, Moll et al[13] and Neveu et al[15] documented no difference between the two groups, but Legro et al[14] observed a significant lower rate in the metformin group versus the C/C alone or combination group. These authors concluded that metformin should not be added to C/C in primary ovulation induction regimens.

In the secondary analysis of our study, we observed that all patients ovulated with a BMI<27. With a BMI>27 there was no difference in ovulation between C/C alone or metformin and C/C. Legro et al[14] observed a significantly higher rate of live births in women with a BMI less than 30 when compared to those with a BMI more than 30. However, in the study by Neveu et al[15], they observed a better outcome when metformin was added in the more obese group, BMI 27-35. This improved outcome on metformin in the more obese patients was also observed in the study by Nestler et al[16]. In the study by Palombo et al[11] they unfortunately excluded women with a BMI more than 30.

As part of the secondary analysis of our study, the Mann Whitney test was performed to test for an association of any of the characteristics and unsuccessful ovulation. From this analysis we can extrapolate that weight/BMI (P=.009) was the major predictive factor. This is a very important finding and supports current literature to optimize the BMI first, loose weight if needed, before commencing with any ovulation induction regimen[17].

Other important factors observed in the current study were SHBG (sex hormone binding globulin) P=.036 and 17hydroxy progesterone (17OH Progesterone) P=.027. The variable SHBG was a significant factor and positively associated with ovulation. The physiological effect of SHBG is a

lowering of the free androgen index. This may lead to an improved ovulation outcome. In a study by Ghazeeri et al[18], rosiglitazone was administered to 25 obese, C/C-resistant, PCOS women who desired pregnancy. They observed a significant improvement in ovulation rates when rosiglitazone was added to C/C in this study. One of the important findings was a significant rise in SHBG in the group of women treated with rosiglitazone. Our study confirms this finding of improved ovulation rates with a higher SHBG level. Several other investigators have similarly observed an increase in SHBG and a decrease in testosterone and androgenicity with improved conception rates in patients with weight loss[19,20]. In a recent Cochrane review it was concluded that metformin significantly reduced androgen levels[9]. This subgroup of women with PCOS and high androgen levels may have an improved outcome when metformin is added for ovulation induction. However, more data are required before it can be concluded that this subgroup is a definite indication for the use of metformin. The variable 17OH-progesterone was also a significant factor and was negatively associated with ovulation. The factors fasting glucose and insulin had no positive or negative association with ovulation.

Based on the results of this trial, we cannot exclude the possibility that the addition of metformin may lead to an increase in the ovulation rate of 6%. This 6% may be a chance effect or it might have been that if the study was bigger, the difference may have been significant. The sample size (n=107) was the biggest limitation of our study. However, two other prospective randomised control trials had similar outcomes to our study[13,14] regarding ovulation outcome. All three authors[13,14,15] concluded that metformin should not be added in primary induction protocols. In a recent meta-analysis, it was found that the addition of metformin is beneficial when added to C/C in the C/C-resistant PCOS women[10]. Based on the results of our study and the trials discussed, we conclude that metformin should not be added to C/C as a primary method for ovulation induction in women with PCOS. The addition of metformin is advised in the C/C-resistant PCOS women. However, it is of utmost importance that all obese PCOS women should first be placed on an active exercise and weight loss programme before any treatment is offered.

4.5 REFERENCES

1. Frank S. Polycystic ovary syndrome. N Engl J Med 1995;333:853-861.
2. Zawadzki JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome: toward a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriman GR, Polycystic ovary syndrome. Boston: Blackwell; 1992;337 – 384.

3. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.
4. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19-25.
5. Hamilton-Fairley D, Taylor A. Anovulation. BMJ 2003;327-546-549.
6. Holtkamp DE, Greslin JG, Root CA, Lerner LJ. Gonadotropin inhibiting and anti-fecundity effects of chloramiphene,1960. Proc Soc Exp Biol Med 105:197-201
7. Hughes E, Collins J, Vanderkerckhove P, Lilford R. Clomiphene citrate for ovulation induction in women with oligo-amenorrhoea. Cochrane Database Syst Rev 2000;CD000056.
8. Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. Hum Reprod Update 1997;3:359-365.
9. Lord JM, Flight IHK, Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 2003;327:951-956.
10. Siebert IT, Kruger, TF, Steyn DW, Nosarka S. Is the addition of metformin efficacious in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome? A structured literature review. Fertil Steril 2006;86:1432-1437
11. Palomba S, Orio F, Falbo A, Manguso F, Russo T, Cascella T, et al. Prospective parallel randomised, double-blind, double-dummy controlled clinical trial comparing Clomiphene Citrate and Metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with Polycystic Ovary Syndrome. J Clin End Metab 2005;90:4068-4074.
12. World Health Organization. World Health Organization Laboratory Manual for Examination of Human Semen. Cambridge: Cambridge University Press; 1999
13. Moll M, Bossuyt PMM, Korevaar JC, Lambalk CB, Van der Veen F. Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double-blind clinical trial. BMJ 2006;332:1485-1489.
14. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, et al. Clomiphene, Metformin, or both for Infertility in the Polycystic Ovary Syndrome. N Engl J Med 2007;356:551-566.
15. Neveu N, Granger L, St-Michel P, Lavoie HB. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction and achievement of pregnancy in 154 women with polycystic ovary syndrome. Fertil Steril 2007;87:113-120.

16. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the Polycystic Ovary Syndrome. N Engl J Med 1998;338:1876-1880.
17. Norman RJ, Davies MJ, Lord J, Moran LJ. The role of lifestyle medication in polycystic ovary syndrome. Trends Endocrinal Metab 2002;13:251-257.
18. Ghazeeri G, Kutteh WH, Bryer-Ash M, Haas D. Effect of rosiglitazone on spontaneous and clomiphene citrate induced ovulation in women with polycystic ovary syndrome. Fertil Steril 2003;79;562-566
19. Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod 1998;13;1502-1505
20. Kiddy DS, Hamilton-Fairley D, Bush A et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 1992;36;105-111.

FIGURE 1: **Study Flowchart**

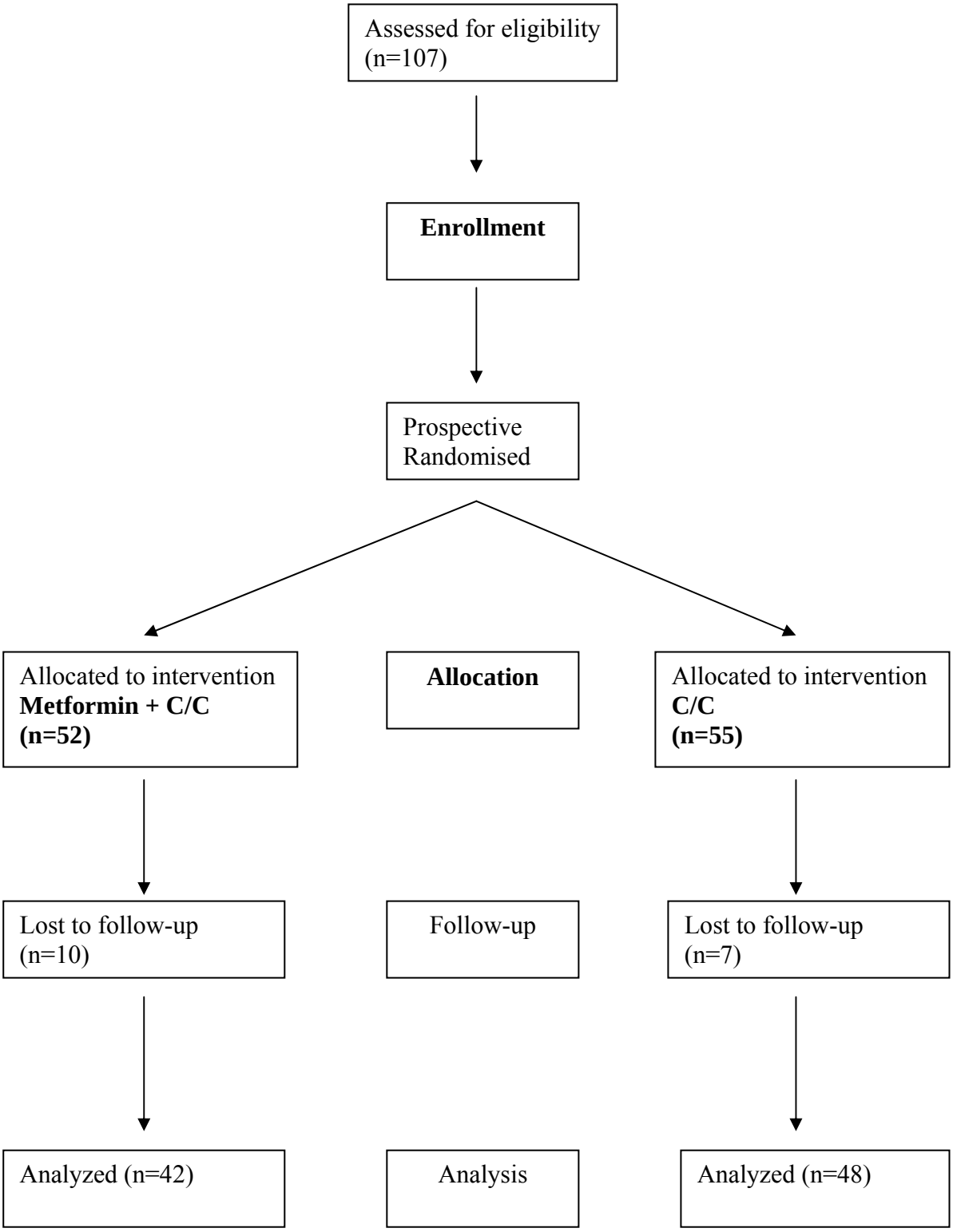


TABLE 1. Intention to treat analysis.

Table 1	**M+C/C***	**C/C****	**TOTAL**
Ovulation (column%)	34 (65.38%)	36 (65.45%)	70 (65.42%)
Non-ovulation (column%)	18 (34.62%)	19 (34.55%)	37 (34.58%)
TOTAL (column%)	52 (100.00%)	55 (100.00%)	107 (100.00%)

*M = Metformin

**C/C = Clomiphene Citrate

TABLE 2: Ovulation rates in the two treatment arms (Unpaired Samples)

	Sample M+C/C	Sample C/C	Total
Number with ovulation	34	36	70
Number without ovulation	8	12	20
Sample Size	42	48	90
Proportions	0.810	0.750	
Difference	0.060		

90% Confidence Interval for the difference -0.087 to 0.199 Recommended (Newcombe) Method

Standard Error of difference 0.087 Normal Value 1.650

M=Metformin

C/C=Clomiphene citrate

TABLE 3: Ovulation outcome: Dosage comparison

Dose	M+C/C (%)	C/C (%)	Difference	90% CI(lower to upper)	p-value
50mg	20/27 (74)	18/23 (78)	4	-31 to 19	.776
100mg	11/12 (92)	11/14 (79)	13	-20 to 49	.566
150mg	3/3 (100)	7/11 (64)	36	-22 to 77	.332
All	34/42 (81)	36/48 (75)	6	-9 to 20	.592

The estimated treatment effect by C/C dosage show an increased effect by dose. However the sample size within each dose is small and a test for a dose by treatment effect is not significant, p=.414. The 90% confidence intervals for the estimated treatment effect is also given for completeness.

CHAPTER 5

HOW DO WE DEFINE MALE SUBFERTILITY AND WHAT IS THE PREVALENCE IN THE GENERAL POPULATION?

5.1 INTRODUCTION

Several semen parameters are used to discriminate the fertile male from the sub-fertile male. The most widely used parameters are sperm concentration, motility, progressive motility and sperm morphology. Of these parameters, the sperm morphology is the single indicator most widely debated in the literature. A large number of classification systems have been used to describe which factors constitute a morphologically normal/abnormal spermatozoon. The most widely accepted classification systems for sperm morphology are World Health Organization (WHO) criteria of 1987 and 1992[1,2] and Tygerberg strict criteria, now also used by the WHO since 1999.[3-6]

Although there is a positive correlation between normal semen parameters and male fertility potential, the threshold values for fertility/sub-fertility according to WHO criteria[1,2] are of little clinical value in discriminating between the fertile and sub-fertile male.[7-11] If these criteria were applied, a great number of fertile males (partners having had pregnancies shortly before, after, or at the time of a spermiogram) were classified as sub-fertile. The predictive values of sperm morphology using strict criteria in *in vitro* fertilization (IVF) and intrauterine insemination (IUI) have been reviewed recently and proved to be useful.[12,13] Much less has been published on the use of this criterion regarding *in vivo* fertility.

5.2 AIM

In this chapter we will evaluate the classification systems for semen parameters after review of the literature published in English on semen parameters and *in vivo* fertility potential. We will also use data from the literature to establish fertility/sub-fertility thresholds for semen parameters and the WHO 1999 guidelines.[3-6] These thresholds should be of clinical value and useful when assessing male fertility potential for *in vivo* conditions in order to identify those males with a significantly reduced chance of achieving success under *in vivo* conditions. In general there is also quite a poor level of understanding and evidence regarding the profile of the semen analysis of the general population.

Therefore, we believe that possibly the best reference group to study the semen profile in a general population is the semen of partners of women who have been diagnosed to have chronic anovulation/PCOS.

5.3 WHO CRITERIA OF 1987 AND 1992 AND MALE FERTILITY POTENTIAL

The semen analysis is used in clinical practice to assess the male fertility potential. To be of clinical value the methods used for semen analysis should be standardized and threshold values for fertility/sub-fertility should be calculated for the different parameters used in standard semen analysis.

Because there are so many different methods for semen evaluation, it would be difficult to standardize the methods used in semen analysis. This applies especially to the assessment of sperm morphology. The two classification systems most widely accepted are the WHO[1,2] and the Tygerberg strict criteria.[3-6] Various methodological problems concerning sperm morphology have been identified. The variants among different methods of morphology assessment have been shown by Ombelet et al.[14-16] and others[17,18] and they recommended standardization of semen analysis methodologies. Some authors recommend that laboratories should adopt the accepted standards such as those proposed by the WHO.[17,18] Another problem identified is the variation in intra-and inter-individual and inter-laboratory sperm morphology assessment.[18,19] This problem could be addressed by using the Tygerberg strict criteria.

Menkveld et al. showed that comparable and reliable results between and within observers could be obtained when using this method.[19] Franken et al. delivered dedicated work on continuous quality control programs for strict sperm morphology assessment and showed that consistent reading could be achieved and thus urged for global quality control measurements in andrology laboratories.[20,21] Cooper et al.[18] also urged for standardization of such quality control programs and that quality control centres should reach agreement with each other.

Previous WHO thresholds of 50% and 30% for sperm morphology were empiric values and not based on any clinical data. Several authors found these values to be of little or no clinical value.[7,9,10] These studies did, however, find a positive correlation between the high proportion of morphologically normal sperms and the increased likelihood of fertility and/or pregnancy. Other studies confirmed this correlation.[22-25]

Van Zyl et al.[25] were the first to show a faster than linear decline in fertilization rate when the proportion of normal forms dropped to less than 4%. Eggert-Kruse et al.[23] found a higher *in vivo* pregnancy rate for higher percentage normal forms at thresholds of 4, 7 and 14% using strict criteria for morphology assessment. Zinaman et al. confirmed the value of sperm morphology (strict criteria) by demonstrating definite decline in pregnancy rates in vivo, when the normal morphology

dropped below 8% and sperm concentration below $30x10^6$/ml.[26] In a study performed by Slama et al.[27], measuring the association between time to pregnancy and semen parameters, it was found that the proportion of morphologically normal sperm influenced the time to pregnancy up to a threshold value of 19%. This value is somewhat higher than that calculated in other studies.

5.4 THE USE OF SEMEN PARAMETERS IN IVF AND IUI PROGRAMS

The percentage of normal sperm morphology (strict criteria) has a positive predictive value in IVF and IUI programs. Normal sperm morphology thresholds produced positive predictive values for IVF success when using the 5% and the 14% thresholds, respectively, with the overall fertilization rate and overall pregnancy rates significantly higher in the group with normal morphology bigger or equal than 5% as compared with the smaller than 5% group.[12] A meta-analysis of the data on IUI programs showed a higher pregnancy rate per cycle in the group with normal sperm morphology of equal to or bigger than 5%. In the group with normal sperm morphology less than 5%, other semen parameters proved to be predictive IUI success.[13] In the IUI analysis, motility[28], total motile sperm count[29] and concentration[30] also played a role in some of the studies evaluated, whilst others[31] stated that sperm morphology alone was enough to predict the prognosis. Because of the high cost of assisted reproduction, males with good or reasonable fertility potential under *in vivo* conditions should be identified on the basis of semen quality. Conversely, males with a poor fertility potential should be identified and introduced to assisted reproduction programs.

5.5 FERTILITY/SUBFERTILITY THRESHOLDS FOR SPERM MORPHOLOGY USING TYGERBERG STRICT CRITERIA, SPERM CONCENTRATION AND SPERM MOTILITY/PROGRESSIVE MOTILITY

In an effort to establish fertility/sub-fertility thresholds for the abovementioned parameters we identified four articles in the published literature. It is our opinion that these articles constitute a representative sample of studies published on the predictive value of sperm morphology, sperm concentration and motility/progressive motility for *in vivo* fertility/sub-fertility. These articles compared the different semen parameters of a fertile and a sub-fertile group. They used either the classification and regression tree (CART) analysis or the receiver operating characteristic (ROC) curve analysis to estimate thresholds for the different semen parameters. The ROC curve was also used to assess the diagnostic accuracy of the different parameters and their ability to classify subjects into fertile and sub-fertile groups.

Using ROC curve analysis, Ombelet et al.[32] calculated the following thresholds: proportion normal morphology 10%, proportion normal motility 45% and normal sperm concentration $34x10^6$/ml. The

sperm morphology was shown to be the best parameter with the highest prediction power (area under curve or AUC 78%). Much lower thresholds were calculated using the 10th percentile of the fertile population, these thresholds being 5% for normal morphology, 28% for motility and 14.3x10^6/ml for sperm concentration (table 1 and 2).[32]

Günalp et al.[33] also calculated thresholds using ROC curve analysis. The thresholds were proportion normal morphology 10%, proportion normal motility 52%, proportion progressive motility 42% and sperm concentration 34x10^6/ml. The two parameters that performed best were progressive motility (AUC 70.7%) and morphology (AUC 69.7%). Assuming 50% prevalence of sub-fertility in the population, the authors used the positive predictive value as indicator to calculate a lower threshold for each parameter. Values of 5% for proportion normal morphology, 30% for proportion normal motility, 14% for proportion progressive motility and 9x10^6/ml for sperm concentration were calculated (Tables 1 and 2).[33]

In the most recent article of the four, Menkveld et al.[34] found much lower thresholds than the others. Using ROC curve analysis, the following thresholds were calculated: 4% for normal morphology and 45% for normal motility. The morphology again showed a good predictive value with an AUC of 78.2%. Although a threshold for sperm concentration was not calculated (a sperm concentration smaller than 20x10^6/ml was used as inclusion criterion), the authors proposed that the cut-off value of 20x10^6/ml could be used with confidence, based on the resultant lower 10t^h percentile of the fertile population. Adjusted cut-off points calculated on the assumption of 50% prevalence of male sub-fertility were as follows: 3% for proportion normal morphology and 20% proportion normal motility (Tables 1 and 2).[34]

In the fourth article by Guzick et al.,[35] the authors used the CART analysis and calculated two thresholds for each semen parameter which allowed for designation in three groups, namely normal (fertile), borderline and abnormal (sub-fertile). The normal (fertile) group had values greater than 12% for morphology, greater than 63% for motility and higher than 48x10^6/ml for sperm concentration. The abnormal (sub-fertile) group had values lower than 9% for morphology, lower than 32% for motility and lower than 13.5x10^6/ml for sperm concentration.

In these four articles the predictive power of the different parameters were calculated as its AUC using the ROC curve. The AUC for sperm morphology ranged from 66-78.2%, confirming the high predictive power of sperm morphology. In fact, it had the best performance of the different semen parameters in two articles.[32,35] The threshold calculated in these two articles were 10% and 9%

respectively while Günalp et al.[33] calculated a threshold of 12% using sensitivity and specificity to analyze their data and the fourth study calculated a 4% predictive cut-off point value. Although sensitivity and specificity for the values are relatively high, the positive predictive values are not. This will result in classifying fertile males as sub-fertile, therefore, probably leading to a degree of anxiety and unnecessary and costly infertility treatment. A second and much lower threshold was calculated in three of the four articles. Ombelet et al.[32]calculated their second and much lower threshold by using the 10th percentile of the fertile population while Günalp et al.[33] screened the population with the positive predictive value as indicator and Menkveld et al.[34] assumed a 50% prevalence of sub-fertility in their study population. The lower threshold ranged from 3 to 5% (Table 2). These lower thresholds have a much higher positive predictive value than the higher thresholds with the negative predictive value not much lower.

We suggest that the lower threshold should be used to identify males with the lowest potential for a pregnancy under *in vivo* conditions. Values above the lower threshold should be regarded as normal. These findings are in keeping with previous publications by Coetzee et al.[12] (IVF data) and Van Waart et al.[13] (IUI data) which showed a significantly lower chance of successful pregnancies in males with normal morphology below their calculated thresholds.

The higher threshold values for percentage motile sperm as calculated in the four articles (using ROC curve or CART analysis) ranged from 32 to 52% while the lower threshold values ranged from 20 to 30%. Motility also had a high predictive power with an AUC of between 59 and 79.1%. Günalp et al.[33] calculated thresholds for progressive motility: a higher threshold of 42%, using the ROC curve, and a lower threshold of 14% with a positive predictive value as indicator. In this study, progressive motility proved to be a marginally better predictor of sub-fertility than sperm morphology with AUC values of 70.7 and 69.7%, respectively.[33] Montanaro Gauci et al.[28] found percentage motility a significant predictor of IUI outcome. The pregnancy rate was almost three times higher in the group with motility bigger than 50% as compared with the group with motility less than 50%.

The higher threshold values for sperm concentrations calculated by Ombelet et al.,[32] Günalp et al.[33] and Guzick et al.[35] ranged from $13.5x10^6$/ml to $34x10^6$/ml while the lower threshold values ranged from $9x10^6$/ml to $14.3x10^6$/ml. An AUC value of between 55.5 and 69.4% served as confirmation of the predictive power of this parameter. Although Menkveld et al.[34] did not calculate a threshold value for sperm concentration (because values of less than $20x10^6$/ml served as inclusion criteria in their study), they suggested a threshold value of $20x10^6$/ml to be used with confidence because it

did not influence the results from their fertile population. The clinical value of motility and sperm concentration serve as confirmations of findings reported in numerous other publications.[7,8,11,22-24]

Although the different parameters had good predictive power, independent of each other, the clinical value of semen analysis increased when the parameters were used in combination. Ombelet et al.[32] found that the differences between the fertile and sub-fertile populations only became significant, when two or all three semen parameters were combined. Bartoov et al.[36] concluded that the fertility potential is dependent on a combination of different semen characteristics. Eggert-Kruse et al.[23] found a significant correlation between the three parameters reviewed in their study. Although the different semen parameters show good individual predictive power, the clinical value of semen analysis increases when the parameters are used in combination. We, therefore, suggest that no parameter should be used in isolation, when assessing male fertility potential. The lower thresholds as discussed in this chapter have a much higher positive predictive value and a high negative predictive value. Therefore, we suggest that these lower thresholds should be used in identifying the sub-fertile male.

As suggested by WHO in 1999, each group should develop their own thresholds based on the population they are working in. Each laboratory should establish these thresholds if possible. It seems as if the sperm morphology threshold of 0-4% normal forms indicates a higher risk group for sub-fertility and fits the IVF and IUI data calculated previously.[12,13] The four articles discussed in the latter half of this chapter[32-35] showed the same trends and can serve as guidelines to distinguish fertile from sub-fertile males.

As far as concentration and motility are concerned, the thresholds are not clear, but a concentration lower than 10million/ml and a motility lower than 30% seem to fit the general data.[32-35] However, more, preferably multi-centre, studies are needed to set definitive thresholds.

5.6 SEMEN PROFILE OF THE GENERAL POPULATION: PARTNERS OF WOMEN WITH CHRONIC ANOVULATION

In general there is quite a poor level of understanding and evidence regarding the profile of the semen analysis of the general population. Many male populations have been proposed to be the mirror image of the semen analysis of the general population. Using donors of a semen donation program for normality is certainly not the best option since this population is positively biased for fertility. Army recruits are biased by age. Husbands of tubal factor patients can be biased by a positive history of infection (tubal factor due to pelvic infection) or a good fertility history (women

with tubal sterilization). Therefore, we believe that possibly the best reference group to study the semen profile in a general population is the semen of partners of women who have been diagnosed to have chronic anovulation/PCOS.

Two different studies, one retrospective and one prospective evaluating the semen analysis of the partners of women presenting with anovulation were selected.

5.6.1 Retrospective study of partners of women presenting with chronic anovulation (> 35 days) at Tygerberg Fertility Clinic

Included in this study were all male partners of patients diagnosed as anovulatory, at the Tygerberg Fertility Clinic. The methods used to examine the semen were according to the WHO guidelines[6] and for sperm morphology Tygerberg strict criteria was used.[3,4,6] The laboratory personnel initially evaluated all slides and each slide was then evaluated by one observer (TFK) according to strict criteria. Sixty-two samples were eventually selected and included in the study (Table 3).

5.6.2 A prospective study of partners of women presenting with PCOS at the Tygerberg Fertility Clinic

Tygerberg Fertility Clinic conducted a study on patients with PCOS. The patients were diagnosed with PCOS according to the recent Rotterdam consensus statement.[37] The aim of this study is to establish factors influencing ovulation induction in this group.

The semen of the partners of all these women was examined. The methods used to examine the semen were according to the WHO guidelines[6] and for sperm morphology Tygerberg strict criteria was used.[3,4,6] The lab personnel initially evaluated all slides and all P-pattern slides were evaluated by one observer (TFK) (Table 4). The thresholds used for subfertility are those suggested by Van der Merwe et al.[38] in their recent review: 0-4% normal forms; <30% motility; <10mill/ml and outlined in the first section of this chapter.

5.7 DISCUSSION

In the two studies (Table 3, retrospective; Table 4, prospective) ±50% of patients had a normal semen analysis. The most common single abnormality was that of teratozoospermia (25.8% retrospective, and 27.8% prospective). Azoospermia occurred in 1.4% to 4.8% respectively of patients with triple parameter defects in only 1.4% to 3.2% of cases (Tables 3 and 4).

The thresholds as calculated above were used in a group of anovulatory women. These thresholds give a reflection of the prevalence of male factor infertility in the general population. It is interesting to note that in both the retrospective and prospective studies the prevalence of teratozoospermia (<4% normal morphology) was 25.8% to 27.8% making it the most common defect in this group. About 50% of all the male patients had normal semen parameters based on these two studies by using the suggested thresholds as calculated based on the four articles discussed.[32-35,38]

It is also important to note that in PCOS patients the clinician needs to take into consideration that not only anovulation needs attention, but also that in up to 50% of these patients the male factor also needs attention to assist in achieving a successful outcome in these couples. These lower thresholds is first of all not absolute, but a continuum guiding the clinician to respond to a semen analysis. The golden rule is to repeat an abnormal semen analysis four weeks after the first abnormal evaluation to ensure that the correct approach will be followed. If again abnormal, a thorough physical examination should be performed and the necessary treatment offered. In the case of PCOS, the female factor (anovulation) should obviously be corrected starting as first line approach with weight loss in women with a BMI >25. Although 50% of these patients had a male factor according to the definition used, it is also important to note that only $\pm$ 5% of these factors were serious (azoospermia and the triple parameter defects) with 8-9.7% with a double defect.

To our knowledge this is the first attempt to use the specific suggested lower thresholds to define the prevalence of the subfertile male in the general population by using an anovulatory group of women. These thresholds will guide the clinician towards a more directive management where indicated.

5.8 REFERENCES

1. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, ed 2. Cambridge, Cambridge University Press, 1987.
2. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, ed 3. Cambridge, Cambridge University Press, 1992.
3. Kruger TF, Acosta AA, Simmons KF, Swanson RJ et al. Predictive value of abnormal sperm morphology in *in vitro* fertilization. Fertil Steril 1988;49:112-117.
4. Kruger TF, Menkveld R, Stander FS, Lombard CJ et al. Sperm morphologic features as a prognostic factor in *in vitro* fertilization. Fertil Steril 1986;46:1118-1123.

5. Menkveld R, Stander FSH, Kotze TJ et al. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 1990;5:586-592.
6. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, ed 4. Cambridge, Cambridge University Press, 1999.
7. Barratt CL, Naceeni M, Clements S, et al. Clinical value of sperm morphology for in-vivo fertility: Comparison between World Health Organization criteria of 1987 and 1992. Hum Reprod 1995;10:587-593.
8. Ayala C, Steinberger E, Smith DP. The influence of semen analysis parameters on the fertility potential of infertile couples. J Androl 1996;17:718-725.
9. Blonde JP, Ernst E, Jensen TK, et al. Relation between semen quality and fertility: A population-based study of 430 first-pregnancy planners. Lancet 1998;352:1172-1177.
10. Chia SE , Tay SK, Lim ST. What constitutes a normal seminal analysis? Semen parameters of 243 fertile men. Hum Reprod 1998;13:3394-3398.
11. Chia SE, Lim ST, Tay SK et al. Factors associated with male fertility: A case-control study of 218 infertile and 240 fertile men. BJOG 2000;107:55-61.
12. Coetzee K, Kruger TF, Lombard CJ. Predictive value of normal sperm morphology: A structured literature review. Hum Reprod Update 1998;4:73-82.
13. Van Waart J, Kruger TF, Lombard CJ et al. Predictive value of normal sperm morphology in intrauterine insemination (IUI): A structured literature review. Hum Reprod Update 2001;7:495-500.
14. Ombelet W, Pollet H, Bosmans E et al. Results of a questionnaire on sperm morphology assessment. Hum Reprod 1997;12:1015-1020.
15. Ombelet W, Wouters E, Boels L. Sperm morphology assessment: Diagnostic potential and comparative analysis of strict or WHO criteria in a fertile and a sub-fertile population. In J Androl 1997;20:367-372.
16. Ombelet W, Bosmans E, Janssen M et al. Multicenter study on reproducibility of sperm morphology assessments. Arch Androl 1998;41:103-114.
17. Keel BA, Stembridge TW, Pineda G, et al. Lack of standardization in performance of the semen analysis among laboratories in the United States. Fertil Steril 2002;78:603-608.
18. Cooper TG, Björndahl L, Vreeburg J, et al. Semen analysis and external quality control schemes for semen analysis need global standardization. Int J Androl 2002;25:306-311.
19. Menkveld R, Stander FS, Kotze TJ et al. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 1990;5:586-592.

20. Franken DR, Smith M, Menkveld R et al. The development of a continuous quality control programme for strict sperm morphology among sub-Saharan African laboratories. Hum Reprod 2000;15:667-671.
21. Franken DR, Barendsen R, Kruger TF. A continuous quality control program for strict sperm morphology. Fertil Steril 2000;74:721-724.
22. Holland-Moritz H, Krause W. Semen analysis and fertility prognosis in andrological patients. In J Androl 1992;15:473-484.
23. Eggert-Kruse W, Schwarz H, Rohr G et al. Sperm morphology assessment using strict criteria and male fertility under in-vivo conditions of conception. Hum Reprod 1996;11:139-146.
24. Dunphy BC, Neal LM, Cooke ID. The clinical value of conventional semen analysis. Fertil Steril 989;51:324-329.
25. Van Zyl JA, Kotze TJ, Menkveld R. Predictive value of spermatozoa morphology in natural fertilization; in Acosta AA, Swanson RJ, Ackerman SB, Kruger TF, Van Zyl JA, Menkveld R (eds): Human Spermatozoa in Assisted Reproduction, Baltimore, Williams & Wilkins, 1990, pp 319-324.
26. Zinaman MJ, Brown CC, Selevan SG et al. Semen quality and human fertility: A prospective study with healthy couples. J Androl 2000;21:145-153.
27. Slama R, Eustache F, Ducot B et al. Time to pregnancy and semen parameters: A cross-sectional study among fertile couples from four European cities. Hum Reprod 2002;17:503-515.
28. Montanaro Gauci M, Kruger TF, Coetzee K et al. Stepwise regression analysis to study male and female factors impacting on pregnancy rate in an intrauterine insemination programme. Andrologia 2001;33:135-141.
29. Cohlen BJ, te Velde ER, van Kooij RJ et al. Controlled ovarian hyperstimulation and intrauterine insemination for treating male subfertility: A controlled study. Hum Reprod 1998;13:1153-1158.
30. Ombelet W, Vandeput H, Van de Putte G et al. Intrauterine insemination after ovarian stimulation with clomiphene citrate: Predictive potential of inseminating motile count and sperm morphology. Hum Reprod 1997;12:1458-1465.
31. Lindheim S, Barad D, Zinger M et al. Abnormal sperm morphology is highly predictive of pregnancy outcome during controlled ovarian hyperstimulation and intrauterine insemination. J Assist Reprod Genet 1996;13:569-572.

32. Ombelet W, Bosmans E, Janssen M et al. Semen parameters in a fertile versus sub-fertile population: A need for change in the interpretation of semen testing. Hum Reprod 1997;12:987-993.

33. Günalp S, Onculoglu C, Gürgan T et al. A study of semen parameters with emphasis on sperm morphology in a fertile population: An attempt to develop clinical thresholds. Hum Reprod 2001;16:110-114.

34. Menkveld R, Wong WY, Lombard CJ et al. Semen parameters, including WHO and strict criteria morphology, in a fertile and infertile population: An effort towards standardization of *in vivo* thresholds. Hum Reprod 2001;16:1165-1171.

35. Guzick DS, Overstreet JW, Factor-Litvak P et al. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med 2001;345:1388-1393.

36. Bartoov B, Eltes F, Pansky M et al. Estimating fertility potential via semen analysis data. Hum Reprod 1993;8:65-70.

37. The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004 Jan;19(1):41-7.

38. Van der Merwe FH, Kruger TF, Oehninger SC, Lombard CJ. The use of semen parameters to identify the subfertile male in the general population. Gynecologic and Obstetric Investigation 2005;59(2):86-91.

TABLE 1. Thresholds: fertile vs. subfertile populations studied

Author	Morphology (%)	Motility (%)	Progressive motility (%)	Concentration (10^6/ml)
Guzick *et al.* 2001[17]	9	32		13.5
Menkveld *et al.* 2001[16]	4	45		20
Günalp *et al.* 2001[15]	10	52	42	34
Ombelet *et al.* 1997[14]	10	45		34

TABLE 2: Possible lower thresholds for the general population to distinguish between subfertile and fertile men based on the assumed incidences of subfertile males in their populations

Author	Morphology (%)	Motility (%)	Progressive motility (%)	Concentration (10^6/ml)
Menkveld *et al.* 2001[16]	3	20		20
Günalp *et al.* 2001[15]	5	30	14	9
Ombelet *et al.* 1997[14]	5	28		14.3

TABLE 3. Retrospective study of partners of women presenting with chronic anovulation (> 35 days) at Tygerberg Fertility Clinic

	Number (<10mill/ml cut off)	% of patients
Normozoopermia	29	46.7
Sperm abnormality		
Single parameter defect		
Azoospermia	3	4.8
Oligozoospermia (O)	3	4.8
Asthenozoospermia (A)	-	0
Teratozoospermia (T)	16	25.8
Polizoospermia (P)	2	3.2
Immunological factor (I)	1	1.6
Double parameter defect		
OA	-	0
OT	4	6.5
AT	-	0
TP	1	1.6
TI	1	1.6
Triple parameter defect		
OAT	2	3.2
Threshold values used		
Concentration/ml = < 10 ml/l		
Motility = < 30%		
Morphology = < 4% normal forms		

TABLE 4. A prospective study of partners of women presenting with PCOS at the Tygerberg Fertility Clinic

	Number (<10mill/ml cut off)	% of patients
Normozoospermia	41	56.9
Sperm abnormality		
Single parameter defect		
Azoospermia	1	1.4
Oligozoospermia (O)	1	1.4
Asthenozoospermia (A)	-	0
Teratozoospermia (T)	20	27.8
Polizoospermia (P)	3	4.2
Immunological factor (I)	-	0
Double parameter defect		
OA	-	0
OT	1	1.4
AT	-	0
TP	3	4.2
TI	1	1.4
OP	-	0
Triple parameter defect		
OAT	1	1.4

CHAPTER 6

OVULATION INDUCTION IN WOMEN WITH PCOS: AN EVIDENCE BASED APPROACH

6.1 INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. In 1935 Steyn and Leventhal[1] described the association of amenorrhoea, obesity, infertility, hirsutism and bilateral enlarged ovaries. To date the diagnosis in use remain topical and controversial. There are two definite schools of thought regarding the diagnosis of PCOS. In the UK the classical ultrasound features[2] are the cornerstone of the diagnosis, which includes the clinical and biochemical presentation. On the contrary, in the USA, PCOS is diagnosed on the clinical and biochemical evidence with the exclusion of CAH, hyperprolactinaemia and hypothyroidism[3].

In 2003 the Rotterdam consensus statement[4] was made to give clinicians guidance in the diagnosis of PCOS. This statement concluded that the diagnosis of PCOS can be made if two of the following three features are present: chronic anovulation; polycystic ovaries on ultrasound; hyperandrogenism/hirsutism with the exclusion of other diseases causing hirsutism.

Familial clustering of cases suggests that genetic factors play an important role in the diagnosis of PCOS. Using a candidate gene approach, Franks et al[5] found evidence for the involvement of two key genes in the aetiology of PCOS. They suggest that the steroid synthesis gene CYP 11a and the insulin VNTR regulatory polymorphism are important factors in the genetic cause of PCOS. It is, however, unlikely that these two are the only genes involved in the aetiology of this complex syndrome.

On the basis of the theory that hyperinsulinaemia negatively effects ovulation and that it is an important role-player in the pathophysiology of PCOS, it is postulated and has been proven that insulin sensitisers may improve the endocrine imbalances and result in normal menses, ovulation and normalisation of hyperandrogenism[3]. However, in our own study, Chapter 4, we could not confirm the above finding. It is also known that obesity on its own, and in association with hyperinsulinaemia, is associated with relative gonadotrophin resistance[6]. By using a simple formula we can isolate the hyperinsulinaemia/insulin resistant patient and commence with a combination of weight loss and insulin sensitisers. At this stage the HOMA (homeostasis model assessment) has been proven to be of great success in identifying insulin resistance[7].

HOMA = $\frac{\text{fasting insulin x fasting glucose.}}{22,5}$

The value of more than 2.5 is generally accepted as insulin resistant, the same is true for a fasting insulin level of more than 17 IU/ml. Obesity is defined as a BMI of greater than 30kg/m2 and is found in 30 – 50% of women with PCOS[8]. As mentioned, obesity on its own is associated with ovulation resistance. A minor weight loss of 5% often result in normalisation of cycles and ovulation[9].

Clomiphene citrate, an anti-estrogen, is the drug most regularly used for ovulation induction. The primary site of action is the hypothalamus where it binds to estrogen receptors and blocks the negative feedback effect of circulating estrogens and ultimately results in an increase in gonadotrophin releasing hormone secretion[10].

The aim of this chapter is to address the approach in managing the patient with PCOS who desires to have a baby. This chapter will summarise the findings of the thesis and is presented as a current opinion.

6.2 THE IMPACT OF OBESITY ON THE REPRODUCTIVE SYSTEM AND THE SUBSEQUENT EFFECT OF WEIGHT LOSS

In a review by Norman et al[11], the association between obesity and women with PCOS was highlighted with the emphasis on the effect of obesity on the reproductive system. Using the classification of body mass index (BMI, weight in kg per height in m^2), in the United States 60% of the adult population are overweight (BMI $\geq$ 25kgm^{-2}) and 22% are obese (BMI $\geq$ 30 kgm^{-2})[12]. This rising prevalence is an important health issue due to the clear association of obesity with an increased risk of impaired psychosocial health, type 2 diabetes mellitus, cardiovascular disease (CVD), osteoarthritis, sleep apnoea and breast and uterine cancer[13].

Reproductive processes are influenced by body weight and reproductive dysfunction will occur in both positive and negative extremes of body weight[14,15]. A direct relationship between menstrual irregularity and the degree of obesity in women of reproductive age was reported by Hartz et al[16]. Furthermore, once conception is achieved and increased risk of pregnancy complications and miscarriage may result with increased weight[17,18].

Women with PCOS constitute a significant proportion of the infertile population. Obesity prevalence estimates in PCOS range from 35% to 63%[19,20]. As a primary treatment modality, weight loss should be the initial treatment aim in all, obese, infertile women. Resumption of ovulation occurred with weight losses of 5,5 - 6,5 kg in anovulatory women[21-23]. The NIH document 'Clinical Guidelines on the identification, evaluation, and treatment of overweight and obesity in adults' recommends a multifaceted approach to treating obesity. (Table 1).[24]

Norman et al[11] concluded that weight loss should be the first course of action in obese, infertile women. This can be difficult to achieve and maintain. Principles identified, to succeed in weight loss, in the general population and in obese infertile women include adoption of healthy eating principles and moderate amounts of low-intensity exercise. Modifying additional factors such as alcohol consumption, smoking, cognitive behaviour therapy, and use of a group environment can increase the long-term success and maintenance of weight loss and reproductive and metabolic improvements.

A prospective randomised controlled trial[25] was conducted to assess the effectivity when metformin is added to C/C, compared to C/C alone in primary ovulation induction protocols(Chapter 4). It was observed that all patients ovulated with a BMI<27. With a BMI>27 there was no difference in ovulation between C/C alone or the combination of metformin and C/C. In a study by Legro et al[26], a significant higher rate of live births in women with a BMI less than 30 was noted when compared to those with a BMI more than 30. Neveu et al[27] observed a better outcome when metformin was added in the more obese group(BMI 27-35). The same observation was seen by Nestler et al[28]. A study by Palombo et al[29] could not be compared, because they excluded women with a BMI more than 30. From these results it is clear that obesity plays a significant role in the fertility prognosis of the infertile patient. In this study[25] performed at our institution, weight/BMI (P=.009) was the major predictive factor in ovulation outcome. In figure 1 it is clear that all patients ovulated when the BMI was less than 27. This is an important finding and supports current literature to optimize the BMI first, loose weight if needed, before commencing with any ovulation induction regimen[30].

Other significant factors observed in this study were SHBG (sex hormone binding globulin) P=.036 and 17hydroxy progesterone (17OH Progesterone) P=.027 (Chapter 4). The variable SHBG was a significant factor and positively associated with ovulation. The physiological effect of an increase of SHBG is a lowering of the free androgen index and this may lead to an improved ovulation outcome. Abdominal fat is related to decreased SHBG and increased androgenicity in infertile women.[11] Increased androgen production and reduced binding of androgens to SHBG contributes to

hyperandrogenism resulting in anovulation through inhibition of follicular maturation. In a study by Ghazeeri et al[31] rosiglitazone was administered to 25 obese, CC-resistant, PCOS women who desired pregnancy. They observed a significant improvement in ovulation rates when rosiglitazone was added to C/C in this study. One of the important findings was a significant rise in SHBG in the group of women treated with rosiglitazone. Several other investigators have similarly observed an increase in SHBG and a decrease in testosterone and androgenicity with improved conception rates in patients with weight loss[30,32].

6.3 METFORMIN vs CLOMIPHENE: WHICH DRUG TO OFFER WHEN?

6.3.1 Is there a place for Metformin as a primary (1st line) drug?

Insulin resistance and concomitant hyperinsulinaemia are frequently found in obese PCOS women (65%)[33]. The incidence of insulin resistance among lean PCOS women is nearly 20%[32]. This results in hyperinsulinaemia and enhances the LH driven production of androgens from ovarian theca cells[33]. Hyperinsulinaemia, insulin resistance and an increase in androgen production are all linked together in the PCOS patient[34,35]. It is also known that patients with PCOS and insulin resistance are more resistant to ovulation induction. Is the answer in the management of infertile PCOS women then the use of insulin sensitisisers?

Recently four prospective randomised controlled trials were published[26,27,29,36]. The primary aim of these studies was to compare C/C with metformin alone or in combination when studied as primary ovulation induction agents in women with PCOS.(See Chapter 4: Discussion) In our study[25] (chapter 4), we prospectively randomised 107 patients and 17 (16,3%) patients were lost to follow up. In the study by Moll et al[36], they lost 63 (27,6%) patients to follow up. In their study more patients were lost in the metformin group, which might have been due to the side effects. In contrast, in our study a similar number of women were lost to follow up in the two groups studied. It was observed that the combination of metformin and C/C had a 6% better ovulation rate as C/C alone, however this finding was not statistically significant. Similar conclusions were drawn by Moll et al[36] , Legro et al[26] and Neveu et al[27] . These authors concluded that metformin should not be added to C/C in primary ovulation induction regimens in patients with PCOS.

6.3.2 What is the proposed role of Metformin in ovulation induction protocols?

As discussed, it was shown that there is currently no benefit for metformin in primary ovulation induction protocols in women with PCOS. Two review articles were published recently[37,38]. In the one review[37] two important articles were not included in their analysis, and in the other[38] only two articles were mentioned in the C/C-resistant group with the data set very heterogenic. (See Chapter

3: Discussion) Based on the above-mentioned facts and the fact that C/C-resistance is a major problem in the handling of the PCOS patient, we performed a meta-analysis with more articles to our disposal and according to the selection criteria as outlined[39] (See Chapter 3). We obtained data from four prospective randomised double blind trials and two prospective randomised (not double blind) trials. The data on the first four articles[40-43] clearly showed a statistical significant effect in favour of ovulation with addition of metformin in the clomiphene resistant patient. When the data of the two prospective randomised articles[44,45] were pooled with the first mentioned data set it further confirmed the positive effect on ovulation with the addition of metformin in the C/C-resistant patient(figure 2), (See Chapter 3, figure 3). Based on this review it can be concluded that metformin is highly effective in achieving ovulation in the C/C-resistant PCOS patient. We also recommend that all obese PCOS patients seeking fertility help should be guided using a lifestyle modification program that should include weight-reducing diet and exercises[23,30]. When this goal is achieved, the patient can be started on C/C, and only if C/C-resistance is present, metformin should be added to achieve ovulation.

6.4 THE FORGOTTEN MALE FACTOR?

In general there is quite a poor level of understanding and evidence regarding the profile of the semen analysis of the general population. Many male populations have been proposed to be the mirror image of the semen analysis of the general population. We believe that possibly the best reference group to study the semen profile in a general population is the semen of partners of women who have been diagnosed to have chronic anovulation/PCOS. The thresholds used for subfertility are those suggested by Van der Merwe et al[46] in their recent review: 0-4% normal forms; <30% motility; $<10^6$/ml.

It is important to note that in PCOS patients the clinician needs to take into consideration that not only anovulation needs attention, but also that in up to 50% of these patients the male factor also needs attention to assist in achieving a successful outcome in these couples. These lower thresholds is first of all not absolute, but a continuum guiding the clinician to respond to a semen analysis. In the case of PCOS, the female factor (anovulation) should obviously be corrected. Although 50% of these patients had a male factor according to the definition used, it is also important to note that $\pm$ 13-14,5% of these factors were serious (azoospermia, triple parameter defects and double defect)[47]. (See Chapter 5)

In PCO studies it is important to take the male factor into account, especially if two groups/treatment modalities are compared prospectively. Even a single sperm defect, e.g. severe

sperm morphology (P Pattern) can have an effect in follow up especially if pregnancy is the endpoint of the study. We seldom observe that the male factor is outlined in PCO research.

6.5 CONCLUSION

The diagnostic criteria of women with PCOS remains controversial. It is however extremely important to adhere to the current Rotterdam consensus statement for clinical and research purposes.

When addressing the issue of women with PCOS who desire to fall pregnant, successful ovulation induction is the first hurdle to conquer. In obese PCOS women, the cornerstone of management is weight loss and an active exercise programme. As little as 5% weight loss results in spontaneous ovulation.

Clomiphene citrate is still the first drug of choice for ovulation induction in women with PCOS. Only when C/C-resistance is present, metformin should be added to achieve successful ovulation.

One important factor frequently ignored or neglected in women with PCOS is the semen analysis. 50% of partners of women with PCOS can have a male factor with 13-14,5% serious defects[47]. We need to address this important factor when managing the women with PCOS, may it be in clinical practise or in research projects, especially where the endpoint is pregnancy outcome.

6.6 REFERENCES:

1. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935;29:181-191.
2. Eden JA. Polycystic ovary syndrome: diagnostic and clinical aspects. Gynaecology Forum 1997:2.
3. Kim LH, Taylor AE, Barbieri RL. Insulin sensitizers and polycystic ovary syndrome: can a diabetes medication treat infertility? Fertil Steril 2000;73:1097-1098.
4. The Rotterdam ESHRE/ASRM sponsored PCOS consensus group. Revised 2003 consensus on diagnostic criteria and long-term health risk related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19(1):41-47.
5. Franks S. The genetic basis of polycystic ovary syndrome. Hum Reprod 1997; 12:2641-2648.

6. Fedorcsak P, Dale PO, Storeng R, Tanbo T, Abyholm T. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome. Hum Reprod 2001;16(6):1086-1091.
7. Haffner SM, Gonzalez C, Miettinen H, Kennedy E, Stern MP. A Prospective Analysis of the Homa Model. Diabetes Care 1996;19:(October)1138-1141.
8. Franks S. Polycystic ovarian syndrome: a changing perspective. Clin Endocrinol 1989; 31:87-120.
9. Huber-Buchholz MM, Carey DGP, Norman RJ. Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab 1999;84:1470-1474.
10. Seli E, Duleba AJ. Optimizing ovulation induction in women with polycystic ovary syndrome. Obstet Gynaecol 2002;14(3):245-254.
11. Norman RJ, Moran LJ. Weight, fertility and management approaches. In: Kruger TF, Van der Spuy Z, Kempers RD (eds): Advances in Fertility Studies and Reproductive Medicine, Juta 2007:24-35.
12. Flegal KM, Carroll MD, Kucsmarski RJ, Johnson. Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord 1998;22:39-47.
13. World Health Organisation. Obesity: preventing and managing the global epidemic. Geneva: World Health Organisation, 1997.
14. Lake JK, Power C, Cole TJ. Women's reproductive health: the role of body mass index in early and adult life. Int J Obes Relat Metab Disord 1997;21:432-438.
15. Rogers J, Mitchell GW. The relation of obesity to menstrual disturbances. N Eng J Med 1953;247:53-55.
16. Hartz AJ, Barboriak PN, Wong A, Katayama KP, Rimm AA. The association of obesity with infertility and related menstrual abnormalities in women. Int J Obes 1979;3:57-73.
17. Hamilton-Fairley D, Kiddy D, Watson H, Paterson C, Franks S. Association of moderate obesity with a poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotrophin. Br J Obstet Gynaecol 1992;99:128-31.
18. Sebire NJ, Jolly M, Harris JP et al. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. Int J Obes Relat Metab Disor 2001;25:1175-82.
19. Kiddy DS, Sharp PS, White DM et al. Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol (Oxf) 1990;32:213-20.
20. Norman RJ, Masters SC, Hague W, Beng C, Pannall P, Wang JX. Metabolic approaches to the subclassification of polycystic ovary syndrome. Fertil Steril 1995;63:329-335.

21. Hollmann M, Runnebaum B, Gerhard I. Effects of weight loss on the hormonal profile in obese, infertile women. Hum Reprod 1996;11:1884-91.

22. Clark AM, Ledger W, Galletly et al. Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod 1995;10:2705-12.

23. Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. Hum Reprod 1998;13:1502-5.

24. National Institute of Health. Clinical guidelines on the identification, evaluation and treatment of overweight and obesity in adults: Evidence report. Journal of Obesity Research 1998;6(Supplement 2).

25. Siebert TI, Kruger TF, Lombard CJ. Evaluating the equivalence of Clomiphene citrate with and without Metformin in ovulation induction in PCOS patients: a randomised controlled trial. Submitted for publication in Fertility and Sterility.

26. Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA et al. Clomiphene, Metformin, or both for infertility in the Polycystic Ovary Syndrome. N Engl J Med 2007;356(6):551-66.

27. Neveu N, Granger L, St-Michel P, Lavoie HB. Comparison of clomiphene citrate, metformin or the combination of both for first-line ovulation induction and achievement of pregnancy in 154 women with polycystic ovary syndrome. Fertil Steril 2007;87(1):113-20.

28. Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the Polycystic Ovary Syndrome. N Engl J Med 1998;338(26):1876-80.

29. Palomba S, Orio F, Falbo A, Manguso F, Russo T, Cascella T et al. Prospective parallel randomised, double-blind, double-dummy controlled clinical trial comparing Clomiphene Citrate and Metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 2005;90(7):4068-74.

30. Norman RJ, Davies MJ, Lord J, Moran LJ. The role of lifestyle medication in polycystic ovary syndrome. Trends Endocrinal Metab 2002;13:251-7.

31. Ghazeeri G, Kutteh WH, Bryer-Ash M, Haas D. Effect of rosiglitazone on spontaneous and clomiphene citrate induced ovulation in women with polycystic ovary syndrome. Fertil Steril 2003;79:562-66.

32. Kiddy DS, Hamilton-Fairley D, Bush A et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 1992;36:105-11.

33. Chang RJ, Nakamura RM, Judd HL and Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:356-9.

34. Dunaif A. Insulin resistance and the polycystic ovarian syndrome: mechanism and implications for pathogenesis. Endocrinol Rev 1997;18:774-800.

35. Burghen GA, Givens JR and Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinemia in polycystic ovarian disease. J Clin Endocrinol Metab 1980;50:113-5.

36. Moll M, Bossuyt PMM, Korevaar JC, Lambalk CB, Van der Veen F. Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double-blind clinical trial. BMJ 2006;332:1485-9.

37. Lord JM, Flight IHK and Norman RJ. Metformin in polycystic ovary syndrome: systematic review and meta-analysis. Brit Med J 2003;327:1-6.

38. Costello MF and Eden JA. A systematic review of the reproductive system effects of metformin in patients with polycystic ovary syndrome. Fertil Steril 2003;79:1-13.

39. Siebert TI, Kruger TF, Steyn DW, Nosarka S (2006) Is the addition of Metformin efficacious in the Clomiphene-resistant PCOS patient? (A structured literature review). Fertility and Sterility 86(5):1432-1437.

40. Hung Yu Ng E, Ming Sun Wat N and Chung Ho P. Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomised, double-blinded placebo-controlled trial. Hum Reprod 2001;16:1625-31.

41. Sturrock NDC, Lannon B and Fay TN. Metformin do not enhance ovulation induction in clomiphene resistant polycystic ovary syndrome in clinical practice. Br J Pharmacol 2002;53:469-73.

42. Kocak M, Caliskan E, Simsir C and Haberal A. Metformin therapy improves ovulatory rates, cervical scores and pregnancy rates in Clomiphene citrate-resistant women with polycystic ovary syndrome. Fertil Steril 2002;77:101-6.

43. Vandermolen DT, Ratts V, Evans WS, Stovall DW, Kauma SW and Nester JE. Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who is resistant to clomiphene citrate alone. Fertil Steril 2001;75:310-5.

44. Nestler J, Jakubowich DJ, Evans WS and Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Eng J Med 1980;25:1876-80.

45. Malkawi HY and Qublan HS. The effect of metformin plus clomiphene citrate on ovulation and pregnancy rates in clomiphene-resistant women with polycystic ovary syndrome. Saudi Med J 2002;23:663-6.

46. Van der Merwe FH, Kruger TF, Oehninger SC, Lombard CJ. The use of semen parameters to identify the subfertile male in the general population. Gynecol Obstet Invest 2005;59(2):86-91.

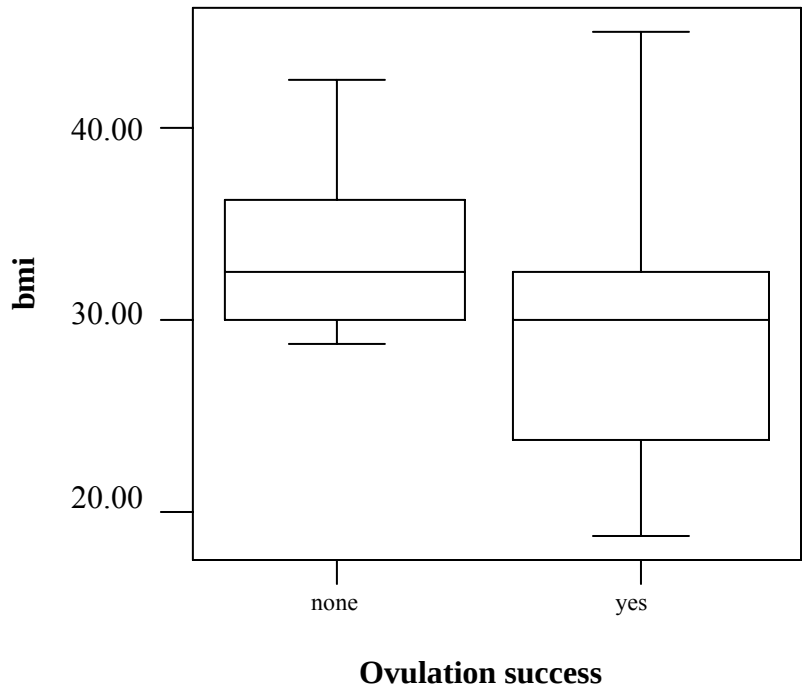
47. Siebert TI, Van der Merwe FH, Kruger TF, Ombelet W. How do we define male subfertility and what is the prevalence in the general population? In: Male Infertility Diagnosis and Treatment: Oehninger SC, Kruger TF (eds), Informa Healthcare 2007:269-76.

Table 1. National Institute of Health Clinical Guidelines for long-term treatment of overweight and obesity

Effective weight loss and long-term results – National Institute of Health Guidelines
1. Sensible diet and changed eating habits for long-term 2. Effective physical activity program sustainable long-term 3. Behaviour modification, reduction of stress, wellbeing 4. Combination of dietary and behaviour therapy and increased physical activity 5. Social support by physician, family, spouse, peers 6. Smoking cessation and reduction in alcohol consumption 7. Avoidance of "crash diets" and short-term weight loss 8. Minor roles for drugs involved in weight loss 9. Avoidance of aggressive surgical approaches for majority 10. Adaptation of weight loss programmes to meet individual needs 11. Long-term observation, monitory and encouraging of patients who have successfully lost weight

Adapted from [24]

FIGURE 1. Effect of BMI on ovulation success



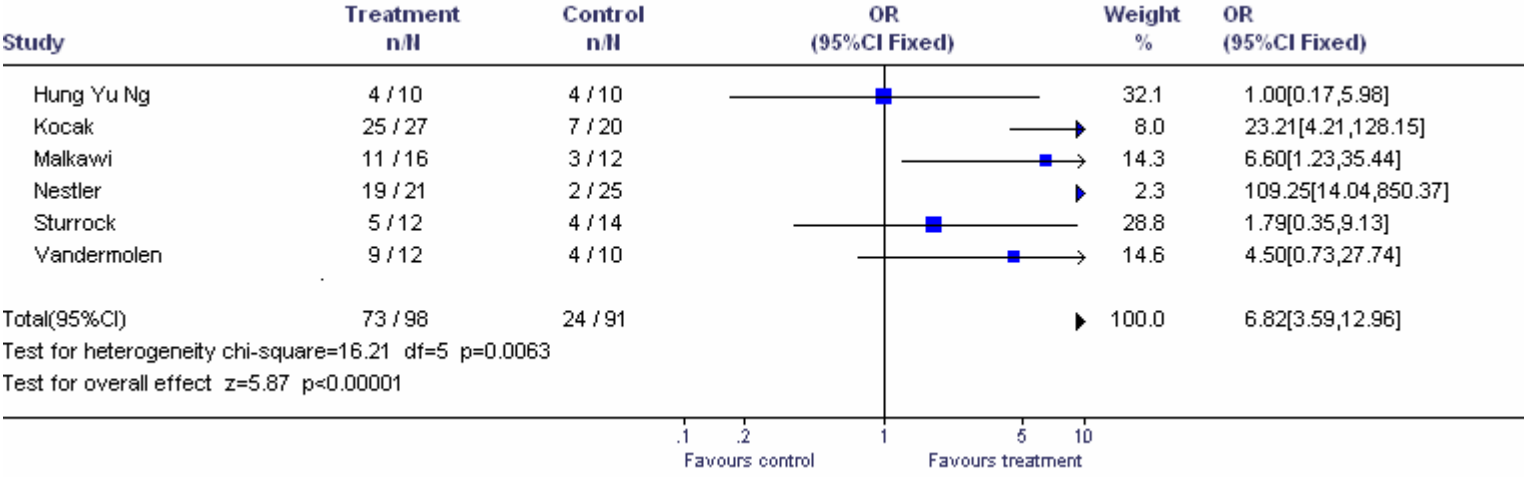
Horizontal axis: ovulation success
Vertical axis: bmi = body mass index

Figure 2.

Group 1: Four trials where the addition of metformin was randomised in a prospective double-blind placebo controlled fashion in the CC resistant patient

Group 2: Two trials where the addition of metformin was prospectively randomised in the CC resistant patient

n/N = the number of women where ovulation induction was achieved / the total number of women in the group



Adapted from [39] (See Chapter 3, figure 3)