FACTORS ASSOCIATED WITH SUCCESSFUL OUTCOME OF INFERTILITY TREATMENT AT HOSPITAL RAJA PEREMPUAN ZAINAB II

By

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LIST OF ABBREVIATIONS

ACC	Ambulatory Care Centre
Adj OR	Adjusted Odds Ratio
AFC	Antral Follicle Count
AMH	anti-Müllerian hormone
ART	Assisted Reproductive Technology
BMI	Body Mass Index
CC	Clomiphene Citrate
CI	Confidence interval
COH	Controlled Ovarian Hyperstimulation
DFOR	Diminished Functional Ovarian Reserve
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin-releasing hormone
HCG	Human chorionic gonadotrophin
IQR	Interquartile Range
IUI	Intrauterine Insemination
IVF	In-Vitro Fertilisation
LH	Luteinizing Hormone
LSCS	Lower Segment of Caesarean Section
MOH	Ministry of Health
MREC	Medical Research Committee
NMRR	National Medical Research Register
OR	Odds Ratio
PS	Power & Sample Size
rFSH	Recombinant follicular stimulating hormone
ROC	Receiving Operating Characteristic
SD	Standard Deviation
SVD	Spontaneous vaginal delivery
SPSS	Statistical Package for Social Science
TVS	Transvaginal Scan
USM	Universiti Sains Malaysia
WHO	World Health Organization

LIST OF SYMBOLS

>	More than
<	Less than
=	Equal to
≥	More than and equal to
≤	Less than and equal to
α	Alpha
ß	Beta
%	Percentage
Δ	Precision

ABSTRAK

Infertiliti bermaksud ketidakupayaan mengandung selepas tempoh satu tahun melakukan hubungan kelamin tanpa mengamalkan sebarang langkah pencegahan kehamilan. Terdapat pelbagai jenis rawatan untuk infertiliti ini bermula dari rawatan pemakanan ubat Clomiphene Citrate hingga ke rawatan invasif seperti bantuan teknologi rawatan pembiakan. Objektif kajian hirisan lintang ini adalah untuk mengkaji faktor yang terbabit dalam menentukan kejayaan seseorang wanita yang mengambil rawatan infertiliti di Hospital Raja Perempuan Zainab II. Seramai 117 wanita yang terlibat dalam kajian ini dengan menggunakan kaedah persampelan keseluruhan populasi. Rekod rawatan pesakit dilihat dan data diambil menggunakan proforma yang mengandungi maklumat tentang latarbelakang pesakit, (umur, bangsa, tahap pembelajaran, status pekerjaan), maklumat wanita (yang merangkumi maklumat tentang haid, masalah infertiliti, hubungan kelamin, sejarah kesihatan dan pembedahan dan siasatan tentang penyebab infertiliti), maklumat suami (yang merangkumi sejarah ketidaksuburan, sejarah kesihatan dan pembedahan, analisa ujian air mani), rawatan bagi infertiliti yang diambil dan pencapaian (berjaya atau gagal). Kejayaan pencapaian dibahagikan kepada kehamilan (samada di dalam rahim atau di luar rahim) dan keguguran. Kehamilan di dalam rahim dilihat dari sudut kaedah bersalin (kelahiran secara normal atau pembedahan), bilangan bayi yang lahir (samada seorang atau lebih). Kaedah deskriptif dan regresi logistic berganda telah digunakan untuk menjawab objektif dan hipotesis kajian ini. Secara keseluruhannya, kadar kejayaan rawatan adalah 20.5% (95% CI:16.8%, 24.2%) yang menghasilkan tiga belas kandungan di dalam rahim, sepuluh keguguran dan satu kandungan di luar rahim. Kesemua kandungan di dalam rahim menghasilkan seorang bayi, tiada kes

kembar yang dicatatkan. Dua daripada bayi yang lahir adalah pramatang dan selebihnya lahir genap tempoh matang. Tujuh kehamilan di dalam rahim ini melalui proses bersalin normal (SVD) dan enam melalui proses pembedahan (LSCS) bagi tujuan kehamilan unggul (dua kes), bayi lemas (2 kes) dan satu kes kedudukan uri dibawah (placenta praevia). Analisis regresi logistic berganda menunjukkan umur wanita semasa haid kali pertama (adj OR= 1.59,95% CI: 1.05, 2.42, p-value= 0.030) mempunyai kaitan yang signifikan apabila dibandingkan dengan umur wanita dan suami, jisim berat badan (BMI), tempoh percubaan untuk hamil, tabiat merokok, karakter air mani dan rawatan yang diambil. Pecahan wanita yang berjaya selepas rawatan secara keseluruhannya memuaskan dan kebolehan untuk berjaya selepas rawatan infertiliti ini lebih cenderung kepada wanita yang mengalami haid yang pertama di umur yang lewat.

Kata kunci: infertiliti, kehamilan di dalam rahim, kehamilan di luar rahim, keguguran, umur semasa haid pertama kali

ABSTRACT

Infertility is defined as the inability to conceive after a year of regular unprotected intercourse. Different treatment modalities are available to treat infertility ranging from simple, non-invasive oral agent such as clomiphene citrate to invasive procedure such as assisted reproductive technology (ART). The objective of this cross-sectional study was to determine the factors associated with the successful outcome of infertility treatment at Hospital Raja Perempuan Zainab II. Out of 429 cases, only 117 cases that fulfilled the inclusion creiteria were included. A retrospective record review was done using a checklist proforma consisting of sociodemography (age, ethnicity, education level and employment status), female variables (menstrual history, infertility history, sexual history, medical and surgical history, investigations, causes and treatment), male variables (smoking status, medical and surgical history, seminal fluid analysis, the last treatment method and its outcome (success or failure). The successful outcome of treatment was categorised into intrauterine pregnancy, abortion or extrauterine pregnancy. The end outcome for intrauterine pregnancy was assessed in terms of mode of delivery (SVD or LSCS) and the fetal outcome (singleton or multiple births and gestational age). The descriptive methods and multiple logistic regression were applied to answer the objectives and hypotheses of this study. The overall successful rate post infertility treatment was 20.5% (95% CI: 16.8%, 24.2%) which resulted in thirteen intrauterine pregnancies, ten abortions and one extrauterine pregnancy. All thirteen pregnancies resulted in singleton babies, seven were delivered via SVD and six delivered via LSCS for precious pregnancy (two cases), fetal distress (2 cases) and one case of placenta praevia. Two of the babies were prematured, and the rest were delivered at

term. Analysis from logistic regression showed that age of menarche (adj OR= 1.59,95% CI: 1.05, 2.42, *p*-value= 0.030) was the only significant factor affecting the successful outcome of infertility treatment when adjusted for age of men and women, BMI, duration of infertility, smoking, sperm characteristics (sperm count, percentage of progressive motility and normal morphology) and treatment options. The proportion of women with successful outcome post infertility treatment was almost similar to other studies and the odds of having a successful outcome are much higher in women with late age of menarche.

Keywords: Infertility, intrauterine pregnancy, extrauterine pregnancy, abortion, age of menarche.

CHAPTER ONE: INTRODUCTION

1.1 Overview

Infertility can be defined by clinical, epidemiological and demographic definition. By clinical definition, infertility is customarily defined as the inability to conceive after 1 year of regular unprotected intercourse (Zegers-Hochschild *et al.* (2009). The infertility evaluation is typically initiated after 1 year of trying to conceive, but in couples with advanced female age (≥ 35 years), most practitioners initiate diagnostic evaluation after an inability to conceive for 6 months (Quaas and Dokras, 2008).

The epidemiologist on the other hand defines infertility as women of reproductive age (15-49 years) at risk of becoming pregnant (not pregnant, sexually active, not using contraception and not lactating) who report trying unsuccessfully for a pregnancy for 2 years or more (Gnoth et al., 2005). This definition is mainly used for monitoring and surveillance in epidemiology.

Both of these definitions however, are not appropriate when making population-based estimates of infertility using household surveys. Clinical definitions are designed for early detection and treatment of infertility (Zegers-Hochschild *et al.*, 2009). A definition and assessment of infertility based on medical histories and diagnostic tests is appropriate for clinical settings, where the aim is to understand causes and provide treatment as soon as it is indicated. However, measuring patterns and trends in infertility at the population level necessitates a measure that may be elicited using a standard set of survey questions (Larsen, 2005). The World Health Organisation (WHO) epidemiologic definition is more closely aligned with clinical practice than demographic definitions are, and may be measured using survey data.

However, few household surveys determine whether a couple is trying to become pregnant, and the majority do not collect information on past pregnancies, only on previous live births (Mascarenhas *et al.*, 2012b).

In terms of demographic definitions of infertility, infertility is defined as an inability of those of reproductive age (15-49 years) to become or remain pregnant within 5 years of exposure to pregnancy (Rutstein and Shah, 2004). This definition is consistent with definition given by Mascarenhas et al., 2012 as an inability to become pregnant with a live birth, within 5 years of exposure based upon a consistent union status, lack of contraceptive use, non-lactating and maintaining a desire for a child (Mascarenhas *et al.*, 2012b). The 5-year time frame was used as the exposure period which is needed to accommodate the time it takes to become pregnant and give birth and helps prevent unreported temporary separations, periods of post-partum sexual abstinence or lactational amenorrhoea from unduly affecting the infertility measure (Mascarenhas et al., 2012). The preferred outcome is birth rather than pregnancies as information on live births is collected more often and reported more accurately than pregnancies in the household survey.

A common definition of infertility is very important for the appropriate management of infertility(Gnoth et al., 2005) as a balanced management of reduced fertility requires appropriate timing of infertility investigations and appropriate timing of starting treatment to avoid both over- and under-treatment (Brosens et al., 2004). As a consequence of that, WHO and the International Committee for Monitoring Assisted Reproductive Technology (WHO-ICMART) has come to an agreement to standardise the definition of infertility based on the clinical definition to harmonize international data collection, and to assist in monitoring the availability,

efficacy, and safety of assisted reproductive technology (ART) being practiced worldwide (Zegers-Hochschild *et al.*, 2009)

Despite the great success in improving maternal and child health globally in the past decade, the focus on infertility has been neglected (Cousens *et al.*, 2011; Cui, 2010; Kassebaum *et al.*, 2014). One of the United Nation's Millennium Development Goals was for universal access to reproductive health care by 2015, and WHO has recommended that infertility be considered a global health problem as the inability to have children can affect both men and women across the globe (Hammarberg and Kirkman, 2013). Infertility can lead to distress and depression, as well as discrimination and ostracism (Cui, 2010; Sembuya, 2010). The main challenges in generating global estimates of infertility are the scarcity of population-based studies and the inconsistent definitions used in the few high-quality studies available (Gurunath *et al.*, 2011). An accurate profile of the prevalence, distribution, and trends of infertility is an important first step towards shaping evidence-based interventions and policies to reduce the burden of this neglected disability globally (Mascarenhas *et al.*, 2012b).

A WHO evaluation of Demographic and Health Surveys (DHS) data (2004), estimated that about one in every four couples is affected by infertility in developing countries (Rutstein and Shah, 2004). Then, in 2010, another WHO study estimated 48.5 million couples worldwide were infertile (Mascarenhas *et al.*, 2012). Data for this study were obtained from DHS, Reproductive Health Surveys, the World Fertility Survey, the Pan Arab Project for Family Health and Pan Arab Project for Child Development, the European Multicenter Study on Infertility and Subfecundity, the Fertility and Family Survey, the United States National Survey of Family Growth

and the China In-Depth Fertility Sample Surveys, involving a total of 190 countries and territories. The result showed that the prevalence of infertility was highest in South Asia, Sub- Saharan Africa, North Africa/Middle East, and Central/Eastern Europe and Central Asia. Apart from declines in primary and secondary infertility in Sub-Saharan Africa and primary infertility in South Asia, the levels of infertility in 2010 were similar to those in 1990 in most world regions. However, due to population growth, the absolute number of couples affected by infertility had increased from 42.0 million in 1990 to 48.5 million in 2010 (Mascarenhas *et al.*, 2012b).

The United Nation World Fertility Data 2012 provides a comparable and up-to-date set of national data on fertility and the timing of childbearing for all 223 countries and areas of the world including Malaysia. Among the key indicators used are age-specific fertility rates, total fertility and mean age at childbearing and children ever born. For each of these indicators and to the extent that data are available, data are presented for five reference dates: 1970, 1985, 1995, 2005 and the most recent data available. Major sources of data on fertility and the timing of childbearing presented in World Fertility Data 2012 are civil registration systems, sample surveys and censuses. Total Fertility Rate (TFR) is defined as the average number of children a hypothetical cohort of women would have at the end of their reproductive period if they were subject during their whole lives to the fertility rates of a given period and if they were not subject to mortality. TFR is expressed as children per woman. According to the World Fertility Data 2012, Malaysia had a total fertility rate of 2.07 within 2005to 2010, a decreasing trend from 6.23 in 1950 to 1955. This is similar to data from Family Health Development Division, Ministry of Health which stated that the TFR in Malaysia latest in 2012 was 2.1 (Dr Majdah

Mohamed of Family Health Development Division, Ministry of Health, personal communication, 17 December 2014).

1.2 Types of infertility

Infertility is regarded as a disability which falls under the Convention on the Rights of Persons with Disability. Infertility was ranked the 5th highest serious global disability (Shakespeare and Officer, 2011). The disability can be categorized into primary infertility and secondary infertility. Primary infertility is defined as the absence of a live birth for women who desire for a child and have been in a union for at least five years, during which they have not used any contraceptives. When a woman is unable to ever bear a child, either due to the inability to become pregnant or the inability to carry a pregnancy to a live birth, she would be classified as having primary infertility (Mascarenhas et al., 2012). The prevalence of primary infertility is calculated as the number of women in an infertile union divided by the number of women in both infertile and fertile unions (Mascarenhas *et al.*, 2012a).

Primary infertility prevalence among child-seeking women varied by region in 2010 from 1.5% in the Latin America/Caribbean region, to 2.6% in the North Africa/Middle East region. The decline in primary infertility was greatest in Sub-Saharan Africa, which experienced a substantial decline in primary infertility, from 2.7% in 1990 to 1.9% in 2010, a decline of 0.8 percentage points over the 20-y period. The lowest estimated prevalence of primary infertility occurred in middle-income countries in Latin America (Peru, Bolivia, Ecuador, and El Salvador) and in Poland, Kenya, and the Republic of Korea (0.9%–1.0%). At the other extreme, 13

countries in Eastern Europe, North Africa/Middle East, Oceania, and Sub-Saharan Africa had prevalence of 3.0% or greater.

Secondary infertility is defined as the absence of a live birth for women who desire a child and have been in a union for at least five years since their last live birth, during which they did not use any contraceptives (Mascarenhas *et al.*, 2012a). Those who repeatedly spontaneously miscarry or whose pregnancy results in a stillbirth, or following a previous pregnancy or a previous ability to do so, are then not able to carry a pregnancy to a live birth would present with secondary infertile (Mascarenhas *et al.*, 2012). The prevalence of secondary infertility is calculated as the number of women in an infertile union divided by the combined number of women in infertile and fertile unions. Women in a fertile union have successfully had at least one live birth in the past five years and, at the time of the survey, have been in a union for at least five years following their first birth (Mascarenhas *et al.*, 2012a).

Global and country patterns of secondary infertility were similar to those of primary infertility, with two notable exceptions: first, the prevalence of primary infertility was high in some countries in the North Africa/Middle East region, notably Morocco and Yemen, with prevalence's greater than 3%, but prevalence of secondary infertility was low in those same countries. Second, the prevalence of primary infertility observed in the Central/Eastern Europe and Central Asia region was low to-intermediate relative to that of other regions, though this region had the highest prevalence of secondary infertility. The prevalence of secondary infertility ranged from 7.2% in the High Income region and 7.2% in the North Africa/Middle East region to 18.0% in the Central/Eastern Europe and Central Asia region. Most

regions experienced non-significant increases in the prevalence of secondary infertility between 1990 and 2010 with the exception of Sub-Saharan Africa, where the prevalence of secondary infertility declined from 13.5% in 1990 to 11.6 in 2010. Like primary infertility, the prevalence of secondary infertility varied by country within each region, particularly in Sub-Saharan Africa.

1.3 Causes of infertility

Infertility has a wide range of causes stemming from three general sources: physiological dysfunctions, preventable causes, and unexplained issues. Anatomical, genetic, endocrinological and immunological problems can all cause or contribute to infertility(Evens, 2004). Physiological causes of female infertility include: tubal blockage, abnormal ovulation, congenital malformation, and endometriosis. Male factors include issues with sperm counts, motility, and quality; and ejaculatory dysfunctions.

Most primary and secondary infertility in developing countries is attributable to infectious disease and subsequent damage or blockage of the fallopian tubes (Evens, 2004). Tubal blockage is responsible for up to two-thirds of infertility in nulliparous women in sub-Saharan Africa, up to one-third of the infertility in other parts of the developing world and up to one-quarter in the developed world. Infection-related infertility can be caused by undiagnosed or poorly treated genital tract infections, sexually transmitted infections (STIs), or postpartum or post abortion infection. Infectious and parasitic diseases such as pelvic tuberculosis, schistosomiasis or malaria can also cause infertility. Infertility can be resulted from genital scarification or cutting as well.

The most common preventable causes of infertility are sexually transmitted infections, especially chlamydia and gonorrhoea(Hull *et al.*, 1985) . Undiagnosed or inadequately treated chlamydia and gonorrhea in women can lead to pelvic inflammatory disease (PID) which can lead to infertility (Zuky *et al.*, 2005). In men, chronic chlamydial genital infection can also possibly lead to infertility (Hull *et al.*, 1985). A prospective study in Universiti Sains Malaysia Hospital between 2002 and 2003 found that, among 150 infertile women, six of them had Chlamydial infection (Zuky *et al.*, 2005). The prevalence of Chlamydial infection worldwide varies depending on the sample of women. But the prevalence rate of 4 % at HUSM at that time was almost similar to the prevalence of Chlamydial infection in the United Kingdom.

The term unexplained infertility usually refers to a diagnosis (or lack of diagnosis) made in couples in whom all the standard investigations such as tests of ovulation, tubal patency and semen analysis are normal. Unexplained infertility is a term that has been applied to as many as 30–40% of infertile couples(Ray *et al.*, 2012). Unexplained infertility may arise in two ways. Some couples may have some subtle, undetected defect in the reproductive process, while in others conception is delayed by chance alone, as the couples fecundity may be on the lower side of the normal distribution. The later cause may provide a better prognosis for spontaneous pregnancy in such couples than in those with diagnosed causes of infertility (Quaas and Dokras, 2008).

1.4 Treatment modalities

There are different treatment modalities available in treating infertility according to the underlying cause. Comparison between centers can be very unreliable because of marked variation in the definitive criteria employed. For the purpose of this study, the treatment regime discussed in this study will be based on the treatment modalities available at Hospital Raja Perempuan Zainab II include

i. Superovulation with clomiphene citrate (clomid) and natural coitus, without intrauterine insemination (IUI)

ii. Superovulation with clomid or Controlled Ovarian Hyperstimulation (COH) with recombinant follicular stimulating hormone(rFSH) or GnRH and IUI

iii. Assisted Reproductive Technology (ART)

Clomiphene Citrate (CC)

Clomiphene Citrate (CC) is a selective oestrogen receptor modulator with both oestrogenic and antioestrogenic properties. It was first approved for use in women with anovulation in 1967, and has been used as a first-line ovulation induction agent for over 40 years (Pritts, 2010). Acting as an antioestrogen, CC competitively inhibits the binding of estradiol to its receptors in the hypothalamus and pituitary, which in turn blocks the negative feedback effect of endogenous oestrogens, including estradiol. This results in an increased secretion of pulsatile gonadotrophin-releasing hormone (GnRH) from the hypothalamus, leading to an increase in follicle-stimulating hormone (FSH) and luteinising hormone (LH)

production and secretion from the pituitary gland. This increase in FSH secretion stimulates follicular growth and estradiol production, thereby inducing a mid-cycle LH surge and subsequent ovulation.

Studies with CC have shown an ovulation rate of 60– 85% and a pregnancy rate of 30–50% after six ovulatory cycles (Kousta et al., 1997). This apparent discrepancy between good ovulation rates and lower pregnancy rates has been attributed to the anti-oestrogenic effects of CC on the endometrium and cervical mucus (Hart and Norman, 2006).

Adverse effects of CC include higher twin and triplet pregnancies, at 5–7% and 0.3%, respectively. The incidence of OHSS is also increased but is less than 1%. It has also been recommended that the lifetime CC exposure of a patient might place the patient at increased risk of ovarian tumours (Cetin et al., 2008).

Intrauterine Insemination (IUI)

IUI is often suggested to infertile couples in which the woman has at least one permeable fallopian tube and the man has partially modified sperm or low sperm quality (Merviel et al., 2010). Other indications for IUI include unexplained infertility factors, cervical factors, ovulatory dysfunction and endometriosis.

The advantage of this approach is that some of the associated with IVF are avoided, particularly those relating to oocyte retrieval. There is however significant risk of ovarian hyperstimulation syndrome (OHSS), multiple pregnancy and a small but finite risk of infection following IUI.

The pregnancy rate depends on sperm parameters, female factors, and the ovarian stimulation methods (Merviel et al., 2010). The overall successful pregnancy rate of IUI varies from as low as 5% to as high as 70% (Badawy et al., 2009). Thereby IUI treatment seems to be far more efficient than natural cycle, clomiphene medication and timed intercourse (Bungum et al., 2004).

Assisted Reproductive Technology (ART)

Many couples, however, fail to conceive after three IUI treatment cycles and more advanced fertility treatment (ART) may subsequently be employed (Bungum et al., 2004). Based on WHO-ICMART, assisted reproductive technology (ART) means all treatments or procedures that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of establishing a pregnancy (Zegers-Hochschild et al., 2009). This includes, but is not limited to, in vitro fertilization and embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and gestational surrogacy. ART does not include assisted insemination (artificial insemination) using sperm from either a woman's partner or a sperm donor (Zegers-Hochschild et al., 2009).

The indications for IVF have expanded to include infertility caused by severe male factor, diminished ovarian reserve, ovulatory dysfunction, severe endometriosis, and infertility of unexplained cause (Huang and Rosenwaks, 2012). This technique also provides a new means of preconception genetic diagnosis and preservation of fertility. And in fact, IVF is the most effective treatment option for couples with multi-factorial infertility problems (Huang and Rosenwaks, 2012).

The increased risk of adverse pregnancy outcomes after ART is a concern as ART is associated with multiple pregnancies in more than 30% of ART pregnancies, small for gestational age infant and preterm delivery as well as high maternal complications such as preeclampsia, gestational diabetes, placenta praevia, placental abruption and caesarean delivery.

1.5 Treatment at Hospital Raja Perempuan Zainab II (HRPZ II)

The Infertility Clinic service at Hospital Raja Perempuan Zainab II is a subdomain of the Obstetrics and Gynaecology Department which is located at the Ambulatory Care Centre (ACC) building since early this year. The Infertility Clinic has been in operation since 2005 by a Consultant Obstetrics and Gynaecologist. This clinic caters for subfertile and infertile women around Kota Bharu and acts as a tertiary clinic that received referral from the whole Kelantan, Hulu Terengganu and Kuala Lipis for further management of infertility. As these patients were already seen by a medical officer or a specialist prior to visit at Infertility Clinic, HRPZ II, initial investigations were already conducted there and patients usually presented to the Infertility Clinic with a baseline result. The baseline investigations are day 2 FSH, day 21 serum progesterone, LH, thyroid function test. The investigation result will be reviewed by the requested doctor prior to referring patient to the Infertility Clinic. Any incomplete investigation or doubtful result usually will be repeated upon visit at the Infertility Clinic, HRPZ II including seminal fluid analysis. A transabdominal or transvaginal ultrasound scan is also done on the first visit to the Infertility Clinic.

All female patients were treated with ovarian stimulation agents initially using clomiphene citrate (CC) alone, prescribed at 100 to 150 mg of clomiphene citrate starting on day 2 to 6 of the cycle whether or not the investigation result was ready. This method of superovulation must coincide with timed coitus. The women will be followed up again at the Infertility Clinic after one month and will be subjected for a trans-vaginal scan and reviewed by a doctor on the next visit. If there is an evidence of pregnancy on scan, the patient will be seen twice before transferred to the antenatal care clinic.

The clomiphene citrate is usually given for 2-3 cycles and during this time, a hysterosalpingogram will be arranged to further investigate the causes of infertility. At this point, patient will be counselled for intrauterine insemination (IUI) either by using clomiphene citrate alone and IUI or in combination of clomiphene citrate and gonadotrophin(Gonal- For Puregon) and IUI. A serial transvaginal scan (TVS) was performed to monitor the growth of thefollicles starting at Day 12 of cycle. Human chorionic gonadotrophin (HCG) was administered to the patient when the size of the follicles reached ≥18 mm.

All semen samples from male patients were collected either by masturbation following an abstinence period of 3-4 days and left to liquefy for less than 1 hour. The samples were assessed according to WHO (2010) criteria. The sperm count before wash were divided into two groups: < 100 million per ml and ≥ 100 million per ml. Puresperm media was used for sperm preparation. In the gradient method, 45% and 90% solutions were prepared and then top layered with the semen samples. The samples were than centrifuged at 1200rpm for 20 minutes, the pellet was left to sink at the bottom and the supernatant discarded. The pellet was then washed with 2ml of media wash and centrifuged again at 2000rpm for 10 minutes. For oligospermia samples, the mini gradient method was used with the same techniques. The final pellet was re-suspended with 0.3ml IVF medium.

The uterine insemination was performed 36-40 hours post HCG injection. The procedure was carried out using a soft IUI catheter (Laboratoire CCD / Gynetics Medical Products/ Select Medical Systems/ Cook K-Jets) with the patient in the dorsal position. Sterility of the procedure was strictly observed. The catheter was gently passed through the cervical canal and the sperm suspension expelled into the

uterine cavity. Insemination volumes ranged from 0.5 to 2 ml. The women remained supine for 15 min after IUI. All patients were given Duphaston given for 1 month. Three weeks after the IUI procedure, if patients remained amenorrhoeic, urine pregnancy test was done and if it was positive, transvaginal ultrasound scan was performed a week later to observe for the gestational sac.

Patients who were warranted for assisted reproductive treatment (ART) will be referred to Hospital Sultanah Nur Zahirah (HSNZ) , Kuala Terengganu as the service was not available at HRPZ II.

Any women who were found to be pregnant by urine pregnancy test and confirmed by unltrasound scan will undergo antenatal follow-up at the Antenatal Clinic,another subdomain of the Department of Obstetrics and Gynaecology, HRPZ II. Patient will be closely monitored until delivery. The method and place of delivery were planned beforehand.

Despite the various forms of treatment available nowadays, studies done previously had looked into the factors associate with the success of specific treatment such as intrauterine insemination and assisted reproductive technology. Many factors have been reported as influencing pregnancy rates after IUI include the woman's age, the length of infertility, type of infertility, the sperm count analysis, the number of mature follicles, the E2 concentration on the day of HCG administration and type of catheter used(Merviel *et al.*, 2010) . Whereas, the key factors affecting success of IVF treatment include selection of the appropriate controlled ovarian hyperstimulation protocol and gonadotropin dosage, close monitoring of follicular growth and serum E2 levels, adjustment of gonadotropin dosage to avoid hyper-response, and individualised timing of hCG injection(Huang and Rosenwaks, 2012).

There is a lack of study done to look at the common factors influencing the success in all infertility treatment modalities of which this study is intended to do.

1.6 Rationale of Study

Infertility is a public health concern. By looking into the factors which may affect the fertility status and its treatment outcome, it is hoped that by doing this study, preventive measures including tackling the modifiable factors that may affect the outcome of infertility can be done at an earlier stage. The population is growing over time, and hence the issues of infertility should be made known to the public and to encourage them to seek help and treatment without delaying any further.

CHAPTER 2: LITERATURE REVIEW

2.1 Age

One of the major predictors for a successful infertility treatment was female age (Azantee *et al.*, 2011; Merviel *et al.*, 2010). Female age is the single most important determinant of spontaneous as well as treatment-related conception, with a gradual decline in fertility especially after the age of 35 years (Menkenet al., 1986; Templeton et al., 1996). Age related fertility problems increase after 35 and dramatically after 40. This could be due to women having had more opportunity to acquire pelvic infections or develop endometriosis or premature menopause.

Demographic studies have shown that more women are delaying childbearing at the present time than previously (Botting Dunnell, 2003). The mean age at first birth and mean age at childbearing was noted to have increased in all developed countries since the 1990s (Evers, 2002; Rutstein and Shah, 2004). As women delay childbearing in favour of pursuing education and vocation opportunities, they face potentially increased difficulty in becoming pregnant (Evens, 2004). Increasing age at childbearing could also increase the prevalence of infertility, as the ability to become pregnant and deliver a live birth reduces with age in all populations. This trend is expected to cause a corresponding rise in the mean age at which women first present with infertility. Similar trend also happened in low- and middle-income countries, although first birth still occurs at young ages, the age at first birth has increased. As Demographic and Health Surverys (DHS) survey done in the 1990s and another survey during 2000–2011 in 40 countries, it was revealed that the overall median of the median age at first birth among women aged 25–49 years increased from 19.8 to 20.3 years old (Rutstein and Shah, 2004). While the age at first birth has

increased, the average number of children has decreased, and thus, the mean age at childbearing has not changed in these countries.

Though the female age is an important predictor for the success of infertility treatment, age also plays a role in men. As men age, they are progressively at greater risk for orchitis; testicular trauma, torsion, and cancer; varicocele; genital inflammation; hormonal changes; systemic diseases, and conditions necessitating surgery, all of which can dramatically reduce male fertility (Petraglia *et al.*, 2013). High levels of gonadotropins associated with low levels of testosterone (T) indicate hypergonadotropic hypogonadism, a state of primary hypogonadism. On the other hand, low or normal levels of gonadotropins associated with low T levels tend to indicate hypogonadotropic hypogonadism, a state of hypogonadism secondary to a dysfunction of hypothalamic and pituitary origin. Varicocele is an abnormal dilation of the veins of the plexus pampiniform and is present in 15% of the male population and approximately 40% of infertile men. A detrimental effect of varicocele on spermatogenesis is found in some men but not all. With age, fertility is reduced in men but not as much as in women. For men younger than 35 years, there is no effect, but starting in the late 30s, the impact of male age becomes pronounced.

Although descriptive studies show declines in fertility with age, this can be attributed by the less frequent sexual intercourse in older couples. In particular, among 35-year-old women, the proportion of many infertile couples will conceive naturally in the second year of trying ranges from 43% to 63% depending on age. However, result from Dunson *et al.* (2004) study on the frequency of sexual intercourse revealed differently. Increasing frequency of sexual intercourse from 2 to 3 times per week had relatively little effect on the number of menstrual cycles

required to conceive. However, time to pregnancy increased substantially for couples having intercourse only once per week. This probably occurs because the fertile interval each menstrual cycle is 5–6 days, so couples having sexual intercourse only once a week can miss it completely. It was concluded that women aged 30–34 years were similar to the 27 to 29-year-olds, but women aged 35–40 years had further reductions in their probabilities of pregnancy (Dunson *et al.*, 2004).

A study done by (Maheshwari *et al.*, 2008) looking into diagnostic profile comparing the older age group of women to the younger age group from a population-based data of 7172 women revealed that there was a reduction in the diagnosis of ovulatory dysfunction by one-third, but a two-fold rise in unexplained and tubal infertility beyond the age of 35 years. Although, the differences are more marked at the age of over 35 years, the increase in incidence of unexplained and tubal factor infertility is evident from the age of 30 years onwards, as is decreased incidence of ovulatory dysfunction. With the declining numbers and quality of oocytes, the female fertility begins to decline sharply around the age of 35 of which the cut-off point for advanced maternal age was taken (Cooke *et al.*, 2012). Thus, these women over 35 years of age have the priority and should be referred early from primary care for investigations and prompt treatment of infertility (Maheshwari *et al.*, 2008).

Given the relationship between advanced maternal age and the decline in fertility, it is not surprising that age is the most important determining factor of success in women undergoing in-vitro fertilisation (Templeton *et al.*, 1996). Although ART may overcome infertility in younger women, it does not reverse the age-dependent decline in fertility. Advanced maternal age is associated with a

decline in the number of oocytes retrieved, embryos available for transfer, and embryo quality, ultimately resulting in lower implantation, pregnancy, and live birth rates. A strong correlation also exists between advanced maternal age and the incidence of chromosomal anomalies. The risk of miscarriage approaches 50% among women over the age of 40 years.

2.2 Duration of infertility

A higher proportion of women in the older group had increased duration of infertility, which would be explained by the fact that women in this population with secondary infertility present later at the clinics (Maheshwari *et al.*, 2008). Duration of infertility became one of the predictors to the success of infertility treatment apart from female age (Hakan E.Duran et al., 2002). But there are different views on whether duration of infertility successfully affecting the outcome of treatment. Study done by Nuojua-Huttunen et al, 1999 found a significant decrease in pregnancy rate with an increasing duration of infertility, which was also similar to the finding of Tay *et al.* (2007). Finding from Krog *et al.* (2014) revealed different views of which duration of infertility has no predictive value towards the outcome of IVF.

2.3 Sperm parameters

Over the past 30 years, the World Health Organisation (WHO) has been attempting on the creation of reference threshold values for semen analysis as male infertility contributes towards 20-30% of infertility cases. This definition of male infertility is made either by diagnosis of a known cause of male infertility or based

solely on semen analyses in idiopathic cases (Murray *et al.*, 2012). If interpreted correctly, the fast and inexpensive semen analysis remains the gold standard for defining a man's role in subfertility (van der Steeg *et al.*, 2011). Since 1987 the WHO has published five editions of the ''WHO Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.'' Prior to the development of WHO Manual in 2010, the reference values were based on the WHO manual 1999 which has its own limitations (Cooper *et al.*, 2010).

The limitations were resulted from data that were derived from imprecisely defined reference populations and obtained from laboratories with unknown comparability with respect to analytical methodologies. These values were limited by the lack of available data on semen variables in recent fathers, and did not define true reference ranges or limits (Cooper *et al.*, 2010). There are no true ''fertile'' or ''normal'' cutoffs for semen parameters. The only way that quantitative parameter terminology can be used is to state a value as ''above'' or ''below'' minimum reference values (Murray et al., 2012). While some centres considered the cited values for characteristics of sperm concentration, morphology and mortality too high, whereas others considered them too low, the discrepancy can result in over- or under- diagnosis and hence leading to inappropriate management. It was hoped that the new development and application of clear reference ranges helps to reduce the incidence of misdiagnosis of fertility problem and improve clinical care.

In the establishment of the new WHO guidelines on semen analysis, produced in 2010, semen samples from over 4500 men in 14 countries in 4 continents were obtained from retrospective and prospective analyses on fertile men whose partners had a time-to-pregnancy (TTP) of ≤12 months, unscreened men of

unknown fertility status from general population (UNSCR), fertile men with no report of when they fathered children (NOTTP) and screened men of normozoospermic based on 1999 WHO standards (SCR).

Men whose partners had a TTP ≤ 12 months were chosen as individuals to provide reference distributions for semen parameters since infertility is currently defined as a failure to conceive after at least 12 months of unprotected intercourse. TTP is a well-known and standardised epidemiological index , defined as the number of months (or cycles) from stopping contraception to achieving a pregnancy.

Data on semen volume, sperm concentration, total sperm number per ejaculate , motility, vitality and normal morphology were included only if they were generated from complete semen samples, obtained following 2-7 days of sexual abstinence.

The men in the reference population are characterised by not only larger semen volumes and higher concentrations and numbers of spermatozoa in their ejaculate, but also by a higher total number of motile and morphologically normal cells per ejaculate than found in the other groups.

A comparison was made between WHO 1999 and WHO 2010 criteria, using 529 consecutive semen samples from 427 men in order to determine how many men with normal screening according to WHO 1999 criteria would be considered having abnormal semen based on 2010 criteria and vice versa (Catanzariti *et al.*, 2013). The study also aimed to determine which parameter of volume, concentration, motility and morphology is the most responsible of this change from normal (defined normal by all parameters) to abnormal (defined abnormal by at least one parameter) using the two WHO criteria. It was found out that 199 (37.83%) were considered normal

and 246 (46.76%) abnormal both according to WHO 1999 and WHO 2010 criteria; 3 men were azoospermic and none of the samples previously classified as normal according to WHO 1999 criteria was considered abnormal after re-evaluation using WHO 2010 criteria. Meanwhile, 82 (15.58%) samples evaluated as abnormal according to 1999 criteria changed to normal according 2010 criteria. The concordance between 1999 and 2010 evaluation was 84.44%. The authors concluded that the changes from WHO 1999 to WHO 2010 criteria did not modify the interpretation of semen quality regardless whether all 3 parameters (count, motility and morphology) were considered or not modify interpretation of semen quality, because comparing the two classifications it was demonstrated that there is a substantial agreement, among the three parameters (count, motility and morphology) all together, and also considering each single parameter. Despite that, almost 16% of the patients considered infertile according to the old criteria, should be evaluated normal by the new classification and they should not need any treatment for infertility.

2.4 Menarche

There are few data available investigating on the relationship of age at menarche and the subsequent fertility. The first menstrual bleeding, menarche, is the best recorded female pubertal milestone defining the beginning of ovulation and fecundity (Guldbrandsen et al., 2014). Girls, who do not have menarche by the age of 16 years, are diagnosed as having delayed menarche. This condition indicates disturbance of the process of maturation of sexual functioning, and so might affect subsequent fertility.

A study done by Komura *et al.* (1992) was the first study investigating on the relationship between the age at menarche and subsequent reproductive functioning. The study was designed as a retrospective survey on 2281 healthy married women who received a medical check-up at the Health Test System between February 1986 and January 1987. The women were each asked on their age at menarche, history of pregnancy and delivery; and whether their menstruations during the first few years after menarche had been regular. For the purpose of analysis, women who had disorders that prevent pregnancy, such as chromosomal abnormality or dysgenesis of the Miillerian duct (which are included in primary amenorrhea), those who had undergone hysterectomy before having a child, those who prevented conception and women who failed to conceive due to male infertility were excluded. Of the total 2278 women surveyed, 115 (5.0%) were infertile. The age at menarche of the fertile group was 13.7 ± 0.1, which was significantly ($P < 0.01$) earlier than that of the infertile group (14.0 ± 0.2). The relationship of infertility with menstrual regularities in the first few years after menarche was investigated. A total of 2042 females (90%) had regular menstruations, and their rate of infertility was 4.7%. In contrast, among the women with irregular menstruations 8.9% were infertile. Thus women who had irregular menstruations had a significantly ($P < 0.01$) higher incidence of infertility.

This study showed that in women who had menarche at the age of 18 or later the incidence of infertility is significantly higher than that in those with menarche before the age of 18. Among the women who had menarche before the age of 17, there was no relationship between the age at menarche and the rate of infertility. Therefore, women who had menarche at the age of 16 or 17, had normal reproductive functioning. This study also looked on the regularity of menses which showed that irregularity of menstruations during the first few years after menarche