# Final Report

## A study on ovulatory dysfunction in an infertile population of Sri Lanka and a prospective randomized comparison between Clomifene citrate and Letrozole, in ovulation induction and augmentation.

**Principal Investigator:** Professor Prasantha S Wijesinghe

**Grant Number:** RG/2007/HS/08

## Section 1

i) **Grant Number**

RG / 2007 / HS / 08

ii) **Title of the Project**

A study on ovulatory dysfunction in an infertile population of Sri Lanka and a prospective randomized comparison between low dose step-up and step-down gonadotropin regimes in controlled ovarian stimulation.

Later changed to

A study on ovulatory dysfunction in an infertile population of Sri Lanka and a prospective randomized comparison between Clomifene citrate and Letrozole, in ovulation induction and augmentation.

(The letter from the NSF approving the change in the research proposal and the revised budget is annexed (Annex 1).

iii) **Principal Investigator**

Professor Prasantha S Wijesinghe

iv) **Co-Investigators**

Professor Harshalal R Seneviratne
Dr. Thilina S Palihawadana

v) **Institute(s) where research was being carried out**

Faculty of Medicine, University of Kelaniya

vi) **Date of award**

03rd December 2007

vii) **Date of completion of Project**

05th April 2011

viii) **Total allocation of funds (Rs):**

Total budget required : Rs. 908,013.00

Total allocation from NSF after the modification : Rs. 607,378.00

An additional Rs. 300,635.00 was obtained as research grants from the University of Kelaniya (Rs. 210,000.00 - Grant no: RP/03/04/10/01/2006) and Kelaniya university reproductive research and development fund (Rs. 90,635.00).

ix) **Total spent from NSF allocation** : Rs 584,506.20
**Balance** : Rs: 22,871.80

x) **Number of Research Students employed**

None

**xi) Post graduate degree completed with dates**

Thesis prepared for submission for an MPhil degree at University of Colombo.

**xii) Number of Technical Assistants and/or labourers employed and period of service**

None

**xiii) Publications/Communications arising from the project during the reporting period**

Letrozole resistance in ovulation induction among subjects with ovulatory dysfunction: A case control study – Abstract submitted for 9th Royal College of Obstetricians and Gynaecologists International Meeting in Athens to be held in September 2011 and accepted (Abstract attached – Annex 2).

## Section 2 : Executive Summary of the Project

**Introduction:** Ovulatory dysfunction (OD causes infertility and Clomifene citrate (CC) is an effective treatment while Letrozole is an alternative. Knowledge on efficacy and factors associated with resistance to drugs in a local population is useful.

**Objectives:** Understand causes of infertility in an infertile population and determine the factors associated with resistance to CC and Letrozole in augmentation of ovulation.

**Method:** The study was carried at the university infertility clinic at teaching hospital, Ragama. Infertile couples seeking treatment were recruited. A subset of subjects underwent induction of ovulation with CC and induction of ovulation with Letrozole was carried out on a sample of subjects with and without clomifene resistance. A randomised trial was undertaken to compare the effects of either drug in augmentation of ovulation in ovulatory infertility.

**Results:** Among 518 couples OD was seen in 53%. SFA abnormalities were noted in 45% while 10% had sexual dysfunction. Tubal disease was noted in less than 10%.
Factors associated with OD included irregular menstruation (OR 94.6), being overweight (OR 1.68) or Obese (OR 3.05), presence of acanthosis (OR 7.19), hirsutism (OR 5.31), polycystic ovarian syndrome (OR 88.3), high TSH levels (OR 1.92), a reversed LH: FSH ratio (OR 3.92) and a high testosterone level (OR 7.56).
Ovulation induction with CC was done in 128 subjects and CC resistance was noted in 23%. Factors associated with CC resistance included a duration of infertility > 3 years (OR 2.06), presence of hirsutism (OR 2.76), a higher antral follicle count, presence of PCOS (OR 2.99) and a reversed LH: FSH ratio (OR 3.11).
Induction of ovulation with Letrozole was studied among 50 subjects and resistance was noted in 50%. This was 24% among those who responded to CC but 76% among those who were resistant to CC. Factors associated with Letrozole resistance included presence of hirsutism (OR 3.86) and CC resistance (OR 10.0).
Augmentation of ovulation with CC compared to Letrozole demonstrated a higher and a prolonged rise in FSH. The endometrial thickness was increased with Letrozole and CC was associated with a non-significant increase in the incidence of multi-follicle development.

**Discussion:** Factors associated with CC resistance should be considered in patient management and counselling prior to treatment. Letrozole seems to be effective only in a minority of patients with CC resistance. Use of Letrozole seems to be favourable for the endometrium while risk of multiple pregnancies may be low.

## Section 3: Report in detail.

This section contains

1. General introduction
2. Definition, prevalence and aetiology on infertility
3. Ovulatory dysfunction: Prevalence and association of underlying causes
4. Ovulation induction
5. Conclusion and Recommendations
6. References

### 1. General introduction

Infertility is defined as failure of a couple to achieve a pregnancy in spite of trying for a considerable time. This is known to affect nearly one in six couples who wish for a pregnancy. Infertility may result due to abnormalities in either partner of an infertile couple. In the male it may be abnormalities in seminal fluid parameters or sexual dysfunction while in the female it could be due to abnormalities in ovulation, pathologies in fallopian tubes or structural abnormalities in the rest of the female genital tract. Much research is being carried out at present to understand the other mechanisms of infertility such as abnormalities in cervical mucus, presence of antibodies for sperms either in the female genital tract or in the seminal fluid and genetic causes in either partner of an infertile couple. However, the definite clinical significance of such aetiologies has still not proven. In a significant proportion of couples, an abnormality may not be found in spite of investigations, thus termed as having unexplained infertility.

Ovulatory dysfunction is one of the main contributory causes of infertility and it may result from many underlying pathologies. Ovulation is a complex process which is controlled by numerous endocrinological pathways. Abnormalities in any of these hormonal control mechanisms or dysfunctions in the ovary, the end organ, may result in ovulatory dysfunction. The prevalence of ovulatory dysfunction and its underlying pathologies may vary in different populations. This is an area that has not been studied in detail, in a Sri Lankan infertile population.

Mainstay of treatment in ovulatory dysfunction is induction of ovulation, which is commonly achieved by oral anti-oestrogen agents such as Clomifene citrate or tamoxifen. Such treatment is commenced after basic investigations of the seminal fluid parameters and establishment of tubal patency in the female. The dose requirements in individual patients vary and there are no definite predictive factors that allow the clinicians to use in counselling patients prior to commencement of treatment. Furthermore, while this treatment is successful in achieving ovulation in many individuals with ovulatory dysfunction, some individuals may not respond to such treatment. There are no definite prognostic factors that could be used to predict resistance to Clomifene citrate therapy.

While Clomifene citrate remains the most commonly used ovulation induction agent worldwide for many years, new research has shown drawbacks in such treatment mainly brought about by the anti-oestrogenic properties of the drug. Newer treatment options have been suggested as alternative Clomifene citrate that would confer the same benefits minus the unwanted negative effects. Third generation aromatase inhibitor Letrozole is one such treatment that is currently being studied worldwide. It has been proposed as an alternative in induction of ovulation in ovulatory dysfunction as well as augmentation of ovulation in unexplained infertility. One main advantage of Letrozole over Clomifene citrate is hypothesized to be the absence of anti-oestrogen activity on the endometrium, thus facilitating implantation.

This study was carried out to understand many aspects related to infertility secondary to ovulatory dysfunction with an emphasis on oral agents for induction of ovulation. The first phase of the study was aimed at determining the causes of infertility in an infertile

population of Sri Lanka and a total of 518 infertile couples were included in this phase of the study which was carried out with a cross-sectional study design. The prevalence of male factor infertility, sexual dysfunction and tubal factor infertility among the couples was assessed.

The ovulatory dysfunction among this infertile population was assessed in a total of 411 female partners of the above study population in a cross sectional study. This was a non-contrived subgroup of the above study population and excluded only those who opted to discontinue participation and those who were lost to follow up. The prevalence of abnormalities in ovulation and the association of underlying pathologies that impair ovulation were determined.

Next stage of the study was aimed at determining the factors that could be used to predict treatment success in induction of ovulation with anti-oestrogen Clomifene citrate and to determine the efficacy of Letrozole in induction of ovulation in patients with ovulatory dysfunction. A sample of 128 subjects from the initial study population who required induction of ovulation was included in a prospective interventional study design to assess the response to ovulation induction with Clomifene citrate. Two groups of subjects selected from the above sample, with 25 subjects in each group, who were sensitive or resistant to induction of ovulation with Clomifene citrate were included in an interventional study design to assess response to ovulation induction with Letrozole.

Furthermore, the study aimed to study the use of Clomifene citrate and Letrozole in augmentation of ovulation. This phase included a randomized interventional study comparing response to augmentation of ovulation with Clomifene citrate and Letrozole among subjects with confirmed regular ovulation. Twenty five subjects each, selected from the original study population, were included in the two treatment groups.

In Sri Lanka infertility is managed mainly in the specialist centres which offer varying grades of investigations and treatment modalities. While the basic investigations and basic to intermediate treatment options are offered by many specialist centres, more advanced treatment modalities such as assisted reproductive techniques are available only in the highly specialised tertiary care centres. The study setting for this study was a specialist treatment centre offering services of basic and intermediate management of infertility. The patient population included those referred by the primary care health service providers such as general practitioners and patients referred by the outpatient department of the hospital. The treatment was based on clinical protocols used in the department for the management of infertility, which were mainly based on the recommendations of the National Institute of Health and Clinical Excellence, United Kingdom, as described in NICE guideline on management of infertility published in year 2004. The study was funded by research grants from the National Science foundation (Grant no: RG/2007/HS/08), from the University of Kelaniya (Grant no: RP/03/04/10/01/2006) and from the Kelaniya University Reproductive Research and Development Fund.

Ethical considerations

Ethical approval for the study was obtained from the Ethics Review committee of the faculty of Medicine, University of Kelaniya, prior to commencement of the study. Written informed consent was obtained prior to participation in the study using a consent form in Sinhalese language. The study was carried out in two phases. In the initial phase, only subjects who required investigations for infertility were included in the study thus avoiding investigations unless clinically indicated. No additional investigations were carried out on the study subjects for the purpose of the research project. All investigations were carried out free of charge and the clinical data were given to study participants in writing to be used in their clinical management. On detection of any abnormality the subjects were offered further evaluation of the abnormality and appropriate treatment.

Study subjects included in the second phases of the study were with a clinical indication for ovulation induction as part of the management of infertility. The subjects had the right to decline participation in the study as well as discontinue follow up. Though not licensed to be used in ovulation induction, Letrozole has been used in clinical trials and no adverse effects have been reported so far. It has been in clinical use for other indications for many years and there are no reported significant adverse effects attributed to its use. There is no known teratogenicity associated with Letrozole. The study was registered with the Clinical trials registry of the Sri Lanka Medical Association. (Trial registration number – SLCTR/2008/015)

## 2. Definition, prevalence and aetiology on infertility

### 2.1 Introduction

Definition of infertility

Only 40-45 years ago infertility was not recognised as a clinical entity. Little was known scientifically and medically, and the topic was hardly discussed socially (Balasch 2000). Within the short period of time since then, the speciality has evolved to be one of the most success stories of the medical sciences.

The National Institute for Health and Clinical Excellence (NICE) define infertility as "failure to conceive after regular unprotected sexual intercourse for two years in the absence of known reproductive pathology" (NICE 2004a). A similar definition has been held by the World Health Organization (WHO) over the years (World Health Organization 1975; 2000), as well as by many other scholars in specialities such as demography and epidemiology (Larsen 2005).

In clinical practice infertility is commonly defined as the absence of conception after twelve months of regular, unprotected intercourse (Evers 2002, Habbema et al. 2004a, Larsen 2005). A shorter time interval has been adopted in this instance to optimise the benefits of treatment and investigations to the couples. This approach has been supported by the definition used by the American Society for Reproductive Medicine (formerly the American Fertility Society) which states "Infertility is a disease. The duration of the failure to conceive should be twelve or more months before an investigation is undertaken unless medical history and physical findings dictate earlier evaluation and treatment" (The Practice Committee of the American Society for Reproductive Medicine 2006). The national institute of clinical excellence (NICE) in United Kingdom also recommends that in routine clinical practice couples should be offered investigations after one year of trying (National Institute for Health and Clinical Excellence 2004a).

The word 'subfertility' has been used synonymous with infertility though it is not a term listed in many medical dictionaries. It has gained recognition due to its use in European Society of Human Reproduction and Embryology publications, abstracts of the Cochrane database of systematic trials and many other related high-impact reproductive medicine journals (Habbema et al. 2004a). Though some have attempted to use the words subfertility and infertility to make a distinction between a relative inability of reproduction to a complete inability, no authority has made it clear how each term differ from the other (te Velde & Cohlen 1999, Habbema et al. 2004b) thus making some experts to propose that word subfertility should be abandoned (Homburg 2005). The term infertility is used throughout this thesis to denote the condition with the time duration as defined in clinical guidelines.

Prevalence of infertility

Infertility is estimated to affect 10-15% couples who are trying to achieve a pregnancy, worldwide (World Health Organization 1991, Evers 2002, Balen & Rutherford 2007). After review of 24 studies done around the world Boivin et al. estimated the prevalence of current infertility rate to be 3.5-16.7% while the lifetime infertility rate to be 5-26.4% (Boivin et al. 2007). It is estimated that less than half of couples suffering either from infertility or have had some difficulty in achieving the desired family size would seek treatment (White et al. 2006, Balen & Rutherford 2007). In recent times, owing to the publicity to infertility treatment and public acceptance of it as a medical condition, the number of couples seeking treatment has increased.

The prevalence of infertility is being studied in many areas of science, other than reproductive medicine, such as demography and epidemiology. The variations in definitions used and the methods employed have given a wide variation in the reported estimations (Larsen 2005). In demography for instance, the childbearing performance is measured disregarding the fact whether the couple wanted a child or not, so that those who were voluntarily childless are also counted (Larsen 2000, Habbema et al. 2004a).

It is estimated that worldwide there are 72.4 million women aged 20–44 and living in married or consensual relationships would experience a delay in conception by more than twelve months. Out of these women, around 40 million are likely to seek medical help for infertility (Boivin et al. 2007).

Prevalence data of infertility is not available in Sri Lanka due to lack of properly conducted epidemiological studies. The primary infertility rate in Sri Lanka was estimated by de Silva in 1995 using the data collected in the demographic health survey of 1987, to be around 5%. While the rate of secondary infertility was not estimated in this study the limitations in calculation of secondary infertility by demographic studies due to the effect of voluntary childlessness was recognised (de Silva 1995). Another study aimed at determining the point prevalence of primary and secondary infertility in the community, carried out in district of Colombo revealed the prevalence of primary infertility to be 4.1% and that of secondary infertility to be 16.1% (Samarakoon 1994).

Aetiology of infertility

Conception is a complex process and involves many key events such as ovum maturation, ovulation, ovum pickup by the fallopian tubes, fertilisation by the sperms and transportation of the embryo to the uterine cavity where implantation takes place. Each of these steps are regulated by numerous physiological and biochemical interactions, thus making it susceptible to abnormalities. Infertility could result from abnormalities in either one or more of these numerous steps. The common abnormalities include sperm dysfunction, ovulation disorders and fallopian tube damage while the lesser common causes comprise of endometriosis and other peritoneal disease, cervical mucus reactions, coital problems and uterine abnormalities. In a significant proportion of couples an abnormality would not be detected in spite of investigations and are therefore termed as suffering from unexplained infertility, which is in fact a diagnosis of exclusion. It is estimated to be found in around 20-25% of couples seeking treatment (Hart 2003).

Aetiology of infertility demonstrates varying patterns in different populations. In a study done in a single health district of England, sperm dysfunction or male infertility as it is commonly known, was found to be the commonest cause (in 24% of couples) of infertility among clinic attendees, and was followed by ovulatory dysfunction (21% of couples) and tubal disease (14% of couples). Other causes such as endometriosis, coital failure, cervical mucus defects and uterine abnormalities combined contributed to less than 15% of all couples (Hull et al. 1985). The data is limited on contribution of different aetiological factors in infertile populations of Sri Lanka as such studies have not been carried out in the country to date. However, few smaller studies have attempted to describe the prevalence of selected abnormalities in different infertile populations.

Sperm dysfunction: Sperm dysfunction is diagnosed by a seminal fluid analysis (SFA) which is most often the first investigatory intervention a male partner of an infertile couple would be offered. The main parameters assessed include the macroscopic assessment of the ejaculate for volume, liquefaction time, pH and the microscopic estimation of sperm concentration, total sperm number, motility, morphology, vitality and the white blood cell count. Abnormalities are described in comparison to the WHO defined reference values (World Health Organization 2000). A well conducted seminal fluid assessment is thought to be a reliable indicator of the fertility of a male, though it has its limitations in prediction of long term prognosis (Iammarrone et al. 2003, ESHRE Capri Workshop Group 2004).

Hull and colleagues who studied a large infertile population in the Bristol area, United Kingdom was able to detect sperm abnormalities in nearly 30% of couples (Hull et al. 1985). Similar figures have been described in certain parts of India where it was found to be 27.6% (Zager et al. 1997) and much higher rates in Mongolia 44.4% (Bayasgalan et al. 2004). However, studies carried out in Sri Lanka have demonstrated much higher rates of seminal fluid abnormalities. A study carried out by Wijeratne et al in 2005 in the Colombo district of

Sri Lanka the prevalence of seminal fluid abnormalities was as high as 67% (260 of 383 subjects) while in another study by Fernando in 2001 demonstrated a prevalence of nearly 60% (Wijeratne et al 2005, Fernando 2001).

Fallopian tube disease: Normal functioning and patency of the fallopian tubes are prerequisites for natural conception. The fallopian tubes are highly specialised organs which have a critical role in ovum pickup as well as transport of the oocyte, sperms and the embryo. In addition they function as the site of sperm capacitation and fertilisation. As the first stages of development of the embryo occur during its journey through the fallopian tubes, it serves as an organ of nutrition which facilitates the development of the embryo (Khalaf 2003). Infertility may result from either complete or partial occlusion of the tubes, damage to the tubal epithelium affecting its function or due to pelvic pathologies such as endometriosis and pelvic infection causing pelvic adhesions which reduces mobility of tubes. Infections of the genital tract involving the tubes remain the leading cause of tubal factor infertility. Chlamydia trachomatis infection is the most prevalent bacterial sexually transmitted infection causing tubal damage throughout the world and is estimated to be responsible for nearly two thirds of cases of tubal infertility (Peipert 2003). Tubal disease has been shown to be the main contributory cause for infertility in 11-30% of infertile couples in previous studies (Hull et al. 1985, Evers 2002). The prevalence of Chlamydia infection is thought to be much less in local populations, and a recent study on a local infertile population demonstrated the disease prevalence in either partner to be 7.5% (Palihawadana et al 2010).

Hysterosalpingography (HSG) and laparoscopy with chromotubation remains the most commonly used investigations to assess tubal morphology and patency. Laparoscopy has the ability to detect pelvic pathology in addition to tubal assessment and has proven to be more reliable in predicting fertility than HSG (Evers 2002). However, the invasive nature of the procedure, the increased cost involved and the higher risk profile do not justify the replacement of HSG in routine clinical practice and it is recommended the two procedures be selected on an individual patient basis (National Institute for Health and Clinical Excellence 2004b).

Endometriosis and other peritoneal disease: Endometriosis is defined as presence of endometrial like tissue outside the endometrial cavity, which includes a chronic inflammatory reaction. It mainly affects the pelvic organs with ovaries being the commonest site followed by pouch of Douglas, round ligament, uterosacral ligaments, fallopian tubes and the myometrium. Extra-pelvic sites such as the umbilicus, lungs and the bowels can also rarely be involved. Endometriosis is estimated to be present in 20-40% of women with infertility in contrast to around 5% of fertile women (Hart 2003). The American Society of Reproductive Medicine, ASRM (formerly the American Fertility Society, AFS) has classified endometriosis in to four stages of disease severity, in which the appearance of lesions, the degree of adhesions and the obliteration of the pouch of Douglas provide a score. Endometriosis contributes to infertility through many pathological processes. Pelvic adhesion formation affecting tubal motility is thought to be the commonest mechanism.

Though a causative association has been demonstrated between moderate and sever forms of the disease and infertility, the relationship of minimal to mild endometriosis and infertility remains controversial. A systematic review carried out by Jacobson et al. in 2002, which included two randomised clinical trials favoured towards treatment of mild to moderate disease and based on this many authorities recommend this approach in routine clinical practice (NICE 2004c, RCOG 2006).

While endometriosis is the commonest peritoneal disease affecting fertility many other inflammatory and infective causes can occur in women of the reproductive age group. Inflammatory conditions of the gastrointestinal tract as well as ascending infections of the genital tract may give rise to pelvic inflammatory disease (PID). Though, pelvic infection

results mainly from ascending infection from the genital tract, occasional occurrence of isolated pelvic infections are seen with pathogens such as Mycobacterium tuberculosis.
Uterine abnormalities: These abnormalities which could be either congenital or acquired are an uncommon cause of infertility. The abnormalities may affect the endometrium or the myometrium. The congenital causes include developmental abnormalities of the mullerian dust system that may affect the normal morphology of the uterus, fallopian tubes, cervix or the upper vagina. They make a spectrum of abnormalities from total absence of uterus and vagina (Mayer-Rokitansky-Kuster-Hauser syndrome) to minor defects such as arcuate uterus and uterine or vaginal septae. Many more abnormalities have been described in literature such as absence of endometrium, but the occurrence of such conditions is extremely rare (Berker et al. 2008). While strong associations have been described between uterine abnormalities and pregnancy wastage and premature delivery, the relationship of such anomalies with infertility is less clear except in complete absence of uterus, cervix, vagina or a combination of these. The acquired causes include growths such as fibroids and endometrial polyps, endometrial damage by infection or secondary to investigatory or therapeutic procedures such as dilatation and curettage leading to endometrial adhesions or synechiae.
Fibroids which is the commonest cause of uterine abnormality seen among infertile patients has been implicated to be contributing to infertility in 5-10% of patients but is estimated to be the sole cause only in 2-3% of patients (Mukhopadhaya et al. 2007). The effects are thought to be brought about by distortion of uterine cavity, reducing implantation, dysfunctional uterine contractility and changes in the endometrial cavity milieu. However the place of surgery in fibroid uterus and which patients would benefit from treatment remains controversial.
Other causes of infertility: While many other causes may be contributory to infertility, the exact contribution of these in isolation is estimated to be very low. Coital problems are a group of disorders that may be brought about by the inability to have frequent, regular penetrative coitus. It is recommended that a couple have sexual intercourse once every two to three days to achieve the maximum fertility potential. Intercourse timed with ovulation is thought to cause excessive stress on the couple and thereby reduce the chance of fertility (NICE 2004a). Cervical mucus and sperm interaction has also been described as an uncommon cause of infertility (Fernando 2001). However, controversy exists with regard to the actual contribution of such interactions in causation of infertility.

## 2.2 Objectives

1. To describe the distribution of an infertile population according to socio-demographic characteristics.
2. To describe the clinical features that were elicited in male and female partners of a population of infertile couples who sought treatment.
3. To determine the prevalence of uterine abnormalities among a female infertile population.
4. To determine the prevalence of seminal fluid parameters among the male partners of an infertile population.
5. To describe the prevalence of sexual dysfunction among a population of infertile couples.

## 2.3 Methods

Study setting and design

This part of the study was carried out at the university infertility clinic of the North Colombo Teaching hospital. This is an infertility treatment facility which provides services of basic andrology and endocrinology investigations, clinical gynaecology investigations as well as treatment up to intermediate level including intrauterine insemination (IUI). The main source

of patient referral was through the outpatient department of the hospital as well as from the primary health care workers of the area. A small proportion of patients were referred by the consultant gynaecologists where the patients were referred for specific investigations or treatments.
A cross sectional study design was carried out to identify prevalence of different causes of infertility among the study population. The study subjects were followed up in the clinic till the investigations were complete.

Method

All new patients presenting to the clinic seeking infertility treatment were recruited to the study after obtaining informed written consent. As the main focus of the study was on ovulatory dysfunction among study subjects, an in depth evaluation of the ovulatory status of the female partners was carried out. Findings in relation to ovulatory status and the contributory causes for anovulation are described in the following section.
Assessment of the couples with regard to other causes of infertility was according to routine clinical protocol. This included a detailed clinical interview of both partners to assess risk factors for reduced fertility, a clinical examination of the female partner, seminal fluid analysis of the male partner in the first presentation. Further investigations were according to the findings of the initial assessment or as required for the treatment offered.
The clinical history and examination were recorded using an observer administered questionnaire and was carried out by one investigator in all subjects. The questionnaire used was pre-tested and modified prior to use in the study. It was used to record basic socio-demographic details of the couple, the risk factors for female and male factor infertility, the sexual history and the previous treatment the couple has undergone.
The sexual function of the couples was assessed with a detailed clinical interview from both partners. The regularity and frequency of intercourse as well as any abnormalities in erection and ejaculation was assessed. The intercourse was considered irregular if taking place irregularly over the preceding month irrespective of whether it was due to lack of sexual desire or due to other reasons such as staying away from home due to demands of the occupation. The frequency of intercourse for a week was recorded. An inability to gain an erection or maintain the erection till ejaculation was considered as erectile dysfunction. Absence of ejaculation or low volume ejaculation was included in ejaculatory dysfunction.
Examination of the female partner included the BMI, evidence of hyperandrogenic status, abnormalities of the thyroid gland, evidence of hyperprolactinaemia and any evidence of pelvic pathology.
All female partners were offered a pelvic ultrasound scan two days after the first day of the menstrual bleeding to evaluate the endometrium, uterine cavity and to exclude any other uterine pathology. Investigations for tubal patency were offered only according to clinical indications. These include infertility of more than three years, a clinical history suggestive of tubal or pelvic pathology or those couples who were to undergo treatment such as ovulation induction or intrauterine insemination. In those whom a pelvic pathology was suggestive either from the history or the examination, diagnostic laparoscopy was undertaken whereas others were offered hysterosalpingography (HSG).
All male partners of the couples were offered seminal fluid analysis (SFA) as the first line investigation and this was interpreted according to the WHO guidelines (WHO 2000). If the initial SFA was abnormal a repeat test was performed after a three month period and the investigation which yielded a higher number of total motile sperm count (volume x concentration x motility) was considered in identifying those with male factor infertility. The reference range used in interpretation of SFA is shown in table 2.1.

**Table 2.1 The normal values for sperm parameters in seminal fluid analysis according to World Health Organization**

| Parameter | Normal range |
|---|---|
| Volume | 2 ml or more |
| pH | 7.2 or more |
| Sperm concentration | 20 million / ml or more |
| Sperm motility | 50% or more with progressive motility (motility grades 'a' & 'b') |
| Sperm morphology | 30% or more with normal morphology |
| Sperm viability | 75% or more viable |

Sample size and study duration

This phase of the study included 518 couples who sought treatment for infertility.

## 2.4 Results

The socio demographic characteristics of the study population

The 518 couples included in the study had a mean (SD) duration of marriage and infertility of 50(37) months and 35(27) months respectively. The duration of marriage ranged from 12 months to 156 months while for the duration of infertility it ranged from 12 months to 144 months. Majority of women (n=378, 73%) had primary infertility while in 140 women (27%) it was secondary infertility. These data is shown in table 2.2.

**Table 2.2 The duration of marriage, duration of infertility and the type of infertility among the study subjects. (n= 518)**

| Characteristic | |
|---|---|
| Duration of marriage; mean(SD, range) in months | 50(37, 12-156) |
| Duration of infertility; mean(SD, range) in months | 35(27, 12-144) |
| Type of infertility; no(%) | |
| Primary | 378(73%) |
| Secondary | 140(27%) |

The mean(SD) age of the female subjects was 29.3(4.9) years with a range of 18 years to 40 years. Most number of women were within 25-30 years (n=207, 40.0%) while nearly 83% of the study population was less than 35 years of age. Five subjects (1%) were less than 20 years, 81(15.6%) between 20 and 25 years , 207 (40.0%) between 25 to 30 years, 139(26.8%) between 30-35 years while 86 subjects (16.6%) were between 35 to 40 years of age. The male partners of the couples were of a more advanced age group with a mean(SD) of 32.54(5.31) years and a range between 21 years to 50 years. Most number of subjects were between 30-35 years (n=182, 35.1%). Twenty subjects (3.9%) were less than 25 years, 148 (28.6%) between 25 and 30 years, 182(35.1%) between 30 and 35 years, 108 (20.8%) between 35 and 40 years, 48 (9.3%) between 40 and 45 years while 12 male partners (2.3%) were 45 years or more of age. The age distribution of the study population is shown in table 2.3.

**Table 2.3 The distribution of the study population according to age category. (n= 518)**

| Age category | Female partners; n(%) | Male partners; n (%) |
|---|---|---|
| Less than 20 years | 5(1%) | - |
| 20 to <25 | 81(15.6%) | 20(3.9%) |
| 25 to <30 | 207(40.0%) | 148(28.6%) |
| 30 to <35 | 139(26.8%) | 182(35.1%) |
| 35 to <40 | 86(16.6%) | 108(20.8%) |
| 40 to <45 | - | 48(9.3%) |
| 45 years or more | - | 12(2.3%) |

A large proportion of the female partners of the study population had an education up to ordinary level (grade 10) or less while three (0.58%) of the study subjects did not have even the primary education. Thirteen subjects (2.5%) had only primary education (up to grade 5 or less), 331(63.8%) between grade five to ordinary level, 141 (27.2%) up to advanced level, 9 (1.7%) up to diploma or technical training while 21(4%) had an education leading to a first degree or more. Among the male partners only one subject (0.2%) was without any formal education and 18(3.47%) had only primary education, 352(67.9%) between grade five to ordinary level, 130(25%) up to advanced level, 10 subjects (1.9%) with a diploma or a technical training while 7 subjects (1.3%) were graduates.

Among the female partners of the study population 147 subjects (28.3%) were employed. Four (2.7%) of them were in occupations of manual labour, 98(66.7%) in non-skilled work, 43 in skilled work (29.3%) while two (1.4%) were professionals. All except one male study subject were employed and 42 (8.1%) of them were engaged in occupations of manual labour, 230 (44.5%) in non skilled work, 239 (46.2%) in skilled work and six (1.2%) were in professional occupations. The monthly household income for the study population was between 6000 to 100,000 rupees per month with a mean(SD) income of Rs.18,213.79 (12,010).

Among the 140 female study subjects who had secondary infertility, spontaneous miscarriage was the most common previous gestation and was seen in 74 subjects (52.9%), followed by viable pregnancy (n=64, 45.7%), termination of pregnancy (n=16, 11.4%) and ectopic pregnancy (n=8, 5.7%) while molar pregnancy was seen only in one study subject (0.7%).

Of the study sample 460 subjects (88.8%) were vaccinated against rubella while one patient (0.2%) had a history of infection in the past and 25(4.8%) were not vaccinated. Thirty two study subjects (6.2%) were unaware of their immune status.

<u>The clinical features elicited in the study population</u>

Majority of women (n=385, 74.3%) described their menstrual flow as normal in relation to amount of flow while 111 (21.4%) of them complained of heavy menstrual loss and 22 (4.2%) of scanty or reduced flow. Seventy eight of the subjects (15.1%) experienced dysmenorrhoea severe enough to either affect their daily activities or make them use medication for pain relief.

Thyroid dysfunction was the most commonly seen pre-existing medical illness that could affect fertility with 12(2.3%) subjects suffering from it followed by diabetes (n=4, 0.8%), pelvic tuberculosis (n=1, 0.2%), pelvic inflammatory disease (n=1, 0.2%) and sexually

transmitted infection (n=1, 0.2%). Fourteen subjects (2.7%) were on regular medication at the time of recruitment with four subjects were on antihypertensives, four on oral hypoglycaemic agents, nine on thyroxine and three subjects on carbimazole. A history of previous abdominal or pelvic surgery was noted among 53 (10.2%) subjects. Seventeen subjects (3.3%) had undergone caesarean section in a previous pregnancy, eleven (2.1%) an ovarian cystectomy, ten (1.9%) appendicectomy, five (0.9%) a salpingectomy, four (0.8%) an unilateral ooprhorectomy, two (0.4%) an endometrial polypectomy while one (0.2%) each had ovarian drilling, adhesiolysis, fimbrial cystectomy or inguinal hernia reapair. The medical disorders and the surgeries in the past among the study subjects are shown in figure 2.1.

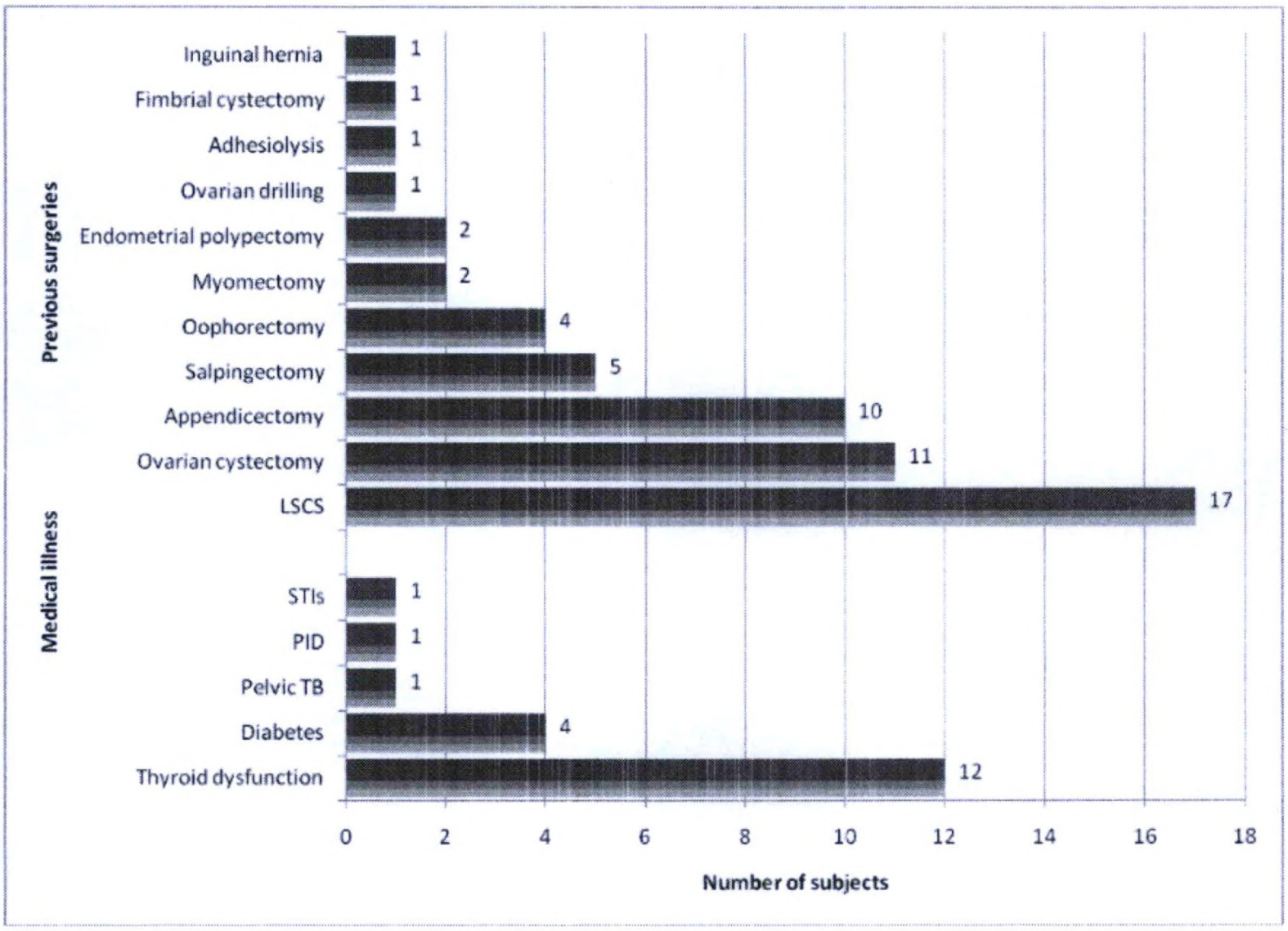



**Figure 2.1 The prevalence of pre-existing medical disorders or a history of significant previous surgeries among study subjects. (n= 518)**

Examination of the female partners revealed that the mean (SD) body mass index (BMI) of the study population was 23.87(4.5) kg/m$^2$ with a range of 14.6 to 37.6 kg/m$^2$. Sixty three subjects (12.2%) were underweight with a BMI less than 18.5 kg/m$^2$ while 253 (48.8%) were within the normal range of 18.5-24.9 kg/m$^2$ while 153(29.5%) being overweight with a BMI of 25.0-29.9 kg/m$^2$ and 49(9.4%) being obese with a BMI of over 30 kg/m$^2$.

Thyroid enlargement was noted in 36 (7.2%) subjects, acanthosis nigricans in 51(10.1%) and hirsutism in 19(17.4%) subjects. Pelvic examination revealed an enlarged uterus in 2 subjects, reduced mobility of the uterus in six and adnexal fullness in 2 subjects. Transvaginal ultrasound scanning to assess the uterus and pelvic cavity demonstrated uterine fibroids in 27 (5.21%) subjects. However, only 3 of them were large enough or were close to the endometrium to cause distortion of the endometrial cavity. Other abnormalities detected included adenomyosis changes in three subjects, endometrial polyps in two subjects, cervical

polyp in one, an unicornuate uterus in one and an uterine septum in one subject. Tubal tests were undertaken in 196 subjects with 139 of them undergoing hysterosalpingogram (HSG) and diagnostic laparoscopy with chromotubation in 57 subjects. Most of the subjects who underwent tubal tests (n=176, 91.3%) had bilateral patent tubes, 18 subjects (9.1%) had unilateral tubal occlusion while two (1%) had bilateral tubal occlusion. Pelvic pathologies noted at diagnostic laparoscopy included mild to moderate endometriosis in 14 subjects, severe disease with frozen pelvis in 2 subjects, arcuate uterus in one subject, unicornuate uterus in one, tubal disease with nodularity and peritubal adhesions in two subjects and an ovarian cyst in one subject.

The clinical features and the seminal fluid analysis in the male partner

The medical illnesses that affect fertility seen among the male partners included diabetes mellitus in four (0.8%), history of sexually transmitted infection in five (1.0%) while 41 subjects (7.9%) had a history of mumps after pubertal age of twelve years. Twenty one (4.1%) male partners had a history of pelvic or abdominal surgery or trauma that may affect fertility. These included appendicectomy in eight subjects, inguinal hernial repair in seven and one each of testicular surgery, penile surgery, varicocelectomy, urethral injury, genital injury and shrapnel injury to the genitalia. Twenty one subjects (4.1%) had a history of exposure to toxic matter in their occupations that may affect spermatogenesis and these included excessive heat (n=8, 1.6%), fumes (n=1, 0.2%), radiation (n=4, 0.8%) and chemicals (n=13, 2.5%). Smoking was seen among 150 (29.0%) of the subjects while 368 (71.0%) were none smokers. Assessment of the alcohol consumption of the study population revealed that 206 (39.7%) male partners did not use alcohol while 19 (3.7%) consumed alcohol on a daily basis, 10 subjects (1.9%) using more than once a week and 283 subjects (54.6%) using it less than once a week.

Among the study population the seminal fluid analysis (SFA) was normal in all parameters in 281subjects (54.2%) whereas at least one parameter was abnormal in 237 (45.7%) subjects. Most common abnormality detected was reduced sperm motility (n=130, 25.1%), followed by low sperm concentration (n=103, 19.8%), low volume (n=100, 19.3%), increased abnormalities in sperm morphology (n=27, 5.2%) and reduced viability (n=22, 4.2%). Azoospermia was detected in 26 subjects (5.0%) while another 33 (6.4%) had severe oligozoospermia (a total motile sperm count of less than 10 million). The remaining 178 subjects (34.3%) with abnormal seminograms had mild to moderate SFA abnormalities. The prevalence of sperm abnormalities among the study population is shown in figures 2.2 and 2.3.

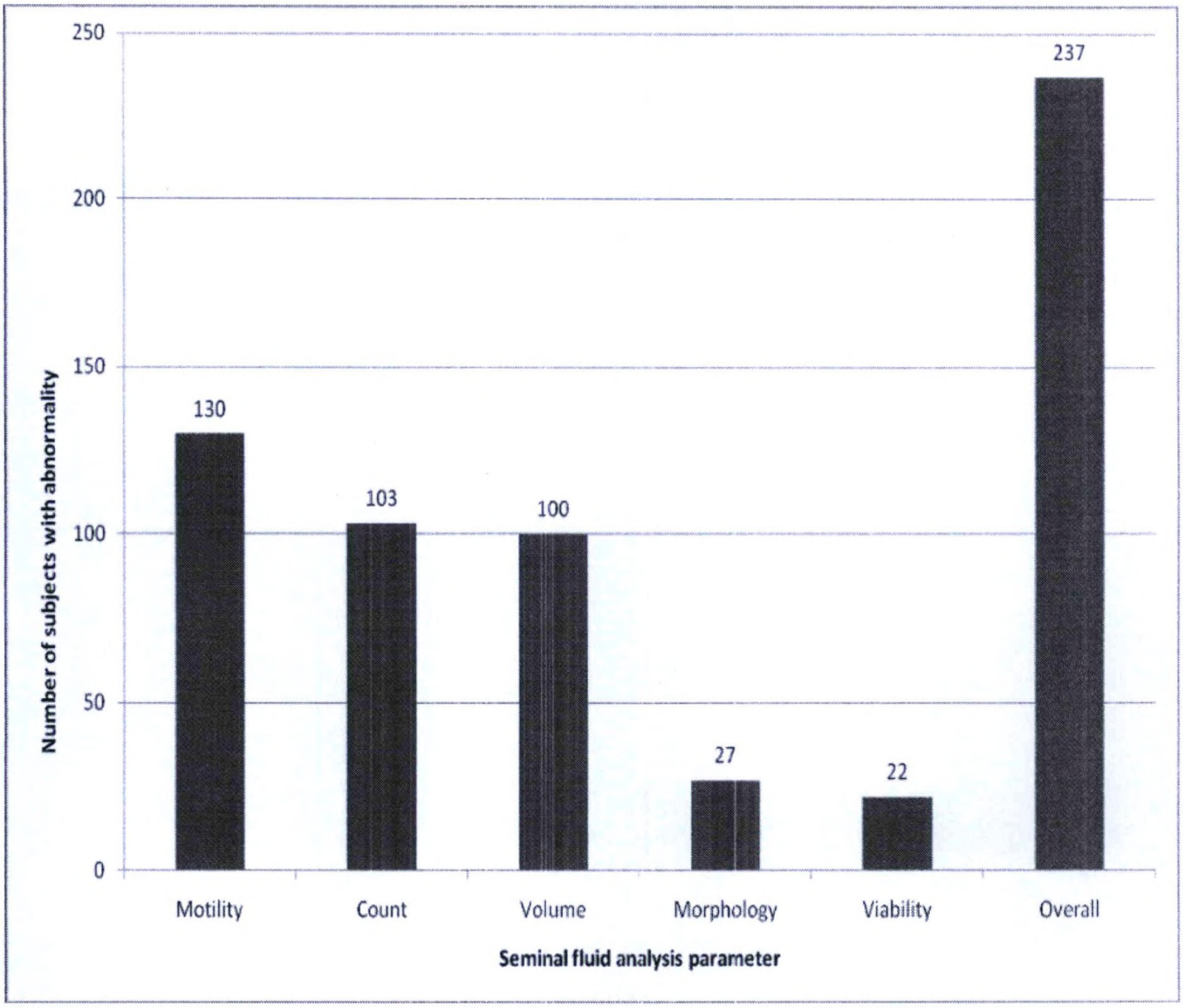



**Figure 2.2 The distribution of the study population according to abnormalities detected in the seminal fluid analysis. (n= 518)**

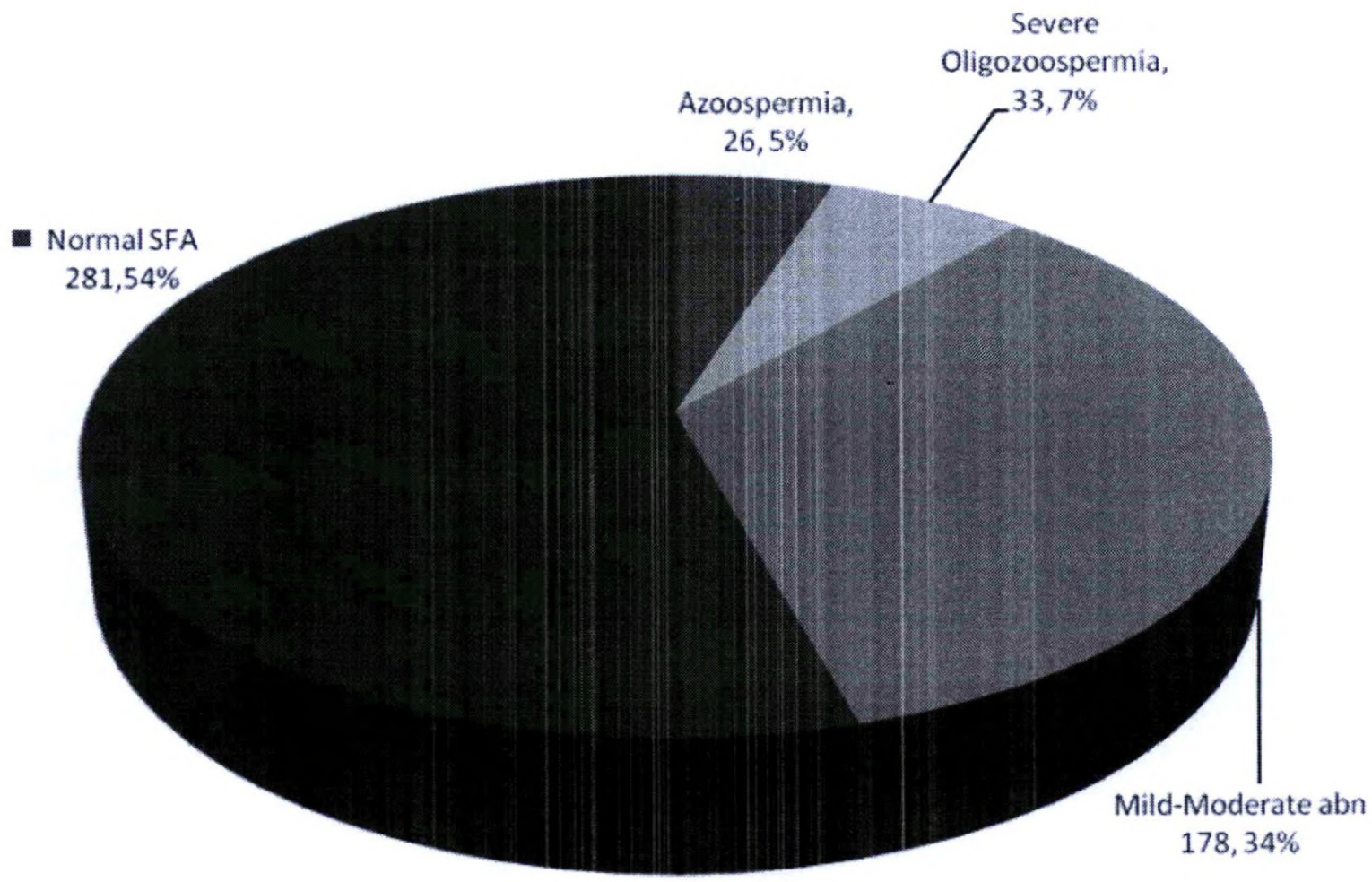



**Figure 2.3 The distribution of study population according to severity of seminal fluid abnormality. (n= 518)**

Abnormalities in the sexual function among the study population

Abnormalities in sexual function were noted among 56 couples (10.8%) of the study population. The most common abnormality detected was irregular coitus which was seen among 36 (6.9%) of subjects. Infrequent coitus, which was defined as less than once a week on average, was seen in 15 (2.8%) couples. Erectile dysfunction and abnormalities in ejaculation were uncommon among the study population being present in only 5 (0.9%) and 4 (0.7%).

## 2.5. Discussion

Infertility is a clinical condition different from many others as it may result from a range of pathologies, in one or both partners of a couple. Therefore, the process of investigation is often more complex than many other clinical scenarios. As the pattern of underlying pathologies varies in different populations, it often causes a dilemma for the clinicians to decide the best sequence of investigations and treatment. An understanding of the prevalence of contributory causes would enable the clinicians to determine the best course of management in a local setting as well as aid in counselling the patients with regard to prognosis.

This study was aimed at describing the socio-demographic characteristics of an infertile population in Sri Lanka, and to determine the prevalence of various underlying causes that may contribute to infertility among them. The main aetiological factors assessed included abnormalities in seminal fluid parameters, abnormalities in sexual function and abnormalities in the female genital tract including fallopian tubes. Abnormalities of ovulation are not described in this chapter as it is discussed in detail in the following section.

The study population studied was a non-contrived sample that presented to the infertility clinic seeking infertility treatment. It was a young population with a mean age of 29 years. The proportion of subjects of an advanced age group, such as over 35 years, was low (16%) and included only 86 subjects. Therefore, the results of the study may not be representative of those that are related to advanced age of the female, which is a known causative factor of reduced fertility (Adamson & Baker 2003).

The mean duration of infertility among the study subjects was around three years. As they were with a long period of infertility it is likely that it included a lesser number of subjects with minor abnormalities which may result in pregnancy with time. Such abnormalities may include causes such as minor ovulatory dysfunctions as well as non-severe abnormalities of seminal fluid parameters. Furthermore, the duration of infertility showed a wide standard deviation, demonstrating that the study population is likely to be heterogeneous in aetiology and severity.

The proportion of subjects with primary infertility in the study population was 73%. This is dissimilar to the findings of a community based study carried out in Sri Lanka, which has shown the proportion of primary infertility to be around 20% (Samarakoon 1994). This discrepancy could be due to higher rate of seeking treatment by subjects with primary infertility compared to those who already have one or more children. Also, this may be due to over representation of secondary infertility in community based studies as some subjects with voluntary childlessness are also categorised as infertile.

The socio-economic indicators of the study population demonstrated that the majority of study participants to be in the middle socio-economic category with the majority educated up to grade 10 and having a mean household monthly income of around 18,000 rupees per month.

In spite of nationwide vaccination programs, nearly 5% of the subjects were not vaccinated against rubella. A further 6% were not aware of their rubella immune status. Rubella infection in early pregnancy is a known cause of serious congenital abnormalities of the offspring. Therefore, it is an important clinical feature that should be elicited in all subjects seeking infertility treatment as it provides an opportunity to offer them vaccination. Screening for rubella immune status should be offered to those who are not aware of their immune status and such interventions are recommended in clinical guidelines (NICE 2004a).

Over 20% of the study subjects interviewed admitted their menstrual flow to be excessive while another 4% complained of scanty flow. Previous studies have shown that the proportion of women who perceive their menstrual flow to be abnormal is significantly high even in the community. One such study carried out in United Kingdom which included over 1500 subjects demonstrated the proportion who perceived their menstrual loss to be abnormal as high as 40% (Shapley et al. 2007). However, in the absence of methods of objective measurement of the menstrual loss it is considered that a significant proportion of such women will not have any underlying pathology and the pelvic ultrasound scanning remains the mainstay of investigation in patients younger than 40 years of age (NICE 2007).

Thyroid dysfunction was seen in over 2% of the study subjects while other conditions such as diabetes, tuberculosis, pelvic inflammatory disease and sexual transmitted infection were much rarer. As the prevalence of these abnormalities is very close to that of the general population, routine screening for them may not be justifiable in clinical practice. While a significant proportion of women had undergone an abdominal or pelvic surgery in the past, which could potentially affect fertility, the exact impact of these surgeries was not studied in this study.

The mean body mass index of the study population was 23 $kg/m^2$ and nearly 50% of the subjects were within the normal range described for Asian populations (18.5-25 $kg/m^2$). The proportion of subjects that was overweight was 29% while 9% were obese. While obesity is a recognised cause of reduced fertility and a predictor of poor response to treatment, the proportion of women with infertility seems to be much lower in this local population

compared to that of the western world where proportion of women with obesity is estimated to be over 15% (Brewer & Balen 2007).

The presence of fibroids was the most common pelvic abnormality that was detected at ultrasound scanning. While over 5% (n=27) of the subjects were found to have fibroids only three of them had fibroids large enough to cause distortion of the uterine cavity. Though many have demonstrated the negative effects of fibroids the exact mechanisms or the characteristics of the fibroids that may have an effect is not completely understood (Farquhar 2004, Griffiths et al. 2006, Hart 2003). Other uterine abnormalities that are known to affect fertility such as uterine septum, unicornuate uterus and endometrial polyps were very rare in this study population. However, a baseline ultrasound assessment of the uterus at the initial presentation is important in detection of these since these are easily treatable causes of infertility. Many authorities have questioned the usefulness of clinical pelvic examination in the era of pelvic ultrasound scanning. However, pelvic examination still remains the mainstay of screening pelvic endometriosis with extensive adhesions where uterus tends to be less mobile.

Nearly 10% (n=20) of the subjects who underwent tubal patency tests were found to have an abnormality. Bilateral tubal occlusion was seen in two subjects while it was unilateral in the others. These tests were undertaken according to the clinical protocols only among subjects who had a clinical history suggestive of pelvic pathology, those who had long standing infertility (infertility >3 years) or those who were planned to undergo treatment such as intrauterine insemination. Therefore, such pathologies would be over-represented as tests have been undertaken in a subgroup with a higher risk of tubal pathology than the total study population.

Though the presence of risk factors for seminal fluid abnormalities was studied the causative association of these was not studied as it was beyond the scope of this study. The most common risk factor was post-pubertal mumps followed by surgery/trauma in the pelvis or genitalia, and exposure to harmful environmental factors. The proportion of males with these risk factors was less than 10% in the total population. Another significant finding of the study population was low proportion of males smoking and consuming alcohol at high levels. Only 29% of subjects admitted to smoking whereas less than 6% of them admitted to using alcohol excessively (more than once a week).

Male factor infertility detected by abnormal seminal fluid parameters was seen among 45% of the study subjects. This was higher than many other studies where it is estimated to be around 30- 40% (Cahill & Wardle 2002, Hull et al. 1985, Adamson & Baker 2003). However, this was much lower than the prevalence rates observed among Sri Lankan populations which have been as high as 60-67% (Wijeratne et al. 2005, Fernando 2001). This dissimilarity is likely to be due to the differences in the study populations studied. Nearly 5% of the male partners had azoospermia at presentation and a further 6.4% was found to have severe SFA abnormalities. This justifies assessment of seminal fluid analysis as an initial investigation of all infertile couples prior to further interventions.

Sexual dysfunction which is often not emphasised adequately in assessment of infertile couples was present in nearly 10% of the population. Among the various abnormalities irregular intercourse was the most common. This was important as many of the sample studied had male partners working away from home preventing them from engaging in intercourse on a regular basis. Other abnormalities such as erectile dysfunction and ejaculatory abnormalities were present in a very small minority of less than 1%.

The above demonstrate that male factor infertility is one of the major contributory factors for infertility. Female factor infertility excluding ovulatory dysfunction is seen in a much less proportion of couples. Also it is important to note that assessment of these factors is important and should be carried out simultaneously with assessment of ovulation in the female partner prior to any treatment.

## 3 - Ovulatory dysfunction: Prevalence and association of underlying causes.

### 3.1 Introduction

Oocyte maturation and Ovulation

Oogenesis has been an interest in medicine for many years. Almost four centuries ago an English physician wrote in ex ovo omnia that "all that is alive comes from the egg" (Yao 2007). Ovulation is the final event of a complex and long process of oocyte development and maturation. This process begins as early as three weeks of fetal life where the primordial germ cells can be identified in the endoderm of the yolk sac. They can be distinguished from the endodermal cells by their large size and the clear cytoplasm which contains fewer organelles (Strauss & Williams 2009). These primordial germ cells originate in the proximal region of the epiblast, close to the extraembryonic endoderm and enter a period of migration and proliferation. In the human they migrate from the yolk sac epithelium to the hind gut by four weeks post-fertilization and then migrate through the dorsal mesentery, finally reaching the genital ridges by approximately six weeks post-fertilization. Germ cells are unable to persist outside the genital ridges and play an indispensible role in induction of the gonadal development. In the absence of germ cells, pregranulosa cells are not maintained and an inert streak gonad containing only stromal cells result. This is seen in females with Turner syndrome (45XO).

The germ cells that arrive at the genital ridges are referred to as oogonia. The modification of genetic imprinting occurs at this stage. By six to seven weeks of intrauterine life the oogonia population expands by mitosis to reach some 10,000 cells and to around 600,000 by the end of eight weeks. From this point onwards the number is influenced by three processes working concurrently; mitosis, meiosis and oogonial atresia. As a result of the combined effect of these the number of germ cells reaches a peak number of six to seven million by 20 weeks of intrauterine life. Starting by around eight weeks of gestation, some of the oogonia enter the prophase of the first meiotic division, which protects them from atresia. The oogonia that enter the prophase invest themselves with granulosa cells and form the primordial follicles whereas those that do not enter the prophase by 28 weeks of gestation and persisting as oogonia undergo apoptotic cell death. Therefore, oogonia do not exist at birth (Espey & Richards 2006).

There are around 700,000 primordial follicles present at the time of birth. This number is further reduced to around 300,000 by the time of puberty. Of these follicles only 400-500 will develop to ovulate during the reproductive life span. The oocytes persist in the prophase of the first meiotic division until just before ovulation, when meiosis is resumed and the first polar body is formed and extruded. The primordial follicles in the human ovary are composed of a late diplotene primary oocyte surrounded by a single layer of flattened granulosa cells. The follicles of this stage of development are not believed to be influenced by gonadotropins. The morphology of the primordial follicle further changes to a primary follicle where a primary oocyte is surrounded by a single layer of cuboidal granulosa cells and a secondary follicle where the primary oocyte is surrounded by several layers of cuboidal granulosa cells. Approximately one year may elapse in the maturation of a primordial follicle to a dominant follicle, of which the follicles are thought to be independent of gonadotropins for much of the time and is influenced by the gonadotropins only in the last 50 days. The granulosa cells are derived from ovarian surface epithelial mesothelium or possibly from the rete ovarii (Espey & Richards 2006).

The initiation of follicle growth is characterized by morphological changes including changes in granulosa cell morphology, from a flattened shape to a cuboidal, proliferation of granulosa cells, enlargement of the oocyte and the formation of the zona pellucida. This process occurs from fifth to sixth month of intrauterine life until menopause. As the secondary follicle is formed, the granulosa cells develop FSH, oestrogen, and androgen receptors. Intra-ovarian factors are believed to play key roles in regulating the early phases of follicular growth. There is little doubt that the transition from secondary follicle to the

antral-follicle stage is promoted by FSH. Antral follicles are rarely observed in humans with FSH deficiency (Strauss & Williams 2009).
Follicle recruitment refers to the process by which the follicle departs from the resting pool to initiate growth. In the early follicular phase, no gross morphological differences exist between the selected follicle and other healthy members of the cohort. The leading follicle can be distinguished from other members of the cohort by its size and high mitotic index of its granulosa cells. Only the leading follicle will have detectable levels of FSH in its follicular fluid. The leading follicle will also contain significant levels of oestradiol (Fauser & Heusden 1997). The follicle dominance refers to the status of follicle destined to ovulate and it attains dominance five to seven days after demise of the corpus luteum of the previous cycle. Studies have demonstrated the selection of the follicle destined to ovulate already has occurred as early as the day eight of the cycle. The chosen follicle is able to synthesise oestradiol in sufficient quantities to result in appreciable passage of this into the general circulation by the fifth to seventh day of the cycle (McGee & Hsueh 2000).
The expression of functional LH receptors on granulosa cells of the pre-ovulatory primary follicle allows LH to substitute for FSH in the promotion of the terminal stages of the maturation. As the midcycle approaches, the rise in oestrogen emanating from the dominant follicle initiates a LH surge and to a lesser extent, a FSH surge. This triggers the resumption of meiosis, ovulation and luteinisation. The pre-ovulatory LH surge precedes the follicular rupture by as much as 36 hours. A conical stigma eventually rises on the surface of the protruding follicle in preparation for rupture. The rupture of the stigma is accompanied by gentle expulsion of the egg and follicle fluid, which suggests that the fluid is not under high pressure. Though it is believed that in primates the ovulation tends to alternate between ovaries due to local action of progesterone produced by the corpus luteum on follicular dynamics, this has not been supported by conclusive evidence (Strauss & Williams 2009).

Gonadotropins and ovulation

Follicle stimulating hormone (FSH) is required for the transition of pre-antral follicles to antral stage. Follicle maturation initiated at the start of a new menstrual cycle is driven by an increase in FSH levels in the late luteal phase, which is associated with a falling progesterone, oestradiol and inhibin A levels. Pre-antral follicles apparently require a threshold of FSH concentration to sustain growth and this threshold level is reached during the late luteal phase. Remarkably the threshold can be crossed with as little as a 10% to 30% increment in FSH, indicating that granulosa cells have a high sensitivity for FSH. This hormone can induce follicular growth to the pre-ovulatory size of 17mm in the virtual absence of LH.
Granulosa cell division is also promoted by FSH, possibly by an indirect mechanism (Strauss & Williams 2009). One of the main functions of FSH is the induction of aromatase in granulosa cells. Therefore, little or no oestradiol can be produced in the FSH un-primed granulosa cells. FSH also induces LH receptors of the granulosa cells of the pre-ovulatory follicle. In the late stages of follicle maturation the LH can facilitate FSH function in propelling follicular maturation. This allows the dominant follicle to complete its maturation in spite of declining FSH levels (Ginther et al. 2001). In addition, the dominant follicle is prepared to respond to the ovulatory LH surge (ESHER 2004).
The luteinizing hormone (LH) stimulates the theca cell steroidogenesis during the follicular phase which provides the androgen substrate for granulosa cell aromatization. The LH is not required for follicular expansion since the FSH can drive follicular development up to the pre-ovulatory state. In the normal menstrual cycle the FSH induced appearance of LH receptors on pre-ovulatory granulosa cells allows the LH to take over the function of FSH in the terminal stages of follicular maturation. This also enables the follicle to respond to the LH surge that initiates the resumption of meiosis, ovulation and subsequent luteinisation of the granulosa and theca cells. These events are only achieved when the threshold level of LH

is reached. Furthermore, the LH action of stimulating the dominant follicle for final maturation indirectly retards the development of smaller follicles. This has an important clinical implication in ovulation induction, where administration of LH or hCG to drive terminal stages of follicular development facilitating monofollicular development.
In addition to these endocrine controls many other chemical and immunological mechanisms are also thought to be involved in the ovulation process (Espey 1980, Richards et al. 1998, Epsey & Richards 2006).

Assessment of ovulation

Detection of ovulation is an important part of investigation and treatment of couples with infertility. In contrast to most other species there are no distinct behavioural changes or obvious physical signs noted with ovulation in the human though many hormonal changes occur throughout the menstrual cycle. Tests used to assess ovulation are broadly divided in to those that predict ovulation and those that are used for confirmation. Furthermore, they are divided as direct and indirect methods where direct methods visualise ovulation commonly by high resolution ultrasound scanning while the indirect methods look at the ovarian or pituitary hormones that are associated with ovulation or their effects. The indirect methods have the advantage of being less expensive and less invasive than direct visualisation. However, they have their own limitations in terms of accuracy.
Since ovulation is preceded by a rise in oestradiol due to production by developing follicles and an LH surge, a rise in either of these hormones in isolation of in combination can be used to predict ovulation. These hormones can be measured in various body fluids such as blood, urine and saliva. Monitoring of blood levels are thought to be more accurate as the magnitude of change is greater than in other body fluids (O'Connor et al. 1998). The main indirect methods of confirming ovulation are the assessment of progesterone or its effects in the luteal phase of the cycle. The detection of its biological effects such as basal body temperature changes and changes in the endometrium are rarely used in clinical practice due to their low accuracy and practical difficulties. Measurement of progesterone in the mid-luteal phase, on the other hand, has a higher accuracy compared to other tests. However, this does not allow differentiating between ovulation and luteinized unruptured follicle (LUF), which can be diagnosed only by serial ultrasound scanning.
Of the various biochemical tests available mid-luteal serum progesterone assay is the best test for confirmation of ovulation, while detection of the pre-ovulatory LH surge is the best single test for prediction where pre-ovulatory LH-Oestradiol combination gives the best prediction overall (ESHER 2000).
Transvaginal ultrasound is an alternative method of monitoring ovulation. Serial ultrasound scans carried out during the follicular phase and peri-ovulatory phase of the menstrual cycle can be used to predict as well as to confirm ovulation. Furthermore, it can detect LUF, which cannot be recognised by biochemistry. It involves an early follicular phase scan to rule out any pre-existing follicles or cysts and then following up with serial ultrasound scans to monitor follicle growth up to pre-ovulatory maturation (18 mm in diameter) and confirmation of ovulation by detection of rupture of the mature follicle and detection of free fluid in the pouch of Douglas. This has been shown to be a consistent finding with ovulation in previous studies (Collins 1991). Though not routinely used in clinical practice due to cost implications, ultrasound monitoring of ovulation is often used as the gold standard test of ovulation in many research settings (Behre et al. 2000).

Aetiology of ovulatory dysfunction.

Ovulatory dysfunction may result from numerous underlying pathologies and the optimal management modality could be determined by the type of abnormality that exists. In order to aid clinical management of patients presenting with ovulatory dysfunction a classification was developed by the world health organization (WHO) as far back as 1973 (WHO 1973).

Though modifications for this classification have been proposed it remains the most widely used classification to date (Dhont 2005). It classifies ovulatory dysfunction in to three broad groups. Firstly, the hypogonadotropic hypogonadism, where the pituitary gonadotropins are low with minimal endogenous oestrogen activity. Second group is comprised of normogonadotropic hypogonadism where the subjects exhibit endogenous oestrogen activity with normal gonadotropin levels in the presence of abnormalities in ovulation. Third group is where end organ (ovarian) failure is evident with low oestrogen activity with pathologically high levels of gonadotropins due to lack of negative feedback.

The prevalence of anovulation and its underlying causes

Ovulatory dysfunction is one of the main causes of infertility and is assumed to be the commonest cause of female infertility (Evers 2002). The prevalence of ovulatory dysfunction has been studied in many populations. While definite data on ovulatory dysfunction is lacking among the general population it is estimated to be around 20-30% among the infertile population (Hull et al. 1985, Hamilton-Fairley & Taylor 2003). Similar data are not available for the Sri Lankan infertile population.

The WHO group I anovulation or hypogonadotropic hypogonadism include hypothalamic and pituitary causes and is characterized by the selective failure of the pituitary gland to produce FSH and LH. The common causes are excessive exercise, a low BMI, or both (Hamilton-Fairley & Taylor 2003). This is thought to be secondary to a physiological reduction in the hypothalamic production of gonadotropin releasing hormone. Sheehan syndrome or panhypopituitarism caused by infarction of the anterior pituitary after massive postpartum haemorrhage or trauma and Kallman syndrome or congenital isolated lack of gonadotropin production by the pituitary are other rare causes of hypogonadotropic hypogonadism. Hyperprolactinaemia is another cause of pituitary abnormality where excessive production of Prolactin is seen with reduced FSH and LH levels. This is commonly seen with a micro-adenoma while some may have a macro-adenoma associated with headache and bitemporal hemianopia.

Ovarian causes with normal gonadotropin levels make up the group of WHO group II anovulation or normogonadotropic hypoganodism. Polycystic ovarian syndrome (PCOS) is the single major contributor to this group and is also the most common cause of ovulatory dysfunction overall. It is estimated to be responsible for around 70% of cases of ovulatory dysfunction in infertile patients (Adamson & Baker 2003). PCOS is currently diagnosed based on the 2003 Rotterdam criteria which requires the presence of at least two of the following criteria; oligo and/ or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries, in the absence of other aetiologies (Rotterdam 2004). The prevalence of PCOS among general population has been mostly studied with previous diagnostic criteria such as National Institute of Health (NIH) criteria, which allowed a diagnosis to be made in the presence of oligo- or anovulation and clinical or biochemical evidence of elevated androgen levels. This did not take in to consideration the morphology of ovaries. Such studies have demonstrated prevalence rates of around 3.4 – 8% in many other parts of the world (Knochenhauer et al.1998, Norman et al. 2007). However, one of the large population-based studies since the introduction of Rotterdam criteria has demonstrated this rate to be as high as 17% among Caucasian populations. Furthermore, this study clearly demonstrated that the underestimation of prevalence by using NIH criteria, which demonstrated a prevalence rate of 6.3% in the same study population (March et al. 2010). A population based study has been carried out in Sri Lanka using the new Rotterdam criteria, and this is one of the earliest studies to use these criteria in a population based study. This demonstrated the prevalence of PCOS in the general population of Sri Lanka to be around 6.5% (Kumarapeli et al. 2008). There have been no large scale studies to assess the prevalence of PCOS in infertile populations of Sri Lanka. Other causes of WHO group II anovulation include endocrinopathies such as thyroid dysfunction and diabetes mellitus.

WHO group III anovulation or hypergonadotropic hypogonadism on the other hand is made up of many conditions that lead to ovarian failure resulting in lack of response from the ovaries to increasing levels of gonadotropins. This is an irreversible condition and could be secondary to Turner syndrome (45XO) which result in development of streaky ovaries, translocations or deletions of the X chromosome, infection of the ovaries such as mumps and tuberculosis, auto-immune conditions or due to iatrogenic insults such as pelvic radiation or chemotherapy (Hamilton-Fairley & Taylor 2003, Sybert & McCauley 2004). The definite population prevalence of such conditions in different populations is not known mainly due to low prevalence.

## 3.2 Objectives

1. To determine the prevalence of ovulatory dysfunction among an infertile population presenting to a tertiary care infertility care setting in a suburban area of Sri Lanka.
2. To describe the association of various contributory causes with ovulatory dysfunction in this study population.
3. To describe the prevalence of known contributory causes of anovulation among subjects with ovulatory dysfunction in this study population.

## 3.3 Method

Study design and setting

This part of the study was carried out as a cross sectional study at the university infertility clinic at the North Colombo teaching hospital, Ragama. This clinic is conducted by the department of obstetrics and gynaecology, faculty of medicine, University of Kelaniya. The study participants were selected from the first time clinic attendees and were included in the study after informed written consent.

Study participants and sample size

The study participants included the female partners of the infertile couples who were attending the clinic for the first time. The inclusion and exclusion criteria considered in selecting subjects to the study are shown in table 3.1.

**Table 3.1 The inclusion and exclusion criteria for inclusion in the study**

Inclusion criteria

- First presentation to the infertility clinic for treatment
- Has been trying for a conception for more than one year and failed, hence fulfil the criteria of infertility investigations
- Age between 18 - 40 years.
- Currently not on any treatment to improve fertility excluding folic acid supplementation.

Exclusion criteria

- Pre-existing medical disorders or medication affecting ovulation
- Previous surgery involving either one or both ovaries, including ovarian drilling
- Medication to induce ovulation within the last six months.

The sample size was calculated to describe the prevalence of ovulatory dysfunction. With an estimated prevalence of 40% and an acceptable difference of 5% at a confidence level of 95%, a minimum sample size of 360 was calculated. The sample was a non-contrived selection from a larger population of 518 couples, described in previous section, after considering inclusion and exclusion criteria.
The study population included in the final analysis consisted of 411 subjects. The 107 subjects excluded from the original study population of 518, included twelve subjects with thyroid dysfunction and on treatment, four with diabetes and on treatment, eleven subjects who had undergone ovarian cystectomy, four subjects with a history of unilateral oophorectomy and one subject with a history of ovarian drilling previously. Fourteen subjects in whose partners were found to have azoospermia or severe SFA abnormalities where IUI was not sufficient treatment opted out of follow up and were excluded from the study. In a further 61 subjects the follow up was not complete thus had to be excluded from the study and were not included in the final data analysis.

Study interventions:
All study participants underwent a clinical interview and an examination on the day of the recruitment. A baseline ultrasound scan was scheduled for the subjects with the subsequent menstruation. A blood sample was collected for the hormonal assays and assessment of ovulation was commenced from the same cycle. Any further investigations were based on the findings of the above.
Clinical interview and examination: A detailed clinical history was obtained from all subjects, and this included baseline information such as age, type and duration of infertility, education level, occupation and monthly household income. Furthermore, details regarding regularity of the menstrual cycle, periods delayed for more than 90 days within the last six months, symptoms of other medical diseases and any medication used were recorded. A clinical examination was carried out to determine the body mass index (measurement of height and weight), hirsutism or an enlarged thyroid gland. All study subjects recruited were offered an early follicular phase transvaginal ultrasound scan (second day of menstrual cycle) to determine the ovarian morphology and to identify any pre-existing ovarian cysts in order to facilitate follicle tracking in the midcycle.

Outcome variables:
Menstrual cycle regularity: A menstrual cycle pattern between 21 to 35 days with a cycle duration difference of not more than 10days in any two consecutive cycles within the last six months was taken as regular menstruation. Anything outside this was considered irregular menstrual cycles and among them any episode where time between two menstrual bleeds was more than 90 days (3 months) within the last six months was also recorded.
Body mass index: This was calculated from the weight and height on the day of recruitment with measurement of height and weight while in light clothing without any footwear. The body mass index (BMI) was calculated using the formula, BMI= weight in kilograms/ Height in meters$^2$.
Hirsutism: This was assessed by observing hair growth in eight body areas (upper lip, chin, chest, upper abdomen, lower abdmen, arms, thighs, upper back and lower back) and each area was given a score from zero to four based on the type of hair growth, zero for absence of terminal hair to four for extensive terminal hair. The modified Ferriman-Gallwey scoring system with a cut off of eight was used to determine significant hirsutism (Archer & Chang 2004).
Thyroid anlargement: This was determined by observation or palpation of an enlarged thyroid gland.
Baseline ultrasound scanning and ovarian morphological assessment: The baseline ultrasound scanning was undertaken on the second day of the menstrual cycle. An ultrasound

scan machine using a transvaginal probe with a transducer of 4.0-8.0 MHz was used for the scanning (LOGIQ 3, GE Healthcare, USA). The settings used were at 5.0MHz frequency routinely, but lowered in obese subjects. The uterus was assessed for uterine growth such as fibroids, or appearance of adenomiosis and the endometrial lining was assessed for any focal growths suggestive of endometrial polyps. Both ovaries were visualised at scanning and three dimensions were recorded by obtaining two planes in the longitudinal and a cross-sectional views. The ovarian volume was calculated using the formula for a prolate ellipsoid; ovarian volume=length x width x depth x 0.523 $cm^3$ (Sample et al. 1977). While many other formulas have been proposed to measure the ovarian volume, this formula has shown to have a good correlation with the ovarian volume measurements obtained by three dimensional ultrasound scanning (Balen et al. 2005). The antral follicle count was determined by counting the number of follicles between 2-9mm in each ovary. If any cysts with a diameter of more than 10mm was present in an ovary, ovarian volume assessment and the follicle count assessment was not carried out in that ovary and this information was recorded. In the instance where the ovary was not clearly visualised in the two planes, this was recorded as an ovary not seen.

Assessment of ovulation: Assessment of ovulation was done with follicle tracking commencing from ninth day of the menstrual cycle. Subsequent scans were undertaken in two to three days intervals depending on the initial ultrasound scan findings. The follow up was carried out till a mature follicle of more than 18mm mean diameter was detected. The mean follicle diameter was calculated by using two dimensions of the follicle in two planes. Once a mature follicle was detected the transvaginal scan was repeated three days later to confirm ovulation. Absence of the mature follicle was taken as evidence of ovulation while additional features such as collection of free fluid in the Pouch of Douglas and visualisation of the corpus luteum were also looked into. If ovulation was not detected, repeat scans twenty four hours apart were undertaken up to five days from the day of detecting a mature follicle. Subjects with a follicle growth less than 1mm growth between two scans done three days apart during the follicle tracking or those who do not show evidence of ovulation five days after detection of a mature follicle were classified as anovulatory in the index cycle.

Study participants with regular menstrual cycles in the last six months with ovulation confirmed in one cycle were classified as ovulatory. Those who have a history of irregular menstrual cycles were followed up with ovulatory assessment for three consecutive cycles. If all three cycles were ovulatory they were termed as ovulatory while those who did not ovulate in all three cycles were termed anovulatory and those who had ovulation only in some of the cycles were termed as having inconsistent ovulation. Study subjects with a history of a cycle length of more than 90 days at least once in the preceding six months with anovulation in the first cycle of ovulation assessment were termed anovulatory. However, those who demonstrate ovulation in the first cycle were followed up for three cycles and classified similar to those with irregular menstrual cycles as described above. Those with anovulation and inconsistent ovulation were classified as having ovulatory dysfunction.

Endocrinological assessment: All subjects underwent an endocrine assessment with serum Follicle stimulating hormone (FSH), Leutinising hormone (LH), Thyroid stimulating hormone (TSH), Prolactin and Testosterone on the second day of menstruation. A FSH level less than 2 IU/L was taken as low and a level more than 20 IU/L was taken as increased with a level between 2-20 IU/L considered to be within the normal range. In those with very high levels of FSH, it was repeated with serum estradiol levels. In those subjects where the FSH was repeated the lower FSH value was taken as the FSH level in the analysis. Where ovarian failure was diagnosed with endocrinology (High FSH with low oestradiol levels), further investigations were not carried out for the purposes of the study to identify the underlying cause, since premature ovarian failure was taken as an end diagnosis. The LH:FSH ratio was calculated and a ratio of more than one was taken as abnormal. TSH level was considered to be normal between 0.4 – 4.0 mIU/L, with levels less than 0.4 mIU/L considered low and

more than 4.0 mIU/L as high. Serum Prolactin level measurement was considered normal at levels less than 500 IU/L and the abnormal values were categorised at levels more than 500 IU/L and at 1000 IU/L. Testosterone level was considered to be normal between 0.06-0.8 µg/mL and levels more than 0.8 µg/mL were considered to be biochemical evidence of hyperandrogenism. All hormonal assays were carried out using an Elycsis® 1010 Automated analyzer from Roche Diagnostic, Germany, which uses elctro-chemiluminescence immunoassay technology. The quality control measures were carried out as specified by the manufacturer using commercially available calibrators and controls.

Polycystic ovarian syndrome: The diagnosis of polycystic ovarian syndrome was made using the modified Rotterdam criteria. According to this, in order to make a diagnosis two of the three criteria needed to be present (Rotterdam 2004). The three criteria included evidence of ovulatory dysfunction noted by irregular menstruation, clinical or biochemical evidence of hyperandrogenism and ovarian appearance of polycystic ovaries in at least one of the ovaries. Presence of hirsutism or an elevated Testosterone level was taken as evidence of hyperandrogenic state. Polycystic appearance of the ovary was detected with ultrasound scanning by demonstrating an ovary with either a volume more than 10 $cm^3$ or having more than 12 antral follicles.

Data analysis

The data collected was entered in an EpiData based electronic database. Statistical tests used in data analysis included mean with standard deviation and percentages for descriptive data and student t-test for comparison of means and odds ratio (OR) with 95% confidence interval (95% CI) for comparison of proportions between the groups with and without ovulatory dysfunction. The data analysis was done using the SPSS v16 (SPSS Inc, Chicago, IL.) and WinPepi (Abramson 2004) softwares.

## 3.4 Results

Description of the study population

The study population consisted of 411 subjects. The mean (SEM) age of the study population of was 29.4 (0.244) years. There were three subjects (0.7%) below the age of 20 years, 64(15.6%) between 20-25 years, 161 (39.2%) between 25-30 years, 115 (28%) between 30-35 years and 68 subjects (16.6%) between 35 - 40 years. Primary infertility was observed in 306 subjects (74.5%) while 105 (25.5%) had a history of a conception and secondary infertility. The mean(SEM) duration of infertility among the study subjects was 35.43(0.12) months with a range between 12 and 132 months. Majority of the study population was with an education level of up to ordinary level or less (n=263, 64%). One subject (0.24%) had no formal education, while 10 subjects (2.43%) were educated only up to grade 5, 252 (61.31%) between grade 5 and up to ordinary level, 122 (29.68%) up to advanced level, eight subjects (1.95%) with a diploma or a vocational training and 18 subjects (4.38%) educated up to a first degree or beyond. 119 (28.95%) subjects were employed while the remaining 292 (71.04%) were unemployed. The monthly household income of the study subjects was between Rs.6000-100,000 with a mean (SEM) of Rs. 17,673 (569). These basic characteristics of the study population are shown in tables 3.2 and 3.3.

**Table 3.2 The basic characteristics of the study population. (n= 411)**

| Study population characteristic | |
|---|---|
| Age; Mean(SEM) | 29.4(0.24) years |
| Age categories; no(%) | |
| <20 years | 3 (0.7%) |
| 20-25 years | 64 (15.6%) |
| 25-30 years | 161 (39.2%) |
| 30-35 years | 115 (28.0%) |
| 35-40 years | 68 (16.6%) |
| Type of infertility; n(%) | |
| Primary | 306 (74.5%) |
| Secondary | 105 (25.5%) |
| Duration of infertility; mean(SEM) | 35.4 (0.12) months |

**Table 3.3 The socio-economic characteristics of the study population. (n= 411)**

| Study population characteristic | |
|---|---|
| Education level; no(%) | |
| No formal education | 1 (0.2%) |
| Primary education | 10 (2.4%) |
| Up to Ordinary level | 252 (61.3%) |
| Up to Advanced level | 122 (29.6%) |
| Up to Diploma / vocational training | 8 (1.9%) |
| Primary degree or beyond | 18 (4.3%) |
| Employment status; no(%) | |
| Employed | 119 (28.9%) |
| Unemployed | 292 (71.0%) |
| Monthly household income; mean(SEM) | Rs. 17,673 (569) |

Ovulatory status of the study population

Assessment of the ovulatory status of the study population demonstrated that 193 subjects (47.0%) had regular ovulation while 61 subjects (14.8%) had inconsistent ovulation. Anovulation was detected in 157 subjects (38.2%). Therefore ovulatory dysfunction, including anovulation and inconsistent ovulation, was present in 218 subjects (53%).

Comparison between study subjects with ovulatory dysfunction and those with regular ovulation

The age of the subjects with and without ovulatory dysfunction showed a significant difference with a mean(SD) age of 28.47 (4.8) years and 30.45 (4.8) years, p=0.004, respectively. Similarly advanced age showed a significantly lesser risk of having ovulatory dysfunction in the study population with an odds ratio of 0.49 (95% CI 0.31 – 0.77) for those beyond 30 but less than 35 years and for those beyond 35 years of age (OR 0.38; 95% CI 0.22-0.66). The association between age and ovulatory dysfunction is shown in table 3.4.

**Table 3.4 The association between age and ovulatory dysfunction among the study subjects. (n= 411)**

| | Ovulatory dysfunction | Regular ovulation | |
|---|---|---|---|
| **Age of the subject; mean(SD) years** | **28.47(4.8)** | **30.45(4.8)** | **Sig: p = 0.004** |
| Age 30 years or less (n=228) | 141 | 87 | |
| **vs. Age between 30-35 years (n=115)** | **51** | **64** | **OR 0.49 (95% CI 0.31-0.77)** |
| **vs. Age more than 35 years (n=68)** | **26** | **42** | **OR 0.38 (95% CI 0.22-0.66)** |

All associations are statistically significant.

The menstrual cycles were regular in 250 study subjects (61%) while it was irregular in 161 (39%). Among those with irregular menstrual cycles 107 subjects had a history of one or more prolonged cycles with cycle duration of more than 90 days within the last six months.

Ovulatory dysfunction was seen only among 24.8% (62/250) subjects with regular menstrual cycles and 91% (52/57) subjects with irregular menstruation and all of the subjects (n=104) with a cycle duration of more than 90 days within the last six months.
However, inconsistent ovulation was seen among 23 of the 53 subjects with irregular menstruation (40.3%) while 29 (50.8%) had anovulation. Among the 104 subjects with a history of a prolonged menstrual cycle of more than 90 days within last six months regular ovulation was detected in none while inconsistent ovulation was detected in four subjects and anovulation in the remaining 100 subjects (96.1%).
Ovulatory dysfunction was associated with irregular menstrual cycles with an odds ratio of 31.54 (95% CI 12.15-81.84) and with a history of cycle duration of more than 90 days with an odds ratio of 630.34 (95% CI 39.11-10,159.20). All menstrual cycle irregularities considered together was associated with ovulatory dysfunction with an odds ratio of 94.61 (95% CI 37.22-240.4). The association of menstrual cycle abnormalities and ovulatory dysfunction is shown in tables 3.5 & 3.6.

**Table 3.5. The distribution of study population according to menstrual cycle abnormalities and ovulatory dysfunction. (n= 411)**

| | Regular menstruation | Irregular menstruation | Irregular menstruation (>90d) |
|---|---|---|---|
| Regular ovulation | 188 (75.2%) | 5 (8.7%) | 0 |
| Inconsistent ovulation | 34 (13.6%) | 23 (40.3%) | 4 (3.8%) |
| Anovulation | 28 (11.2%) | 29 (50.8%) | 100 (96.1%) |
| Total | 250 | 57 | 104 |

**Table 3.6. The association between menstrual cycle abnormalities and ovulatory dysfunction among the study subjects. (n= 411)**

| | Ovulatory dysfunction | Regular ovulation | |
|---|---|---|---|
| Regular menstrual cycle (n=250) | 62 | 188 | |
| **vs. Irregular menstrual cycles (n=57)** | **52** | **5** | **OR 31.5 (95% CI 12.1-81.8)** |
| **vs. Cycles >90 days (n=104)** | **104** | **0** | **OR 630.4 (95% CI 39-10,159)** |
| **vs. All menstrual cycle abnormalities (n=161)** | **156** | **5** | **OR 94.6 (95% CI 37.2-240.4)** |

All associations are statistically significant.

Use of menstrual cycle abnormalities in detection of ovulatory dysfunction had a sensitivity of 70.8% (95% CI 63.9-76.3) and a specificity of 96.3% (91.3-98.4).

The body mass index (BMI) was normal in 195 subjects (47.4%), while 47 (11.4%) were underweight, 130 (31.6%) were overweight and 39 (9.4%) subjects were obese. The mean BMI of the study subjects with ovulatory dysfunction was 24.92 (SD 4.44) $kg/m^2$ while it was 22.96 (SD 4.33) among those with regular ovulation, p=0.002. Being overweight and obese were significantly associated with ovulatory dysfunction compared to those with a normal BMI with odds ratios of 1.68 (95% CI 1.07-2.64) and 3.05 (95% CI 1.42-6.55) respectively. A BMI of less than 18.5 (underweight) was not significantly associated with ovulatory dysfunction (OR 0.54, 95%CI 0.28-1.05).

An enlarged thyroid gland was detected in 27 (6.5%) study subjects. Among them 11(40.7%) had ovulatory dysfunction while 16(59.2%) had regular ovulation. There was no significant association between detection of an enlarged thyroid gland and ovulatory dysfunction (OR 0.59; 95% CI 0.27-1.28). Acanthosis nigricans was detected among 40 study subjects, of whom 35 had ovulatory dysfunction showed a significantly association with an OR of 7.19 (95%CI 2.79-18.54). Hirsutism was detected among 78 (18.9%) of study subjects and ovulatory dysfunction was noted in 64(82.1%) of them. Presence of hirsutism was significantly associated with ovulatory dysfunction with an OR of 5.31 (95% CI 2.88-9.81). The associations between the examination findings and ovulatory dysfunction among the study subjects is shown in table 3.7.

**Table 3.7 The association between clinical examination findings and ovulatory dysfunction among the study subjects. (n= 411)**

| | Ovulatory dysfunction | Regular ovulation | |
|---|---|---|---|
| **Body mass index; mean(SD)** | **24.92(4.44)** | **22.96(4.33)** | **Sig: p=0.002** |
| Normal BMI (n=195) | 95 | 100 | |
| vs. Underweight (n=47) | 16 | 31 | OR 0.54 (95% CI 0.28-1.05) |
| **vs. Overweight (n=130)** | **80** | **50** | **OR 1.68 (95% CI 1.07-2.64)** |
| **vs. Obese (n=39)** | **29** | **10** | **OR 3.05 (95% CI 1.42-6.55)** |
| Normal thyroid gland (n=384) | 207 | 177 | |
| vs. Enlarged thyroid gland (n=27) | 11 | 16 | OR 0.59 (95% CI 0.27-1.28) |
| Absence of acanthosis (n=371) | 183 | 188 | |
| **vs. Presence of acanthosis (n=40)** | **35** | **5** | **OR 7.19 (95%CI 2.79-18.54)** |
| Absence of hirsutism (n=333) | 154 | 179 | |
| **vs. Presence of hirsutism (n=78)** | **64** | **14** | **OR 5.31 (95% CI 2.88-9.81)** |

Significant associations shown in bold.

Polycystic ovarian syndrome was diagnosed in 130 (31.6%) subjects. Among the study population 161 subjects had irregular menstruation and 181 subjects had at least one ovary with an appearance of polycystic ovaries. Clinical or biochemical evidence of increased androgens was noted in 85 study subjects. Fifty five subjects had all three criteria present, while another 75 had two criteria fulfilled. The distribution of the study sample according to features of PCOS is shown in Figure 3.1.

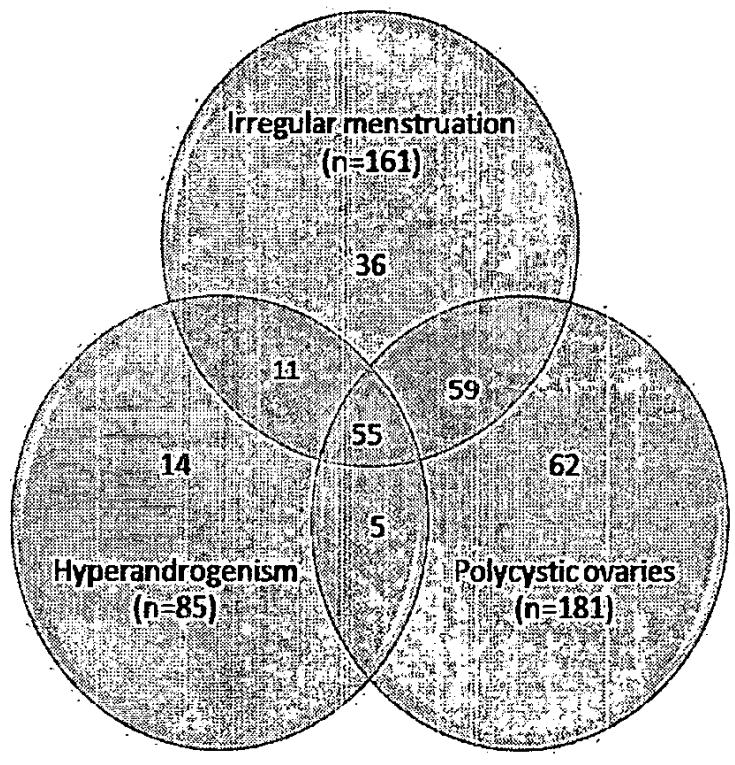



**Figure 3.1 The distribution of the study population according to the presence of diagnostic features of polycystic ovary syndrome. (n= 411)**

Among the subjects with polycystic ovary syndrome, 127 (97.6%) were found to have ovulatory dysfunction while 3 (2.3%) subjects had regular ovulation. Presence of polycystic ovarian syndrome was significantly associated with ovulatory dysfunction with an odds ratio of 88.39 (95% CI 27.5-284.0).

The hormonal assessment of the subjects revealed low FSH levels (<2 IU/L) in one subject. She was with prolonged periods of amenorrhoea and no evidence of ovulation. The FSH level was between 2-10 IU/L in 276 (67.1%) subjects, between 10-20 IU/L in 120 (29.1%) subjects and high (over 20 IU/L) in 14 (3.4%) subjects. Among those with FSH within 2-10 IU/L, 163 (59.0%) were found to have ovulatory dysfunction while 113 (40.9%) subjects had regular ovulation. Among those with FSH between 10-20 IU/L, 47 (39.1%) subjects had ovulatory dysfunction and 73 (60.8%) had regular ovulation. Among the subjects with high FSH levels, only four had consistently low oestradiol levels suggestive of premature ovarian failure. Another three subjects had ovulatory dysfunction, thus a total of 7 out of the 14 (50%) with high FSH levels having ovulatory dysfunction. The remaining 7 subjects (50%) had regular ovulation in spite of having raised FSH levels. The mean FSH level in the groups with ovulatory dysfunction and those with regular ovulation did not differ significantly, with the mean (SD) FSH level being 9.53(8.1) vs. 10.4(5.7) IU/L; p=0.217, respectively. The distribution of the study population according to FSH level on second day of menstruation is shown in Table 3.8.

**Table 3.8. The distribution of the study population according to FSH level on second day of menstruation. (n= 411)**

| **FSH level on the second day of the menstruation** | |
|---|---|
| FSH level; Mean (SEM) IU/L | 9.97 (0.38) |
| Sample distribution; n (%) | |
| <2 IU/L | 1 (0.2%) |
| 2-10 IU/L | 276 (67.1%) |
| 10-20 IU/L | 120 (29.1%) |
| >20 IU/L | 14 (3.4%) |

A reversed LH:FSH ratio (LH:FSH >1) was seen among 102 (24.8%) of the study subjects. Of them 78 (76.4%) subjects had ovulatory dysfunction while 24(23.5%) subjects had regular ovulation. A reversed LH:FSH ratio was significantly associated with ovulatory dysfunction with an odds ratio of 3.92 (95% CI 2.36-6.52).

The thyroid stimulating hormone (TSH) level was normal in 361 (87.8%) while 2 (0.4%) subjects had low TSH levels and 48 (11.6%) had high TSH levels. An enlarged thyroid gland was noted in 19% (8/50) of subjects with TSH abnormalities while only 5.2% (19/342) of subjects with a normal TSH level had the same. Therefore a subject with an enlarged thyroid gland was 3.43 times more likely to have TSH abnormalities than those who did have a normal thyroid gland. Detection of goitre had a high specificity (94.7%) and a low sensitivity (16.0%) for abnormal TSH levels in this study population. The relationship between detection of goitre and abnormalities in TSH hormone suggestive of thyroid dysfunction is shown in Table 3.9

**Table 3.9 The relationship between detection of a goitre and abnormalities in TSH hormone suggestive of thyroid dysfunction. (n= 411)**

| | Thyroid goitre (n=27) | Normal thyroid gland (n=384) | |
|---|---|---|---|
| Abnormal TSH (low or high); n=50 | 8 (29.6%) | 42 (10.9%) | **OR 3.43 (95% CI 1.42-8.26)** |
| Normal TSH level; n=361 | 19 (70.3%) | 342 (89.0%) | **Sensitivity = 16.0%** **Specificity = 94.7%** |

The association is statistically significant

Ovulatory dysfunction was noted among 184 (44.7%) subjects with normal TSH, 2(100%) subjects with low TSH and 32(66.6%) subjects with high TSH levels. Ovulatory dysfunction was significantly associated with both a raised TSH level (OR 1.92; 95% CI 1.03-3.61) and a low TSH level and this association is shown in Table 3.9.

**Table 3.9 Association between thyroid stimulating hormone abnormalities and ovulatory dysfunction among study population. (n= 411)**

| | Ovulatory dysfunction | Regular ovulation | |
|---|---|---|---|
| Normal TSH levels (n= 361) | 184 (50.9%) | 177 (49%) | |
| Low TSH levels (n=2) | 2 (100%) | 0 | - |
| **High TSH levels(n=48)** | **32 (66.6%)** | **16 (33.3%)** | **OR 1.92 (95% CI 1.03-3.61)** |

Statistically significant associations shown in bold

Prolactin level was within normal range (<500 IU/L) in 372 (90.5%) subjects while it was between 500-1000 IU/L in 32(7.7%) subjects and over 1000 in 7 (1.7%) subjects. Ovulatory dysfunction was noted in 192 (51.6%) subjects with normal Prolactin levels, 21(65%) subjects with levels between 500-1000 and 5(71.4%) subjects with levels higher than 1000 IU/l. Raised Prolactin levels did not show a significant association with ovulatory dysfunction at levels over 500 (OR 1.87; 95% CI 0.94-3.73). No association was detected in subset of subjects with prolactin levels of 500-1000 (OR 1.79; 95% CI 0.96-3.35) or over 1000 IU/L (OR 2.34; 95% CI 0.65-8.48).

The testosterone level of the study population was normal in 393 (95.6%) subjects while it was raised in 18 (4.3%) subjects. None of the subjects had low testosterone levels. Ovulatory dysfunction was noted among 88.8% (16/18) of subjects with high testosterone and 51.3% (202/393) of subjects with normal testosterone levels. The distribution of the study sample according to abnormalities in hormone profile and the associations between hormone abnormalities and ovulatory dysfunction are shown in tables 3.10 - 3.11 and figure 3.2.

**Table 3.10 The distribution of study population according to abnormalities in LH: FSH ratio, Prolactin and Testosterone level. (n= 411)**

| Hormone studied | No (%) |
|---|---|
| LH: FSH ratio | |
| 1 or less | 309 (75.1%) |
| More than 1 | 102 (24.8%) |
| Thyroid stimulating hormone(TSH) | |
| Low (< 0.04 mIU/L) | 2 (0.4%) |
| Normal (0.04-4 mIU/L) | 361 (87.8%) |
| High (>4 mIU/L) | 48 (11.6%) |
| Prolactin | |
| Normal (up to 500 IU/L) | 372 (90.5%) |
| Raised (>500 IU/L) | 39 (9.4%) |
| 500-1000 IU/L | 32 (7.7%) |
| >1000 IU/L | 7 (1.7%) |
| Testosterone | |
| Low (<0.06 µg/mL) | 0 |
| Normal (0.06-0.8 µg/mL) | 393 (95.6%) |
| High (>0.8 µg/mL) | 18 (4.3%) |

**Table 3.11. The association between hormone abnormalities and ovulatory dysfunction in the study population. (n=411)**

| The hormone studied | Ovulatory dysfunction | Regular ovulation | |
|---|---|---|---|
| Mean FSH level; mean(SD) | 9.53(8.1) | 10.4(5.75) | Sig: p=0.217 |
| Normal FSH level (2-20 IU/L); (n=396) | 210 | 186 | |
| vs. Low FSH level (<2 IU/L); (n=1) | 1 | 0 | OR 0.89 (95% CI 0.37-2.10) |
| vs. High FSH level (> 20 IU/L); (n=14) | 7 | 7 | |
| LH : FSH ratio = 1 or less; (n=309) | 140 | 169 | |
| **vs. LH:FSH > 1; (n= 102)** | **78** | **24** | **OR 3.92 (95% CI 2.36-6.52)** |
| Thyroid stimulating hormone(TSH) normal; (n=361) | 184 | 177 | |
| vs. low TSH (<0.04mIU/L); (n=2) | 2 | 0 | |
| **vs. High TSH (>4mIU/L); (n=48)** | **32** | **16** | **OR 1.92 (95% CI 1.03-3.61)** |
| Prolactin levels normal (<500 IU/L); (n=372) | 192 | 180 | |
| vs. Prolactin >500 IU/L; (n=39) | 26 | 13 | OR 1.87 (95% CI 0.94-3.73) |
| vs. Prolactin 500-1000 IU/L; (n=32) | 21 | 11 | OR 1.79 (95% CI 0.96-3.35) |
| vs. Prolactin >1000 IU/L; (n=7) | 5 | 2 | OR 2.34 (95% CI 0.65-8.48) |
| Normal Testosterone level (0.06-0.8 µg/mL); (n=393) | 202 | 191 | |
| **vs. High Testosterone (>0.8 µg/mL); (n=18)** | **16** | **2** | **OR 7.56 (95% CI 1.79-32.0)** |

The significant associations are shown in bold.

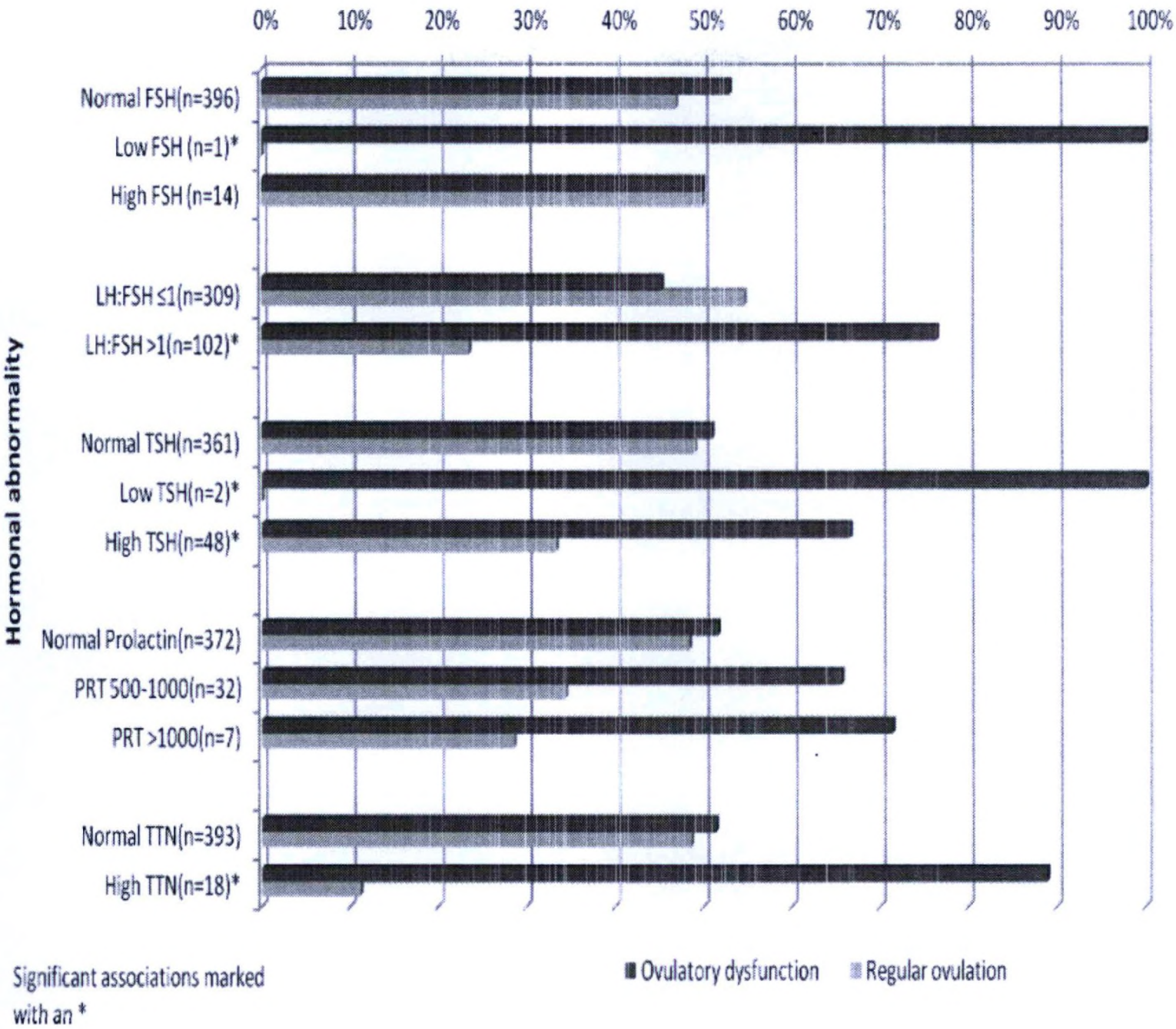



**Figure 3.2. The association between hormonal abnormalities and ovulatory dysfunction among study population(n= 411).**

One subject (0.24%) of the study population had a very low FSH level on two occasions suggestive of hypogonadotropic hypoganadism. Her FSH level on the second day of the menstrual cycle was 0.10 IU/L and 0.12 IU/L. She also had a low LH level of 0.6 IU/L. She had secondary amenorrhoea with no evidence of ovulation. Her BMI was 22.3 kg/m2 and there was no history of excessive physical exercise.

Fourteen subjects (3.4%) had FSH levels higher than 20 IU/L. Seven of them were between 35 to 40 years of age while one was below 25 years, three between 25-30 and another three between 30-35 years. In spite of such high FSH levels, seven subjects had regular cycles and ovulation was confirmed on assessment of ovulation. One subject who was found to have irregular menstrual cycles had inconsistent ovulation. The other six subjects had oligomenorrhoea or amenorrhoea with no evidence of ovulation. Serum oestradiol levels were low in four of these subjects while it was within the normal range in others. Therefore, a diagnosis of ovarian failure could be made in four of the study subjects (0.97%).

The prevalence of underlying pathologies leading to ovulatory dysfunction

In this study population the presence of known underlying causes of ovulatory dysfunction among those with the latter was as follows; a low BMI (<18.5 kg/m2) in 16(7.3%), Obesity in 29 subjects (13.3%) and polycystic ovarian syndrome in 132 (60.5%). Among the endocrine abnormalities that were detected, a low FSH (<2 IU/L) was seen in 1 subject and a high FSH level (>20 IU/L) in 7 subjects, out of which four (1.8%) were found to have ovarian failure. Low TSH levels suggestive of hyperthyroidism were seen among 2 (0.9%) subjects with ovulatory dysfunction while 32 subjects (14.6%) had high TSH levels suggestive of hypothyroidism. Raised Prolactin levels of over 500 IU/L were seen among 26 subjects (11.9%), of whom 5 subjects had levels over 1000 IU/L. The prevalence of the underlying causes of ovulatory dysfunction among the subjects with the condition is shown in able 3.12.

**Table 3.12. The prevalence of known underlying causes affecting ovulation among the study subjects with ovulatory dysfunction (n=218).**

| **The underlying causes studied** | **No (%)** |
|---|---|
| Hypogonadotropic hypogonadism | |
| Low FSH levels (<2 IU/L) | 1 (0.4%) |
| Normogonadotropic hypogonadism | |
| Low BMI (<18.5 kg/m$^2$) | 16 (7.3%) |
| Obesity (>30 kg/m$^2$) | 29 (13.3%) |
| Polycystic ovarian syndrome | 132 (60.5%) |
| High FSH level (>20 IU/L) | 7 (3.2%) |
| Hyperthyroidism (TSH < 0.04 mIU/L) | 2 (0.9%) |
| Hypothyroidism (TSH > 4 mIU/L) | 32 (14.6%) |
| Raised Prolactin levels (>500 IU/L) | 26 (11.9%) |
| Prolactin >1000 IU/L | 5 (2.2%) |
| Hypergonadotropic hypogonadism | |
| Ovarian failure | 4 (1.8%) |

## 3.5 Discussion

This study was carried out on a study population selected from a larger group described in the previous section. It comprised approximately 80% of the original study. Subjects with a history of any treatment or surgery that could have an effect on the ovulation were excluded from the study. This included subjects who were on treatment for various endocrinopathies as well as those who had a history of surgery involving the ovaries. Other causes for non inclusion were those who opted out from follow up due to severe abnormalities in seminal fluid parameters of the partner, thus requiring more advanced treatment modalities. These couples were discontinued from the follow up and were referred to centres that offered such treatment options. A further 61 had incomplete follow up or incomplete data thus were

excluded from the final analysis. None of the factors that could have an effect on ovulation was used in selection or exclusion of subjects for the study thus avoiding any selection bias.
The study population was a relatively young population, with a mean age of around 30 years, representing the age group that frequently seek infertility treatment. Those who were over 40 years were not included in the study. Though the study subjects were recruited at first presentation to the infertility clinic at the study centre they were not necessarily without prior treatment. Many had undergone investigations and treatment prior to attending this clinic. Such subjects were included if they had not received any treatment aimed at inducing ovulation within the last six months.
The study of socio-economic data suggested the sample population to be of the middle social class with majority having an education level up to GCE ordinary level or less and nearly 70% of the population being unemployed. The mean monthly household income also was low. This was likely due to the fact that the study centre was a government institute providing non fee levying health services.
The objectives of this study were aimed at gaining knowledge in two important clinical areas. Firstly, to gain an understanding of the clinical features in the clinical history and examination that increases the risk of ovulatory dysfunction in a patient. Such knowledge would be useful in identifying those who require further investigations and interventions in management of infertility. Secondly, the study was aimed at understanding the prevalence of ovulatory dysfunction and the underlying pathologies in a local population. This would enable the clinicians to decide the common pathologies to seek and determine the initial clinical investigations in patients with ovulatory dysfunction. Since the prevalence of specific pathologies differ in different populations it is important to carry out such studies in local populations.
Assessment of ovulation in a subject could be done in many different ways. While the different methods will have varying degrees of accuracy in detecting ovulation in a particular cycle, no test would have 100% accuracy in determining the overall ovulatory status of an individual. Except in a few pathologies such as ovarian failure and hypogonadotropic hypogonadism, women are known to have sporadic ovulation in many other conditions. Therefore, in assessment of ovulation the best that could be done is to determine whether a woman is likely to have regular ovulation or some dysfunction in ovulation. Since it is recommended to manage ovulatory dysfunction with induction of ovulation, this is the distinction that is important to make on a clinical viewpoint too. The two acceptable methods of assessing ovulation in a given cycle are serum progesterone assay in the midluteal phase of the cycle and follicle tracking with transvaginal ultrasound scanning. Follicle tracking was employed in this study as it has the advantage of assessment of ovulation in subjects with very irregular cycle lengths, where determining the midluteal phase of the cycle would be difficult. Follicle tracking also has the advantage over serum progesterone assay in ruling out luteinized unruptured follicle (LUF). In assessment of ovulation the subjects were initially categorised in to three groups of regular ovulation, inconsistent ovulation and anovulation as described in methods above. However, since this was an arbitrary classification with significant overlap between groups, two groups were identified in analysis as those with regular ovulation and those with ovulatory dysfunction. These are the two groups that are important to identify in the clinical management of these subjects. The study demonstrated that more than half the subjects had ovulatory dysfunction. This included 38% of subjects with anovulation and 15% with inconsistent ovulation. The high proportion of subjects with 'anovulation' was due to the fact that those with a history of prolonged cycles (>90 days) with no evidence of ovulation in the first cycle were termed as anovulatory. Previous studies have demonstrated varying prevalence rates for ovulatory dysfunction, ranging between 20-30% (Hamilton-Fairley & Taylor 2003, Evers 2002, Adamson & Baker 2003). This variation in prevalence is likely due to the difference in the methods used to detect ovulatory dysfunction and the patient profile studied. Many studies have used either epidemiological

data or assessment in one menstrual cycle. Such an assessment would categorise a subject with ovulation in that cycle as ovulatory. A proportion of such subjects may have inconsistent ovulation thus termed as having ovulatory dysfunction in this study. There may have been some variation in sample selection too, since this study included patients at their first presentation prior to having any infertility treatment. In clinical settings where basic measures in ovulation induction are undertaken at the primary care level, minor abnormalities in ovulation could be corrected thus underestimating the prevalence at tertiary centres (Evers 2002).

The clinical features in clinical interview and examination were compared between the subjects with regular ovulation and ovulatory dysfunction in order to identify the relevant clinical features that could be used to identify high risk subjects. Advancing age is a known risk factor for ovulatory dysfunction. However, in this study it was observed that the mean age of the subjects with ovulatory dysfunction was significantly lower than those with regular ovulation. Furthermore, ovulatory dysfunction was significantly lower among subjects over the age of 30 years as well as over the age of 35 years. The likely cause of this paradox is that subjects with advanced age with a longer period of infertility may have had treatment earlier, which would have corrected minor abnormalities of ovulation. Therefore, the observed prevalence of ovulatory dysfunction among these subjects would be lower.

The menstrual cycle pattern is a clinical feature easily elicited at the clinical interview. While majority of women had regular menstrual cycles, nearly 40% of the study population had irregular menstruation. Ovulatory dysfunction was very strongly associated with menstrual cycle abnormalities affecting over 95% of subjects with cycle irregularities. In the presence of a history of a prolonged cycle of more than 90 days within the last six months, the prevalence of ovulatory dysfunction was nearly 100%. The body mass index (BMI) comparison between the two groups showed a significantly elevated BMI among subjects with ovulatory dysfunction. Furthermore, ovulatory dysfunction was raised in overweight and obese subjects by 1.68 and 3.05 times, respectively, than those with a normal BMI. A low BMI did not show a significant association with ovulatory dysfunction in this population. Other clinical features examined in the study population included the presence of an enlarged thyroid gland, presence of acanthosis and significant hirsutism. While an goitre was not associated with ovulatory dysfunction the presence of acanthosis had a risk 7 times than those without and hirsutism had a risk of over 5 times. Therefore, in the initial clinical assessment of the female the important features to identify in the history and examination include the irregularities in the menstrual cycles, a raised BMI and presence of acanthosis or hirsutism.

Polycystic ovarian syndrome is known to be one of the commonest causes of ovulatory dysfunction among infertile populations. In the population studied it was present in nearly 31% of subjects. This was by far the commonest pathology detected among subjects with ovulatory dysfunction, seen in around 60% of them. These findings were similar to those of previous studies; though in some studies PCOS had been shown to be present in nearly 75% of subjects with ovulatory dysfunction (Homburg 2004). Since the recent modifications in diagnostic criteria of the PCOS (Rotterdam criteria), comparison of prevalence has been difficult with previous work. The new criteria are assumed to increase the prevalence among infertile populations compared to the previous NIH criteria. In this study population 23% of subjects diagnosed with PCOS had regular ovulation. This is an important finding for the clinical practice, since the presence of PCOS by itself does not warrant treatment. However such a finding would indicate assessment of ovulation as ovulatory dysfunction was strongly associated with PCOS.

In order to make a diagnosis of PCOS three criteria should be assessed. These include evidence of ovulatory dysfunction, hyperandrogenism and polycystic ovaries. Ovulatory dysfunction is commonly noted by irregularities in the menstrual cycles. In this study population menstrual abnormalities had a high sensitivity (70.8%) and very high specificity

(96.3%) for ovulatory dysfunction. However, nearly 24% of subjects with regular menstruation also had ovulatory dysfunction. Therefore, in detecting ovulatory dysfunction, menstrual irregularities alone may not be sufficient. The raised androgen levels in the subjects were assessed in two methods. Firstly, presence of hirsutism noted by a score of more than 8 in the modified Ferriman-Gallwey scoring system was taken as clinical evidence of hyperandrogenism. The presence of acne and alopecia were not considered as they have been shown to be poor predictors of hyperandrogenism, when taken alone, in previous studies (Rotterdam 2004). Secondly, the serum testosterone was measured in this study. However, it is known that testosterone alone has limitations in detecting hyperandrogenic states and combination of other androgens as well as calculation of androgen index has shown to improve the sensitivity of the test. Polycystic ovaries were detected in a large proportion (44.0%) of subjects. However, a significant proportion (34.2%) of them had neither hyperandrogenism nor irregular menstruation, thus not having polycystic ovary syndrome. Therefore, detection of polycystic ovaries on transvaginal scanning alone should not be considered an abnormality.

Endocrinopathies are known to be associated with ovulatory dysfunction. While some patients may be known to suffer from such conditions some others may be diagnosed during investigations for infertility. A reduced FSH level suggestive of hypogonadotropic hypogonadism is very rare and was seen only on one subject in this study population. Raised FSH levels of over 20 IU/L were seen in over 3% of subjects. Such levels are known to be associated with ovarian failure. However, to make a diagnosis of ovarian failure it is important to demonstrate low ovarian activity, commonly done by detecting low serum oestradiol levels. In the study population 14 subjects had raised FSH with levels over 20 IU/L. However, only four of them (28.5%) had low oestradiol levels thus highlighting the importance of combining the two hormonal assessments in diagnosis of hypergonadotropic hypogonadism. Isolated FSH elevation may have other clinical implications in treatment since it may suggest reduced ovarian reserves of an individual. The FSH level (10 IU/L or more) by itself did not significantly correlate with ovulatory dysfunction. Neither did a level over 20 IU/L. The latter may be due to the small sample size with FSH at that range.

In the early follicular phase of the cycle the FSH is expected to be much higher than that of leutinizing hormone level. A reversed ratio, often described as a feature of polycystic ovary syndrome was noted in nearly one quarter of the population. Such an abnormality increases the risk of ovulatory dysfunction by more than three times than with those with a normal ratio. This is likely to be due to underlying polycystic ovary syndrome that give rise to the elevated LH levels.

Thyroid stimulating hormone (TSH) is not generally recommended as a first line investigation of infertility. It is estimated that thyroid dysfunction among people with fertility problems is no higher than those in the general population and such investigations are only reserved for people with symptoms of thyroid disease (NICE 2004b). However, in this study the presence of goitre demonstrated a very low sensitivity (16%) for abnormal TSH levels. As expected, presence of goitre was significantly associated with abnormal TSH levels with an odds ratio of 3.43. Furthermore, TSH abnormalities, both low and high TSH levels, were significantly associated with ovulatory dysfunction. Therefore, it seems worthwhile investigating for TSH abnormalities in subjects with ovulatory dysfunction since this is an abnormality that is easily correctable by medication.

Raised serum prolactin levels are a recognised cause ovulatory dysfunction and a milder rise in prolactin (between 500-1000 IU/L) could be secondary to other endocrinopathies such as PCOS as well as it could be due to physiological response to stress. Higher levels (over 1000 IU/L) are often associated with increased prolactin secretion secondary to either a microadenoma or a macroadenoma of the pituitary gland. While raised Prolactin levels showed a trend towards increased prevalence of ovulatory dysfunction among this study group, it failed to demonstrate a significant association. The number of subjects with raised

prolactin levels over 1000 IU/L was very low (n=7), thus explaining the likely absence of a significant association.

Though abnormalities in testosterone by itself is not a cause of ovulatory dysfunction it may be part of a pathology such as polycystic ovary syndrome or abnormalities in androgen secretion found in conditions such as congenital adrenal hyperplasia. In the population studied, raised testosterone levels were associated with a 7.5 times increased risk of ovulatory dysfunction. Furthermore, assessment of testosterone level would be important in making a diagnosis of PCOS in some subjects since they may not demonstrate clinical evidence of hyperandrogenism in spite of raised androgen levels. However, which androgen to be assayed in the clinical setting requires further evaluation and was beyond the scope of this study.

In describing the underlying pathologies leading to ovulatory dysfunction among this local population, the pathologies were categorised in three broad groups of hypogonadotropic hypogonadism, normogonadotropic hypogonadism and hypergonadotropic hypogonadism. Only one subject (0.4%) was found to have persistently low FSH levels with anovulation suggestive of hypogonadotropic hypogonadism. This subject experienced long periods of amenorrhoea with a normal BMI (between 18.5-25 $kg/m^2$) and with no history of excessive physical activity. She did not have any other endocrinopathies and no anosmia suggestive of Kallman syndrome (Isolated GnRH deficiency).

As expected many subjects were in the category of normogonadotropic hypogonadism. Commonest cause in this category was polycystic ovary syndrome which was seen in over 60% of subjects with ovulatory dysfunction. Previous studied in western countries have demonstrated prevalence of PCOS as high as 75% among subjects with ovulation abnormalities (Homburg 2004). While history and examination may point towards the underlying pathology it is important to carryout assessment of androgen levels and an early follicle phase pelvic ultrasound examination to assess ovarian morphology to make a definite diagnosis. Since PCOS is a very common pathology among women with ovulatory dysfunction, it is of paramount importance to offer all patients these investigations early in the investigations of infertility. Endocrinopathies are an uncommon cause of ovulatory dysfunction and was seen rarely in this study population with the exceptions of raised TSH and marginal rises in serum prolactin levels. Both these abnormalities are known to be associated with PCOS and may explain the high rate of prevalence. In the presence of PCOS such abnormalities does not require specific treatment unless they are associated with symptoms of hypothyroidism or galactorrhoea.

Hypergonadotropic hypogonadism is a rare cause of ovulatory dysfunction and affected less than 2% of the subjects with ovulatory dysfunction. In the presence of raised FSH levels it is important to assess the serum oestradiol levels to make a definite diagnosis of this condition and such investigations may need to be carried out more than once in some cases, especially in the presence of irregular menstruation. It is important to identify people with ovarian failure since the condition is irreversible and requires alternative treatment modalities such as oocyte donation with assisted reproductive techniques to achieve a pregnancy. There are reported cases of spontaneous pregnancies in spite of detecting raised FSH levels and low oestradiol levels and therefore it should be borne in mind that the diagnostic accuracy of the condition may not be 100%.

There were few limitations in this study. Firstly, the study population was not a non-contrived sample representing the general population. As it was a tertiary care hospital setting many subjects were with previous investigations and treatment for infertility including ovulation induction. Such a sample tends to have a lesser representation of reversible mild pathologies as such patients would have responded to basic treatment modalities earlier. Therefore an over-representation of pathologies resistant to basic treatment could be expected.

Secondly, the study did not attempt to identify the effect of individual underlying causes in the presence of multiple pathologies. While a multiple regression model analysis would have allowed this, it may have required a much larger sample size due to small number of many underlying causes. Such a sample would be difficult to be recruited in one prospective study and best done with retrospective data analysis from medical records.

Another limitation of the study was incomplete assessment of andrological assessment of the study subjects. Addition of other indices of hyperandrogenism such as free androgen index may have improved the detection rate of hyperandrogenism among study subjects thus increasing the prevalence rate of PCOS. However, such investigations were not feasible to be carried out in this study.

Furthermore, while the study was able to achieve the study objectives, it failed to clearly demonstrate the associations with certain endocrinopathies due to limitations in sample size. Since the prevalence of endocrine abnormalities in general population is very low, the sample size was too small to describe association between these abnormalities. Such limitations were observed with low FSH, low TSH and prolactin levels over 1000 IU/L, where a trend towards a positive association was observed, the sample size was not enough to carry out a statistical analysis. A different study design such as a case-control study would be required to describe such associations in the local population.

## 4. Ovulation induction

### 4.1 Introduction

Effective treatment of abnormalities in ovulation was non-existent till the early 1960s. However, newer drugs and methods of inducing ovulation became available in the 1960s and 1970s due to pioneering work of researchers from various parts of the world. The first of such work was published by Greenblatt et al. in 1961 in the Journal of the American Medical association, where they described the results of the use of Clomifene citrate (then Known as MRL-41) in treatment of anovulation (Greenblatt et al. 1961). Around the same time pituitary gonadotropins came to light as effective treatment in inducing ovulation with reporting of success in ovulation induction as well as reporting of the first pregnancy after such treatment by Gemzell et al. (Cross reference from Homburg 2002 – Gemzell et al. 1958, 1960). Other developments included introduction of gonadotropin releasing hormone and Prolactin-inhibiting agents which were introduced to clinical practice in the 1970s (Homburg 2002). Laparoscopic ovarian drilling (LOD) was introduced in the 1980s by Gjonnaess as a method of ovulation induction in patients with PCOS (Gjonnaess 1984), which replaced wedge resection of ovaries. Many people with infertility secondary to abnormalities in ovulation have benefited from such treatment over the years. The treatment modalities have also undergone many changes and developments during this period. These changes include the areas of indications for treatment, drug regimes and monitoring facilities with limited advancements in introducing newer therapeutic agents.

Ovulation induction in present day clinical practice is mainly aimed at restoring ovulation among those who do not ovulate and in regularising ovulation among those who have irregular ovulation. It is also recommended as a mode of facilitating fertility among those with ovulatory infertility as adjuvant therapy for intrauterine insemination or solely in the treatment of unexplained infertility (NICE 2004d). Ovarian stimulation with superovulation is a process where the ovary is stimulated to produce numerous mature oocytes, which are harvested and used in in vitro fertilization (IVF) and embryo transfer. This differs from ovulation induction carried out in people with ovulatory dysfunction, since it attempted to mimic an exaggerated ovarian response compared to the near physiological response achieved in the controlled ovarian stimulation. Such ovarian stimulation for IVF treatment cycles is not discussed in this thesis.

Ovulation induction is desirable in all forms of ovulatory dysfunction but is not achievable with hypergonadotropic hypogonadism (pure gonadal failure) as the ovaries do not respond to endogenous or exogenous gonadotropins. Hypogonadotropic hypogonadism where the primary defect is lack of sufficient amounts of gonadotropins, supplementation of such would be effective in achieving ovulation. These patients generally are resistant to other forms of medication and require pituitary gonadotropins in the treatment. The WHO group II anovulation or those with normogonadotropic hypogonadism is responsive to various forms of treatment interventions and do have a high success rate in achieving ovulation. Since PCOS is seen among a majority of patients in this group, the treatment options vary and often include more than one intervention.

Augmentation of ovulation is used in couples with regular ovulation as a mean of improving fertility potential. While development of more than one oocyte is thought to increase the fertility potential in an individual, some believe the improvement in fertility potential is due to correction of minor hormonal imbalances that exist in spite of ovulation. This form of treatment has been supported by meta-analysis that have shown a benefit of treatment with ovulation induction in cases of unexplained infertility (Hughes 1997, Hughes et al. 2000). Therefore, it is recommended in clinical practice to offer controlled ovarian stimulation to couples with unexplained infertility (NICE 2004d).

While ovulation induction has shown benefit to many patients and helped millions of women to achieve a pregnancy, it carries some unwanted effects too. The two most commonly

known unwanted effects, ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies are related to body's loss of control of ovarian function leading to multiple follicle development. This is mainly through lack of negative feedback mechanisms that regulate ovarian stimulation. However, these unwanted effects are not always predictable or preventable. Ovarian hyperstimulation syndrome (OHSS) is a potentially fatal condition when many follicles are stimulated, leading to ascites, pleural and pericardial effusion, haemoconcentration and coagulopathy (Aboulghar & Mansour 2003). Multiple pregnancy, also resulting from multiple oocyte maturation, can cause serious pregnancy complications in the woman with possible long term consequences in the offspring. Other less common but probable side effects of ovulation induction include an increased risk of ovarian cancer and prion diseases transmission during the use of human gonadotropins. While there is evidence to demonstrate a higher risk of ovarian tumours among women who have undergone ovulation induction compared to those who have not, evidence on risk of ovarian cancer is not very clear (Nugent et al. 1998, Venn et al. 1999, NICE 2004d). There is conflicting evidence of the actual causal relationship. Prion disease transmission risk exists whenever a drug contains material of human or bovine origin. Since FSH produced by human menopausal urine contain human urine products and recombinant FSH uses bovine derived reagents, both forms of medications have a theoretical risk of prion disease transmission. However, there is no case reported of such disease transmission to date (NICE 2004d).

Therapeutic effects of ovulation induction agents

The therapeutic effects of ovulation induction agents are described by two main concepts: FSH threshold and FSH window. Following demise of the corpus luteum the oestrogen production falls and the FSH levels start to rise from the end of the luteal phase of the menstrual cycle. This rise in FSH is closely related to ovulation and begins 12 days from the LH surge of the preceding menstrual cycle. The initiation of growth of primordial follicles occurs continuously and in a random fashion. Only follicles that happen to be at a more advanced stage of development during the inter-cycle rise in FSH will gain FSH dependence and develop while all other will cease to grow and undergo atresia. The concept of this FSH rising above a certain level during this critical period is referred to as the 'FSH threshold' and was first described by Brown in 1975 (Fauser & Heusden 1997). While this is a critical level required for follicle development, higher doses are known to be associated with multifollicle development.

On the other hand, in follicles less than 10mm in size the aromatase enzyme activity is poorly expressed and intrafollicular oestradiol levels are low. The fate of these developing antral follicles is closely associated with their ability to create an oestrogen-rich intra-follicular environment. It is proposed that the follicle selected to gain dominance is the one that has most rapidly acquired the highest sensitivity for FSH. However, the serum FSH level steadily decreases during the mid to late follicular phase of the menstrual cycle and the follicle that has gained dominance is less dependent on the high FSH levels and continue to develop in spite of the falling FSH levels. Thus this limited period of high FSH levels above the threshold is an important factor in uni-folicular development and is referred to as the 'FSH window'. It has been used to describe the importance of duration of elevated FSH levels, instead of the magnitude of the FSH rise (Fauser & Heusden 1997). These concepts form the basis of ovulation induction with uni-follicular development.

Anti-oestrogens in induction of ovulation

Oral anti-oestrogens are the main medications used in ovulation induction worldwide. Clomifene citrate is the most widely used anti-oestrogen agent for ovulation induction while Tamoxifen is used less frequently in clinical practice. Both agents are structurally comparable and have similar modes of action.

Clomifene is a triphenylethylene derivative distantly related to diethylstilboestrol. The commercially available form of Clomifene is the dihydrogen citrate salt (Clomifene citrate) and is absorbed by the gastrointestinal tract. Fifty percent of the oral dose is excreted after five days, but radioactivity from labelled Clomifene appears in the stools up to six weeks after administration. However, the pharmacologic effect of Clomifene citrate is believed to be more brief (Seli & Arici 2006). It acts by competitive non-selective oestrogen receptor binding at the level of the hypothalamus and pituitary interfering with endogenous oestrogen negative feedback. This results in an increased production of gonadotropins which is usually followed by follicle growth and ovulation (Messinis 2005, Santbrink & Fauser 2006). Clomifene citrate is generally administered from day 2 of the menstrual cycle for 5 days. Some researchers have attempted starting Clomifene citrate as late as fifth day of the cycle. While there are some studies which demonstrate that there is no difference in follicular response and endometrial quality between commencement of Clomifene citrate on day 2 and day 5 of the cycles (Cheung et al. 2002) other studies have demonstrated a significant advantage in starting it on the second day and hence early commencement is widely regarded as more effective (Dehbashi et al. 2006, Homburg 2002, NICE 2004d). Many different doses have been described from 50 mg up to 150 mg per day. The minimum dose required to achieve ovulation is generally recommended in order to minimise the unwanted anti-oestrogenic effects of Clomifene citrate. The dose required vary widely between individuals hence it is recommended to commence treatment with the lowest dose (50 mg per day) and increase by 50mg increments in subsequent months if ovulation is not evident. Once ovulation is achieved the same dose would be used in the subsequent treatment cycles. Women who do not respond to a dose of 150mg per day are considered to be Clomifene citrate resistant (NICE 1004d). The optimal dose is not clear when Clomifene citrate is used to augment ovulation in ovulatory women who undergo intra-uterine insemination and in those couples with unexplained infertility. Many authors suggest a dose of 100 mg per day in such patients in order to have a significant effect while minimising adverse effects of the treatment and this is the most commonly used treatment regime in previous studies as shown in a meta-analysis by Costello (Homburg 2002, Costello 2004).

Review of the work of many researchers made Kousta et al. to conclude that ovulation induction with Clomifene citrate among individuals with WHO group II anovulation would be able achieve an ovulation rate of 60-85%. However, the overall pregnancy rate was as low as 30-40% (Kousta et al. 1997). In a study carried out by Santbrink and Fauser comparing the efficacy of Clomifene citrate and gonadotropins in ovulation induction in WHO group II anovulation, 240 women were randomized to have either of these treatment modalities. Treatment with Clomifene citrate was successful in achieving ovulation in 77% of subjects but only 47% of the Clomifene induced women were able to achieve a pregnancy (Santbrink & Fauser 2003). A study by Milsom et al. involving 82 subjects demonstrated an ovulation rate of 88% and a cumulative pregnancy rate of 67% at the end of 12 months (Milsom et al. 2002). Other authors also agree on similar success rates (Lambalk et al. 2005) and such rates were observed in a meta-analysis carried out comparing Clomifene citrate versus placebo in anovulatory infertility by Beck et al. (Beck et al. 2007). The wide difference between ovulation rate and pregnancy rate is thought to be brought about by the unwanted anti-oestrogenic effects of the drug on the endometrium and the cervical mucus (Lambalk et al. 2005). Ovarian hyperstimulation syndrome is rare with oral anti-oestrogenic agents including Clomifene citrate and in the instance that it occurs, it tends to be of mild severity. One meta-analysis which reviewed over 135 Clomifene citrate induced cycles, failed to report any case of hyperstimulation (Athaulla et al. 2002). The multiple pregnancy rates reported vary between different studies, likely due to differences in study populations and the treatment regimes used. Santbrink et al. reported a rate of 2% in their study while others report rates as high as 5% (Santbrink & Fauser 2003, van Dop PA 2005).

Exogenous gonadotropins in induction of ovulation

Gonadotropins are the second most common agents used in ovulation induction. It has been in clinical practice for over 40 years and is used more widely in present day than anytime before. Gonadotropins used in ovulation induction include follicle stimulating hormone (FSH) and leutinizing hormone (LH), which are glycoproteins composed of two non-covalently linked polypeptide chains, namely α and β subunits. The α subunits of these hormones are identical whereas the β subunit is hormone specific. They are available for clinical use in two basic preparations. Human menopausal gonadotropins (hMG) are produced by isolation of gonadotropins from urine of post menopausal women and thus contain both FSH and LH with an FSH:LH activity ratio of 1:1 (Fauser & Heusden 1997) . More purified preparations with very little LH content are now available in the market. The second preparation of FSH is the recombinant FSH (rFSH) preparation, which are produced by recombinant technology and contains only FSH. In production of rFSH the genes encoding the α and β subunits of FSH are isolated and introduced in to the genome of a Chinese hamster ovary cell line, which then synthesis and secretes FSH (Amer 2007).

The use of gonadotropins in ovulation induction is described in two distinct clinical settings. Firstly, FSH and LH are used as substitution therapy in patients with hypogonadotropic hypogonadism, where the patient is deficient in the two gonadotropins. In many clinical settings this is preferred over pulsatile gonadotropin releasing hormone (GnRH) therapy. Secondly, FSH is used in treatment of Clomifene resistant anovulation and Clomifene failure as a second line ovulation induction agent (Messinis 2005). Previous studies have reported pregnancy rates up to 30% with gonadotropin therapy in WHO group I anovulation while some have reported cumulative pregnancy rates as high as 90% after six months of treatment (Homburg 2002). Since individuals with hypogonadotropic hypogonadism lack both gonadotropins, preparations with both FSH and LH are required in treatment. However, the pregnancy rates are much lower in WHO group II anovulation treated with gonadotropin therapy. Santbrink et al. studied success of ovulation induction in women with Clomifene citrate failure and resistance involving 240 subjects. They demonstrated an ovulation rate of around 82% and a pregnancy rate of 56% (Santbrink et al. 2003). In a randomised controlled trial carried out by Bayram et al. comparing laparoscopic ovarian drilling and ovulation induction with gonadotropins, 85 women underwent FSH induction and pregnancy rate achieved was 57% (Bayram et al. 2004). Other studies have demonstrated ovulation rates of 82% and pregnancy rates of 17% per cycle with cumulative pregnancy rates around 36-63% for gonadotropin induction (Cross reference from Homburg 2002: Howles 1993, Hamilton-Fairley and Frank 1990). Though higher pregnancy rates have been described with the use of gonadotropins for ovulation induction compared to Clomifene citrate it is not recommended as a first line treatment option due to the higher incidence of OHSS and multiple pregnancy rates compared to Clomifene citrate (NICE 2004d). The risk of multiple pregnancy among people undergoing ovulation induction was up to 20% for twins and 8.8% for higher order pregnancies in analysis of data of over 441 such clinical pregnancies (Gleicher et al. 2000). The incidence of OHSS has been described to be between 5-36% depending on the treatment regime that was used (Christin-Maitre & Hugues 2003) and nearly 1% develops the severe form of this condition (Amer 2007). Both these complications are a result of an exaggerated ovarian response to treatment which results in a disruption of FSH window that facilitate mono-follicle development. Though, it has been proposed to develop individualised FSH regimes to mimic FSH threshold and FSH window (Fauser & Heusden 1997, Koning et al. 2004) such treatment regimes are not used in clinical practice at present. Clinical and research evidence suggest that there is no superiority of one gonadotropin, hMG or rFSH over the other and that they are equally effective. A prospective study carried out by Matorras et al. compared the effectiveness of recombinant FSH and

purified urinary FSH in an IUI program. Recombinant FSH was offered to 45 subjects in 139 treatment cycles and the pregnancy rate observed was 57.8%, which was comparable with a pregnancy rate of 52.2% for 46 subjects in 155 treatment cycles, who received purified urinary FSH. Similar results have been reported by other researchers as well (Gleicher et al. 2003, Amer 2007). Ng et al. demonstrated in a study with 40 subjects who underwent ovarian stimulation for IVF that there was no significant difference in the oocyte and embryo quality between subjects who received rFSH and hMG (Ng et al. 2001).

Aromatase inhibitor Letrozole in induction of ovulation

In search for newer treatment modalities that would be able to improve the rate of unwanted adverse effects of existing medication, Aromatase enzyme inhibitors have been proposed as an alternative treatment. Aromatase is a microsomal cytochrome p450 haemoprotein-containing enzyme and catalyzes the rate-limiting step (conversion of androstenedione and testosterone to oestrone and oestradiol) in the production of oestrogens. The enzyme is active in many tissues including the ovaries, brain, breasts and adipose tissue (Casper & Mitwally 2006). Aromatase inhibitors have been in clinical practice for more than three decades and have been mainly used in treatment of breast cancer in women after menopause. The agents have undergone many changes and currently third generation agents (Letrozole, Anastrozole and Exemestane which is a steroidal agent) are in use for the last 10 years. The main drawbacks of first (Aminoglutethimide) and second generation (Fadrozole, Formestane) agents were the high side effect profile, low potency and non-specificity to aromatase enzyme (Casper & Mitwally 2006).

Letrozole, which is a third generation aromatase inhibitor has been tried in clinical trials as a potential ovulation induction agent with promising results (Casper 2007, Quintero et al. 2007, Gregoriou et al. 2007). It is a reversible competitive aromatase inhibitor with considerable potency compared to second generation agents. It is completely absorbed after oral administration and has a mean half life around 45 hours (range 30-60 hours). The clearance is mainly by the liver. Mild gastrointestinal disturbances account for most of the adverse effects and seldom severe enough to discontinue therapy (Casper & Mitwally 2006). Letrozole is assumed to block oestrogen negative feedback without depletion of oestrogen receptors, as occurs with Clomifene. Both circulating oestrogen (produced by the ovarian follicles and the peripheral conversion of androgens in the adipose tissue) and locally produced oestrogens in the brain exert a negative feedback on gonadotropin release. Administration of an aromatase inhibitor in the early part of the menstrual cycle will block oestrogen production from all sources by inhibiting aromatization. This in turn will release the hypothalamic-pituitary axis from the oestrogen negative feedback and will cause a rise in the gonadotropin secretion which facilitates follicle development (Casper & Mitwally 2006). Since it does not deplete the oestrogen receptors the central negative feedback mechanisms remain intact. With the development of the dominant follicle the oestrogen levels rise and negative feedback mechanisms will come in it to play and cause a suppression of FSH secretion. This causes atresia of smaller follicles and facilitates uni-follicle development.

Though it has not been extensively studied due to the limited period of use, Letrozole has been used in clinical trials in various clinical situations. It has been studied as a second line ovulation induction agent following Clomifene citrate resistance and Clomifene failure. Among patients with Clomifene resistance use of Letrozole demonstrated ovulation in 75% of subjects with a higher endometrial thickness. Subjects who were ovulatory but had a very thin endometrial thickness (<5mm) with Clomifene showed higher endometrial thickness (>7mm) with Letrozole (Casper & Mitwally 2006). Another study compared Letrozole and FSH in ovarian stimulation among subjects with Clomifene failure and Clomifene resistance. Letrozole was administered in 99 treatment cycles in 52 subjects and FSH in 65 treatment cycles in 33 subjects. The pregnancy rate per cycle was significantly lower in the Letrozole group (9% vs. 28%) in subjects with Clomifene failure. However, in subjects who had

undergone less than 3 Clomifene induced cycles the pregnancy rates were comparable among the two groups (Quintero et al. 2007). Similar results have been demonstrated by Gregoriou et al. in a randomized study with 50 subjects who had Clomifene failure after three treatment cycles, where the pregnancy rate per cycle was 8.9% with Letrozole compared to 14% in those who received FSH (Gregoriou et al. 2007). All these studies have demonstrated that Letrozole therapy is an acceptable alternative in the presence of Clomifene resistance and failure. However, cost analysis studies are needed to evaluate its use as an alternative to gonadotropin in these conditions since in spite of significantly low pregnancy rates it has a better side effect profile and requires less monitoring during treatment cycles. Recent studies have shown it to be safe with no evidence of teratogenic effects on animal studies (Casper 2007). The short half life of the drug will clear the agent from the body before the implantation takes place. Since the use of Letrozole outside clinical trials is very limited, information on the incidence of multiple pregnancy and OHSS is lacking. However, in clinical trials it has shown to induce mono-follicle development, most likely due to minimal interference with the 'FSH window' (Casper 2007). Letrozole has also been shown to be effective as an adjuvant to gonadotropin treatment in superovulation in IVF. Goswami et al. compared the use of Letrozole as an adjuvant in 38 poor responders to FSH in a randomized controlled trial with 13 subjects receiving Letrozole and FSH and 25 receiving FSH alone for ovarian stimulation. Letrozole was shown to be effective in reducing the FSH total dose requirement with comparable pregnancy rates (Goswami et al. 2004). In a prospective randomized study carried out by Barroso et al. involving 41 subjects, the subjects were assigned to receive either Clomifene citrate or Letrozole as adjuvant to FSH . The pregnancy rates were comparable between the two groups (20% vs, 23%), but the endometrial thickness was significantly higher among subjects who received Letrozole compared to those who received Clomifene citrate (9.5mm vs. 7.3), thus demonstrating Letrozole as an acceptable adjuvant for FSH treatment (Barroso et al. 2006).

Other modalities of ovulation induction

Other modes of ovulation induction are either adjuvant to the above mentioned or are used only in specific pathologies. These interventions vary between life style modifications to medication and surgical interventions such as ovarian drilling. Weight loss is considered as one of the most effective non-pharmacological interventions in inducing ovulation as it not only improves ovulatory status in some women but also improve the response to treatment to many ovulation induction agents (NICE 2004d). In a study examining the effect of weight loss, 67 anovulatory obese women who failed to conceive with conventional treatment were made to lose weight. 60 of them achieved ovulation and 52 achieved a pregnancy and 18 of them became pregnant without any medication (Clark et al. 1998). A reduction of 5-10% of the body weight is estimated to have significant improvement in the ovulation rate (Messinis 2005). In losing weight, both diet as well as exercise is thought to be useful interventions and such interventions are recommended prior to commencement of ovulation induction, especially in obese women with PCOS (Messinis 2005).

Insulin sensitizers have been proposed as an adjuvant therapy in women with PCOS and insulin resistance. The most widely studied and used insulin sensitizing agent is metformin. It increases the sensitivity to insulin by inhibiting hepatic glucose production and by increasing glucose uptake and utilization in muscles. These actions result in reduced insulin resistance, lower insulin secretion and reduce the serum insulin levels (Macklon et al. 2006). Many studies have published the effects of metformin in doses of 1500-2000mg per day in women with PCOS. The restoration of regular menstruation has been observed in a large majority of studies and return of ovulation has been reported in 78-96% of patients (Homburg 2002). Furthermore, it has been studied as an adjuvant therapy in improving response to Clomifene citrate by patient known to be Clomifene resistant. One such study

was able to demonstrate ovulation in 19 out of 21 such subjects with metformin compared to 2 out of 25 in the placebo group (Nestler et al. 1998). Currently metformin is recommended to be used in the subgroups of patients who are resistant to Clomifene citrate and have a body mass index of more than 25, since it improves the ovulation and pregnancy rates (NICE 2004d).

Laparoscopic ovarian drilling (LOD) is a surgical method of ovulation induction exclusively used in patients with PCOS who are resistant to Clomifene citrate. The drilling could be carried out using diathermy or laser. The surface of each ovary is punctured between 4 to 10 places in to a depth of 4-10mm using unipolar coagulating current or laser (Homburg 2002). An analysis which looked in to 35 earlier reports which included more than 940 patients who underwent the procedure, 82% ovulated and 63% conceived either spontaneously or with the same treatment they were resistant to prior to LOD (Donesky and Adashi 1996). Furthermore, a large study comparing LOD with gonadotropin treatment for subjects resistant to Clomifene citrate, similar pregnancy rates were observed in both groups after one year with lower multiple pregnancy rates with LOD (Bayram et al. 2004). In addition to inducing ovulation, LOD has been shown to improve the hyperandrogenism seen in patients with PCOS (Santbrink & Fauser 2006). However, in a study which included 120 women with Clomifene resistance, comparison of metformin and LOD demonstrated similar ovulation rates but significantly higher pregnancy and live birth rates with metformin in obese women with PCOS (Palomba et al. 2004).

Pulsatile GnRH therapy has been proposed as a mode of treatment for hypogonadotropic hypogonadism. However, high pregnancy rates observed among this group with exogenous gonadotropin therapy and the convenience of such treatment than pulsatile GnRH has made gonadotropin the preferred choice of treatment of this condition in clinical practice. Therefore, GnRH agonists and antagonists are currently being used only as adjuvant therapy in ovarian stimulation protocols of the assisted reproductive treatment modalities (Messinis 2005).

Resistance and failure in ovulation induction with oral anti-oestrogens

In the use of anti-oestrogen preparations such as Clomifene citrate for ovulation induction, two major problems anticipated are the Clomifene resistance and Clomifene failure. Clomifene resistance is defined as the failure of an individual to respond to Clomifene citrate. The maximum dose used in routine clinical practice is 150mg per day, as many clinicians agree that higher doses may not be useful among patients who do not respond to this dose. Clomifene failure, on the other hand, is defined as failure to achieve a pregnancy in spite of ovulation with Clomifene citrate treatment over a period of time. The number of Clomifene citrate induced cycles that should be attempted prior to diagnosis of Clomifene citrate failure is controversial. Though British national formulary limits Clomifene citrate use for less than six consecutive cycles due to increased risk of ovarian malignancy, many authorities recommend its use up to 12 cycles (NICE 2004d). However, shorter durations of treatment have been used by researchers in clinical trials to define Clomifene failure.

Prediction of Clomifene resistance in initial assessment is useful in determining the optimal treatment modality for an individual patient as well as discussing the prognosis of treatment. Many researchers have attempted to study the predictive factors for Clomifene citrate among subjects with anovulation. In a study carried out by Santbrink et al. involving 240 subjects noted age, body mass index, free androgen index, irregularities of menstrual cycles and presence of polycystic ovary syndrome as predictors of Clomifene resistance (Santbrink et al. 2003). In a similar study by Imani et al. which studied 201 subjects with PCOS noted free androgen index, BMI, cycle irregularity, serum androgen levels and mean ovarian volume to be significantly associated with Clomifene resistance (Imani et al. 1998). Similar findings were noted in a study by Madani et al., where they studied the predictors of non-responders for tamoxifene induction, high Leutinizing hormone level, a raised LH: FSH ratio and a BMI

over 27 were noted to be significantly associated with tamoxifene resistance. However, Age, duration and fasting insulin levels were not significantly different between the groups (Madani et al. 2006). Other factors that have been studied include insulin resistance and glycaemic control after a glucose dose. Karabayashi et al. studied 59 subjects with administration of Clomifene citrate at a dose of 100 mg per day and noted that 1 hour and 2 hour blood glucose levels in a standard 75g glucose tolerance test to be abnormal in subjects with Clomifene resistance. The fasting blood glucose and fasting insulin levels were not significantly different between subjects with and without Clomifene resistance (Kurabayashi et al. 2006). In the presence of Clomifene resistance various methods have been attempted to improve the sensitivity to the drug. Use of oral contraceptive pill prior to commencement of Clomifene citrate was studied by Branigan & Estes in patients with Clomifene resistance. It was a study with a small sample size with 24 subjects assigned to receive the oral contraceptive pill and another 24 to receive Clomifene citrate alone. Administration of an oral contraceptive containing 0.03mg of ethinyl oestradiol and 0.15mg desogestrel for 42-50 days prior to Clomifene demonstrated a higher ovulation rate (71% vs. 8%) and a higher cumulative pregnancy rate (54% vs. 4%) compared to those who had Clomifene alone (Branigan & Estes 2003). Metformin has been proven to be another adjuvant that is effective in Clomifene resistance. A randomized controlled study by Nestler et al. compared the use of metformin as adjuvant to Clomifene citrate in induction of ovulation with Clomifene and placebo with 21 and 25 obese (BMI > 28) subjects in the two groups respectively. Addition of metformin to Clomifene citrate demonstrated a higher ovulation rate (90% vs. 8%) than the controls (Nestler et al. 1998). Similar results were observed by Hwu et al. with a shorter course of metformin in a sample of patients with Clomifene resistance. Metformin was administered at a dose of 1500mg per day up to 12 days prior to starting Clomifene in 40 subjects while another 40 subjects received only Clomifene. The ovulation rate in the group that received metformin and Clomifene citrate was higher than those who received only Clomifene (42.5% vs.12.5%). The pregnancy rate was also higher in the metformin group with an ovulation rate of 15% compared to none in the control group (Hwu et al. 2005). However, the effectiveness of metformin as an adjuvant in non-obese subjects has not been proven. A randomized controlled trial carried out by Ng et al. on 20 subjects with Clomifene resistance, the subjects were assigned to either receive metformin 1500mg per day or placebo for a period of 3 months prior to Clomifene. The ovulation rates and pregnancy rates did not show a significant difference between the two study groups (Ng et al. 2001b). Therefore, in a majority of patients with Clomifene citrate resistance, the mainstay of treatment is the use of alternative treatment such as gonadotropins or laparoscopic ovarian drilling. Aromatase inhibitors such as Letrozole have been proposed as another alternative recently.

Need for newer therapeutic modalities for ovulation induction persists in spite of all advances that have taken place in the field. Such a treatment modality is desired to be effective in Clomifene resistance, free of anti-oestrogenic effects of Clomifene, less expensive than gonadotropin therapy and has a lesser incidence of side effects such as multiple pregnancy and OHSS which requires close monitoring. Aromatase inhibitors share some of these characteristics, though the efficacy has not been established in clinical trials.

## 4.2 Objectives

1. To describe the pattern of response to ovulation induction with Clomifene citrate among a population of infertile women with ovulatory dysfunction.
2. To determine the factors associated with Clomifene resistance among a sample of women undergoing ovulation induction with Clomifene citrate.
3. To describe the efficacy of Letrozole, in induction of ovulation among subjects with WHO group II ovulatory dysfunction that are Clomifene citrate sensitive.
4. To describe the effectiveness of Letrozole, in ovulation induction among subjects with Clomifene citrate resistance.
5. To compare the efficacy of Letrozole and Clomifene citrate in augmentation of ovulation in women with ovulatory infertility.

## 4.3 Method

Study design and setting

The study included two study populations. First group included subjects with ovulatory dysfunction who underwent ovulation induction with Clomifene citrate. They commenced treatment with the lowest dose (50mg per day) and dose was increased to a maximum (150 mg per day) till ovulation was achieved. The Clomifene dose required to achieve ovulation was noted for subjects who responded to Clomifene stimulation. Those who fail to ovulate in spite of a maximum dose of 150 mg per day of Clomifene citrate were termed as Clomifene resistant. The characteristics in subjects who are Clomifene sensitive and resistant were compared. After a drug free interval of two months a sample of subjects underwent ovulation induction with Letrozole at a dose of 2.5mg per day.

Second group of study subjects were with ovulatory infertility, where regular ovulation was confirmed in previous ovulation assessment. They were randomly allocated to two groups and received either Clomifene citrate at a dose of 100mg or Letrozole 2.5mg per day for augmentation of ovulation.

The study method is described in these two study populations separately as phase I of the study (subjects with ovulatory dysfunction undergoing ovulation induction with Clomifene citrate and subsequently Letrozole) and phase II of the study (subjects with confirmed regular ovulation undergoing augmentation of ovulation with either Clomifene citrate or Letrozole).

The study was carried out at the infertility clinic of the Colombo North Teaching Hospital, Ragama, conducted by the university gynaecology unit of the University of Kelaniya. Study subjects for this study were recruited from a larger study population described in the previous section.

Study participants and sample size

Phase I of the study: The study participants included subjects with WHO group II ovulatory dysfunction with normal FSH, TSH and Prolactin levels and in whom the induction of ovulation was indicated as a part of the clinical management of infertility.

At the commencement of the study, with a 25% estimated rate of Clomifene resistance, 100 study subjects were planned to be recruited to the study in order to include a minimum of 25 subjects with Clomifene resistant. The study included 128 subjects who underwent ovulation induction, and 29 subjects (22.7%) were identified as Clomifene resistant.

Phase II of the study: Second part of the study included subjects with confirmed regular ovulation, in whom augmentation of ovulation was indicated in the management of infertility. They were with normal FSH, TSH and Prolactin levels. A sample size calculation was carried out prior to commencement of the study with endometrial thickness as the main outcome measure. Previous studies have described endometrial thickness after ovulation induction with Clomifene to be 8.46mm (SD 1.15) and with Letrozole to be 7.5mm (SD 0.2)

(Haritha & Rajagopalan 2003, Quintero et al. 2007). To demonstrate a difference of 0.8mm or more with a significance level of 5% and a study power of 80%, a sample size of 18 in each group was calculated. The sample size calculation was done using R statistical computer software. With an assumed dropout rate of less than 30%, 25 subjects were recruited to each study arm.

Study interventions

Phase I of the study: The subjects were recruited for the study amongst the subjects from the study described in the section 3, who have undergone assessment of ovulation. Those with ovulatory dysfunction with normal FSH levels (2-20 IU/L), normal TSH and Prolactin levels who required ovulation induction as part of the treatment plan were recruited. After obtaining informed written consent the subjects underwent ovulation induction with Clomifene citrate. It was commenced with a starting dose of 50mg per day and the dose was increased by increments of 50mg in subsequent months up to a maximum of 150mg per day. Clomifene citrate was administered for five days from the second day of menstruation. Once ovulation was achieved the dose required to achieve ovulation was noted. If ovulation was not observed in spite of a maximum dose of 150 mg per day, those subjects were named as Clomifene citrate resistant. Subjects were categorised in to two groups as Clomifene sensitive or Clomifene resistant.

Twenty five subjects who responded to Clomifene citrate and 25 with Clomifene resistance underwent ovulation induction with Letrozole 2.5mg per day for five days starting from the second day of the menstruation after a two month drug free interval. During ovulation induction with Letrozole the subjects had FSH and LH assays done on $2^{nd}$, $5^{th}$ and $9^{th}$ day of the menstrual cycle as well as on the day of detecting a mature follicle. Oestradiol levels were assessed on the $9^{th}$ day of the menstrual cycle and the day of detecting a mature follicle. Furthermore, they underwent a baseline ultrasound scan to assess ovarian morphology on $2^{nd}$ day of the menstrual cycle and follicle tracking was commenced from the $9^{th}$ day of the menstrual cycle. Endometrial thickness was assessed on the $9^{th}$ day and the day of detecting a mature follicle in all subjects. Ovulation was confirmed by transvaginal ultrasound scanning as described below.

Phase II of the study: The study population consisted of subjects with confirmed regular ovulation and normal FSH (2-20 IU/L), normal TSH and prolactin levels as described in the section 3. Augmentation of ovulation in these subjects was indicated in the clinical management of infertility. Following informed written consent to participate in the study, the subjects were randomly assigned to two treatment groups to receive either Clomifene citrate or Letrozole for augmentation of ovulation. Random allocation of the subjects was done on the second day of the menstrual cycle and was done using opaque envelops which contained instructions on the medication to be prescribed. This was carried out by a research assistant and the drug used was indicated with a code number in the data collection sheet. The FSH and LH levels were assessed in the subjects on the $2^{nd}$, $5^{th}$, $9^{th}$ day of the menstrual cycle and the day of detecting a mature follicle. All subjects underwent a baseline transvaginal ultrasound scan to assess ovarian morphology on the $2^{nd}$ day of the menstrual cycle. Follicle tracking was started on the $9^{th}$ day of the menstrual cycle and transvaginal ultrasound scanning was used to confirm ovulation. On the $9^{th}$ day of menstrual cycle and the day of detecting a mature follicle, the endometrial thickness was assessed. In addition, on the day of detecting a mature follicle the number of follicles more than 15mm in size was also noted. The serum oestradiol levels were assessed on the $9^{th}$ day of the menstrual cycle and the day of detecting a mature follicle.

Outcome variables

Age: The age was recorded as the number of completed years at the last birthday.

Raised BMI: The weight and height of the study participants were measured at the recruitment to the study and BMI was calculated with the formula; BMI=weight in kg/height in meters$^2$. A BMI over 25 kg/m$^2$ (overweight and obese) were classified as a raised BMI.

Menstrual irregularities: A menstrual cycle pattern between 21 to 35 days with a cycle duration difference of not more than 10 days in any two consecutive cycles within the last six months was taken as regular menstruation. Anything outside this was considered irregular menstrual cycles and among them any episode where time between two menstruations was more than 90 days (3 months) in the preceding six months was termed as episodes of amenorrhoea.

Hirsutism: The hair growth in eight body areas (upper lip, chin, chest, upper abdomen, lower abdomen, arms, thighs, upper back and lower back) were observed and each area was given a score from zero to four based on the type of hair growth, zero for absence of terminal hair to four for extensive terminal hair. The modified Ferriman-Gallwey scoring system with a cut off of eight was used to determine significant hirsutism (Archer & Chang 2004).

Transvaginal ultrasound scanning for ovarian morphology and assessment of ovulation: The method used for this was similar to that described in the previous section. Phase II of the study also included measurement of the endometrial thickness and the number of potentially mature follicles with assessment of ovulation. Measurement of the endometrial thickness included measurement of the maximum antero-posterior diameter of the endometrium in the fundus of the uterus. Furthermore, the number of follicles more than 15 mm in mean diameter, calculated by two measurements in two planes, was recorded on the day of detecting a mature follicle. These were considered as follicles with maturing potential.

The hormonal assessment and interpretation of serum testosterone level were similar to described in the previous section.

Data analysis

Data analysis was carried out according to the study objectives.

Study objective 1 included describing the population according to the minimum Clomifene citrate dose required to achieve ovulation. The proportions were described as number of subjects and percentage.

In study objective 2, a comparison was carried out between subjects who were Clomifene resistant and sensitive. Student t test with chi square test was used to compare continuous variables while odds ratio with 95% confidence interval was used to compare dichotomous data.

In study objectives 3 and 4 descriptive data presentation was carried out with proportions and percentage. In study objective 5, a comparison was carried out between subjects who received Clomifene citrate and Letrozole. Student t test with chi square test was used to compare continuous variables while odds ratio with 95% confidence interval was used to compare dichotomous data. A statistical significance level of 5% was used in all statistical analysis.

Data entry was carried out using an EpiData data entry form and analysis was carried out using SPSS v16 (SPSS Inc, Chicago, IL.) and WinPepi (Abramson 2004) statistical computer software.

## 4.3 Results

Response to ovulation induction with Clomifene citrate among subjects with ovulatory dysfunction

The study population of the first phase of the study consisted of 128 patients with ovulatory dysfunction, in whom ovulation induction was indicated in the clinical management. The mean age of this study population was 28.38 (SEM 0.43) years with a range of 18-40years. Two subjects (1.6%) were below 20 years, 27 (21.2%) were between 20-25 years, 55(43.0%) between 25-30, 30 subjects (23.4%) between 30-35 years while 14 subjects (11.0%) were between 35-40 years of age. The mean duration of infertility for the study population was 38.1 (SEM 2.3) months.

The menstrual cycles were regular in 34 subjects (26.6%) while 94 subjects (73.4%) had irregular menstruation. Among the subjects with irregular menstrual cycles 62 subjects (48.4% of total) had experienced a period of amenorrhoea longer than 90 days within the last six months thus termed as having episodes of amenorrhoea.

The mean (SEM) BMI of the study population was 25.4 (0.45) $kg/m^2$. Five subjects (3.9%) were underweight with a BMI less than 18.5$kg/m^2$ at presentation while 54 subjects (42.1%) were with a normal BMI (18.5-25.0 $kg/m^2$). Fifty subjects (39.0%) were overweight with a BMI between 25-30 $kg/m^2$ while another 19 subjects (14.8%) were obese with a BMI over 30 $kg/m^2$. Therefore, 69 subjects (53.8%) were with a raised body mass index.

Hirsutism was observed in 39 study subjects (30.5%). The ovarian volume of the left ovary was measured in 115 subjects while in six subjects it was not measured due to presence of a cyst of more than 10mm at time of scanning and in another seven subjects as a clear view of the ovary could not be obtained. The volume of the right ovary was measured in 114 subjects. A cyst was noted and the right ovarian volume was not measured in 12 subjects and a clear view was not obtainable in another 2 subjects. The mean ovarian volume was derived by calculation of the average of the volumes of the two ovaries. The mean (SEM) ovarian volume of the study population was 7.78 $cm^3$ and the mean antral follicle count (AFC) was 13.9 (SEM 0.615). Polycystic ovary syndrome was diagnosed in 85 (66.4%) with the modified Rotterdam criteria. The mean FSH level on the second day of the menstrual cycle was 8.4 IU/L (SEM 0.37) among the study population with 26 subjects (20.3%) having levels over 10 IU/L. The LH: FSH ratio was raised (LH: FSH >1) in 48 (37.5%) of the subjects while the testosterone levels were raised (>0.8 µg/L) in 10 subjects (7.8%). The sample distribution according to above characteristics is shown in tables 4.1- 4.2.

**Table 4.1. The basic characteristics of the study population. (n= 128)**

| | |
|---|---|
| Age of the subject; mean(SEM) in years | 28.3 (0.43) |
| Distribution of the study population according to age; no (%) | |
| <20 years | 2 (1.6%) |
| 20-25 years | 27 (21.1%) |
| 25-30 years | 55 (43.0%) |
| 30-35 years | 30 (23.4%) |
| 35-40 years | 14 (11.0%) |
| Duration of infertility; mean (SEM) in months | 38.1 (2.3) |

**Table 4.2. The clinical features and the investigatory findings of the study population. (n= 128)**

| The clinical features and the investigatory finding | |
|---|---|
| Pattern of menstrual cycles; no (%) | |
| Regular cycles | 34 (26.6%) |
| Irregular cycles(<90 days) | 32(25%) |
| Irregular with amenorrhea (>90 days) | 62 (48.4%) |
| Body mass index of the study subjects; mean (SEM) in $kg/m^2$ | 25.4 (0.45) |
| Distribution of the study subjects according to BMI; no (%) | |
| Underweight (<18.5 $kg/m^2$) | 5 (3.9%) |
| Normal (18.5 -25.0) | 54 (42.1%) |
| Overweight (25.0-30.0) | 50 (39.0%) |
| Obese (over 30.0) | 19 (14.8%) |
| Presence of hirsutism; no(%) | 39 (30.5%) |
| Mean ovarian volume among study subjects; mean(SEM) in $cm^3$ | 7.78 (0.30) |
| Mean AFC in early follicle scan among study subjects; mean(SEM) in no. | 13.9 (0.61) |
| Polycystic ovarian syndrome; no (%) | 85 (66.4%) |
| FSH level on the second day of menstrual cycle; mean(SEM) IU/L | 8.4 (0.37) |

Following induction of ovulation with Clomifene citrate, ovulation was detected in 41 subjects (32.03%) with a dose of 50 mg per day, in 40 subjects (31.25%) with 100mg per day and in 18 subjects (14.06%) with 150 mg per day. Twenty nine study subjects of the sample did not achieve ovulation in spite of a maximum Clomifene dose of 150 mg per day thus termed Clomifene resistant. Therefore, 99 subjects (77.3%) responded to ovulation induction with Clomifene citrate and the prevalence of Clomifene citrate resistance in this study population was 22.7% (n=29).

The study of known factors associated with Clomifene resistance was carried out between those who were resistant and those who ovulated in response to ovulation induction with Clomifene citrate at a dose of 150 mg per day or less.

The mean(SD) age of the subjects who were resistant to Clomifene citrate was not significantly different from those who responded (28.34 (4.2) vs. 28.38(5.0) yrs; p= 0.969). Clomifene resistance was observed among six out of 29 subjects less than 25 years (20.7%), 14 out of 35 subjects less than 30 years (40.0%) and nine out of 44 subjects (20.5%) over 30 years of age. An advanced age of the subject over 30 years did not show a significant association with Clomifene resistance (OR 0.82; 95% CI 0.34-1.98).

The mean (SD) duration of infertility among the subjects with Clomifene resistance was 41.6(27.8) months while it was 37.2(25.6) months for responders and this difference was not statistically significant (p=0.428). Thirteen out of a total of 41 (31.7%) subjects with a duration of infertility over three years were Clomifene resistant while only 16 out of 87 (18.4%) subjects with a duration of infertility less than three years were resistant. This association was not statistically significant (OR 2.06; 95% CI 0.89-4.79).

An irregular menstrual cycle with periods of amenorrhoea of 90 days or more within the last six months was significantly associated with Clomifene citrate resistance (OR 3.71; 95% CI 1.51-9.14).

The mean (SD) body mass index of the subjects who did not respond to Clomifene citrate and those who responded was not significantly different (26.2(4.29) kg/m$^2$ vs. 25.2 (4.13) kg/m$^2$; p=0.26). Clomifene citrate resistance was observed among none of the five subjects (0%) with a BMI less than 18.5 kg/m$^2$, twelve out of the 54 subjects (22.2%) with a normal BMI, thirteen out of the 50 subjects who were overweight (26.0%) and four out of the 19 subjects (21.0%) who were obese. A BMI of more than 25 kg/m$^2$ was not significantly associated with Clomifene resistance (OR 1.28; 95% CI 0.56-2.94). The presence of hirsutism was significantly associated with Clomifene citrate resistance (OR 2.76; 95% CI 1.18-6.46). The association of these clinical features and Clomifene citrate resistance is shown in figure 4.1.

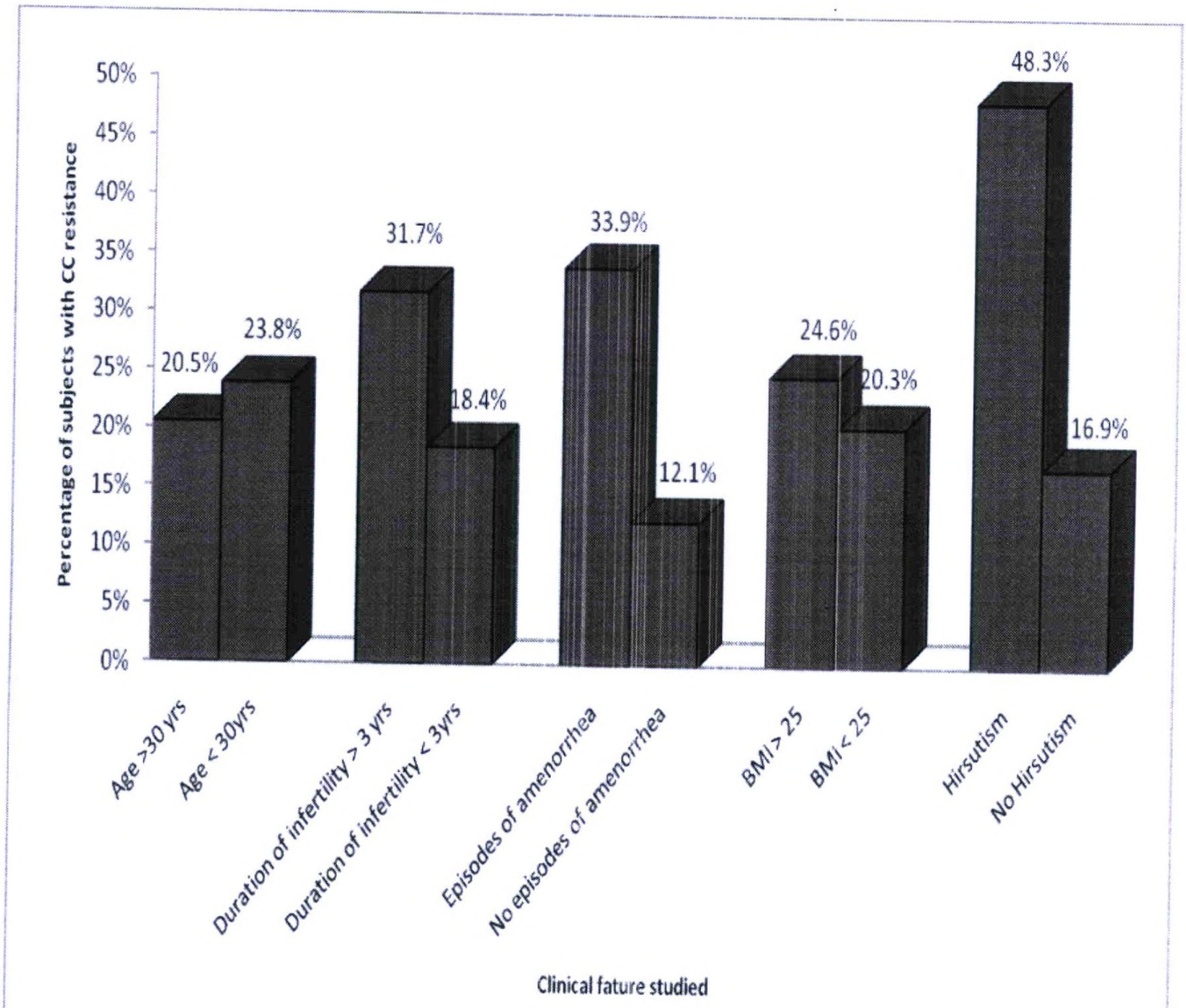



**Figure 4.1. The association of various clinical features with Clomifene citrate among the study subjects with Clomifene citrate resistance. (n=128)**

Though the mean(SD) ovarian volume was higher among non-responders compared to those who ovulated with clomifene citrate this difference was statistically not significant (8.46(3.19) $cm^3$ vs. 7.57(3.37); p=0.208).

A higher mean(SD) antral follicle count was associated with non-response to ovulation induction with clomifene citrate comapred to those who ovulated (16.05(7.17) $cm^3$ vs. 13.32(6.16); p=0.045).

Clomifene resistance was observed among 24 out of the 85 subjects with polycystic ovarian syndrome (28.2%) and five out of the 43 subjecs (11.6%) without the condition. The presence of polycystic ovary syndrome incresed the risk of Clomifene citrate resistance among study subjeccts (OR 2.99; 95% CI 1.06-8.41).

Among the 48 subjects with an LH:FSH ratio of more than one, clomifene reistance was observed in 17 subjects (35.42%) while only 12 out of the 80 subjects (15.0%) with a normal LH:FSH ratio had clomifene resistance. A raised LH:FSH ratio of over one was signifincatly associated with clomifene citrate resistance (OR 3.11; 95% CI 1.33-7.24). The association between above clinical features, these investigatory findings and clomifene resistance is shown in the table 4.3.

**Table 4.3. The associtaion between clinical features, investigatory findings and clomifene resistance among the study population. (n= 128)**

| **Clinical feature/ investigatory finding** | **CC resistance n=29** | **CC responders n=99** | **Significance** |
|---|---|---|---|
| Age of the subjects; mean(SD) yrs | 28.34(4.2) | 28.38(5.0) | p=0.969 |
| Age categories; no(%) | | | |
| ≤25 yrs | 6 | 23 | |
| ≤30 yrs | 14 | 41 | |
| ≤35 yrs | 7 | 23 | |
| ≤40 yrs | 2 | 12 | |
| Age of subject ; n(%) | | | |
| > 30 yrs; n=44 | 9(20.5%) | 35(79.5%) | OR 0.82 |
| ≤ 30 yrs; n=84 | 20(23.8%) | 64(76.2%) | 95% CI 0.34-1.98 |
| Duration of infertility; mean(SD) months | 41.5(27.8) | 37.2(25.7) | p=0.428 |
| **Duration of infertility; n(%)** | | | |
| **> 3 yrs; n=41** | **13(31.7%)** | **28(68.3%)** | **OR 2.06** |
| **≤ 3 yrs; n=87** | **16(18.4%)** | **71(81.6%)** | **95% CI 1.51-9.14** |
| Body mass index; mean(SD) in $kg/m^2$ | 26.2(4.29) | 25.2(4.13) | p=0.26 |
| Body mass index categories; n(%) | | | |
| Underweight; n=5 | 0 | 5(100%) | |
| Normal BMI; n=54 | 12(22.2%) | 42(77.8%) | |
| Overweight; n=50 | 13(26.0%) | 37(74.0%) | |
| Obese; n=19 | 4(21.0%) | 15(79.0%) | |
| Raised BMI; n(%) | | | |
| BMI > 25 $kg/m^2$; n=69 | 17(24.6%) | 52(75.6%) | OR 1.28 |
| BMI ≤ 25 $kg/m^2$; n=59 | 12(20.3%) | 47(79.7%) | 95% CI 0.56-2.94 |
| **Hirsutism; n(%)** | | | |
| **Present; n=39** | **14(35.9%)** | **25(64.1%)** | **OR 2.76** |
| **Absent; n=89** | **15(16.8%)** | **74(83.1%)** | **95% CI 1.18-6.46** |
| Mean ovarian volume; mean(SD) $cm^3$ | 8.46(3.19) | 7.57(3.37) | p=0.208 |
| **Mean antral follicle count; mean(SD) in no.** | **16.0(7.17)** | **13.3(6.16)** | **P=0.045** |
| **Polycystic ovarian syndrome; n(%)** | | | |
| **PCOS present; n=85** | **24(28.2%)** | **61(71.8%)** | **OR 2.99** |
| **PCOS absent; n=43** | **5(11.6%)** | **38(88.4%)** | **95% CI 1.06-8.41** |
| **LH: FSH ratio; n(%)** | | | |
| **LH:FSH ratio > 1; n=48** | **17(35.4%)** | **31(64.6%)** | **OR 3.11** |
| **LH:FSH ratio ≤ 1; n=80** | **12(15.0%)** | **68(85.0%)** | **95% CI 1.33-7.24** |

Statistically significant associations are shown in bold.

Response to ovulation induction with Letrozole among subjects with ovulatory dysfunction

The total study sample included 50 subjects of which 25 responded to Clomifene citrate and rest were Clomifene resistant. The minimum dose of Clomifene citrate required to achieve ovulation among subjects who responded to Clomifene was 50mg per day in 11, 100mg in eight and 150 mg in six subjects.

The mean (SEM) age of the total study population was 29.46 (0.508) years. One subject (2%) was less than 20 years with four subjects (8%) being between 20-25 years, 21 (42%) between 25-30 years, 20 (40%) between 30-35 years and four subjects (8%) between 35 - 40 years.

Menstrual cycles were regular in 11(22%) study subjects while the other 39 subjects (78%) had irregular cycles. Among those with irregular menstrual cycles 25 subjects (50%) had a history of amenorrhoea of more than 90 days within the preceding six months thus termed as having episodes of amenorrhoea.
The mean(SEM) body mass index of the population was 24.07 (0.32) $kg/m^2$ and 27 subjects (54%) had a normal BMI. Three subjects (6%) of the sample were underweight, 19 (38%) overweight while one subject (2%) was obese. Hirsutism was noted among 28 study subjects (56%) and polycystic ovary syndrome in 33 (66%) subjects.
The mean(SEM) ovarian volume of the study subjects was 9.31(0.48) $cm^3$ while the mean(SEM) antral follicle count among the subjects was 15.28(1.02) per ovary.
The mean(SEM) FSH level on the second day of the menstrual cycle in the study subjects was 7.66 (0.40) IU/L. A LH:FSH ratio of more than one was observed in 27 (54%) of subjects while it was less than one in 23(46%) study subjects. These characteristics of the study population are shown in table 4.4.

**Table 4.4. The clinical features and the investigatory findings of the study population (n= 50)**

| **The characteristic studied** | |
|---|---|
| Age of the subjects; mean(SEM) yrs | 29.46(0.50) |
| Distribution according to age; n (%) | |
| ≤ 25 years | 5 (10%) |
| ≤ 30 years | 21 (42%) |
| ≤ 35 years | 20 (40%) |
| ≤ 40 years | 4 (8%) |
| Menstrual cycle pattern; n(%) | |
| Regular cycles | 11(22%) |
| Irregular cycles | 14 (28%) |
| Irregular with episodes of amenorrhea | 25 (50%) |
| Body mass index of the population; mean(SEM) $kg/m^2$ | 24.07(0.32) |
| Distribution according to BMI; n(%) | |
| Underweight | 3 (6%) |
| Normal BMI | 27 (54%) |
| Overweight | 19 (38%) |
| Obese | 1 (2%) |
| Presence of hirsutism; n(%) | 28 (56%) |
| Presence of polycystic ovary syndrome; n(%) | 33(66%) |
| Mean ovarian volume; mean(SEM) $cm^3$ | 9.31(0.48) |
| Mean antral follicle count; mean(SEM) in no. | 15.28(1.02) |
| A LH: FSH ratio of >1; n(%) | 27(54%) |
| Response to IOO with Clomifene citrate; n(%) | |
| Ovulatory with 50mg per day | 11(22%) |
| Ovulatory with 100mg per day | 8 (16%) |
| Ovulatory with 150mg per day | 6 (12%) |
| Clomifene citrate resistant | 25 (50%) |

Induction of ovulation with Letrozole was successful in 25 (50%) subjects. In subjects who were known to be sensitive to a Clomifene citrate dose of 150 mg per day or less, ovulation was noted in 19 out of 25 (76%) subjects while six (24%) subjects did not achieve ovulation. However, among the subjects who were resistant to Clomifene citrate, with failure to ovulate in response to a dose of 150mg per day, ovulation was noted only in six subjects out of 25 (24%) while 19 (76%) remained anovulatory. The response to ovulation induction with Letrozole by the study subjects is shown in figure 4.2.

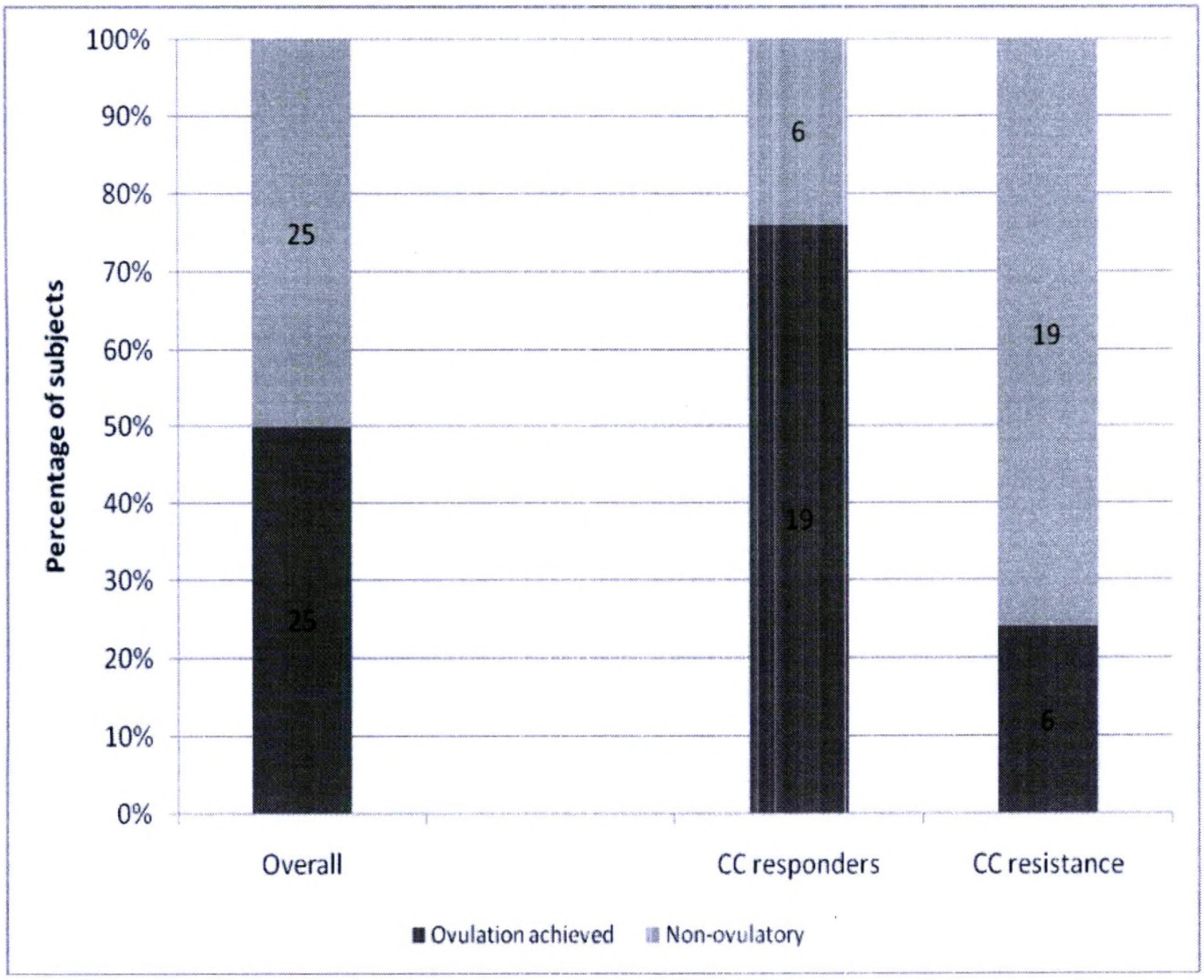



**Figure 4.2. The response of the study subjects to ovulation induction with Letrozole. (n= 50)**

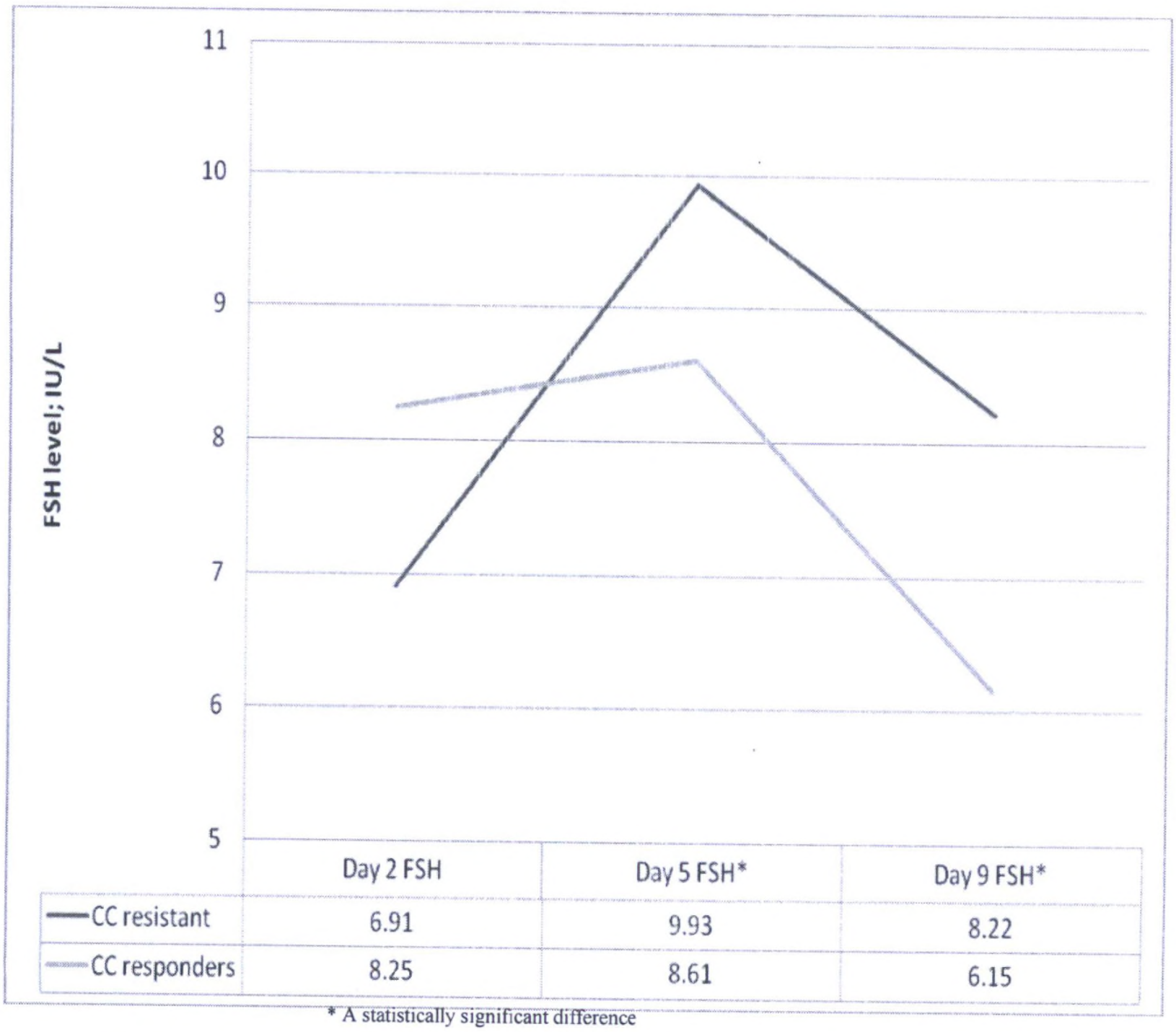
The mean (SD) age was not significantly different between the subjects who did not respond to Letrozole and those who responded (29.60(2.86) years vs. 29.32(3.37)years; p=0.80). Among the 24 subjects with an age of more than 30 years 12 subjects (50%) were non-responsive to treatment while 13 of the 26 subjects (50%) with an age less than 30 years also failed to do so. All of the four subjects with an age of more than 35 years (100%) and 21 of the 46 subjects (45.6%) with an age less than 35 years failed to respond to Letrozole. Advanced age was not significantly associated with a non-response to Letrozole at 30 years (OR 1; 95% CI 0.34-2.97) as well as at a level of 35 years (OR10.67; 95% CI 0.74-154.79).
The mean(SD) BMI was not significantly higher among subjects who did not respond to Letrozole compared to those who ovulated with Letrozole (23.81(1.69) vs. 24.34(3.91); p=0.537). Among the 20 subjects with a BMI more than 25, eight subjects (40%) and 17 of the 30 subjects (56.6%) with a BMI less than 25 did not respond to ovulation induction with Letrozole. A BMI higher than 25 was not significantly associated with non-response to such treatment (OR 0.51; 95% CI 0.17-1.57).
Among the 28 subjects who had significant hirsutism, 18 subjects (64.3%) did not respond to ovulation inductions with Letrozole while only seven out of the 22 (31.8%) who did not have hirsutism failed to respond. Presence of hirsutism was significantly associated with non-response to Letrozole (OR 3.86; 95% CI 1.21-12.30).
Nineteen of the 33 subjects (57.5%) with PCOS did not respond to Letrozole while six of the 17 subjects (35.3%) without PCOS also failed to respond. Presence of PCOS was not significantly associated with non-response to Letrozole induction (OR 2.49; 95% CI 0.76 – 8.10).
Non-response to treatment with Letrozole was noted among 14 of the 27 subjects (51.8%) with a LH:FSH ratio of more than one. In the subjects with a ratio of less than one, a non-response was noted in 11 of the 23 subjects (47.8%). A LH:FSH ratio of more than one was not significantly associated with a higher likelihood of not responding to Letrozole in the study subjects (OR 1.17; 95% CI 0.39-3.50).
19 of the 25 subjects (76%) with Clomifene resistance did not respond to Letrozole while only six of the 25 subjects (24%) who were sensitive to Clomifene failed to respond. Clomifene resistance was significantly associated with a non-response to Letrozole among the study subjects (OR 10.03; 95% CI 2.81-35.77).
The mean(SD) FSH level on second day of the menstrual cycle among the subjects who responded to Letrozole and those who did not were 8.25(2.90) IU/L and 6.91(1.80) IU/L; p=0.055, respectively. The association of above features with resistance to Letrozole ovulation induction is shown in table 4.5.

**Table 4.5 The association of clinical features and investigatory findings with resistance to ovulation induction with Letrozole. (n= 50)**

| Characteristic studied | Resistant to Letrozole | Responsive to Letrozole | Significance |
|---|---|---|---|
| Age of the subject; mean(SD) yrs | 29.6(2.86) | 29.3(3.37) | p=0.803 |
| Advanced age; no(%)<br>> 30 yrs; n=24<br>≤ 30 yrs; n=26 | <br>12(50%)<br>13(50%) | <br>12(50%)<br>13(50%) | <br>OR 1.0<br>95% CI 0.34-2.97 |
| BMI of subjects; mean(SD) | 23.8(1.69) | 24.3(3.91) | p=0.537 |
| High BMI; n(%)<br>> 25 kg/m$^2$; n=20<br>≤ 25 kg/m$^2$; n=30 | <br>8 (40.0%)<br>17(56.6%) | <br>12(60.0%)<br>13(44.4%) | <br>OR 0.51<br>95% CI 0.17-1.57 |
| **Hirsutism; n(%)**<br>**Present; n=28**<br>**Absent; n=22** | <br>**18(64.3%)**<br>**7(31.8%)** | <br>**10(36.7%)**<br>**15(69.2%)** | <br>**OR 3.86**<br>**95% CI 1.21-12.3** |
| Mean ovarian volume; mean(SD) cm$^3$ | 10.23(4.13) | 8.39(2.32) | p=0.058 |
| Mean antral follicle count; mean(SD) | 17.1(5.85) | 13.4(8.12) | p=0.067 |
| Polycystic ovarian syndrome; n(%)<br>PCOS present; n=33<br>PCOS absent; n=17 | <br>19(57.5%)<br>6(35.3%) | <br>14(52.5%)<br>11(64.7%) | <br>OR 2.49<br>95% CI 0.76 – 8.10 |
| LH: FSH ratio; n(%)<br>LH:FSH ratio > 1; n=27<br>LH:FSH ratio ≤ 1; n=23 | <br>14(51.8%)<br>11(47.8%) | <br>13(48.2%)<br>12(52.2%) | <br>OR 1.17<br>95% CI 0.39-3.50 |
| **Response to clomifene citrate; n(%)**<br>**CC resistant; n=25**<br>**CC sensitive; n=25** | <br>**19(76%)**<br>**6(24%)** | <br>**6(24%)**<br>**19(76%)** | <br>**OR 10.03**<br>**95% CI 2.81-35.7** |
| Day 2 FSH level; mean(SD) IU/L | 6.91(1.80) | 8.25(2.90) | p=0.055 |

Statistically significant associations are shown in bold

The mean(SD) FSH level of the subjects who responded to Letrozole and those who did not were 8.61(3.02) IU/L vs. 9.93(1.30) IU/L; p=0.05 on the fifth day and 6.15(1.57)IU/L vs. 8.22(1.50) IU/L; p=0.0001 on the ninth day of the menstrual cycle. The change in FSH level during follicular phase of the cycle in subjects who responded and did not respond to Letrozole treatment is shown in figure 4.3.




* A statistically significant difference

**Figure 4.3. The change in FSH level during follicular phase of the cycle in subjects who responded and did not respond to Letrozole. (n=50)**

The mean(SD) LH level among study subjects who responded and did not respond to Letrozole was 9.75(4.78) IU/L vs. 7.28(2.30) IU/L; p=0.02 respectively on second day of the menstrual cycle, 13.95(7.55) IU/L vs. 12.05(4.27) IU/L; p=0.28 on fifth day of and 12.84(5.47) IU/L vs. 11.88(2.77) IU/L; p=0.43 on ninth day of the menstrual cycle.
The oestradiol level among the subjects who responded to Letrozole was significantly higher compared to those who did not, on the fifth day of the menstrual cycle 28.50(3.39) pg/mL vs. 7.49(3.62) pg/mL; p=0.0007 as well on the ninth day of the menstrual cycle 142.04(76.22) pg/mL vs. 28.10(12.8) pg/mL; p=0.0001.

Response to augmentation of ovulation with Letrozole among subjects with regular ovulation

This phase of the study included 50 treatment cycles in 50 study subjects who were known to be ovulating regularly. They were randomly allocated to two treatment groups to receive either Clomifene citrate 100mg per day from $2^{nd}$ to $6^{th}$ day of the menstrual cycle (group 1) or Letrozole 2.5mg per day on the same days (group 2) for augmentation of ovulation.
The mean(SD) age of the study subjects in groups 1 and 2 were 30.08(3.26) years and 30.40(3.92) years; p=0.755. The mean(SD) duration of infertility was 30.36(24.46) months vs. 34.40(23.32) months; p=0.553 between the two groups and the mean(SD) BMI was 23.18(3.23) $kg/m^2$ vs. 22.87(2.73) $kg/m^2$; p=0.716. The mean (SD) ovarian volume of the

treatment groups 1 and 2 were 8.01(2.95) $cm^3$ vs. 6.85(3.88) $cm^3$; p=0.239 and the mean(SD) antral follicle count between the two groups were 9.06(1.32) follicles and 6.84(4.07) follicles; p=0.798. The mean(SD) FSH on the second day of the menstrual cycle was 8.75(3.19) IU/Lvs. 8.79(2.50) IU/L; p=0.960 in the treatment groups 1 and 2, respectively. None of the subjects included in this phase of the study were diagnosed to have polycystic ovary syndrome. The basic characteristics of the two treatment groups are shown in table 4.6.

**Table 4.6. Comparison of the basic characteristics of the two treatment groups. (n= 50)**

| Characteristic | Treatment group 1 (CC); n=25 | Treatment group 2 (Letrozole); n=25 | Significance |
|---|---|---|---|
| Age of the subjects; mean(SD) years | 30.08(3.26) | 30.40(3.92) | p=0.755 |
| Duration of infertility; mean(SD) months | 30.3(24.6) | 34.4(23.32) | p=0.553 |
| BMI; mean(SD) $kg/m^2$ | 23.2(3.23) | 22.8(2.73) | p=0.716 |
| Mean ovarian volume; mean(SD) $cm^3$ | 8.01(2.95) | 6.85(3.88) | p=0.239 |
| Mean antral follicle count; mean(SD) | 9.06(1.32) | 6.84(4.07) | p=0.798 |
| Day 2 FSH level; mean(SD) IU/L | 8.75(3.19) | 8.79(2.50) | p=0.960 |

At the completion of the treatment cycle ovulation was confirmed in 22 of 25 subjects (88%) who received Clomifene citrate (group1) and 23 of 25 subjects (92%); p=0.826, who received Letrozole for augmentation of ovulation. Among subjects who received Clomifene citrate, one subject did not develop a mature follicle while ovulation was not detected in spite of a mature follicle in two subjects. Of the subjects who received Letrozole one subject each failed to develop a mature follicle and to ovulate after detection of a mature follicle.

The mean(SD) FSH levels of the study subjects in group 1 and 2 on the fifth day of the menstrual cycle were 10.69(4.66) IU/L vs. 8.73(2.09) IU/L; p=0.061, and on the ninth day of the menstrual cycle were 7.28(4.24) IU/L vs. 6.45(2.52) IU/L; p=0.404, respectively. The change in the FSH level during the follicle phase of the menstrual cycle in the two treatment groups is shown in figure 4.4.

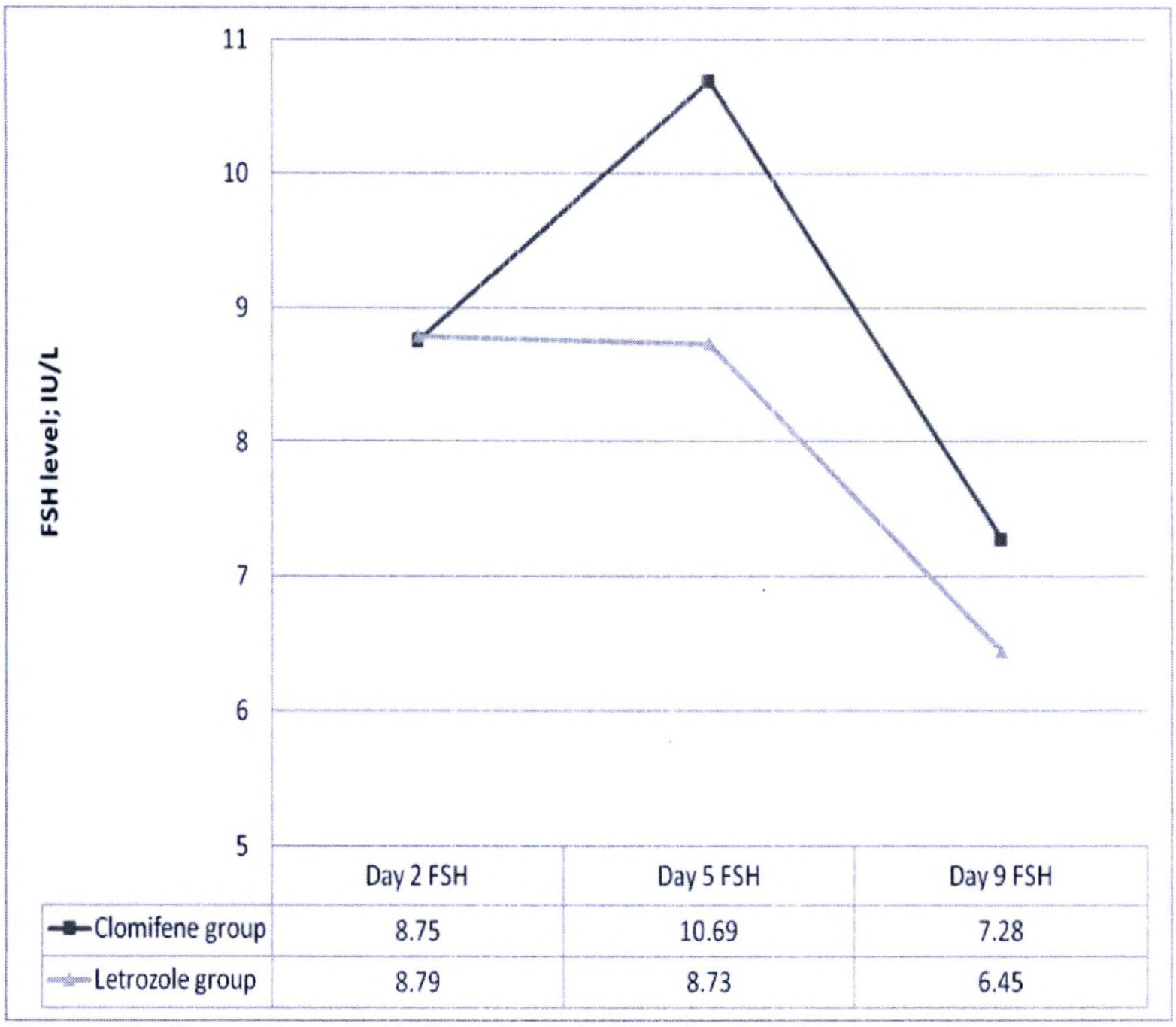
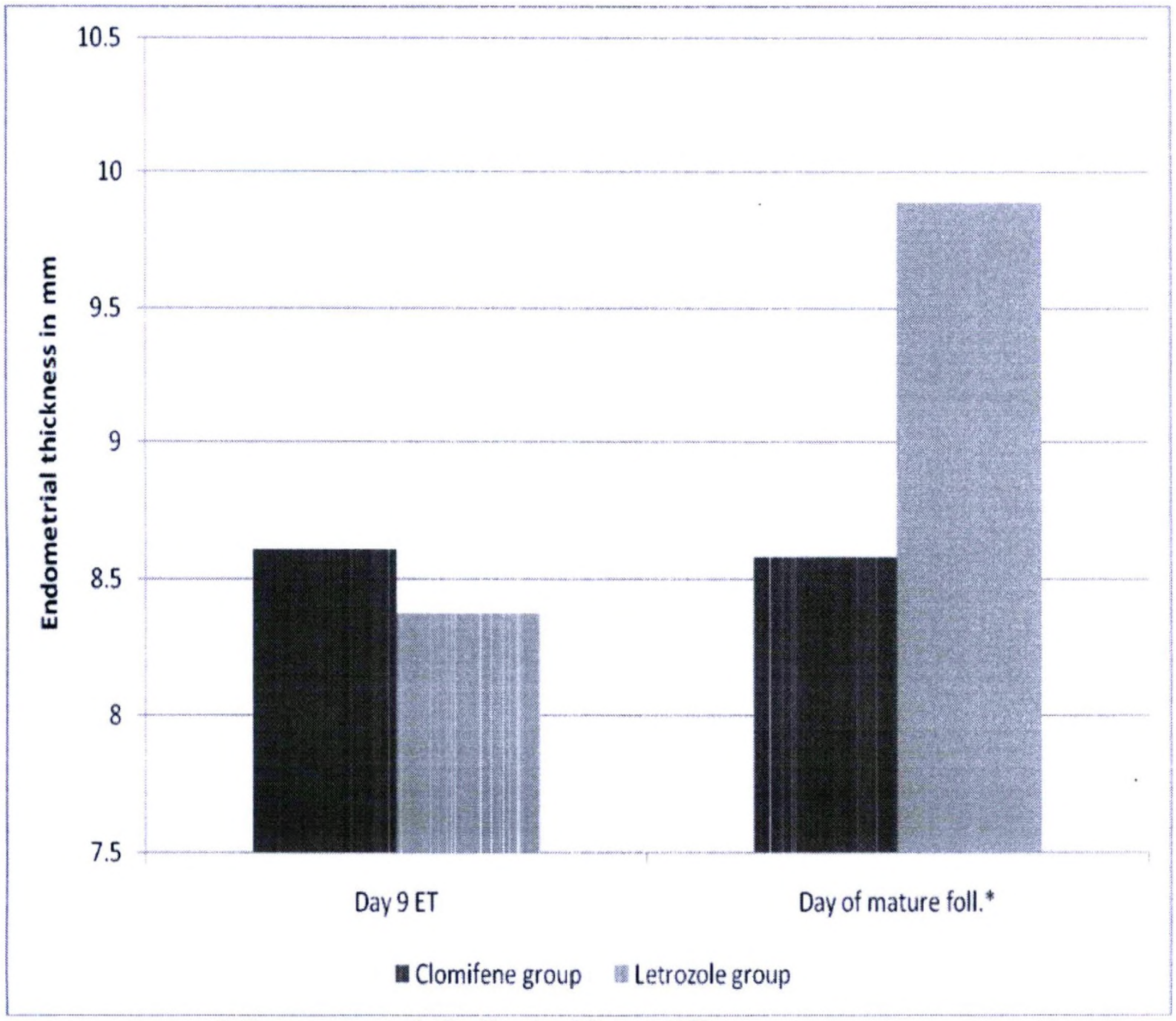



**Figure 4.4. The changes in the FSH level during the follicle phase of the cycle among subjects who received Clomifene citrate and Letrozole. (n=50)**

The mean(SD) LH levels of the study subjects on the fifth day of the menstrual cycle in the study groups 1 and 2 were 10.12(2.88) IU/L vs. 8.35(1.83) IU/L; p=0.013 and 9.73(5.35) IU/L vs. 7.03(2.09) IU/L; p=0.027 on the ninth day of the menstrual cycle.

The serum oestradiol level on the fifth day of the menstrual cycle in treatment groups 1 and 2 were 342.03(304.44) pg/mL vs. 80.14(70.49) pg/mL; p<0.001, whereas on the ninth day of the menstrual cycle they were 897.06(661.21) pg/mL vs. 143.55(79.56) pg/mL; p<0.0001. The mean(SD) serum oestradiol levels on the day a mature follicle was detected were 810.60(329.85) pg/mL in the 22 subjects who ovulated with Clomifene citrate and 102.56(89.09) pg/mL in the 23 subjects who ovulated with Letrozole, p<0.0001.

The mean(SD) endometrial thickness on the ninth day of the menstrual cycle was 8.61(1.21) mm and 8.37(2.13) mm; p=0.666, in the treatment groups 1 and 2. On the day of detecting a mature follicle it was 8.58(1.32) mm in the 22 subjects who ovulated with Clomifene citrate and 9.89 (2.02) mm in the 23 subjects who ovulated with Letrozole, p=0.021. The comparison of endometrial thickness between the two treatment groups is shown in figure 4.5.




* Denotes a statistically significant difference

**Figure 4.5. Comparison of endometrial thickness in the two treatment groups (n=50)**

The number of potentially mature follicles (diameter>15mm) noted on the day of detecting a mature follicle (diameter>18mm) in the 22 subjects who ovulated with Clomifene citrate was one follicle in nine subjects, two follicles in seven, three follicles in five subjects and four follicles in one subject. In the 23 subjects who ovulated with Letrozole it was one follicle in 14 subjects, two follicles in six subjects and three follicles in three subjects. The mean(SD) number of potentially mature follicles in the treatment groups on the day of detecting a mature follicle were 1.92(0.9) and 1.52(0.7) for the two groups; p=0.143. Though multifollicle development (two or more dominant follicles) was more common with use of Clomifene citrate (13 out of 25) compared to those who used Letrozole (nine out of 25), the difference was not statistically significant (OR 1.93; 95% CI 0.63 – 5.83).

## 4.5 Discussion

Induction and augmentation of ovulation are common procedures undertaken in management of infertility in present day clinical practice. These interventions are used alone or in combination with other procedures such as intrauterine insemination. Clomifene citrate is the most commonly used therapeutic agent worldwide. While many studies have attempted to identify factors that are associated with resistance to Clomifene therapy, little is known about the relevant factors in the local population.

One objective of this study was to identify the proportion of women with ovulation dysfunction who would respond to ovulation induction with Clomifene citrate. This was studied in 128 subjects who underwent ovulation induction with Clomifene citrate. The study sample selected from a larger study population described in an earlier section, was of a young age group and were without any endocrinopathies, except polycystic ovary syndrome.

Among the 128 subjects who underwent ovulation induction, 99 subjects (77.3%) responded to Clomifene citrate and achieved ovulation. While 41% of them responded to 50mg of Clomifene citrate per day and another 40% required up to 100mg per day. Thus, over 80% of women who would respond to Clomifene citrate, will do so at doses of 100mg per day or less. The proportion of women resistant to the maximum dose of Clomifene citrate (150mg per day) in this population was 22.7%.

The features that were associated with a non-response to Clomifene citrate were also studied among these study subjects. Such information is useful in predicting response to treatment and can be used in counselling of patients in clinical setting. An understanding of the features that are associated with Clomifene citrate resistance would also be useful in identifying subjects who should be considered for alternative treatment modalities.

Advanced age is known to be associated with resistance to all forms of ovulation induction and this is thought to be due to a reduction in ovarian reserve. However, in this population the age was not significantly higher among women who did not respond to Clomifene citrate and being over 30 years or 35 years did not significantly increase the risk of Clomifene resistance.

Factors associated with Clomifene resistance in this population included the clinical features such as a history of episodes of amenorrhoea of more than 90 days within the preceding six months (OR 3.71) and presence of hirsutism (OR 2.76). While subjects with Clomifene resistance had a higher mean antral follicle count than those who responded (16.0 vs. 13.3 per ovary), presence of polycystic ovary syndrome was 2.99 times more likely to be associated with Clomifene resistance. A reversed LH: FSH ratio of more than one was 3.11 times more likely to be associated with Clomifene resistance.

Above factors are important to be considered in discussing prognosis with patients prior to commencing treatment as presence of above may indicate that the subjects would require higher doses of the drug or may not respond to Clomifene citrate at all. Furthermore, it should be noted that some factors, such as periods of amenorrhoea, a reversed LH:FSH ratio and PCOS, are more strongly associated with resistance to Clomifene than the others.

There are many others factors that have been described as factors that are associated with Clomifene resistance. These include an advanced age, high BMI, raised FSH levels and raised testosterone levels. Such factors were not seen to be associated with Clomifene resistance in the population studied.

Letrozole, the third generation aromatase inhibitor, has been proposed as an alternative for Clomifene in the field of ovulation induction and was studied for use in Clomifene resistance as well as in augmentation of ovulation in ovulatory infertility. Effectiveness of Letrozole in ovulation induction was studied in 50 subjects that included 25 subjects who were responsive to Clomifene citrate as well as 25 who were resistant. The subjects with sensitivity to Clomifene included 11 subjects who responded to 50mg of Clomifene citrate, eight subjects

who required up to 100mg and six subjects who required 150mg. Induction of ovulation with 2.5mg of Letrozole given for five days from the second day of the menstrual cycle was successful in 25 (50%) of the subjects. The success among those who were Clomifene sensitive was 76% (19 out of 25) while it was only 24% (6 out of 25) for those with Clomifene resistance.

The factors associated with resistance to Letrozole included presence of hirsutism (OR 3.86) which was associated with Clomifene resistance too. Subjects with Clomifene citrate resistance were 10 times more like to be resistant to Letrozole treatment.

The hormonal changes among the subjects during the follicular phase of the cycle were significantly different in the two groups who responded and did not respond to Letrozole stimulation. The FSH levels on the fifth and ninth days of the cycle were significantly higher among the subjects who failed to respond in comparison to those who did respond. This explains the lack of negative feedback brought about by the oestrogen produced by developing follicles. This is further explained by the significantly lower levels of oestradiol in subjects who failed to respond to Letrozole.

Another main objective of this study was to assess the response of subjects with confirmed ovulation to augmentation of ovulation with either Clomifene citrate or Letrozole. Use of Clomifene citrate has been proven to be effective in increasing the pregnancy rate among such patients. However, the undesired effects of Clomifene citrate, that causes a significant discrepancy between ovulation and pregnancy rates, are likely to be present in the use of the drug for this indication too. Therefore, newer agents such as Letrozole which does not have the anti-oestrogenic properties may be able to give the benefits of ovulation augmentation without the undesired anti-oestrogenic effects of Clomifene citrate.

While evaluation of the pregnancy rates would be a better comparison of the two treatment modalities, such a study requires a large sample size as well as strict standardization with regard to the rest of the treatment protocol. As this study population was much heterogeneous with regard to the male factor infertility and the other treatment interventions undertaken, only secondary features were evaluated. The main outcome compared was the endometrial thickness on the day of detecting a mature follicle. The anti-oestrogenic properties of Clomifene citrate are thought to act on the endometrium with a reduction in the thickness and therefore making it less favourable for implantation. Other outcome variables that were assessed included the incidence of multi dominant follicle development, which could be a contributor for multiple pregnancy as well as the hormonal changes that take place during the follicular phase of the menstrual cycle.

The two study groups were comparable with regard to the age, duration of infertility, BMI, mean ovarian volume, mean antral follicle count and the FSH level on the second day of the menstrual cycle. Ovulation was confirmed in 88% (22 out of 25) of the subjects who received Clomifene citrate and 92% (23 of 25) of subjects who received Letrozole. Follicle development was not noted in one subject in each group. Two subjects in the Clomifene citrate group and one subject in Letrozole group failed to ovulate in spite of development of a mature follicle. Further analysis was done with the subjects in whom ovulation was confirmed (22 in the Clomifene citrate group and 23 in the Letrozole group).

The FSH levels on the $5^{th}$ day of the menstrual cycle were significantly higher among the subjects who received Clomifene citrate suggesting a prolonged rise in the gonadotropin following treatment with Clomifene compared to Letrozole treatment. The rise remained up to the ninth day of the menstrual cycle and was statistically significant. The LH levels, which were comparable on the second day of the cycle, also were higher among subjects receiving Clomifene than those who received Letrozole both on the fifth and ninth days of the menstrual cycles. The oestradiol levels showed a marked rise among the subjects undergoing augmentation with Clomifene citrate than those receiving Letrozole and this rise was evident

both on the ninth day of the menstrual cycle as well as on the day of detecting a mature follicle.

The above observation of raised gonadotropin levels persisting for a longer time as well as raised oestradiol levels among the subjects who received Clomifene citrate compared to those who received Letrozole suggests the inhibition of negative feedback mechanisms of oestrogen by the anti-oestrogen properties of Clomifene. The negative feedback mechanism of natural cycles is thought to be preserved in Letrozole augmented cycles. Lack of negative feedback control has the risk of multiple pregnancies as it predisposes to multi-follicle development from altering the FSH threshold and FSH window. In this study the subjects who received Clomifene citrate showed a trend towards a higher number of potentially dominant follicles. The likelihood of multi-follicle development was higher with the use of Clomifene citrate compared to using Letrozole. However this association did not demonstrate a statistical significance. The small sample size with a large proportion of women achieving mono-follicle development in both treatment groups is a possible explanation.

The endometrial thickness on the day of detecting a mature follicle, the main outcome measure, was significantly higher among the study subjects who received Letrozole compared to those who received Clomifene citrate. While a thickened endometrium is considered favourable for conception the exact thickness that would be considered as unfavourable is not clear. Thickening of the endometrium is due to proliferation in response to increased oestrogen activity in the follicle phase of the menstrual cycle. Previous research has demonstrated that anti-oestrogens such as Clomifene and tamoxifen could impair the proliferation of the endometrium making it thinner and less favourable for implantation. This is postulated to be one of the reasons for the discrepancy between ovulation and pregnancy rates following ovulation induction with Clomifene citrate. While a higher endometrial thickness resulting with the use of Letrozole may seem as more favourable for conception, such a finding could only be proven with studies aimed at assessing pregnancy rates as the final outcome measure. Such a study would have the inherent difficulties in design with regard to large sample size required as the pregnancy rate would be low in either treatment modality. Furthermore, the study population need to be controlled for many other factors such as male factor infertility and other pathologies in order to make comparisons. This study sample comprised of a population heterogeneous with regard to the seminal fluid parameters and other contributory factors. Therefore, comparison of pregnancy rates was not feasible.

Letrozole has shown to have a higher endometrial thickness in previous studies. However, evidence that correlate improved pregnancy rates with a higher endometrial thickness is lacking.

This study demonstrates that Letrozole is an alternative to Clomifene citrate in augmentation of ovulation and is associated with an increased endometrial thickness. Furthermore, Letrozole shows a statistically non-significant trend towards lesser incidence of multi-follicle development (< 3 dominant follicles) when compared with ovulation augmentation with Clomifene citrate. This is likely due to non-alteration of FSH changes in early part of the menstrual cycle.

## 5. Conclusion and Recommendations

The pattern of underlying causes of infertility in this population demonstrated that ovulatory dysfunction to be the most common cause of infertility, seen in over 53% of study subjects. However many of them showed evidence of inconsistent ovulation while irreversible causes such as ovarian failure was seen in less than 2% of subjects with ovulatory dysfunction.

This study was able to describe the factors associated with ovulatory dysfunction. Knowledge of such factors could be used in clinical practice to determine the patients who require assessment of ovulation early in the work up of infertility management. Furthermore, the study was able to describe the endocrinological abnormalities that are associated with ovulatory dysfunction such as thyroid dysfunction, raised testosterone levels and abnormal gonadotropin ratios. Investigations to detect such abnormalities should be undertaken in patients known to have abnormalities in ovulation since some of these endocrinopathies are easily correctable.

Male factor infertility demonstrated by abnormal SFA parameters was the second most common cause of infertility in this population and was seen in nearly 45% of couples. Seminal fluid analysis should be offered to all the couples in the initial stages of infertility work up to exclude those with severe SFA abnormalities.

Sexual dysfunction was evident in nearly 10% of couples with irregular intercourse being the most common. This was often due to demands of occupation which required the partners to live separately. Due to this high prevalence it should be assessed in all patients presenting for infertility treatment and proper advice should be provided.

Other causes of infertility such as abnormalities in female genital tract were rare in this population. Assessment of tubal patency was carried out only in a selected sample with a clinical indication and therefore it is expected to be overestimated than that of total population.

This study demonstrates that ovulatory dysfunction is highly prevalent among infertile women of this local population and therefore it is of utmost importance to assess ovulation in the basic investigatory work up of infertile couples. Many features in the history such as menstrual abnormalities, obesity, acanthosis and hirsutism may indicate the presence of such abnormalities in ovulation. In the presence of ovulatory dysfunction the individuals should be systematically investigated to identify the underlying pathologies. It is justifiable to offer all such women a baseline ultrasound in the early follicle phase to assess ovarian morphology. However, the presence of polycystic ovaries by itself does not give rise to ovulatory dysfunction and other diagnostic features of PCOS such as hyperandrogenism should be assessed.

Endcocrinological assessment should be offered to women with ovulatory dysfunction and this should include gonadotropins (FSH, LH), TSH, Prolactin and androgen levels. The presence of a goitre demonstrated a high specificity for TSH abnormalities but a low sensitivity. Therefore absence of a goitre does not exclude abnormalities in TSH levels and it is sensible to carry out a TSH assessment in all those with ovulatory dysfunction.

Clomifene citrate was able to induce ovulation in over 77% of subjects with ovulatory dysfunction where 22% of subjects were resistant to a maximum dose of anti-oestrogens. The study was able to demonstrate the features that are associated with resistance to Clomifene citrate which could be used in patient counselling prior to commencing treatment. Letrozole at a dose of 2.5mg per day was able to induce ovulation in over 75% of subjects who responded to Clomifene citrate and 24% who were resistant to Clomifene. Therefore, Letrozole may have a place in ovulation induction in some of the patients who are resistant to

Clomifene citrate. However, resistance to Letrozole was strongly associated with Clomifene citrate resistance suggesting some common associated factors.

Use of Letrozole in augmentation of ovulation resulted in a higher endometrial thickness compared to Clomifene citrate. This is promising as the reduced thickness in the endometrium with Clomifene citrate therapy is thought to be due to the unwanted anti-oestrogen effects of the drug on the endometrium. Furthermore, Clomifene citrate therapy demonstrated a non-significant trend towards a higher incidence of multifollicle development which increases the risk of multiple pregnancies.

While this study was able to achieve many of the objectives, it had certain limitations too. The study population was not completely non-contrived as many were with previous treatment. Therefore, an over representation of more severe pathologies is expected as patients with less severe pathologies would have responded to previous treatment and not present to a tertiary care setting.

The study population that was included was young with a mean age of 29 years and therefore the effects of advanced age was not clearly evident due to the small sample sizes in group over 35 years of age.

In study of Letrozole only one dose (2.5mg per day for 5 days) was studied and the efficacy of higher doses was not studied as the safety of such doses are not well established. Further studies should be aimed at study of treatment efficacy of Letrozole at such doses.

A main limitation of the study was that ovulation was taken as the outcome variable in assessment of treatment efficacy in induction and augmentation of ovulation. Though pregnancy rate would have provided a better outcome measure, it could not be used in this study due to requirement of a larger sample size and the inability to control other areas of the clinical management protocol.

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## Section 4: Impact of Research results

**i) Relevance of results achieved to scientific advancement**

This study was aimed at assessing factors contributing to infertility in a large Sri Lankan population. Not many studies have been carried out in the country before. Furthermore, it studied the contributory causes of ovulatory dysfunction in a sample of infertile couples in Sri Lanka including the association of endocrinopathies. This knowledge would be useful in counseling patients at the commencement treatment as well as to decide on a rational approach to investigation. This would enable the clinicians to identify the patients who would benefit from assessment of ovulation and to decide the investigations to be undertaken in patients with ovulatory dysfunction.

Clomifene citrate is a drug that is commonly used in clinical practice in Sri Lanka. While such treatment is successful in achieving ovulation in nearly three fourths of patients others show resistant to ovulation induction to clomifene citrate. The factors that are associated with clomifene resistant have not been studied in a Sri Lankan population to date. The knowledge gained from this study in this area would be useful for clinicians in counseling patients prior to commencement of treatment as well as to decide on alternative treatments. Furthermore, the this information would be useful for future researchers in identifying subjects for further studies that would be aimed at finding alternative treatment protocols of ovulation induction.

Third generation aromatase inhibitor, Letrozole, is a new therapeutic agent that has been introduced for the indication of ovulation induction and it is still been studied in clinical trials in many parts of the world. This is the first study that studied its efficacy in ovulation induction in Sri Lanka. Furthermore the study was able to identify the factors that are associated with resistance to Letrozole and was also able to compare it with Clomifene citrate in augmentation of ovulation. Not many studies have been reported in this regard in the scientific literature.

**ii) Relevance of results achieved to national / socio-economic development**

In this era of evidence based guideline based clinical management the information gained from this study would be useful for national regulating bodies to develop national clinical guidelines on the management of infertility that are more suited for a local population. While this will improve the treatment success it will also reduce the cost of treatment by reducing the number of unnecessary investigations. Furthermore, this will allow the policy planners to divide the limited resources in a more cost effective manner.

The new therapeutic agent Letrozole, that was shown to be effective in a significant proportion of patients with clomifene resistance, has many advantages in the national setting. This is a low cost treatment modality that requires minimal monitoring during treatment and is thought to be associated with a lesser incidence of complications such as multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). This would have many economic advantages to Sri Lankan population as the alternative treatments of Clomifene resistant such as ovarian drilling and FSH treatment are costly and are associated with a higher complication rate. Use of Letrozole in the clinical practice would enable many patients to continue infertility treatment after a diagnosis of clomifene resistant, whom would have been otherwise unable to afford to high cost treatments such as FSH.

The implementation of the study was able to develop skills in many areas of infertility treatment including use of Letrozole, ultrasound scanning and development of skills in infertility laboratory management that would be a sustainable development in service providence in this clinical area.

**iii) Dissemination/application of research project**

The findings of the study would be published as an MPhil thesis of the University of Colombo. One abstract has already been submitted to a prestigious international conference and has been accepted for presentation in September 2011.

The other findings would be presented as journal publications in international and national scientific journals. The manuscripts are currently being developed.

The findings of the study would be used in the development of clinical protocols of patient management of patients at the infertility clinic of the Teaching hospital, Ragama while they would be presented for development of guidelines and protocols at national level.

## Section 5: Miscellaneous

i) **List of major equipment acquired during the project period and their functionality**
None

ii) **List of publications/communication arising from the project and/or presentations made at seminars, workshops etc. (please attach copies)**
Letrozole resistance in ovulation induction among subjects with ovulatory dysfunction: A case control study – Abstract submitted for 9th Royal College of Obstetricians and Gynaecologists International Meeting in Athens to be held in September 2011 and accepted (Abstract attached – Annex 2).

**Section 6:** Summary statement of expenditure

**RG/2007/HS/03**

**Pro P.S. Wijesinghe**

**Financial Statement 30.06.2011**

| | Budget | Funds Received | Expenses | Balance |
|---|---|---|---|---|
| Personnel (TA) | 50,400.00 | 33,600.00 | 33,600.00 | - |
| Consumable | 2,337,447.00 | 464,163.00 | 457,986.20 | 6,176.80 |
| Equipment | 99,615.00 | 99,615.00 | 92,920.00 | 6,695.00 |
| Miscellaneous | 23,000.00 | 10,000.00 | - | 10,000.00 |
| Advance | | | - | - |
| | 2,510,462.00 | 607,378.00 | 584,506.20 | 22,871.80 |
| | | | | |

Assistant Bursar

Faculty of Medicne

Ragama

S. P. D. Peiris
Assistant Bursar
Faculty of Medicine
University of Kelaniya
Ragama

**Section 7:**

i) Grantees' signature

Professor Prasantha S Wijesinghe .....

Dr. Thilina S Palihawadana .....

Professor Harshalal R Seneviratne .....

ii) Comments of the Head of the department / signature

Dr. Asantha de Silva
Head / Dept of Obstetrics & Gynaecology
Faculty of Medicine, University of Kelaniya.

.....
Head,
Department of Obstetrics
& Gynaecology,
Faculty of Medicine,
Ragama.

iii) Head of the Institution's signature

Professor Sarath Amunugama
Vice Chancellor / University of Kelaniya

.....
Professor Sarath Amunugama
Vice - Chancellor
University of Kelaniya
Kelaniya - Sri Lanka

**nnex 1**

[redacted]

Copy

Prof. PS Wijesinghe
Department of Obstetrics and Gynaecology
Faculty of Medicine
University of Kelaniya
Kelaniya

07/04/2010

Dear Prof. Wijesinghe,

**Re: Modification of the research proposal**

Study title: A study on ovulatory dysfunction in an infertile population of Sri Lanka and a prospective randomized comparison between low dose step-up and step-down gonadotrophin regimes in controlled ovarian stimulation

Grant No: RG / 2007 / HS / 08

Your request under the above subject was tabled at the meeting of the NSF Research Committee on Health Sciences for its recommendation.

The Committee accepted the revised study protocol and recommended that the investigators should be permitted to obtain funds from the University of Kelaniya to continue work, so that the study would not be interrupted.

As per the rules and regulations of the NSF, the investigators are expected to submit the Final Report according to the NSF format, along with the final financial report, at the end of the project period as mentioned in the Contractual Agreement.

Thank you.

Yours sincerely,

Dr. Sarath Abayawardana
Director

NATIONAL SCIENCE FOUNDATION

Director
National Science Foundation
47/5, Maitland Place
Colombo 07

Annex 2

**Letrozole resistance in ovulation induction among subjects with ovulatory dysfunction: a case control study.**

**Palihawadana TS, Wijesinghe PS, Seneviratne HR.**

**Introduction:** Letrozole has been proposed for induction of ovulation in anovulatory infertility. Its efficacy has not been adequately studied in clinical trials. Since the mechanisms of action differ, factors associated with letrozole resistance are expected may differ from that of anti-oestrogens. This study was aimed at identifying factors that are associated with non-response to a standard dose of letrozole.

**Methods:** A case-control study was carried out among 50 subjects with anovulatory infertility undergoing ovulation induction at University infertility centre, University of Kelaniya, Sri Lanka. 25 responders to clomifene and 25 subjects with clomifene resistance were included. All subjects had a FSH level in the range of 2-20 IU/L and no previous ovarian surgery. Letrozole 2.5mg from $2^{nd}$-$6^{th}$ days of the cycle was used and ovulation was confirmed by follicle tracking.

**Results:** Ovulation rate was 76% (19/25) among clomifene responders and 24% (6/25) with clomifene resistance. No association was demonstrated with advanced age and a high BMI between non-responders and responders.

Presence of hirsutism (OR 3.86; 95% CI 1.21-12.30), a LH: FSH ratio of more than one (OR 1.17; 95% CI 0.39-3.50) and clomifene resistance (OR 10.03; 95% CI 2.81-35.77) were significantly associated with a non-response to letrozole. The mean (SD) LH level on Day 2 of the cycle was significantly higher among non-responders (9.75IU/L (4.78) vs. 7.28(2.30); p=0.02).

**Conclusions:** Both letrozole and clomifene seem to share some factors that are associated with resistance. Small number of subjects in this study with advanced age and high BMI limited the ability to study the association of these abnormalities. Since a significant proportion of subjects with clomifene resistance responded to letrozole, it may be useful in treatment of some patients with clomifene resistance.

Funded by grants from National Science foundation (RG / 2007 / HS / 08) and University of Kelaniya.

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