POSSIBLE CAUSES OF INFERTILITY IN PATIENTS VISITING AN IVF CLINIC AND THE USE OF BASAL GONADOTROPHINS AS PREDICTIVE MARKERS OF OVARIAN RESPONSE IN IVF CLIENTS

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Assisted Conception, LISTER HOSPITAL AND FERTILITY CENTRE, ACCRA. This work has not been submitted for any other degree.

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DEDICATION

This work is dedicated to the entire YAKASS family (Mr. and Mrs. Yakass, Sylvester, Sylverine, Gladys, Roland, Doris, Emelia, Dorothy and Gifty) for their diverse contribution to my education and livelihood. Again I dedicate this work to Caroline.

ABSTRACT

Background - Assisted reproduction is expensive, time-consuming and stressful for patients. The development of sufficient number of follicles during ovarian stimulation for IVF is a very important step towards a successful outcome. The accurate determination of ovarian reserve continues to be a challenge for reproductive physicians. Basal hormonal markers may contribute to the prediction of ovarian reserve and as a reflection of the number of oocytes retrieved after ovarian stimulation. Therefore, a major challenge to the IVF team is to predict prospective patients who will be low responders and to appropriately counsel women who are potential candidates for assisted reproduction.

Aims - The aim of this study was to assess basal hormonal markers; LH, FSH and prolactin and obesity markers; BMI and WHR as predictive markers of ovarian response in IVF clients. Again it was to determine the effect of some lifestyle and behavioural patterns like exercise and smoking on measurable outcomes of ovarian stimulation by subjects who seek assisted conception.

Methods - A total of 104 subjects were recruited at the Lister hospital and fertility centre in Accra - Ghana for this study. Anthropometric measurements performed included body mass index (BMI) and waist to hip ratio (WHR). Other lifestyle features like exercise and smoking patterns were assessed from responses to a provided questionnaire. Blood samples were drawn on second day of their menstrual cycle in the month prior to the IVF procedure and basal LH, FSH and prolactin assayed. A ratio of FSH/LH was calculated.

Results – Age, basal FSH and FSH/LH ratio of subjects showed a very significant negative correlation with measured outcomes of ovarian stimulation. Poor responders were significantly older than normal responders (median age; 40 vs. 36, P = <0.01). Basal FSH was significantly higher in poor responders as against normal responders (median FSH; 12.7 vs. 9.7, P = <0.05 respectively). In response to lifestyle, all subjects who engaged in moderate to high forms of exercise recorded a normal response after the ovarian stimulation protocol. Number of retrieved oocytes and ovarian capacity were negatively correlated to both obesity indices; WHR and BMI.

Conclusion - By way of predicting ovarian response, our study reveals that basal FSH levels and FSH/LH ratio better predict response of subjects after ovarian stimulation protocol. Obesity negatively impacts on outcomes of in-vitro

fertilization process as it has a negative effect on the number of oocytes aspirated. This could probably be due to the fact that in obese persons higher doses of ovarian stimulation drugs are needed to generate a sizable pool of follicles for fertilization.

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

AFC	Antral Follicle Count
AH	Assisted Hatching
AMH	Anti-Mullerian Hormone
ANOVA	ANalysis Of VAriance
ART	Assisted Reproductive Technology
ASRM	American Society of Reproductive Medicine
BMI	Body Mass Index
CCCT	Clomifene Citrate Challenge Test
COS	Controlled Ovarian Stimulation
DNA	De-oxyriboNucleic Acid
EDC	Endocrine Disrupting Chemicals
EFORT	Exogenous Follicle stimulation hormone Reserve Test
FSH	Follicle Stimulating Hormone
GAST	Gonadotrophin releasing hormone Agonist Stimulation Test
GIFT	Gamete Intra-Fallopian Transfer
GnRH	Gonadotrophin Releasing Hormone
HCG	Human Chorionic Gonadotrophin
ICSI	Intra-Cytoplasmic Sperm Injection
IVF	In Vitro Fertilization
LH	Leutinizing Hormone
OHSS	Ovarian HyperStimulation Syndrome
PCOS	PolyCystic Ovary Syndrome
PGD	Preimplantation Genetic Diagnosis
STD	Sexually Transmitted Disease
TRH	Tyrotrophin Releasing Hormone

UDFA	Ultasound Directed Follicle Aspiration
WHO	Worl Health Organisation
WHR	Waist-to-Hip Ratio

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

The decline in female fecundity with age is caused mainly by decreased oocyte quality in older women, as indicated by the superior results of oocyte donation from younger to older women (Cohen *et al.*, 1999; Faber *et al.*, 1997). In women over 35 years of age, decreased oocyte quality is associated with a prolonged time to pregnancy and an increased risk of spontaneous abortions. Since the decline in oocyte quality with age does not occur at the same rate in all women, there is a demand for clinical tests identifying women with decreased oocyte quality at an early age, in order to provide diagnostic and prognostic information (van Montfrans *et al.*, 2004).

Determination of 'ovarian reserve' is important before any expensive IVF treatment is undertaken. Identification of both low and high responders prior to treatment may decrease cycle cancellation rate and side effects, such as ovarian hyperstimulation syndrome (OHSS) (Eldar-Geva *et al.*, 2005).

The recruitment of an optimal number of follicles during an IVF treatment is fundamental to the success of a treatment cycle. A poor ovarian response with a small number of oocytes collected and even a smaller number of embryos available for transfer, reduces the success rate (Loumaye *et al.*, 1990; Roest *et al.*, 1996). On the other hand, an excessive ovarian response to stimulation increases the risk for ovarian hyperstimulation syndrome (OHSS)(Navot *et al.*, 1992).

The availability of a screening test to predict the ovarian response would provide a valuable means of assisting clinicians in selecting appropriate dose for stimulation, individually tailored for each patient.

Prediction of ovarian response to stimulation for assisted reproductive techniques is one of the major challenges awaiting the reproductive endocrinologist. While age is the only demographic prognosticator of ovarian response, its predictive value is limited (Check *et al.*, 1994; Develioglu *et al.*, 1999; Toner *et al.*, 1991). Accordingly, a variety of tests, including dynamic tests such as the clomiphene citrate challenge test (Develioglu *et al.*, 1999), the gonadotrophin releasing hormone agonist stimulation test (GAST) (Winslow *et al.*, 1991), the exogenous follicle stimulating hormone ovarian reserve test (EFORT) (Fanchin *et al.*, 1994), and more recently measurement of day 3 inhibin concentrations (Seifer *et al.*, 1997) have all been advocated as means of prediction of ovarian response.

Measurement of basal serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and oestradiol, however, remains currently the most commonly employed method (Develioglu *et al.*, 1999).

1.2 MAIN AIM

To use hormonal markers and some lifestyle and behavourial patterns to predict ovarian response in IVF clients

1.3 OBJECTIVES

1. To assess basal FSH, LH and FSH/LH ratio as predictive markers of ovarian response in a controlled ovarian stimulation (COS) of an IVF cycle

2. To determine the relation between antral follicle count and retrieved oocyte number following stimulation and ovarian hyperstimulation syndrome (OHSS) in a COS

3. To assess pregnancy possibility above a set upper limit basal FSH level beyond which subfertile patients are denied self IVF programs for fear of poor ovarian response
4. To determine the effects of some lifestyle factors such as smoking, regular exercise pattens, caffeine intake, body weight, etc on IVF success

1.4 JUSTIFICATION

Infertility and its associated problems have been with us for a long time and for our part of the world (Africa), at times, superstitious reasons are assigned to this. Assisted reproductive techniques are also gradually catching up in Ghana. This procedure however has its own attending woes, not mentioning the heavy financial burden involved. Unsuccessful clients, most of the times go through a lot of emotional trauma.

The ovarian hyperstimulation syndrome (OHSS) is a dramatic iatrogenic complication of gonadotrophin treatment, and can sometimes be fatal (Delvigne *et al.*, 2001).

This study therefore seeks to predict favourably or otherwise the outcome of IVF treatment by use of basal hormonal markers (basal LH and FSH).

Chapter 2

LITERATURE REVIEW

2.1 FEMALE REPRODUCTIVE PHYSIOLOGY

When a female child is born, each ovum is surrounded by a single layer of granulosa cells; the ovum, with this granulosa cell sheath, is called a primordial follicle. Throughout childhood, the granulosa cells are believed to provide nourishment for the ovum and to secrete an oocyte maturation-inhibiting factor that keeps the ovum suspended in its primordial state in the prophase stage of meiotic division. Then, during and after puberty, when FSH and LH from the anterior pituitary gland begin to be secreted in significant quantities, the ovaries, together with some of the follicles within them, begin to grow. The hypothalamus secretes GnRH, which causes the anterior pituitary gland to secrete LH and FSH (Guyton & Hall, 2006).

The normal reproductive years of the female are characterized by monthly rhythmical changes in the rates of secretion of the female hormones and corresponding physical changes in the ovaries and other sexual organs. This rhythmical pattern is called the female monthly sexual cycle (or, less accurately, the menstrual cycle). The duration of the cycle averages 28 days. The ovarian changes that occur during the sexual cycle depend completely on the gonadotropic hormones FSH and LH, secreted by the anterior pituitary gland. In the absence of these hormones, the ovaries remain inactive, which is the case throughout childhood, when almost no pituitary gonadotropic hormones are secreted. At age 9 to 12 years, the pituitary begins to secrete progressively more FSH and LH, which leads to onset of normal monthly sexual cycles (Guyton & Hall, 2006).

During the first few days of each monthly female sexual cycle, the concentrations of both FSH and LH secreted by the anterior pituitary gland increase slightly to moderately, with the increase in FSH slightly greater than that of LH and preceding it by a few days. These hormones, especially FSH, cause accelerated growth of 6 to 12 primary follicles each month. After the early proliferative phase of growth, lasting for a few days, the mass of granulosa cells secrete a follicular fluid that contains a high concentration of estrogen, one of the important female sex hormones. The early growth of the primary follicle up to the antral stage is stimulated mainly by FSH alone. Then greatly accelerated growth occurs, leading to still larger follicles called vesicular follicles. This accelerated growth is caused by the following:

(1) Estrogen is secreted into the follicle and causes the granulosa cells to form increasing numbers of FSH receptors; this causes a positive feedback effect, because it makes the granulosa cells even more sensitive to FSH (Guyton & Hall, 2006)

(2) The pituitary FSH and the estrogens combine to promote LH receptors on the original granulosa cells, thus allowing LH stimulation to occur in addition to FSH stimulation and creating an even more rapid increase in follicular secretion (Guyton & Hall, 2006)

(3) The increasing estrogens from the follicle plus the increasing LH from the anterior pituitary gland act together to cause proliferation of the follicular thecal cells and increase their secretion as well. After a week or more of growth— but before ovulation occurs—one of the follicles begins to outgrow all the others; the remaining 5 to 11 developing follicles involute (a process called atresia), and these follicles are said to become atretic. (Guyton & Hall, 2006)

Ovulation in a woman who has a normal 28-day female sexual cycle occurs 14 days after the onset of menstruation. LH is necessary for final follicular growth and

ovulation. About 2 days before ovulation, the rate of secretion of LH by the anterior pituitary gland increases markedly, rising 6- to 10-fold and peaking about 16 hours before ovulation. FSH also increases about 2-fold to 3-fold at the same time, and the FSH and LH act synergistically to cause rapid swelling of the follicle during the last few days before ovulation. The LH also has a specific effect on the granulosa and theca cells, converting them mainly to progesterone-secreting cells. Therefore, the rate of secretion of estrogen begins to fall about 1 day before ovulation, while increasing amounts of progesterone begin to be secreted. It is in this environment of (1) rapid growth of the follicle, (2) diminishing estrogen secretion after a prolonged phase of excessive estrogen secretion, and (3) initiation of secretion of progesterone that ovulation occurs. Without the initial preovulatory surge of LH, ovulation will not take place. (Guyton & Hall, 2006)

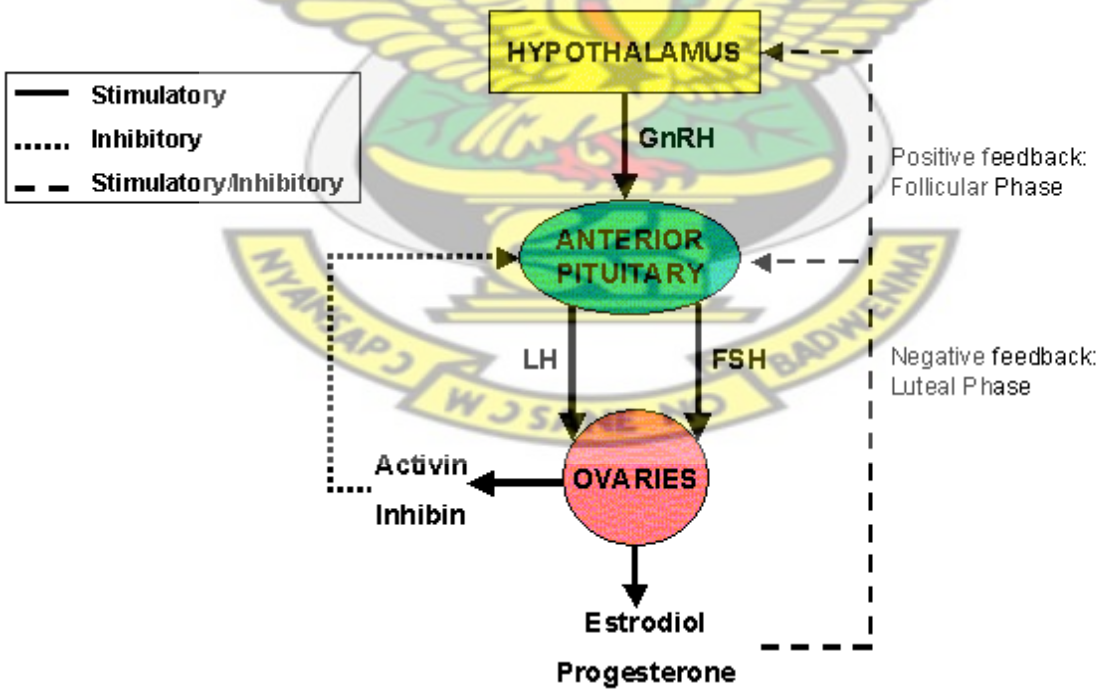


Figure 2.1 the female reproductive axis

Estrogen in small amounts has a strong effect to inhibit the production of both LH and FSH. Also, when progesterone is available, the inhibitory effect of estrogen is multiplied, even though progesterone by itself has little effect. These feedback effects seem to operate mainly on the anterior pituitary gland directly, but they also operate to a lesser extent on the hypothalamus to decrease secretion of GnRH, especially by altering the frequency of the GnRH pulses. (Guyton & Hall, 2006)

In addition to the feedback effects of estrogen and progesterone, other hormones seem to be involved, especially inhibin which is secreted along with the steroid sex hormones by the granulosa cells of the ovarian corpus luteum. This hormone inhibits the secretion of FSH and, to a lesser extent, LH by the anterior pituitary gland. Therefore, it is believed that inhibin might be especially important in causing the decrease in secretion of FSH and LH at the end of the monthly female sexual cycle (Guyton & Hall, 2006). Inhibins are multifunctional molecules involved in the control of pituitary FSH secretion. A body of observational and experimental evidence from several species, including the human, supports the concept that inhibins are gonadal messengers that exert a physiological negative feedback control on FSH release at the pituitary gland (Luisi *et al.*, 2005).

The main physiological functions of the Gonadotrophins are well known. FSH stimulates follicular maturation and granulosa cell oestrogen production in the ovary. LH stimulates theca cell androgen production in the ovary, providing a substrate for granulosa cell oestrogen production, triggers ovulation, and maintains the progesterone production of the corpus luteum (Huhtaniemi, 2000).

Current research indicates that anti-mullerian hormone (AMH) might play a role in folliculogenesis. Anti-Mullerian Hormone (AMH) is a glycoprotein hormone that belongs to the transforming growth factor β superfamily and is chiefly expressed in the fetal testis to drive differentiation of the mammalian reproductive tract. In women, granulosa cell production of AMH is barely detectable at birth and reaches the highest values after puberty. During adulthood although AMH continues to be expressed at basal and similar levels by both the Sertoli and granulosa cells, its biological role is poorly understood (Fanchin *et al.*, 2003)

Basic research data obtained from the adult ovary indicate that AMH is likely to be involved in the regulation of follicular steroidogenesis. In addition, growing evidence indicates that AMH mainly is expressed in pre-antral and early antral follicles and has either direct or indirect roles in various phases of folliculogenesis, from the primordial to FSH-sensitive follicular stages, probably via specific AMH type II receptors that are expressed in granulosa and theca cells. This suggests that, in contrast to other hormonal markers of ovarian function, AMH secretion might concurrently reflect the activity of pre-antral and early antral follicles, which makes it a promising parameter in the evaluation of ovarian follicular reserve (Fanchin *et al.*, 2003).

Studies investigating age-related changes in the hypothalamic– pituitary–ovarian axis have consistently demonstrated physiological and endocrine changes associated with reproductive ageing. One of the earliest signs of reproductive ageing is the FSH rise observed throughout the menstrual cycle, but most prominently noted in the early follicular phase of older ovulatory women (Burger *et al.*, 1998; Klein *et al.*, 1996; Lee *et al.*, 1988; Rannevik *et al.*, 1995). The pituitary gland secretes FSH and luteinizing hormone (LH) in a pulsatile manner in response to GnRH. While the FSH rise occurs relatively early in reproductive ageing, a subtle rise in LH is only observed at a later

stage and to a lesser degree, and LH does not demonstrate a sustained increase until after the menopause (Kim *et al.*, 1997)

2.2 INFERTILITY

Infertility is defined as the inability to conceive after one year of regular unprotected intercourse and accounts for one in six couples wishing to start a family (Shah *et al.*, 2003). One of the most important and underappreciated reproductive health problems in developing countries is the high rate of infertility and childlessness (Shah *et al.*, 2003). Infertility can be classified as primary; infertility of nulliparous women and secondary; infertility of parous women (Larsen, 2000).

2.2.1 Aetiology and consequences of infertility

Infertility can be hormonal, related to age, exercise, obesity or infectious disease; it can be immunological, psychological, result from surgery or blockage, or be associated with defined abnormalities in the gametes (e.g. aberrant semen parameters). Perhaps the most common 'cause' of infertility is simply 'unexplained' and this accounts for about 20% of couples (Uehara *et al.*, 2001).

The World Health Organization reported that African couples have a pattern of infertility different from those in other developing regions or the developed countries. They are more likely to have secondary infertility for a longer duration, a history of sexually transmitted diseases or pregnancy complications (Elussein *et al.*, 2008). Among Tanzanian couples, infertility was primary in 37.1% and secondary in 62.9% (Larsen *et al.*, 2006). In Nigeria, secondary infertility predominated (78.3%) according to one report (Olatunji and Sule-Odu., 2003) and primary infertility (65.0%) in another (Ikechebelu *et al.*, 2003). In a report from Ghana 40% of the couples suffered from primary and 60% from secondary infertility (Fiander, 1990).

In most parts of the Africa, superstitious reasons are sometimes assigned to infertility and most often than not it is the women who are blamed for it all. For some time it has been clear that male subfertility/infertility may contribute substantially to the overall incidence of infertility in the general population (Aitken, 1997; Fraser, 1999; Hull *et al.*, 1985; Jeremias *et al.*, 1996). A study in a sudanese population showed, that, 257 (36.2%) of infertility cases was due to male factor and 350 (49.3%) to female factors: there was no identified cause in 92 (13.0%) of the couples (Elussein *et al.*, 2008). In Tanzania, female-only factor infertility was identified in 65.9% of the couples, male-only factor infertility in 6.8%, male and female factors in 15.2% and unexplained factors in 12.1% (Larsen *et al.*, 2006). Olatunji and Sule-Odu reported that in Nigeria, the male factor was the only cause in 26.8%, the female factor in 51.8% and both male and female factors were contributory in 21.4% (Olatunji and Sule Odu., 2003). Yet, Ikechebelu *et al.*, (2003) reported a positive male-only factor in 133 (42.4%) and female-only in 81 (25.8%) couples in their report from Southern Nigeria. The male factor constituted 45% of infertility and tubal damage was responsible for 23% of female factor diagnosis in a report from Ghana (Fiander, 1990) . In Mongolia 45.8% of couples suffered infertility due to a female factor and 25.6% due to a male factor, 9.8% of infertility had no demonstrable cause: 18.8% had an infertility diagnosis in both partners (Bayasgalan *et al.*, 2004). This goes to show that male factors contribute immensely to infertility no matter where the study is done; hence the practice where in-laws despise and sometimes sack women from their marital homes here in Ghana on the grounds of barreness is not scientifically proven.

Causes of infertility may be grouped as; anovulatory, tubal, uterine or endometriosis, unexplained and male factors. Previous epidemiological studies have shown that in a subfertile population, 25–35% couples have male factor infertility, 14–22% have tubal factor, 10–27% have ovulatory dysfunction, 10–17% have unexplained infertility and

5–6% have endometriosis (Maheshwari *et al.*, 2008). It has been propounded that a diagnosis of ovulatory dysfunction was more common in younger age group and again showed a trend towards an increased prevalence of unexplained infertility in older women. Diminished ovarian reserve has been suggested as a putative cause of unexplained infertility. If this is indeed the case, it might be clinically helpful to distinguish women with true 'unexplained infertility' from those with ovarian senescence by means of more precise diagnostic tests (Maheshwari *et al.*, 2008). Other possible causes of unexplained infertility in older women can be higher body mass index (BMI) (BMI is known to be increased with age), decreased coital frequency and other lifestyle factors, such as smoking and stress (Homan *et al.*, 2007).

A larger proportion of older women, particularly those presenting with secondary infertility, have a diagnosis of tubal factor infertility. This could be due to the increased risk of acquiring pelvic infection with age (Bewley *et al.*, 2005) or the effect of previous salphingectomy due to ectopic pregnancy (Maheshwari *et al.*, 2008).

In a large study performed by the WHO Task Force on the Diagnosis and Treatment of Infertility, 8504 infertile couples in 33 different countries were examined through a standard approach in all participating centres. In Africa, over 85% of women had an infertility diagnosis attributable to an infection compared with 33% of women worldwide. In another study from sub- Saharan Africa, a history of sexually transmitted diseases (STDs) was reported by 46% of participating men. A study of 5800 couples in 33 World Health Organization centres in 25 countries showed that almost 50% of the African couples and 11–15% of other patients in other parts of the world had infectious tubal disease (1987; Ombelet *et al.*, 2008)

The inability to procreate is frequently considered a personal tragedy and a curse for the couple, impacting on the entire family and even the local community. Negative psychosocial consequences of childlessness are common. In many cultures, womanhood is defined through motherhood and infertile women usually carry the blame for the couple's inability to conceive. Moreover, in the absence of social security systems, older people are economically completely dependent on their children. Childless women are frequently stigmatized, resulting in isolation, neglect, domestic violence and polygamy (Leke *et al.*, 1993; Ombelet *et al.*, 2008; Umezulike *et al.*, 2004).

2.2.2 *Changing patterns in modern society*

While there is no universally accepted definition of advanced reproductive age, 35 years is considered as a watershed in fertility terms. On average, female fertility declines from the age of 30 years onwards (van Noord-Zaadstra *et al.*, 1991) or even earlier. In so-called natural breeding populations when no contraceptive measures are used, the end of fertility is reached at a median age of 40–41 years. Menstrual cycles, however, continue to be remarkably regular until a mean age of 45–46 years and it takes another 5 years before menopause is reached (van Zonneveld *et al.*, 2003), but importantly, there is wide variation between women in the number and rate of depletion of follicles (Penarrubia *et al.*, 2005a). The postponement of childbearing, which is a demographic trend in all Western countries (Maheshwari *et al.*, 2008; van Zonneveld *et al.*, 2003), is gradually creeping into some developing countries like Ghana and contributes considerably to the increasing proportion of subfertile couples (Fiander, 1990).

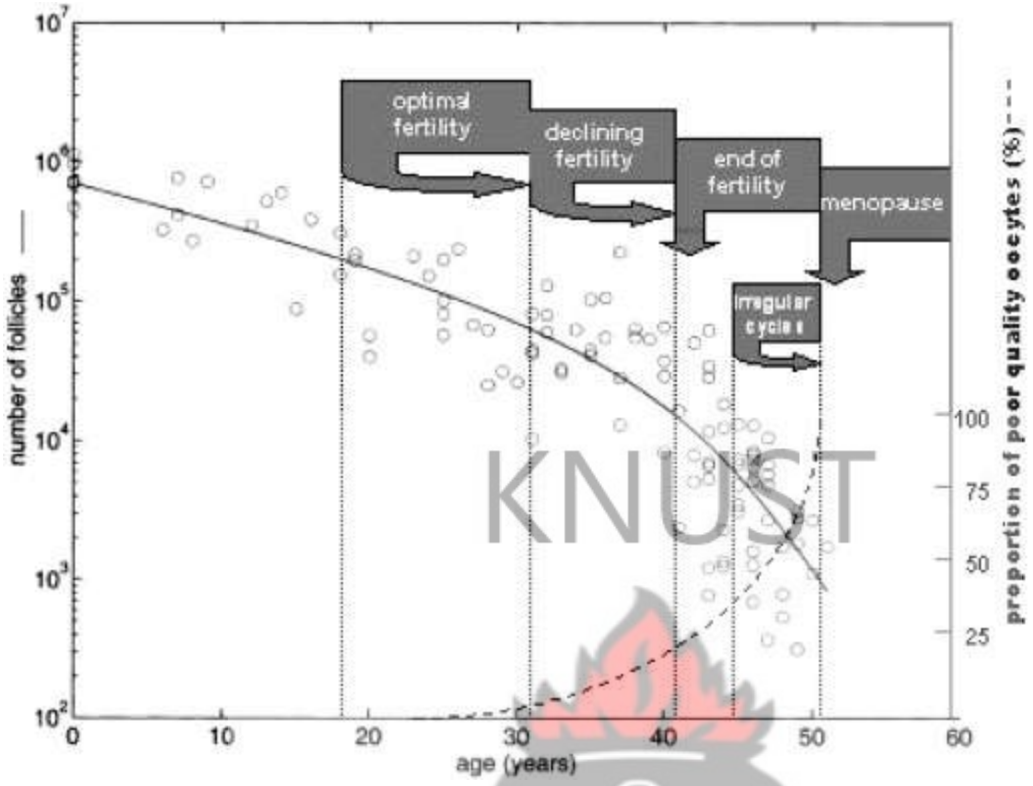


Figure 2.2 Quantitative (solid line) and qualitative (dotted line) decline of the ovarian follicle pool, which is assumed to dictate the onset of the important reproductive events (Broekmans *et al.*, 2006)

Nowadays most young Ghanaian women are postponing childbearing for the sake of educational and professional development till late thirties when fertility and childbearing gets complicated. Demographic studies have shown that more women are delaying childbearing at the present time than previously. It has been suggested that older women may be more likely to be diagnosed with unexplained infertility and that this is due to the negative effect of age on ovarian reserve (Maheshwari *et al.*, 2008). Ageing in women is associated with depletion of the primordial follicle pool, disorganization of the oocytes meiotic spindle and a marked decrease in fertility (Hansen *et al.*, 2005).

These societal changes in family planning have caused a significant increase in the incidence of unwanted infertility due to female reproductive ageing (Broekmans *et al.*, 2006).

2.3 HYPERPROLACTINEMIA AND INFERTILITY

Prolactin is a pituitary-derived hormone that plays a pivotal role in a variety of reproductive functions. It is an essential factor for normal production of breast milk following childbirth. Prolactin negatively modulates the secretion of pituitary hormones responsible for gonadal function, including luteinizing hormone and follicle-stimulating hormone. Like most anterior pituitary hormones, prolactin secretion is regulated by hypothalamic hormones delivered through the hypothalamic–pituitary portal circulation. Under most conditions the predominant signal is inhibitory, preventing prolactin release, and is mediated by the neurotransmitter dopamine. The stimulatory signal is mediated by the hypothalamic hormone thyrotropin-releasing hormone (TRH). The balance between the 2 signals determines the amount of prolactin released from the anterior pituitary gland. Furthermore, the amount cleared by the kidneys influences the concentration of prolactin in the blood (Serri *et al.*, 2003).

It is well recognized that stress and infertility are linked. Serum prolactin, a known stress marker, is commonly elevated in infertile women. Hyperprolactinaemia affects gonadotrophin secretion or may directly affect the ovary (Harlow *et al.*, 1996).

2.4 LIFESTYLE AND INFERTILITY

Lifestyle factors are behaviours and circumstances that are, or were once, modifiable and can be a contributing factor to subfertility.

2.4.1 Nutrition and diet

For females, reproduction involves much greater energy expenditure than for males, and as a protective mechanism against undernutrition, the reproductive axis is closely linked to nutritional status. Consequently, eating disorders leading to loss of weight are associated with reduced frequency or cessation of ovulation. Since energy balance more than absolute weight loss is the key factor, there may be a return of ovulation after no more than a small percentage change in body weight recovery. The reproductive system is extremely sensitive to influences from the external environment. Most animals adjust their pattern of reproduction so that the chances of their offspring surviving are maximal. A common strategy involves timing of conception by photoperiod and/or rainfall which usually ensures that birth takes place in a season when food and climatic conditions are favourable (Homan *et al.*, 2007).

Eating a healthy diet consisting of appropriate composition and caloric intake is fundamental to maintaining a state of optimum physical and psychological health. It is also important in preventing diseases such as obesity, cardiovascular disease, diabetes, osteoporosis and some cancers (Homan *et al.*, 2007). Diet mediates body weight and composition and should be considered fundamental to reproduction. However, although a link has been demonstrated between maternal nutritional status and adverse pregnancy outcomes (Keen *et al.*, 2003), the effect of a woman's nutritional status prior to pregnancy has rarely been studied (Chapin *et al.*, 2004). Studies directly relating dietary components to the chance of conceiving are sparse in humans (Homan *et al.*, 2007). However, there is strong evidence that a well-balanced healthy diet is

beneficial for general well-being and optimum body functioning (Sanders, 2004) and it has been suggested that diet before pregnancy may influence fetal well-being (Moore *et al.*, 2005). Therefore, reproductive performance should be positively influenced by the consumption of a healthy varied diet.

2.4.2 Weight

Menstrual periods often cease after a 10–15% decrease in normal body weight. In theory the mechanisms include altered regulation of gonadotropin-releasing hormone secretion and changes in the dopaminergic and opioid systems (The ECWG, 2006). However, undernutrition is not a reliable predictor of conception among infertile women. In 244 cycles of GnRH treatment for oligoamenorrhoea in 48 women, pregnancy rates were not affected by patients' weight or weight loss (Braat *et al.*, 1991).

The prevalence of overweight defined as BMI 25.0–29.9 and obesity defined as BMI ≥ 30 kg/m^2 is increasing around the world. WHO considers that obesity is an epidemic, as more obese people are found in developed and developing countries, among children as well as adults and elderly and among men and women (The ECWG, 2006). Stein and Leventhal were the first to recognize the relation between obesity and reproductive disturbances. They described what nowadays is known as syndrome 'O': overnourishment, overproduction of insulin, ovarian confusion and ovulation disruption. Treatment of anovulatory infertility of overweight women requires increased concentrations of clomiphene citrate and higher doses of gonadotrophins to induce an ovulatory follicle. Treatment of overweight women on IVF programs shows a much lower pregnancy rate (Crosignani *et al.*, 1994; Fedorcsak *et al.*, 2001; Wang *et al.*, 2000). There is also evidence that in using donor eggs the body mass of the recipient is more important than the body mass of the donor. This is largely due to the miscarriage rate which has been shown to increase four-fold in the obese (Bellver *et al.*, 2003).

Several studies have shown that there is an increased risk of spontaneous abortion in obese women, irrespective of the coexistence of polycystic ovarian syndrome (PCOS) (Hamilton-Fairley *et al.*, 1992; Roth *et al.*, 2003; Wang *et al.*, 2001).

Obesity has been shown to decrease the probability of pregnancy for women undergoing assisted reproductive technologies (ART). A large Australian study of 3586 women who underwent ART found that pregnancy rates were halved for very obese women in comparison with women with a normal BMI (Wang *et al.*, 2000).

The distribution of body fat also impacts on reproductive performance but the mechanism for this effect is unclear (Norman *et al.*, 2004). A prospective study of 500 apparently normal women undergoing donor insemination found that increasing waist–hip ratio was negatively associated with the probability of conception (Zaadstra *et al.*, 1993).

Clinical observations on the effects of body weight during IVF are more controversial. Several investigators have shown a decrease in pregnancy and live birth rates in overweight and obese compared with normal weight women (Fedorcsak *et al.*, 2004; Ferlitsch *et al.*, 2004; Lintsen *et al.*, 2005; Nichols *et al.*, 2003; Wang *et al.*, 2000), whereas others have found no difference (Dokras *et al.*, 2006; Frattarelli *et al.*, 2004; Lashen *et al.*, 1999; Spandorfer *et al.*, 2004; Wittemer *et al.*, 2000). Possible reasons for the differences between obese and normal weight women include: increased gonadotrophin requirement during ovarian stimulation, fewer retrieved oocytes (Fedorcsak *et al.*, 2004; Wittemer *et al.*, 2000), decreased serum estradiol concentrations (Dokras *et al.*, 2006; Lashen *et al.*, 1999), increased cycle cancellations (Dokras *et al.*, 2006; Fedorcsak *et al.*, 2004; Spandorfer *et al.*, 2004) and lower fertilization rates (van Swieten *et al.*, 2005) in the obese population.

In a study by Sneed *et al.*, (2008), they demonstrated that the effect of BMI on IVF success appeared to be related to age. At younger ages, a higher BMI has a pronounced negative influence on pregnancy rate, but this effect is attenuated as age increased. When examined as a main variable alone, BMI did not appear to have a significant effect on IVF responses or outcomes, but when BMI and age interaction was analyzed, there was a marked decrease in pregnancy rates with increasing BMI for younger patients. The BMI and age interaction was significant for retrieved, mature and fertilized oocytes, and for implantation, chemical pregnancy, clinical pregnancy and live birth rates. Thus, the effects of BMI are highly dependent on age. BMI has a much less profound impact on fertility as patients reach the age of 36 and older. These findings continue to underscore the importance of the dominating effect of age on IVF success, particularly above the age of 35.

2.4.3 Smoking

Studies have demonstrated that smoking in women significantly decreases the chance of conception (Augood *et al.*, 1998; Homan *et al.*, 2007; Hughes *et al.*, 1996; Younglai *et al.*, 2005b). There is strong evidence of the adverse effects of smoking on fertility operating through a range of pathways in both the general and infertile population. In males, smoking negatively affects sperm production, motility and morphology and is associated with an increased risk of DNA damage (Kunzle *et al.*, 2003; Zenzes *et al.*, 1999). In the female, the constituents of cigarette smoke may affect the follicular microenvironment and alter hormone levels in the luteal phase. Cotinine and cadmium have been detected in the follicular fluid of female smokers and whose partner smokes, thus affecting the developing follicle (Younglai *et al.*, 2005a). Menopause has been reported to occur 1–4 years earlier for women who smoke compared to nonsmokers (Baron *et al.*, 1990). A recent study demonstrated an

increased thickness of the zona pellucida in smokers, which may make it more difficult for sperm penetration (Shiloh *et al.*, 2004). In one study (Lintsen *et al.*, 2005), women who smoked had a significantly higher abortion rate than non-smoking women. Furthermore the effect of smoking was comparable to an increase in female age with 10 years, from age 20 to 30 years.

A study of 159 women undergoing IVF found that smokers did not respond well to stimulation as non-smokers, fertilization was lower and none of the regular smokers became pregnant (Crha *et al.*, 2001). A recent study of 225 women undergoing IVF found that fertilization rates were similar for smokers, passive smokers and non-smokers, whereas pregnancy rates were significantly decreased for smokers (19.4%) and passive smokers (20%), compared with non-smokers (48.3%) (Neal *et al.*, 2005a). A study of 301 couples undergoing IVF or ICSI found that male smoking significantly decreased ICSI and IVF success rates (Zitzmann *et al.*, 2003). In some studies, maternal smoking resulted in decreased fertilization rates (Elenbogen *et al.*, 1991; Roseveare *et al.*, 1992), decreased number of oocytes, decreased pregnancy rates (Elenbogen *et al.*, 1991; Harrison *et al.*, 1990; Van Voorhis *et al.*, 1996) and increased miscarriage rates (Klonoff-Cohen *et al.*, 2001a). In another study, they demonstrate reduced fertility in female smokers and expands the literature by demonstrating that the reproductive consequences of sidestream/passive smoking are as great as those seen in mainstream smokers (Neal *et al.*, 2005b).

In contrast, in other studies there was no effect of smoking on fertilization (Trapp *et al.*, 1986; Weigert *et al.*, 1999) and pregnancy rates (Hughes *et al.*, 1996; Weigert *et al.*, 1999) in an IVF population. Again in another study, though they controlled for day 3 FSH, age, BMI, embryo quality, number of oocytes retrieved, caffeine and alcohol use in

their large cohort, they still could not demonstrate even a small effect of smoking on IVF outcome (Wright *et al., 2006*).

According to (Lintsen *et al.*, 2005) the effect of smoking and overweight was largest among women with unexplained subfertility. These results suggest that women, and in particular those with unexplained subfertility, may be able to improve the outcome of subfertility treatment by quitting smoking and losing weight (Lintsen *et al.*, 2005).

Tobacco smoke contain several hundred substances including, nicotine, carbon monoxide and mutagens (eg, radioactive polonine, benzo[a]pyrene, naphthalene and methylnaphthalene) (Klonoff-Cohen *et al.*, 2001b). In a review on the genetic damaging effects from smoking and its components on germinal cells, evidence was found that smoking affected the quantity and quality of oocytes and that it leads to an early age of menopause (Zenzes, 2000).

2.4.4 Exercise

Regular exercise affects an individual's general health and wellbeing and provides some protection from obesity, cardiovascular disease, hypertension, diabetes, osteoporosis and psychological stress (Homan *et al.*, 2007).

The evidence for the effects of weight on reproduction from observational studies has given rise to a number of significant intervention studies. Lifestyle modification programmes that include exercise have been shown to assist women to lose weight, improve their fitness, increase psychological well-being and improve reproductive functioning (Clark *et al.*, 1995; Clark *et al.*, 1998).

The results of an Australian study of 87 obese (BMI ≥30kg/m^2) infertile women attending a weekly programme to promote lifestyle changes demonstrate that a relatively small amount of weight loss (average of 6.5 kg) was associated with resumption of ovulation (Clark *et al.*, 1998). The women in this study attended a weekly programme for 6 months that included an exercise component and education relating to diet and psychological issues associated with being overweight. Although the number of women taking part in the study was relatively low, the positive effects of participating in the programme were outstanding. On average, the women lost 10.2 kg/m^2 in BMI. At the beginning of the study, 80% of the women were anovular and at the end of 6 months 90% of these women were ovulating spontaneously. Of the 67 women who completed the study, 77.6% became pregnant and 67% achieved a live birth. The women who did not become pregnant smoked, attended less than two-thirds of the sessions or had a BMI which remained >40kg/m^2.

Rich-Edwards *et al.*, (2002) found that exercise was associated with a reduction in risk of ovulatory infertility.

However, there is accumulating evidence that regular strenuous exercise alters menstrual function. The frequency of amenorrhea or oligomenorrhea among women participating in a variety of strenuous sports varies from 2 to 51 per cent as opposed to 2 to 5 per cent of other women. In a prospective study of women with previously normal ovulation and luteal function, 87 per cent experienced abnormalities of these reproductive functions while engaging in a strenous exercise program (Green *et al.*, 1986). In their study, there was evidence of an increased risk of infertility in nulligravid women who reported exercising vigorously 60 minutes or more per day, however, women who had previously been pregnant were not at increased risk of infertility at any duration of vigorous exercise (Green *et al.*, 1986).

2.4.5 Alcohol and Caffeine consumption

Alcohol is a known teratogen (Chaudhuri, 2000; Randall, 1987) and its consumption has been reported to decrease fertility, although the level of consumption associated with risk is unclear. Alcohol consumption at the extreme level is known to be dangerous to the unborn child (Astley *et al.*, 2000; Goransson *et al.*, 2003; Krulewitch, 2005) but the effect at lower levels is less certain. The mechanisms by which alcohol could impair conception are unclear but may include an alcohol-induced rise in estrogen, which reduces FSH secretion suppressing folliculogenisis and ovulation. It may also have a direct effect on the maturation of the ovum, ovulation, blastocyst development and implantation (Eggert *et al.*, 2004; Gill *et al.*, 2000). Moderate levels of alcohol consumption (seven to eight drinks per week) have been associated with reduced fertility and an increased risk of spontaneous abortion (Grodstein *et al.*, 1994; Windham *et al.*, 1992). Levels as low as one drink per week have also been associated with reduced conception (Hakim *et al.*, 1998).

A prospective study of 221 couples undergoing IVF or GIFT at six Californian fertility clinics, demonstrates female alcohol drinking during the year prior to treatment was associated with a 13% ($P = 0.02$) decrease in the number of oocytes retrieved for each additional drink per day (Klonoff-Cohen *et al.*, 2003). In contrast, a study of women undergoing donor insemination found no association between alcohol consumption and female fecundity(Zaadstra *et al.*, 1994).

The stimulant properties of caffeine have led to its widespread use as a beverage (coffee, tea and soft drinks) and in some foods such as chocolate. Its consumption has been reported to prolong the time to pregnancy; although the mechanism for this is unclear, caffeine may affect female reproduction by targeting ovulation and corpus

luteal function through alterations to hormone levels and has been associated with higher early follicular E_2 levels in females (Lucero *et al.*, 2001).

2.4.6 Environmental pollutants

The potential for environmental and occupational exposures to chemicals and pollutants to adversely affect fertility is not surprising, as environmental and lifestyle factors are said to be key factors in human disease (Homan *et al.*, 2007).

Adverse effects of radiation on male and female reproduction have been demonstrated in various animal species as well as human beings (Kumar, 2004). The reproductive system in males and females are sensitive to radiation causing temporary or permanent sterility dependent on dose, duration and dose rate (Kumar, 2004; Parker *et al.*, 1999; Schieve *et al.*, 1997). Exposure to pesticides and solvents have been associated with sperm threshold values below normal (Kumar, 2004; Oliva *et al.*, 2001). In a study of 726 couples undergoing IVF in the Netherlands (Tielemans *et al.*, 2000), a reduced implantation rate was found in women whose partners worked in occupations with high levels of organic solvent exposure. Men exposed to pesticides and welding have been shown to be at risk for oligozoospermia (Wong *et al.*, 2003).

Endocrine-disrupting chemicals (EDC) are synthetic and naturally occurring chemicals that cannot be classified by any unique physical or chemical properties but are characterized by their ability to mimic the effects of endogenous hormones. Specifically, endocrine disrupters can mimic (Soto *et al.*, 1995) and antagonize the actions of endogenous hormones (Kelce *et al.*, 1997; Kelce *et al.*, 1995), induce changes in steroidogenic enzyme expression and/or activity (Chapin *et al.*, 1997; Crellin *et al.*, 2001), and alter circulating steroid hormone levels (Chapin *et al.*, 1997; Diawara *et al.*, 1999; Lindenau *et al.*, 1994; You *et al.*, 2001). These characteristics of EDC have led to concerns that exposure to these compounds may be linked to adverse health effects in

humans. Endocrine disrupters have deleterious effects in wildlife and fish populations (Damstra, 2002), though adverse health effects in the human population have not been clearly demonstrated. To date, epidemiological studies fail to support an association between exposure to endocrine disrupters and infertility or decreased fecundity (Foster, 2003). However, quantification of endocrine toxicants in human ovarian follicular fluid and their association with IVF outcomes (Younglai *et al.*, 2002) together with observed adverse effects in animals and in vitro studies (Gray *et al.*, 2001), support concerns that exposure to endocrine toxicants has the potential to adversely impact human ovarian function(Younglai *et al.*, 2005b).

It has been demonstrated that maternal exposures to toluene, xylene and formalin (Taskinen *et al.*, 1994), chloroform (Wennborg *et al.*, 2000) and ethylene glycol ethers can decrease fertility. In contrast, there was no increased risk when males were exposed to such chemicals (Correa *et al.*, 1996).

2.5 ASSISTED CONCEPTION

In order to understand assisted reproduction and how it can help infertile couples, it is important to understand how conception takes place naturally. For natural conception to occur, the male must ejaculate his semen, the fluid containing the sperm, into the female's vagina around the time of ovulation, when her ovary releases an egg. Ovulation is a complex event controlled by the pituitary gland, which is located at the base of the brain. The pituitary gland releases follicle-stimulating hormone (FSH), which stimulates follicles in one of the ovaries to begin growing (ASRM, 2008).

The follicle produces the hormone estrogen and contains a maturing egg. When an egg is mature, the pituitary gland sends a surge of luteinizing hormone (LH) that causes the follicle to rupture and release (ovulate) a mature egg (ASRM, 2008).

Following ovulation, the egg is picked up by one of the fallopian tubes. Since fertilization usually takes place inside the fallopian tube, the man's sperm must be capable of swimming through the vagina and cervical mucus, up the cervical canal into the uterus, and up into the fallopian tube, where it must penetrate the egg in order to fertilize it (ASRM, 2008). The fertilized egg continues traveling to the uterus and implants in the uterine lining, where it continues to develop. (ASRM, 2008).

Fortunately, assisted reproductive techniques such as In Vitro Fertilization (IVF) can help. IVF is used in the treatment of various forms of infertility including endometriosis, ovulatory dysfunction, pelvic adhesions, cervical factor, tubal disease, luteal defects, immunological causes, male factor, and unexplained infertility (Klonoff-Cohen, 2005).

2.5.1 Overview of IVF procedure

IVF is a method of assisted reproduction in which a man's sperm and a woman's eggs are combined outside of the body in a laboratory dish. One or more fertilized eggs (embryos) may be transferred to the woman's uterus, where they may implant in the uterine lining and develop. Excess embryos may be cryopreserved (frozen) for future use. The basic steps in an IVF treatment cycle are ovarian stimulation, egg retrieval, fertilization, embryo culture, and embryo transfer (ASRM, 2008).

2.5.1.1Ovarian Stimulation

During ovarian stimulation, also known as ovulation induction, medications or "fertility drugs," are used to stimulate multiple eggs to grow in the ovaries rather than

the single egg that normally develops each month. Multiple eggs are stimulated because some eggs will not fertilize or develop normally after fertilization. Timing is crucial in an IVF cycle. The ovaries are evaluated during treatment with vaginal ultrasound examinations to monitor the development of ovarian follicles. Blood samples may be drawn to measure response to ovarian stimulation medications. Normally, estrogen levels increase as the follicles develop. Using ultrasound examinations and blood testing, the physician can determine when the follicles are ready for egg retrieval. Generally, eight to 14 days is required. When the leading follicles measures about 14mm, hCG injection is given. The hCG replaces the woman's natural LH surge and causes the final stage of egg maturation so the eggs are capable of being fertilized. The eggs are retrieved before ovulation occurs, usually 34 to 36 hours after the hCG injection is given (ASRM, 2008).

IVF cycles may be cancelled for a variety of reasons, usually due to an inadequate number of follicles developing. Occasionally, a cycle may be cancelled to reduce the risk of ovarian hyperstimulation syndrome (OHSS) (ASRM, 2008).

2.5.1.2Egg retrieval

Egg retrieval is usually accomplished by transvaginal ultrasound aspiration; a minor surgical procedure. Some form of analgesia is generally administered. An ultrasound probe is inserted into the vagina to identify the follicles, and a needle is guided through the vagina and into the follicles. The eggs are aspirated from the follicles through the needle connected to a suction device (ASRM, 2008).

2.5.1.3Fertilization and embryo culture

After the eggs are retrieved, they are examined in the laboratory for maturity and quality. Mature eggs are placed in an IVF culture medium and transferred to an incubator to await fertilization by the sperm. Sperm is separated from semen by

centrifugation with special sperm media. Fertilization may be accomplished by insemination, where motile sperm are placed together with the oocytes and incubated overnight or by intracytoplasmic sperm injection (ICSI), where a single sperm is directly injected into each mature egg. ICSI is usually performed when there is a likelihood of reduced fertilization, i.e., poor semen quality, history of failed fertilization in a prior IVF cycle (ASRM, 2008).

Visualization of two pronuclei the following day confirms fertilization of the egg. One of the pronuclei is derived from the egg and the other from the sperm. Two days after the egg retrieval, the fertilized egg has divided to become a 2- 4 cell embryo. By the third day, a normally developing embryo will contain approximately 6 to 10 cells. By the fifth day, a fluid cavity forms in the embryo, and the placenta and fetal tissues begin to separate. An embryo at this stage is called a blastocyst. Embryos may be transferred to the uterus at any time between one to six days after the egg retrieval. If successful development continues in the uterus, the embryo hatches from the surrounding zona pellucida and implants into the lining of the uterus approximately six to 10 days after the egg retrieval (ASRM, 2008).

Assisted hatching (AH) is a micromanipulation procedure in which a hole is made in the zona pellucida just prior to embryo transfer to facilitate hatching of the embryo. Although AH has not been demonstrated definitively to improve live birth rates, AH may be used for older women or couples who have failed prior IVF attempts. Preimplantation genetic diagnosis (PGD) is performed at some centers to screen for inherited diseases (ASRM, 2008).

2.5.1.4Embryo transfer

The next step in the IVF process is the embryo transfer. The physician identifies the cervix using a vaginal speculum. One or more embryos suspended in a drop of culture medium are drawn into a transfer catheter, a long, thin sterile tube with a syringe on one end. The physician gently guides the tip of the transfer catheter through the cervix and places the fluid containing the embryos into the uterine cavity. The number of embryos transferred is largely based on the age of the woman (ASRM, 2008).

Extra embryos remaining after the embryo transfer may be cryopreserved (frozen) for future transfer (ASRM, 2008).

2.5.2 Risks of controlled ovarian stimulation in IVF

Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening complication of pharmacological ovarian stimulation. Severe forms of OHSS complicate about 1% of IVF cycles and are characterized by a massive ovarian enlargement together with a fluid shift into extravascular compartments responsible for the development of ascites, sometimes pleural and/or pericardial effusion, hypovolaemia, oliguria, and hydroelectrolytic disorders. In the most marked cases, thromboembolic phenomena may occur as a result of haemoconcentration and coagulation disturbances (Hollemaert *et al.*, 1996). It usually involves patients with an explosive response to the ovarian stimulation and is more frequent in patients suffering from polycystic ovary syndrome (Navot *et al.*, 1992). The pathophysiology of the syndrome has not been completely elucidated yet. Exclusively post-ovulatory, the vascular fluid leakage is thought to result from an increased capillary permeability of mesothelial surfaces under the action of one or several vasoactive ovarian factor(s) (Elchalal *et al.*, 1997).

Human chorionic gonadotrophin (hCG) is thought to play a crucial role in the development of the syndrome: severe forms are indeed restricted to cycles with exogenous hCG (to induce ovulation or as luteal phase support) or with endogenous pregnancy-derived hCG (Delbaere *et al.*, 2004).

In a recent study, they identified a mutation in the FSH receptor gene in a patient presenting spontaneous OHSS during each of her four pregnancies. The mutation consisted of a substitution of an adenine for a guanine at the first base of codon 567 in exon 10 of the follitropin receptor gene, resulting in the replacement of an aspartic acid with an asparagine. When tested in vitro, the functional response of the mutant receptor displayed an enhanced basal activity and an increased sensitivity to hCG (Smits *et al.*, 2003).

During pregnancy, the expression of FSH receptor decreases drastically in the corpus luteum, but remains constant in granulosa cells of developing follicles (Simoni *et al.*, 1997). These receptors are usually not or only very weakly stimulated during pregnancy, as pituitary gonadotrophins fall to very low or undetectable levels in serum. The mutated FSH receptor expressed in the developing follicles may be hyperstimulated by the pregnancy-derived hCG. Accordingly, the follicles may start growing, enlarge and finally acquire LH receptors on granulosa cells which may also be stimulated by hCG, inducing follicular luteinization together with the secretion of vasoactive molecules responsible for the development of the syndrome (Delbaere *et al.*, 2004).

Because ARTs are expensive, time-consuming, and stressful for patients, there is a need for predicting ovarian response.

2.6 PREDICTORS OF OVARIAN REPONSE

Predictors of ovarian capacity are either static: age (Hughes *et al.*, 1989; Meldrum, 1993; Navot *et al.*, 1994; Scott *et al.*, 1995), basal FSH (bFSH) (Cahill *et al.*, 1994; Hansen *et al.*, 1996; Pearlstone *et al.*, 1992; Toner, 1993), basal estradiol (bE2) (Evers *et al.*, 1998), basal inhibin B (bInhB)(Lahlou *et al.*, 1999); or dynamic: clomiphene citrate challenge test (CCCT) (Scott *et al.*, 1993), exogenous FSH ovarian reserve test (EFORT) (Fanchin *et al.*, 1994), GnRH agonist stimulation test (GAST) (Kwee *et al.*, 2003).

All tests predict the response to ovarian hyperstimulation and the prognosis for pregnancy in IVF treatment. In turn these allow for careful counseling of women with regard to their chances of a successful outcome (Ranieri *et al.*, 2001). Although women with a reduced ovarian reserve may have higher chances of achieving a pregnancy in an oocytes donation programme, a significant proportion will achieve conception with their own oocytes (Faber *et al.*, 1998; Surrey *et al.*, 1998).

2.6.1 Age

Ageing of the ovary plays the major role in reproductive ageing and is related to the gradual reduction in the number of primordial follicles. The number of follicles leaving the pool of the so-called resting follicles to enter the growth phase towards the antral stages of development decreases with increasing age, leading to a stock at menopause estimated at between <100 and 1000 primordial follicles in the pool (Gougeon, 1996; Gougeon *et al.*, 1994). It is generally known that reproductive ageing is related to both a quantitative and a qualitative reduction of the primordial follicle pool (Hendriks *et al.*, 2005c). The decline in female fecundity with age is caused mainly by decreased oocyte quality in older women, illustrated by the superior results of oocyte donation from younger to older women (Cohen *et al.*, 1999; Faber *et al.*, 1997). In women over 35 years of age, decreased oocyte quality is associated with a prolonged

time to pregnancy and with an increased risk of spontaneous abortions (van Montfrans *et al.*, 2004). The ovarian dynamics from birth to menopause implies a progressive reduction in oocytes stored, along with a change of sensitivity to gonadotrophin stimulation (Faddy *et al.*, 1995).

Female age was shown to be superior to basal inhibin B at predicting pregnancy following IVF and embryo transfer (Creus *et al.*, 2000). The age-related effect on female fertility has also been shown in numerous reports on the results of IVF treatment in infertile couples. The probability of live birth obtained through IVF treatment clearly decreases after the age of 35 and the same has been shown to be true for the implantation rate per embryo. In fact, female age has consistently been shown to be an important predictor of success in IVF treatment (Broekmans *et al.*, 2006).

The association between advanced female age and reproductive senescence is well established (Oosterhuis *et al.*, 2002). Nevertheless, in women of the same age, individual variations exist with regard to their ovarian responses. Chronological female age is universally seen as indicative of qualitative alterations of the remaining follicle pool, but cannot serve as the sole marker of ovarian status. Moreso, the decline in oocyte quality with age does not occur at the same rate in all women (Weghofer *et al.*, 2005).

The limited predictive value of age alone in estimating fecundity rates and response to the exogenous ovarian stimulation led to the search for better tests (Hendriks *et al.*, 2005c).

2.6.2 Dynamic tests

The best documented provocative test, Clomiphene Citrate Challenge Test (CCCT) for assessing ovarian reserve, was first described by (Navot *et al.*, 1987) as a means of assessing ovarian reserve in women aged ≥35 years. This test consisted of measuring serum FSH concentrations on cycle day 3 (basal FSH) and then again on day 10 (stimulated FSH) after the administration of 100 mg of clomiphene citrate (CC) from day 5 to day 9. An exaggerated FSH response and/or an elevated basal FSH value is interpreted as a sign of diminished ovarian reserve. The test has been shown to be of value in unmasking poor responders to controlled ovarian stimulation (COS) who would not have been detected by basal FSH screening alone. Moreover, an abnormal test is associated with a reduced chance of pregnancy (Hendriks *et al.,* 2005a). Several groups have evaluated the predictive value of the CCCT in IVF patients and stated that the CC-provoked response of FSH reliably predicts ovarian response and the probability of pregnancy in IVF (Csemiczky *et al.*, 2002; Loumaye *et al.*, 1990; Tanbo *et al.*, 1992; Yanushpolsky *et al.*, 2003). So far, most studies on the diagnostic and prognostic significance of the CCCT in IVF have used a selected group of patients, likely to respond poorly to gonadotrophins and with a reduced prospect for pregnancy (Hendriks *et al.*, 2005a).

Exogenous FSH ovarian reserve test *(EFORT)* involves the measurement of basal FSH, estradiol and estradiol response 24 h after a 300 IU FSH injection on day 3. The addition of the dynamic component to the day 3 FSH concentration might be an improvement of the predictive value of good response to ovarian stimulation (Fanchin *et al.*, 1994). EFORT has not been studied for prediction of pregnancy in an IVF population (Maheshwari *et al.*, 2006).

GnRH agonist stimulation test (GAST) evaluates the serum estradiol concentration change from cycle day 2 to day 3 after the administration of a supraphysiological dose of a GnRH agonist. A prompt estradiol response may be associated with better ovarian reserve. Earlier ART studies did not show any significant benefit in the prediction of ovarian response (Padilla *et al.*, 1990; Winslow *et al.*, 1991); however, later studies did (Hendriks *et al.*, 2005b; Ranieri *et al.*, 1998). Although, when compared with the predictive accuracy and clinical value of the day 3 AFC and inhibin-B measurement, GAST did not perform better. In addition, its predictive ability towards ongoing pregnancy is poor (Hendriks *et al.*, 2005b).

However, it would be of greater clinical value to be able to predict follicular cohort size prior to starting GnRH analogue/ FSH treatment

2.6.3 Basal markers

2.6.3.1 Basal FSH

The cycle day 3 FSH level is one of the most commonly used tests for predicting success in IVF treatment. Some studies have demonstrated that women with an elevated cycle day 3 FSH had reduced ovarian reserve and several others have shown that women with an elevated FSH level, independent of age, have a poor response to ovarian stimulation, leading to a lower pregnancy rate with ART (Abdalla *et al.*, 2004). Recently, however, (El-Toukhy *et al.*, 2002) argued that young age does not protect against the adverse effects of reduced ovarian reserve, suggesting that an elevated day 3 basal FSH level is associated not only with a low response, but also with poor quality oocytes leading not only to a reduction in pregnancy rate but also to a rise in miscarriage rates

Day 3 FSH is an indirect measure of the size of the follicle cohort and is regulated by various factors, including inhibins, activins, estradiol and follistatins (Maheshwari *et*

al., 2006). Recent evidence indicates that basal FSH level is the better predictor of egg production capacity whereas age is the better predictor of egg quality (Toner, 2003; van Rooij *et al.*, 2003).

Some controversy has arisen about the clinical utility of basal-FSH measurements (Jurema *et al.*, 2003; Scott, 2004; Toner, 2004; Wolff *et al.*, 2004). Abdalla *et al.*, (2004) depicted reduced, yet still clinically sound pregnancy and live birth rates in young women with elevated basal-FSH levels of up to 20mU/ml. They therefore concluded that the predictive value of basal-FSH concentrations should be restricted to the counselling of patients on the probability of achieving pregnancy, but should not be used to exclude them from fertility treatment.

In a study by Hendriks *et al (2005)*, the FSH level on cycle day 10 in the single CCCT appeared to be a sensitive test in assessing ovarian reserve (ROC_{AUC} 0.79) if the ovarian response to hyperstimulation is used as outcome. However, the predictive accuracy was not clearly better than that of basal FSH (ROC_{AUC} 0.82), whereas in combined use the cd10 level did not add significant information to basal FSH. From these results it seems that existing literature on the value of the CCCT in IVF settings cannot be confirmed. Hence, from a clinical point of view there is little or no advantage in performing a CCCT (either single or repeated) over the model basal FSH (Hendriks *et al.*, 2005a).

2.6.3.2 FSH/LH Ratio

Similar to FSH, LH secretion increases with age (Cramer *et al.*, 2002). LH levels, alone, apparently do not demonstrate predictive value for IVF outcomes (Bjercke *et al.*, 2005). However, Weghofer *et al* reported that in women with diminished ovarian reserve (defined by basal FSH levels of 10.1–15.0 mIU/ml), high-normal LH levels (i.e. a low FSH/ LH ratio) represented a good prognostic indicator and low-normal LH levels

(high FSH/LH ratio) represented a comparatively poor indicator of oocyte yield (Weghofer *et al.*, 2005). Liu and Greenblatt (2008) confirmed this initial report by demonstrating that a high FSH/LH ratio was significantly associated with poorer IVF cycle outcomes and increased cancellation rates (Liu and Greenblatt., 2008). These data, like the so-called threshold concept of Palermo and associates, who suggested that normal FSH levels, in the presence of unusually high LH, lead to lower pregnancy chances (Palermo, 2007), point towards a rather obvious need for 'appropriate' FSH/LH ratios to affect appropriate steroidogenesis (Weghofer *et al.*, 2009). Shrim et al., also, describes the FSH/LH ratio as an integral marker for the assessment of ovarian reserve (Shrim *et al.*, 2006).

A recent study in older ovulatory women showed a decreased LH response to GnRH stimulation compared with younger women (Fujimoto *et al.*, 1996). This finding suggests that LH synthesis and secretion may be different in older ovulatory women, despite the fact that no significant changes in basal serum LH levels were demonstrated. This being the case, both FSH and LH may contribute information relevant to ovarian reserve. In support of this concept, it has recently been reported that day 3 FSH: LH ratio is useful in predicting the outcome of an in-vitro fertilization (IVF) cycle in women with a normal day 3 FSH (Mukherjee *et al.*, 1996). Another study has shown a subtle increase in LH with age (Ahmed Ebbiary *et al.*, 1994).

2.6.3.3 Anti-Mullerian Hormone (AMH)

One group (de Vet *et al.*, 2002) demonstrated that serum AMH levels on cycle day 3 decreases progressively along with age and become undetectable after menopause. This suggests that peripheral AMH levels are a valuable parameter to monitor the relative follicular exhaustion due to ovarian ageing. Consistently, others (Seifer *et al.*, 2002) showed that day 3 AMH levels are positively related with the number of

oocytes retrieved after controlled ovarian hyperstimulation (COH). Taken together, these results indicate that circulating AMH levels reflect the number of selectable follicles during the early follicular phase. Indeed, the early antral follicle count has been shown to reliably predict the fertility potential of women and their responsiveness to COS. However, the question of whether serum AMH measurements on cycle day 3 reflect ovarian follicular status better than the usual hormonal parameters remains unanswered (Fanchin *et al.*, 2003).

2.6.3.4Serum Oestradiol

Elevated basal estradiol may predict the poor response even when basal FSH is normal (Evers *et al.*, 1998). In regularly menstruating women between the ages of 24 and 50 years, no differences in basal estradiol levels have been demonstrated according to age (Lee *et al.*, 1988). No relationship has been found between serum estradiol levels and pregnancy rates (Scott *et al.*, 1989).

It might be anticipated that direct hormonal products of the ovary would be better markers of ovarian response than indirect markers such as age and FSH. The rise in estradiol concentrations after FSH (Fanchin *et al.*, 1994) or GnRH analogue (Winslow *et al.*, 1991) administration has been shown to be predictive of IVF success, although high basal estradiol concentrations were associated with increased IVF cycle cancellation and lower pregnancy rates (Yong *et al.*, 2003).

2.6.3.5Inhibin-B

Inhibin-B is mainly produced by the granulosa cells in growing follicles and offers a more immediate assessment of ovarian activity than other serum tests. A fall in day 3 inhibin-B levels may predict poor ovarian reserve before the expected rise in day 3 FSH (Danforth *et al.*, 1998; Fried *et al.*, 2003; Seifer *et al.*, 1999). However, other studies

do not support its use as a predictive marker in IVF (Creus *et al.*, 2000; Hall *et al.*, 1999). Inhibin-B levels are influenced by the amount of fat in an individual (Tinkanen *et al.*, 2001), suggesting that the follicles of obese women do not produce as much inhibin-B as those of lean women

Most ovarian reserve tests (ORTs) are quite adequate in predicting ovarian response, but often fail to correctly predict the occurrence of pregnancy, especially if only one IVF cycle was studied. Because a benchmark for ovarian reserve status in the sense of quantity and quality is lacking, the occurrence of poor ovarian response to maximal stimulation and the occurrence of pregnancy in IVF are used as parameters to assess the accuracy of the test (Broekmans *et al.*, 2006).

2.6.4 Sonography

Another group of ORTs consists of sonographic parameters, such as the antral follicle count (AFC) and measurement of the ovarian volume. The way an AFC is performed differs between centers. Most often follicles of 2–5 mm or 2–10 mm are counted (Haadsma *et al.*, 2007). Recently, AFC, as visualized by transvaginal ultrasound scan, has attracted considerable interest as a test of ovarian reserve. An age-related decline in the AFC has been observed (Ng *et al.*, 2003; Ruess *et al.*, 1996). A systematic review has demonstrated the superiority of AFC over basal FSH in the prediction of poor ovarian response (Maheshwari *et al.*, 2006)

2.7 OVARIAN RESPONSE AFTER CONTROLLED OVARIAN STIMULATION

Poor ovarian response may be associated with poor pregnancy rates and many of these cycles are cancelled without proceeding to oocyte retrieval. On the other hand, exaggerated ovarian response leads to an increased risk of ovarian hyperstimulation

syndrome (OHSS) and the resulting high serum estradiol (E_2) concentrations may adversely affect the outcomes of the IVF treatment (Ng *et al.*, 2005).

2.7.1 Number of retrieved oocytes

A maximum of four oocytes at oocyte retrieval is considered 'poor ovarian response'(Weghofer *et al.*, 2005) or as cancellation due to impaired i.e < 3 follicles or complete absence of follicular growth in response to ovarian hyperstimulation. This definition has been adopted because, at a mean fertilization rate of 50–60% in IVF, ≥ 4 oocytes are necessary to obtain ≥2 embryos, which is the intended number to be transferred in most women (Bancsi *et al.*, 2002). The same definition was used in many other studies (El-Toukhy *et al.*, 2002; Surrey *et al.*, 1998) and seems to be the most widely used definition of poor response. Moreover, defining poor responders at a slightly higher or lower threshold, yields comparable proportions of poor responders and will produce similar predictive values (van Rooij *et al.*, 2003).

2.7.2 Ovarian capacity

The development of a sufficient number of follicles during ovarian stimulation for IVF is a very important step towards a successful outcome (Ranieri *et al.*, 2001). Ovarian follicular development is a continuous process throughout the reproductive lifespan. It is estimated that in a young woman, about 10-20 follicles leave the pool of resting primordial follicles each day and start to develop as growing follicles. Since only one follicle is destined to complete maturation and undergo ovulation during each menstrual cycle, the vast majority of oocytes will become atretic. The number of growing follicles at any time-point is believed to be related to the total number of follicles present in the ovary, although the proportion growing increases as the pool of primordial follicles falls approaching the menopause. The pre-menopausal ovary therefore always contains cohorts of growing follicles that may potentially be

stimulated to mature. Treatment with supraphysiological doses of FSH stimulates the development of many large antral follicles by preventing atresia of those secondary follicles that would normally not ovulate. The number of follicles developing under such circumstances reflects the total growing pool and thus the total number of follicles present in the ovary (Yong *et al.*, 2003).

2.8 PREDICTING OVARIAN REPONSE

Assisted reproduction is expensive, time-consuming and stressful for patients. Evaluations of IVF/ICSI performance rarely consider cancelled cycles, which usually result from an inadequate ovarian response to the stimulation treatment. The cycle cancellations further increase the cost and duration of therapy. Therefore, a major challenge to the IVF team is to predict prospective patients who will be low responders and to appropriately counsel women who are potential candidates for assisted reproduction (Penarrubia *et al.*, 2005b). The accurate determination of ovarian reserve continues to be a challenge for reproductive physicians. In the context of IVF treatment, ovarian reserve testing can be predictive of both response to gonadotrophin stimulation and chances of success with treatment (Pellicer *et al.*, 1987). The various available measures of ovarian reserve are better at predicting response to gonadotrophin stimulation than the chance of pregnancy (Creus *et al.*, 2000).

Rather than concentrating on attempting to predict pregnancy, it may be more helpful to use ovarian reserve testing in the prediction of cycle cancellation and poor response to stimulation. Avoiding gonadotrophin treatment for women destined not to respond to stimulation would help to reduce cancellation rates, treatment costs and emotional stress for the patient. Pretreatment counselling for predicted poor responders may ameliorate subsequent disappointment and distress (McIlveen *et al.*, 2007).

The number of mature follicles and oocytes that can be obtained in response to maximal stimulation using exogenous Gonadotrophins is an index of total ovarian follicular capacity and correlates inversely with FSH levels independent of age (Akande *et al.*, 2002).

In a study by Weghofer et al, (Weghofer *et al.*, 2005) within all age groups, women in the highest b-FSH quartiles showed significantly higher b-LH levels than patients in the lowest b-FSH quartiles. Their study however, clearly demonstrates that in women aged 25–35 years, b-FSH levels, even within a normal range, matter with regard to oocyte numbers. Higher age-specific FSH levels lead to fewer eggs.

Wolff *et al.* recently suggested that patients with elevated basal-FSH levels should not be excluded from IVF treatment. Instead, these authors presented that b-FSH values, among other parameters, such as chronological age and follicular phase inhibin B, should be utilized to establish an age-specific likelihood ratio for ovarian response (Wolff *et al.*, 2004).

2.8.1 Ovarian capacity, basal FSH and Age correlation

FSH concentrations in the early follicular phase show an inverse relationship to the size of the follicular cohort. In a study by Yong *et al*, in 2003 early follicular FSH concentration was weakly negatively correlated with AFC.

Research in various fertile and IVF-treated populations demonstrates a close relation of AFC with age (Chang *et al.*, 1998; Muttukrishna *et al.*, 2005; Ruess *et al.*, 1996; Scheffer *et al.*, 1999).

According to Yong *et al*, 2003, there was a signifcant relationship between age and AFC in the luteal and down-regulated phases. In another study, the number of large

follicles developing after stimulation with rFSH was significantly greater in the younger controls compared with that of the older group (group effect P< 0.05) (Hansen *et al.*, 2005). In yet another by Scheffer *et al.*, (2003) both the numbers of antral follicles and the total follicular volume decrease with increasing age. In same study, the number of antral follicles correlated much better with the age of the women evaluated, than other presumed basal markers for reproductive age, including FSH, inhibin B, E_2 and ovarian volume.

2.8.2 Number of retrievd oocytes, basal FSH and Age correlation

Weghofer *et al.*, (2005), demonstrated that at young ages, b-FSH, even if within normal ranges, may contribute to the prediction of ovarian reserve, as a reflection of the number of oocytes retrieved: Higher age-specific FSH levels led to fewer eggs. Yong *et al.*, (2003) also showed that, the concentration of FSH in the early follicular phase showed a highly significant negative correlation with the number of oocytes recovered, but there were no significant correlations with the other basal hormones measured in this phase. Basal FSH emerged as the most significant contributor to the number of oocytes retrieved. In the same study, there was only a poor relationship between age and the number of oocytes recovered (r = -0.18, P = 0.22).

Consequently, b-FSH should not serve as a marker to exclude patients from fertility treatment, but should be interpreted according to patient's age and not in absolute terms, even within the generally considered normal range of ≤10mIU/ml. An age-specific definition of ovarian reserve will allow physicians to stimulate patients in a more individualized fashion, which, in turn, will maximize IVF outcomes.

2.8.3 Age and basal FSH, LH and FSH/LH ratio

Early follicular FSH concentration is widely used in many IVF units to predict the ovarian response and is a better predictor of ovarian response than the age of women (Cahill *et al.*, 1994; Sharif *et al.*, 1998).

Ng *et al* in 2005 demonstrated that AFC by sonography achieved the best predictive value, followed by basal FSH concentration, body mass index (BMI) and age of women in a prospective study of 128 infertile patients undergoing their first IVF cycle using a standard regimen of ovarian stimulation.

Basal FSH concentration was positively correlated with age of women but was negatively correlated with the number of oocytes (Ng *et al.*, 2005). A metaanalysis showed that the performance of basal FSH concentration for predicting poor response was moderate and the performance for predicting no pregnancy was poor (Bancsi *et al.*, 2003). In a study by Haadsma *et al.*, (2007) age is significantly positively correlated with basal FSH ($r = 0.201$; $P < 0.01$).

The rise in FSH begins more than a decade before the menopause and several years before any rise in LH (Akande *et al.*, 2002). From that same study, correlation analysis revealed a reduction in the number of oocytes retrieved with advancing age and rising FSH. The partial correlation coefficients changed very little, suggesting that both age and FSH independently affected the number of oocytes.

In a study by Kim *et al.*, (1997), in the FSH: LH ratio, the only significant main effect was age, with the ratio being consistently higher in older versus younger women. In their study, the serum LH level was significantly elevated in the older group on cycle day 1. It was suggested, that, this finding may be related to the fact that there is an earlier onset of the inter-cycle FSH rise (Klein *et al.*, 1996) associated with earlier

dominant follicle development and ovulation in older ovulatory women. While older ovulatory women have more advanced follicular development on any given follicular phase cycle day, this finding may be due to either earlier onset or an accelerated rate of follicle development (Klein *et al.*, 1996).

Examining LH on cycle day 1, in addition to FSH, may improve predictive value even further, but not when examined as the FSH:LH ratio. Because the LH difference on cycle day 1 was in the same direction as the age difference in FSH values on day 1, this age difference was not reflected by the FSH:LH ratio on day 1. This finding contradicts a recent report in the medical literature that prognosis was generally poor in patients undergoing IVF cycles if the day 3 FSH:LH ratio was elevated to 3.6, even though the day 3 FSH value was normal (Mukherjee *et al.*, 1996).

2.9 MARKERS OF PREGNANCY

It is important to remember that there are several factors that contribute to the occurrence of pregnancy other than ovarian reserve, such as embryo transfer technique and number of embryos replaced. Even in young women with normal reserve, the chances of non-pregnancy remains at least at the 50% level. So, a non-pregnancy state after IVF may even be attributed to unknown, yet non-ovarian reserve related factors (Broekmans *et al.*, 2006).

Several tools are available to diagnose pregnancy: the level of human chorionic gonadotropin (hCG) in the serum, the presence of hCG in the urine (through either highsensitivity or low-sensitivity tests), pelvic ultrasound and a missed menstrual period. These diagnostic methods may detect pregnancy at different stages of progression, and not all pregnancies progress to delivery of a baby. Clinical investigators have options regarding how to best diagnose pregnancy. The choice of

test might be based on availability or cost (Schreiber *et al.,* 2009). But the impact that the timing and accuracy of the pregnancy diagnosis may have should also be considered.

The reality is that the results of many of these positive pregnancy tests do not indicate the beginning of a true clinical pregnancy but instead are chemical pregnancies characterized by a transient rise in the pregnancy hormone level only. The term chemical pregnancy is used to describe a transiently positive hCG level not associated with the development of an embryo or even a gestational sac (clinical pregnancy). In the absence of routine use of ultrasound, a chemical pregnancy could be defined by the peak in hCG (<100 mIU/mL) (Schreiber *et al.,* 2009).

Chapter 3

MATERIALS AND METHODS

3.1 SUBJECTS

A total of 104 subjects were selected for this study. Subjects' ages ranged between the ages of 21 and 50 years. All subjects were seeking assisted conception and reported at the fertility centre of Lister Hospital in Accra. Recruitment for the study spanned between February 2010 and March 2011. A criterion of age being from 20 to 50 years and undergoing self in-vitro fertilization (IVF) was used. Subjects undergoing donor IVF programs were excluded. 50 extra subjects with age range 22--43 years who had delivered spontaneously or naturally within the study period without assisted conception were recruited as controls. Subjects who were selected for this study, all did so willingly without coercion. Informed written consent was sought from subjects before been enrolled.

3.2 SAMPLE COLLECTION

About 3-5mL of blood was drawn from subjects and dispensed into serum separator tubes. Blood samples were taken on the second day of their menses in the month prior to the oocyte stimulation regimen. Samples were spun in a centrifuge at 8000rpm for 5 minutes to yield serum. The yielded serum was used to assay for the basal levels of LH, FSH and prolactin in subjects. All blood samples were drawn and assayed on the same day it was taken at the laboratory of Lister hospital and fertility centre, Accra-Ghana. Assays were performed using the statfax 2200 semi-automated analyzer from Awareness Technologies.

3.3 ANTHROPOMETRY

Anthropometric measurements included weight, height, waist and hip circumferences. The weight and height of subjects were measured as part of regular out-patient

department routines in the hospital. Body weight was measured to the nearest kg using a bathroom scale and the height was measured with a graduated meter rule. Subjects stood upright on the scale without shoes as their weights were taken and with their backs to the graduated meter rule and their heights measured. The Body Mass Index (BMI) was calculated as weight in Kg divided by height in meters squared (Kg/m^2). Four categories of BMI (≤18.5, 18.5 – 24.9, 25–29.9, and ≥30 kg/m^2) were identified. The categories were selected according to WHO recommendations to define individuals with a healthy weight (BMI 18.5 – 24.9), *overweight* (BMI 25–29.9) and *obese* (BMI≥30). Individuals with a BMI ≤18.5 kg/m^2 were classified as underweight (Molarius *et al.*, 1999).

Their waist and hip circumferences in centimeters (cm) were measured and recorded. The Waist to Hip ratio (WHR) was then calculated as waist circumference divided by the hip circumference. Subjects with WHR of < 0.80, 0.80 – 0.84 and ≥ 0.85 were classified as normal weight, overweight or obese respectively. (Molarius *et al.*, 1999)

Anthropometric data of pregnant controls were not taken since their pregnant status will impair their true measurements.

3.4 ESTIMATION OF FERTILITY HORMONES

3.4.1 Enzyme-linked immunosorbent assay sandwich method for prolactin, luteinizing hormone and follicle stimulating hormone

The NoviWell™ assay kits (HySkill Diagnostics, Bahlingen, Germany) were used in determining the serum levels of luteinizing hormone (LH), prolactin (PRL) and follicle-stimulating hormone (FSH) using the sandwich enzyme-linked immunosorbent assay (ELISA) method. The manufacturer's instructions for the performance of the assays were rigorously followed. The principle underlying the assay is the simultaneous

binding of hormone to two monoclonal antibodies; an immobilized one on a microplate and the other a soluble one conjugated with horseradish peroxidase (HRP). Briefly, 50 µl aliquots of standards and samples were dispensed into their respective wells in ready-to-use microtitre plates pre-coated with the corresponding hormones' anti-hormone IgG antibodies. After the addition of 100 µl of anti-hormone-HRP conjugate to each well, the plates were incubated for 60 minutes at room temperature in the dark. The contents of the wells were then decanted and the wells washed three times with 300 µl of distilled water.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/hydrogen peroxide system) or substrate into each well. The microtitre plates were then incubated for 15 minutes at room temperature. The reaction was stopped by the addition of 100 µl of 0.15 M Sulphuric acid (H_2SO_4). The end-point colour developed is directly proportional to the quantity of hormone. Absorbances were measured at 450 nm and the concentrations calculated from a standard curve generated using standards of known concentrations in a Stat Fax 2200 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assays coefficient of variations were 6.1% for FSH and PRL and 5.4% for LH, The analytic sensitivities of the assays were 1.0 mIU/ml for FSH, LH and 1.0 ng/ml for PRL as indicated by the manufacturer.

Cut-off values for b-FSH and LH levels, measured on cycle days 2 or 3, immediately before the commencement of oral contraception, were considered as follows; basal FSH ≤10 mU/ml and basal LH ≤12 mU/ml.

3.5 LIFESTYLE PATTERN

A mixed questionnaire of mostly closed end type questions and some few open end questions were administerd to find smoking habits and exercise patterns, alcohol and caffeine intake, exposure to chemicals like pesticides and welding materials etc

3.6 OVARIAN STIMULATION

In this study, one of two different oocyte stimulation protocols were followed depending on the age and basal FSH levels of the subjects measured on day 2 of menses. Subjects with high basal FSH levels follow the first protocol (long protocol) which is as follows;

On the 2nd or 3rd day of menses, they were started on a 0.5mL of *Burserelin* injection for about 2-3 weeks and trans-vaginal scans done in between. The scans were done to ensure that the endometrium was thin enough and that there were no cysts formed. If there were cysts formations, the protocol was discontinued or the cyst drained, protocol discontinued and regular menses allowed to flow. However, if there are no cysts, 450IU of *Gonal F* was given in addition to the *burselin* injection for 10 days. Trans-vaginal scans were done on the 8th and 11th day. On the 11th day, *burselin* and or *Gonal F* were not given, but rather 10,000 IU of hCG injection was given to mature the developing oocytes before they were collected. Egg collection was done some 36 hours after the hCG injection.

The second protocol (short protocol) was performed on subjects with normal basal FSH levels or below the age of 35 years. In this protocol, decapeptyl pessaries were administered on the 21st day of previous menstrual cycle. Normal menses is allowed to flow. On day 2 of the following menses, 175 – 300 IU of FSH or *Gonal F* injection was administered for 10 days. Trans-vaginal scans were performed on the 8th and 11th day.

10,000 IU of hCG injection was given on the 11th day and egg collection done 36 hours after the hCG injection.

All follicles 2–10mm in size were considered antral follicles, counted and labeled as the ovarian capacity (antral follicle count-AFC)

In both protocols, *cyclogest* pessaries were administered after egg collection and continued till up to about 3 months if pregnancy develops or discontinued when there is no pregnancy. This was done to prepare and maintain the endometrium ready for implantation of the embryo.

Figure 3.1 Ultrasound scan of developing follicles

3.7 OOCYTE RETRIEVAL, FERTILIZATION AND EMBRYO TRANSFER

Oocytes were collected some 36 hours after the hCG injection was administered. The ultrasound directed follicle aspiration (UDFA) technique was used with a 7.5 MHz transducer vaginal probe. The retrieved oocytes were analysed for quality and quantity and placed in the incubator at 37^0C. Throughout the collection and analysis stages, a constant temperature of 37^0C was maintained.

Semen from the right corresponding partner was collected into a sterile container by masturbation. Collected semen was processed by gradient centrifugation. 1 mL of 80% semen preparation medium is poured into a conical tube. 1mL of 40% semen preparation medium was layered onto it carefully to prevent mixing. 1-2mL of the semen was also layerd onto it carefully to prevent mixing. The tube was centrifuged at 5000 rpm for 20 minutes. The supernatant was aspirated off leaving the buttom 0.5mL which is a concentrate of sperm cells. The 0.5mL deposit is pipetted into 3 mL of universal IVF medium and spun at 5000rpm for 5 minutes. The supernatant was aspirated leaving the buttom 0.5mL, a pure concentrate of sperm cells. The pure concentrate of sperm cells was used to fertilize the retrieved oocytes.

Those indicated for the conventional IVF procedure had their oocytes incubated together with a pipetted amount of the sperm cells in a petri dish at 37^0C overnight. Intracytoplasmic sperm cell injection was performed for those with low sperm count using the *Nikon* inverted microscope and incubated at 37^0C overnight. The oocytes were checked for fertilization the following morning which was indicated by the presence of two pro-nuclei; one from the oocyte and the other from the sperm cell. Fertilized oocytes were grouped together and incubated again at 37^0C till the third day after egg collection.

About three very well dividing oocytes were transferred (except in cases where fewer eggs were fertilized) into the uterus with a transfer catheter using an abdominal scan as

a guide. Subjects were allowed about an hour of bed-rest after the transfer and subsequently detained in the hospital for about 2-3 days for constant monitoring before they are discharged to go home. Success or otherwise of the procedure was established by measuring the rising levels of β-hCG, 14 days after embryo transfer.

3.8 ESTABLISHING PREGNANCY

Enzyme-linked immunosorbent assay sandwich method for β-hCG

The same NoviWell™ assay kits (HySkill Diagnostics, Bahlingen, Germany) were used in determining the serum β-hCG levels 14 days after the embryo transfer. The test is based on the simultaneous binding of human β-hCG to two monoclonal antibodies, one immobilized on microwell plates and the other conjugates with horseradish peroxidase (HPR). The kit comes with a concentrated conjugate that needs to be diluted. 10µl of the concentrated conjugate is added to 100µl of the incubation buffer.

The quantity of diluted conjugate was proportional to the number of tests done at any point in time. 25µl aliquots of standards and samples were pipetted into pre-coated microtitre wells. 100µl of the diluted conjugate was added and incubated in the dark for 60 minutes. The contents of the wells were then decanted and the wells washed three times each with 300 µl of distilled water. The enzyme reaction was started by the addition of 100 µl of substrate (chromogen) into each well. The microtitre plates were then incubated for 15 minutes at room temperature. The reaction was stopped by the addition of 100 µl of 0.15 M Sulphuric acid (H_2SO_4).

The end-point colour developed is directly proportional to the quantity of hormone. Absorbances were measured at 450 nm and the concentrations calculated from a standard curve generated using standards of known concentrations in a Stat Fax 2200 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). The kit has a lowest detectable concentration of β-hCG that can be distinguishable from

the zero standard of 0.5mIU/mL at the 95% confidence limit as stated by the manufacturer.

3.9 QUESTIONNAIRE ADMINISTRATION

A simple questionnaire finding the exercise patterns, alcohol and caffeine intake, exposure to chemicals etc of subjects was administered. Some subjects were interviewed whiles they were monitored in the wards after their embryo transfer and others were interviewed on telephone and answers obtained.

By exercise we mean going for walks, jogging, swimming, or working out. Occupational activity where subjects walk as part of their work or any activity in relation to occupation was not recognized by our classification of physical activity.

Responses were taken on the frequency, duration and intensity of the exercise subjects were engaged in at least 3 months prior to the procedure month. However more emphasis was placed on the frequency of exercise. Exercise patterns were classified into three groups in a similar fashion as that documented by Augestad *et al.*, (2004). From answers provided in the questionnaire, subjects who did ≤ 1 day per week exercise for ≤ 10 minutes were classified as LOW exercisers. Those who exercised for 20 – 60 minutes every 2 – 3 days in a week were put together as MODERATE exercisers and those who engaged in ≥ 60 minutes for nearly every day were classified as HIGH. However, those who did ≥ 60minutes for 1 day in a week were also classified as LOW and those who did about 20 – 60 minutes nearly every day for the week were classified as HIGH.

The number of times subjects took in any alcoholic beverage or smoked cigarette in the month prior to and in the very month of the procedure was also documented.

Chapter 4

RESULTS

4.1 GENERAL CHARACTERISTICS

The total of 104 subjects studied was of a mean age of 36.25 years and age ranged from 23 – 48 years. A mean age of 34 years was recorded for 50 controls and age ranged from 22 – 43 years. Age of subjects and controls were statistically different with a p value of 0.0048 (Figure 4.1). Subjects within the age range of 31 – 40 were the mode with a total of 73. Subjects within the age bracket of 41 – 50 recorded the highest mean figures of 0.82 and 28.0 kg/m^2 when WHR and BMI were used as obesity indices respectively indicative of overweight. When considered as a whole, a mean FSH of 12.48 IU/L was recorded, with subjects within the age range of 31 – 40 recording the highest mean FSH of 13.41 IU/L whilst those in the age range 21 – 30 recorded the least mean FSH of 7.64 IU/L. Subjects within the age range of 21 – 30 yielded the highest number of follicles and consequentially the most retrieved oocytes compared to their older counterparts within the age range of 41 – 50 yielding the least follicles and retrieved oocytes (Mean values; 20.92 and 20.31 vs. 8.44 and 7.28 respectively), (Table 4.1).

There were no embryo transfers in 5 cases. Three (3) of them had no oocytes, one had 1 oocyte and the other 3 oocytes but could not be fertilized. There were six (6) cases of OHSS. In all six cases, ovarian capacity was ≥ 30 follicles and oocytes were ≥ 32. Four (4) out of those six got pregnant after treatment of OHSS followed by embryo transfer.

Comparing basal hormonal markers of controls against study subjects; only basal FSH showed a significant difference between the means of these groups (9.5 vs 13.4

respectively, p = 0.002). Basal LH and prolactin showed no significant difference between controls and study subjects (Figure 4.2)

Table 4.1 Characteristics of whole study population

	Age stratification			
	21 – 30	31 – 40	41 – 50	ALL
Number (n)	13	73	18	104
Age (yrs)	28.08 ± 0.65	36.18 ± 0.35	42.44 ± 0.47***	36.25 ± 0.47
Duration of infertility (mths)	52.23 ± 11.37	74.59 ± 1.71	117.3 ± 13.22^{ns}	79.19 ± 4.61
WC (inches)	32.0 ± 1.0	35.0 ± 0.43	34.0 ± 0.83^{ns}	34.0 ± 0.36
HC (inches)	41.0 ± 1.2	44.0 ± 0.44	41.0 ± 0.84**	43.0 ± 0.39
WHR	0.79 ± 0.01	0.79 ± 0.0	0.82 ± 0.01*	0.79 ± 0.0
Weight (Kg)	69.0 ± 1.6	71.0 ± 0.98	73.0 ± 1.7^{ns}	71.0 ± 0.78
Height (m)	1.6 ± 0.02	1.6 ± 0.01	1.6 ± 0.01^{ns}	1.6 ± 0.01
BMI (Kg/m^2)	25.0 ± 0.76	27.0 ± 0.40	28.0 ± 0.70^{ns}	27.0 ± 0.33
LH (IU/L)	6.76 ± 0.87	7.42 ± 0.43	6.99 ± 0.93^{ns}	7.26 ± 0.36
FSH (IU/L)	7.64 ± 1.21	13.41 ± 0.91	12.21 ± 1.24*	12.48 ± 0.71
Prolactin (ng/mL)	13.97 ± 1.48	13.55 ± 0.81	13.98 ± 1.96^{ns}	13.68 ± 0.68
FSH/LH	1.23 ± 0.16	2.23 ± 0.20	2.74 ± 0.64^{ns}	2.19 ± 0.18
Ovarian capacity/ follicle count	20.92 ± 2.63	13.62 ± 0.96	8.44 ± 1.16***	13.63 ± 0.83
Retrieved oocytes	20.31 ± 3.30	11.99 ± 1.04	7.28 ± 2.03***	12.21 ± 0.96

Data are presented as mean ± SEM or number (n) by count, BMI = body mass index, WHR= waist-to-hip ration, LH=leutinizing hormone, FSH = follicle stimulating hormone. *=P<0.05, **=P<0.001, ***=P<0.0001. One way ANOVA was performed.

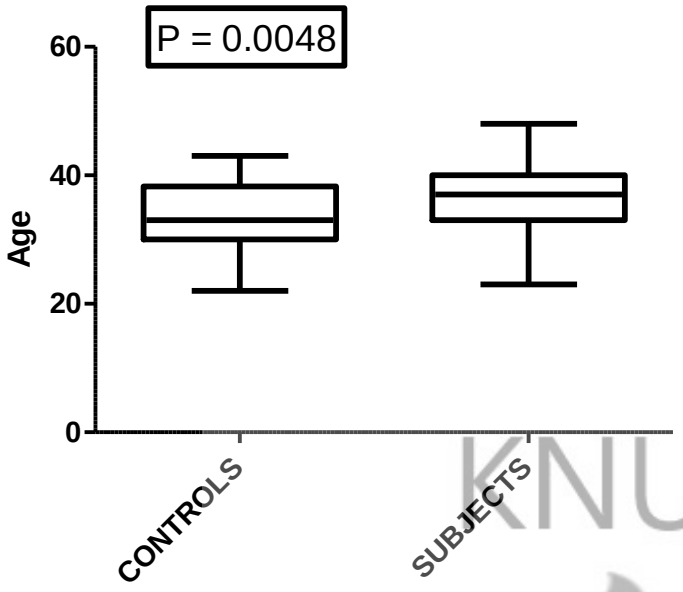
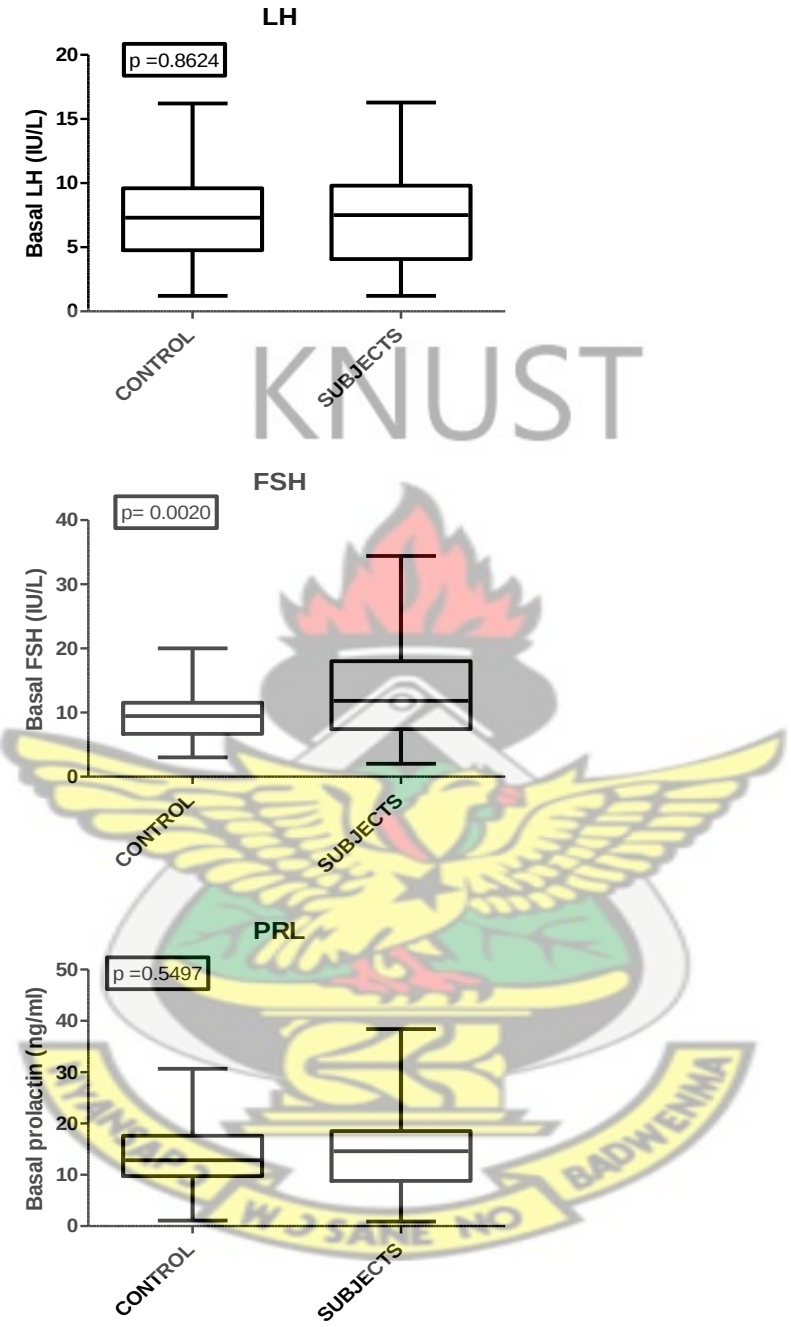


Figure 4.1 Age comparison of controls and subjects

4.2 HORMONAL COMPARISON OF CONTROLS AND TEST SUBJECTS

Blood hormonal levels of women who have conceived naturally were retrieved from the database of the study site. When hormonal levels of infertile subjects were compared to these controls, there was no significant difference in means of LH and prolactin. Means of FSH was however statistically significant with a p-value of 0.002 between controls and subjects. Study subjects were not compared to pregnant controls on basis of anthropometry because controls were already pregnant and this will alter their weight and other anthropometric measurements (Figure 4.2)



Comparison of basal hormonal markers between controls and study subjects (excluding male factor only causes)

Figure 4.2 Comparison of basal hormonal markers between controls and study subjects (ecluding male factor only causes)

4.3 CORRELATION BETWEEN AGE AND OUTCOMES OF OVARIAN STIMULATION

For both measured outcomes of ovarian stimulation; ovarian capacity and number of retrieved oocytes, age showed a significant negative correlation with correlation coefficients r = -0.48 and -0.38 respectively (Figure 4.3).

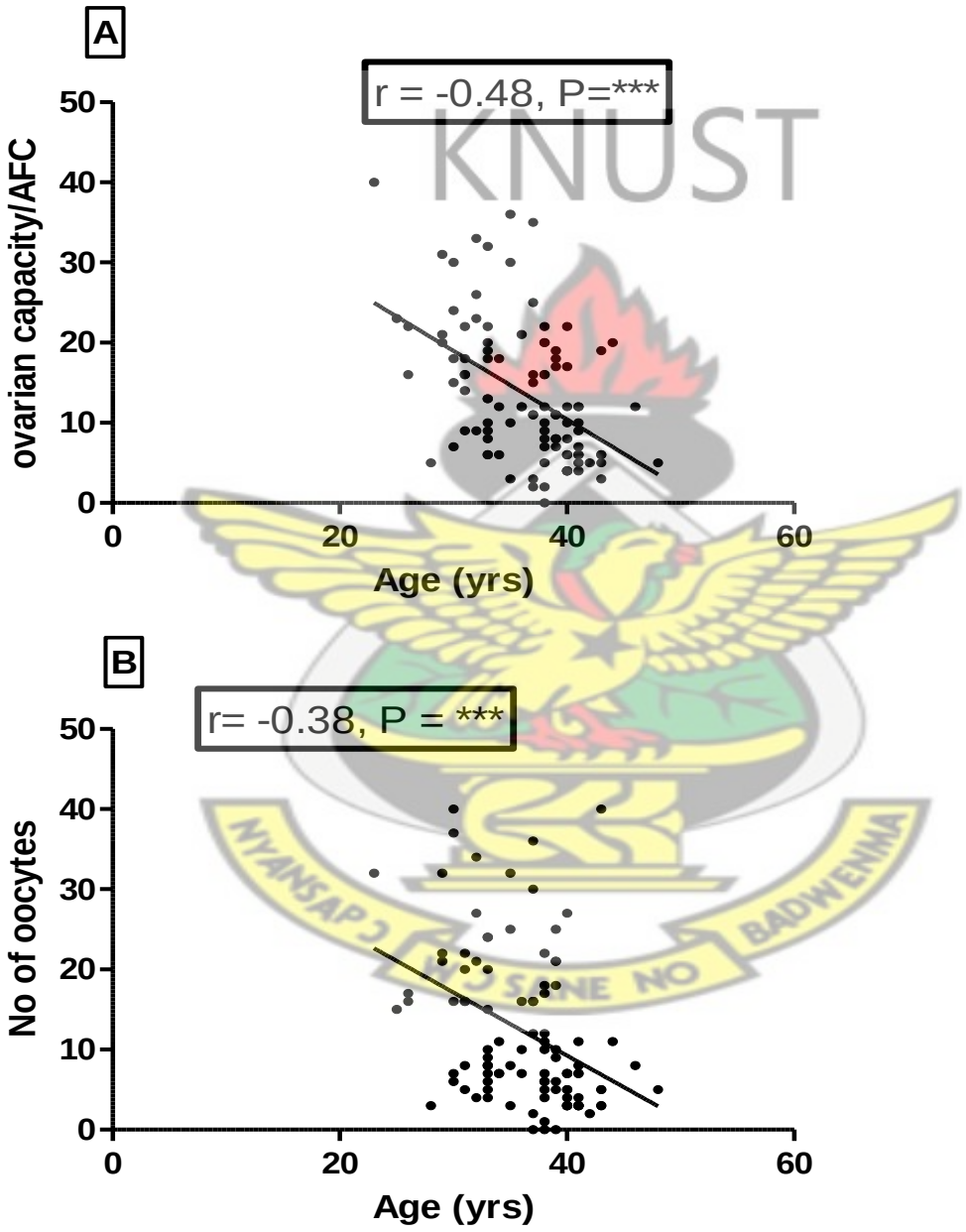


Figure 4.3 Correlation between Age and outcomes of ovarian stimulation

4.4 ASSOCIATION BETWEEN OBESITY INDICES AND MEASURED OUTCOMES OF STIMULATION

When any of the two obesity indices (body mass index; BMI or waist-to-hip ratio; WHR) was used, measured outcomes after ovarian stimulation followed a similar pattern. When BMI was used, mean number of ovarian capacity and retrieved oocytes dropped consistently with increasing BMI. Using BMI, subjects ranked normal recorded a mean ovarian capacity and retrieved oocytes of 20.0 and 16.0respectively, those ranked overweight recorded a mean ovarian capacity and retrieved oocytes of 13.0 and 12.0 respectively, whereas thos ranked obese recorded 5.8 and 5.1 respectively. When WHR was used, mean retrieved oocytes was 15.0 in normal weight subjects, 10.0 in overweight subjects whereas the obese subjects had 7.0 mean oocytes (Table 4.2).

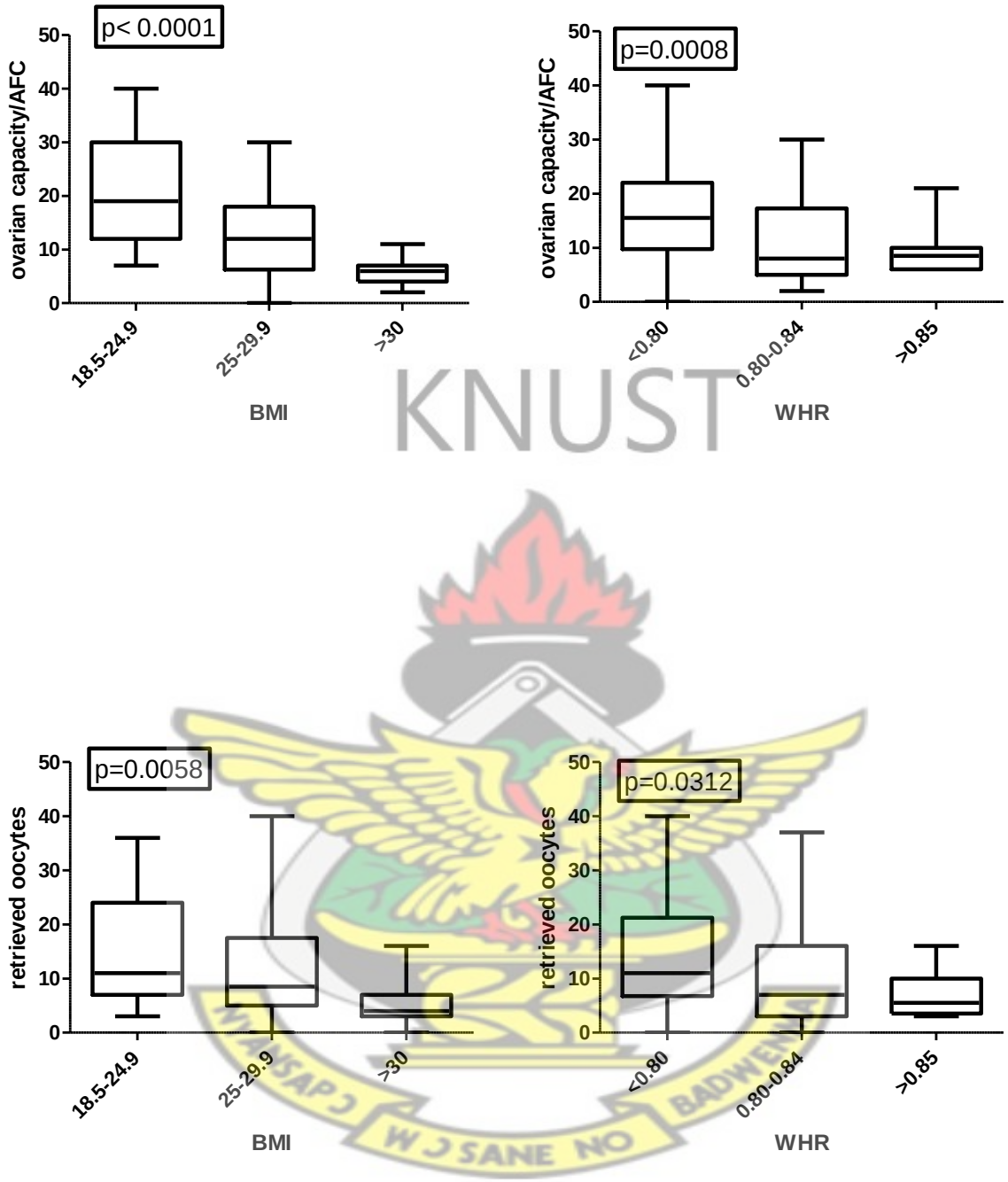
A one way ANOVA of obesity classifications in relation to measured outcomes of stimulation shows statistical difference between means of normal, overwight and obese groupings. Using BMI against ovarian capacity and retrieved oocytes showed a p value < 0.0001 and 0.0058 respectively across the three classifications of normal, overweight and obese subjects (Figure 4.4).

When BMI was used as the obesity index, majority of subjects 61.5% were overweight whereas when WHR was used, a little over half of subjects 51.9% had normal weight. (Table 4.2)

Table 4.2 Effects of obesity on measured outcomes of ovarian stimulation

	BMI				WHR			
Weight	*Grade*	*% (n)*	*Ovarian capacity*	*Retrieved oocytes*	*Grade*	*% (n)*	*Ovarian capacity*	*Retrieved oocytes*
Normal	18.5 - 24.9	26.0 (27)	20.0 ± 1.9	16.0 ± 2.0	<0.80	51.9 (54)	17.0 ± 1.2	15.0 ± 1.4
Overweight	25.0 - 29.9	61.5 (64)	13.0 ± 0.9	12.0 ± 1.2	0.80 - 0.85	40.4 (42)	11.0 ± 1.1	10.0 ± 1.4
Obese	>30	12.5 (13)	5.8 ± 0.7	5.1 ± 1.1	>0.85	7.7 (8)	9.5 ± 1.8	7.0 ± 1.6

Data are presented as percentages (number by count) or mean ± SEM



1-way ANOVA of obesity classifications and measured outcomes of ovarian stimulation

Figure 4.4; One way ANOVA of obesity classifications and measured outcomes of ovarian stimulation

4.5 LIFESTYLE OF SUBJECTS IN ASSOCIATION WITH OUTCOMES OF OVARIAN STIMULATION

Majority of subjects attended with primary infertility; 80.7% vs. 19.3% attending with secondary infertility. (Table 4.3)

Female only factors accounted for 33.7% of infertility causes. 15.4% were of unexplained causes and 11.5% were of both male and female factors. Of the female factors anovulation was the predominant cause.

Subjects with positive β-hCG had significantly higher mean ovarian capacity and retrieved oocytes than their counterparts with negative β-hCG (Ovarian capacity; 16.58 vs. 12.27, retrieved oocytes; 16.12 vs. 10.39) (Table 4.3). However, mean retrieved oocytes of subjects with negative β-hCG were adequate for embryo transfer.

A one-way ANOVA analysis was performed to assess the influence of some lifestyle factors on measured outcomes of ovarian response. On exercise, subjects were classified into three groups (low, moderate and high) according to their exercise patterns. There was no significant difference in ovarian capacity or retrieved oocytes across all three exercise classifications (P = 0.2417 and 0.1148 respectively). However moderate to high exercise showed a progressive increase in mean follicular count/ovarian capacity from low to moderate to high; 13.07 to 16.55 to 18.25 respectively. Subjects who engaged in regular moderate exercises had the highest mean retrieved oocytes of 17.82 (Table 4.3)

There was a significant difference between their WHR; an obesity index across the three exercise patterns (P = <0.05). Subjects who engaged in a higher grade of exercises had the lowest WHR of 0.74 (Table 4.3)

No one in the study population had ever smoked but one had a partner who smoked. All subjects were found to be low on alcohol intake and no one took any alcoholic drink in the month prior to the procedure and within the month of the IVF procedure.

4.6 COMPARING OUTCOMES OF SUBJECTS WITH NORMAL AND HIGH FSH LEVELS

Mean age of subjects with higher than normal FSH levels (37.23years) was significantly higher than their counterparts with normal FSH levels (35.10 years) with a p-value <0.05. Measured outcomes of both classifications of subjects followed the same pattern as subjects with normal FSH had a better and favorable response than their counterparts with high FSH. Mean ovarian capacity of normal FSH was 16.71 as against 11.0 follicles by those with high FSH levels with a p-value <0.001. Mean retrieved oocytes was significantly different between subjects with a normal FSH and those with high FSH with a p- value <0.0001 (16.17 vs. 8.82 oocytes). More cases of pregnancy were recorded amongst subjects with normal FSH levels (Table 4.4).

Table 4.3 Effects of obesity on measured outcomes of ovarian stimulation

PARAMETER	% (n)			
Primary infertility	80.7 (84)			
Secondary infertility	19.3 (20)			
Cause of infertility				
Male	39.4 (41)			
Anovulatory	14.4 (15)			
Tubal	7.7 (8)			
Uterine	11.5 (12)			
Unexplained	15.4 (16)			
mixed/combined	11.5 (12)			
Pregnancy	*Positive*	*Negative*		
% (n)	34.4 (33)	65.6 (63)		
AFC	16.58 ± 1.48	12.27 ± 0.97 *		
no of oocytes	16.12 ± 1.85	10.39 ± 1.05**		
Smoking (self);	*Currently*	*Before*	*Never*	*P*[a]
% (n)	-	-	100 (104)	
Smoking (partner);				
% (n)	-	0.96 (1)	99.04 (103)	
Alcohol intake;	*Low*	*Moderate*	*High*	
% (n)	100 (104)	-	-	
Exercise;	*Low*	*Moderate*	*High*	
% (n)	85.6 (89)	10.6 (11)	3.8 (4)	
AFC	13.07 ± 0.93	16.55 ± 1.45	18.25 ± 4.38	0.2417
no of oocytes	11.43 ± 1.02	17.82 ± 3.34	14.25 ± 3.01	0.1148
BMI	26.92 ± 0.30	27.47 ± 1.80	25.33 ± 1.99	0.5492

WHR	0.79 ± 0.01	0.79 ± 0.02	0.74 ± 0.02	0.023*

Data are presented as mean ± SEM. *=P<0.05, **=P<0.001, *a* = one way ANOVA analysis performe

Table 4.4 Outcomes of subjects with high and normal FSH levels

	Normal FSH	High FSH	*P*
Number (n)	48	56	
Age (yrs)	35.10 ± 0.79	37.23 ± 0.52	<0.05
AFC	16.71 ± 1.34	11.00 ± 0.91	<0.001
Retrieved oocytes	16.17 ± 1.60	8.82 ± 0.95	<0.0001
Positive B-HCG n (%)	17 (38.6%)	16 (30.8%)	

Data are presented as mean ± SEM or n(%)

Table 4.5 Basal gonadotrophins and other parameters in predicting ovarian response

Variables	Total	Normal responders	Poor responders	P
Number	104	81	23	
Age	37(23.00 - 48.00)	36 (23.00 - 48.00)	40 (28.00 - 43.00)	<0.01
Dur. of infertility (mths)	72(7.000 - 240.0)	72 (7.000 - 180.0)	84 (12.00 - 240.0)	0.0761
Primary infertility %(n)	84	76.2 (64)	23.8 (20)	
Secondary infertility %(n)	20	85.0 (17)	15.0 (3)	
Anthropometry				
BMI	26.5 (20.1 - 44.4)	26.5 (20.1 - 44.4)	26.9 (23.5 - 36.1)	0.754
WHR	0.79 (0.69 - 0.93)	0.80 (0.69 - 0.93)	0.79 (0.70 - 0.87)	0.3829
Basal markers				
LH (IU/L)	7.3 (1.2 - 16.3)	7.3 (1.2 - 16.3)	7.0 (1.2 - 14.9)	0.9469
FSH (IU/L)	10.6 (2.0 - 34.4)	9.7 (2.0 - 34.4)	12.9 (6.4 - 33.9)	<0.05
Prolactin	13.5 (0.9 - 38.4)	13.6 (0.9 - 30.7)	13.4 (2.7 - 38.4)	0.5835
FSH/LH	1.62 (0.35 – 11.36)	1.5 (0.35 – 11.36)	2.39 (0.75 – 7.83)	<0.01
Exercise pattern [%(n)]				
Low	89	74.2 (66)	25.8 (23)	
Moderate	11	100 (11)	-	
High	4	100 (4)	-	
Pregnancy [%(n)]	31.7 (33)	35.8 (29)	17.4 (4)	

Data are presented as median (range) or percentage (number by count). Mann-Whitney test was performed

4.7 PREDICTING RESPONSE

Subjects were classified into poor responders and normal responders according to the quantity of their retrieved oocytes. Subjects with 4 or less oocytes were classified as poor responders whereas those with retrieved oocytes more than 4 were said to be normal responders. A Mann-Whitney test was performed to assess the association between response status as classified by number of retrieved oocytes and basal hormonal markers (Table 4.5).

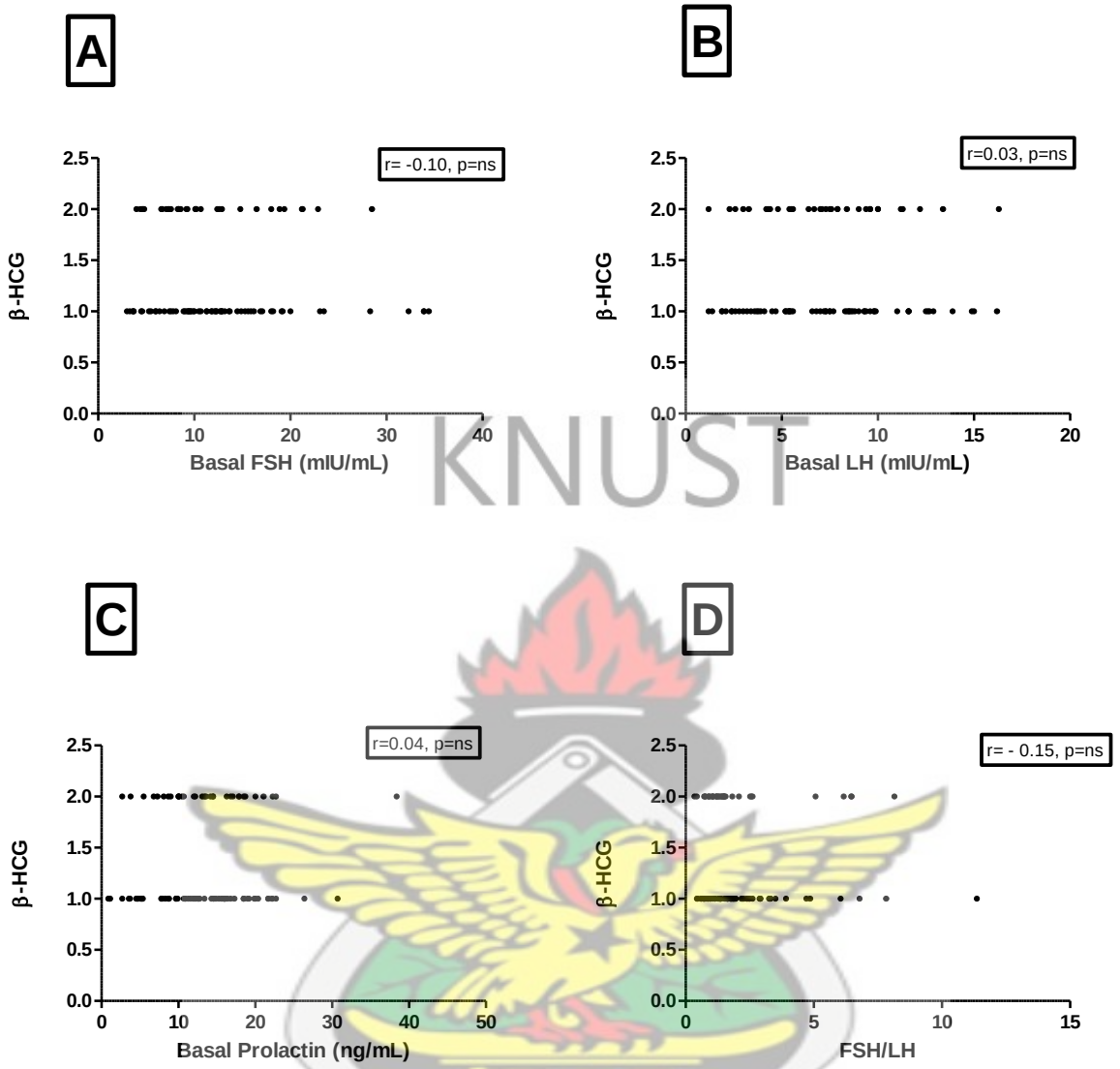
Majority of subjects (81) had a normal response i.e. ≥ 4 retrieved oocytes as against 23 with poor response (<4 oocytes). Poor responders were significantly older than normal responders (median age; 36 vs. 40, P = <0.01). There was no significant difference in both obesity indices when subjects were classified into normal and poor responders (Table 4.5).

Basal FSH was significantly higher in poor responders as against normal responders (median FSH; 9.7 vs. 12.7, P = <0.05 respectively). When basal LH and prolactin were compared independently, there was no significant difference in medians between normal and poor responders. However, when FSH and LH were used together in a ratio as FSH/LH, poor responders had a higher ratio compared to normal responders (median FSH/LH; 2.39 vs. 1.5, P = <0.01 respectively) (Table 4.5).

All subjects who engaged in moderate to high forms of exercise recorded a normal response after the ovarian stimulation protocol. 25.8% of subjects who engaged in no or low exercise regimens recorded a poor response. 17.4% of poor responders recorded a positive β-HCG contrasting twice as much pregnancy rate (35.8%) in normal responders (Table 4.5).

4.8 CORRELATION BETWEEN BASAL HORMONE MARKERS AND CHEMICAL PREGNANCY

Pregnancy was established by a positive β-HCG. There was no significant correlation between any of the basal hormonal markers; LH, FSH and prolactin and the presence of pregnancy. FSH/LH ratio also showed no significant correlation to pregnancy, though it yielded a negative correlation coefficient of r = -0.15. Again, although there was no significant correlation when basal FSH was compared to the incidence of pregnancy; it showed a negative correlation coefficient (r) of -0.10 (Figure 4.4).



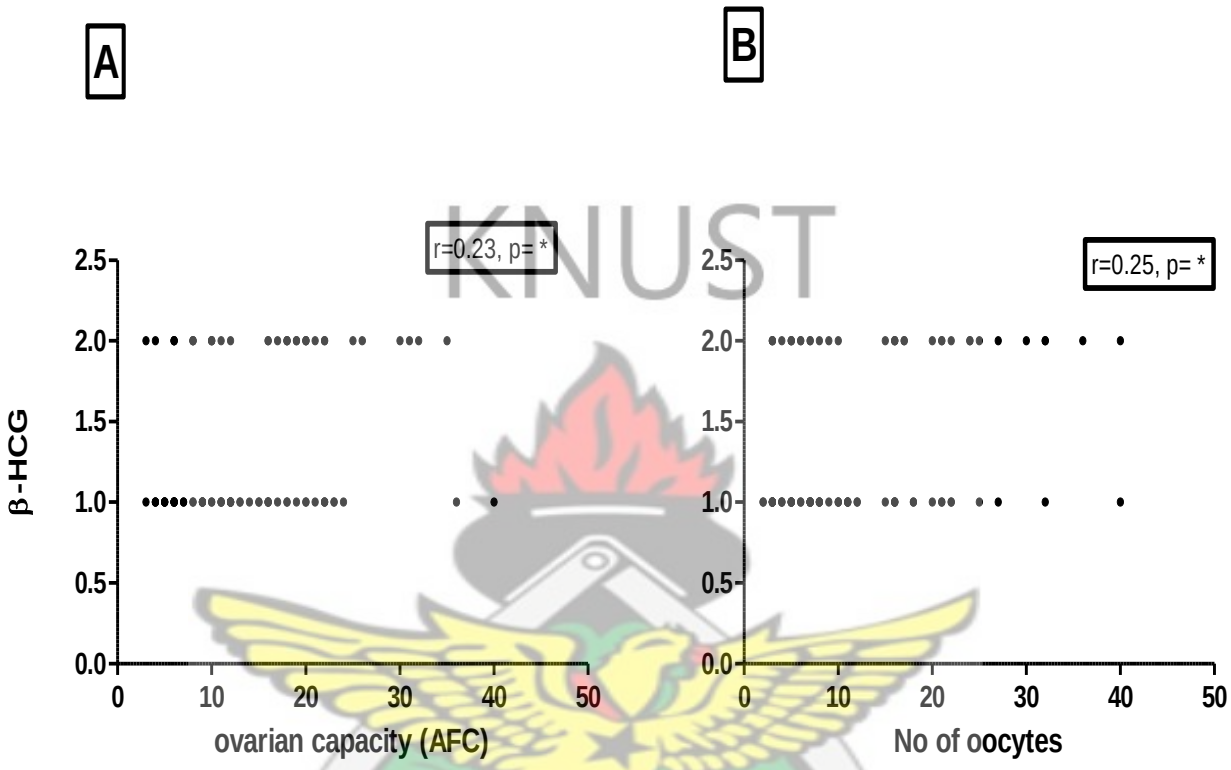
Correlation between (A) basal FSH and βHCG (B) basal LH and β-HCG (C) basal Prolactin and β-HCG (D) LH and β-HCG.
Note; on graph point 1=negative β-HCG, 2= positive β-HCG

Figure 4.5 Correlation between basal hormonal markers and chemical pregnancy

4.9 CORRELATION BETWEEN MEASURED OUTCOMES OF OVARIAN STIMULATION AND PREGNANCY

Both measured outcomes of ovarian stimulation; ovarian capacity/antral follicle count and number of retrieved oocytes showed significant correlation (P = <0.05) with a

positive correlation coefficient (r) of 0.23 and 0.25 respectively when compared with the presence or otherwise of pregnancy (Figure 4.6).



Correlation between (A) antral follicle count (AFC)/ovarian capacity and β-HCG (B) number of retrieved oocytes and β-HCG
Note; point 1= negative β-HCG, point 2 = positive β-HCG

Figure 4.6 Correlation between measured outcomes of ovarian stimulation and pregnancy

4.10 LINEAR CORRELATION BETWEEN BASAL MARKERS AND MEASURED OUTCOMES OF OVARIAN STIMULATION

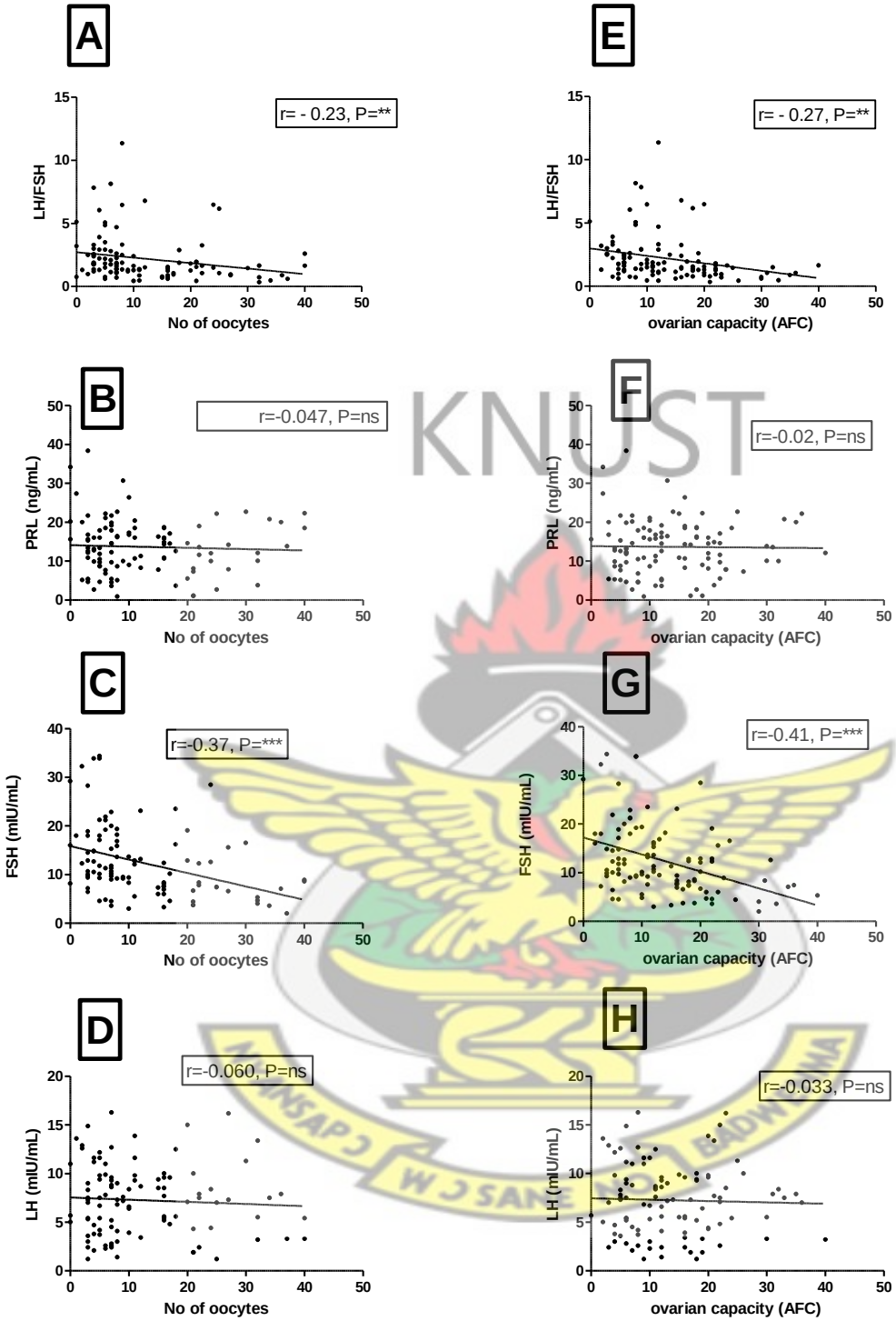
Basal FSH showed statistical significance when compared to both number of retrieved oocytes and ovarian capacity with $P < 0.001$ (Figure 4.6). Basal FSH showed negative correlation coefficients; r = -0.37 and -0.41 when compared with number of retrieved oocytes and ovarian capacity respectively (Graph C & G). FSH/LH ratio also showed significance ($P < 0.01$) when compared with both measured outcomes; number of

retrieved oocytes and ovarian capacity. FSH/LH ration however showed a negative correlation to number of oocytes and ovarian capacity with correlation coefficients r = -0.23 and -0.27 respectively (Graph A & E) (Figure 4.7).

On the other hand, basal LH and prolactin showed no significance when compared with both measured outcomes of ovarian stimulation; number of retrieved oocytes and ovarian capacity (Graphs B, D, G and H) (Figure 4.7).

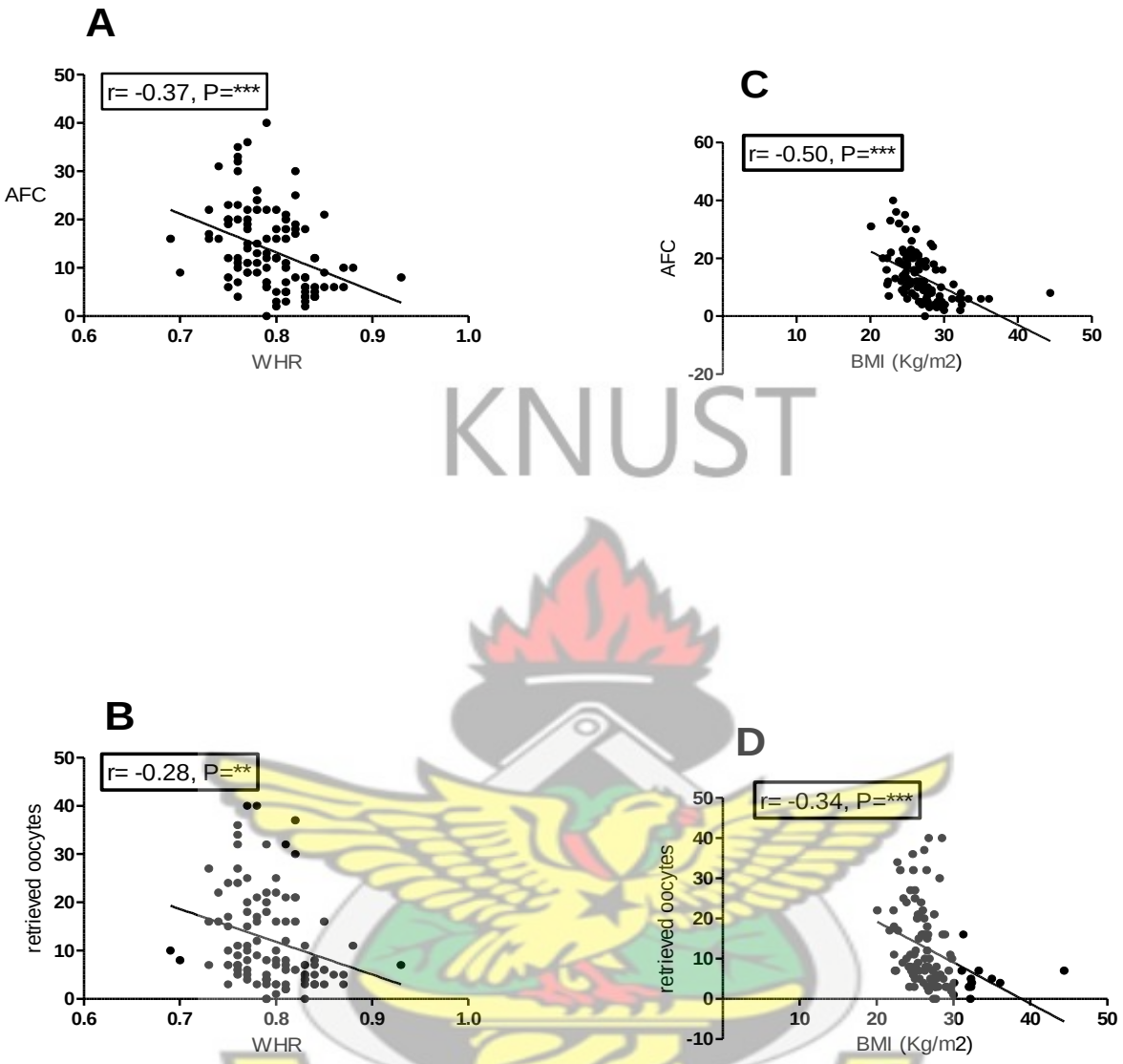
4.11 REGRESSIONAL ANALYSIS BETWEEN OBESITY INDICES AND MEASURED OUTCOMES OF OVARIAN STIMULATION

There was significant correlation between obesity indices and antral follicle count/ovarian capacity and number of retrieved oocytes. Measured outcomes of ovarian stimulation; number of retrieved oocytes and ovarian capacity were negatively correlated to both obesity indices; waist-to-hip ratio (WHR) and body mass index (BMI). AFC compared to WHR and BMI gives a correlation coefficient of r = -0.37 and -0.50 respectively. Number of oocytes when compared to WHR and BMI gives correlation coefficient of r = -0.28 and -0.34 respectively (Figure 4.8).



Linear correlation between basal hormonal markers; (A, E)LH/FSH (B, F) PRL (C, G) FSH (D, H) LH and number of retrieved oocytes (A - D) and ovarian capacity/antral follicle count (E - F)

Figure 4.7 Correlation between basal hormonal markers and chemical pregnancy



Linear regression analysis between measureed outcomes of ovarian stimulation; number of retrieved oocytes (B & D), antral follicle count (A & C) and obesity indices; waist-to-hip ratio (WHR) and body mass index (BMI)

Figure 4.8 Correlation between measured outcomes of ovarian stimulation and chemical pregnancy

Chapter 5

DISCUSSION

A mean age of 36.25 years in our study population is in line with other studies like one by van Noord-Zaadstra *et al.* (1991) which set 35 years as a watershed in fertility terms and that on average, female fertility declines from the age of 30 years onwards. In the recent past, most Ghanaian women gave birth at younger ages but currently more and more women are postponing child bearing to pursue education and other economic feats until later years when fecundity becomes poor due to a decrease in quantity and quality of eggs. Similar geographic trends were found by Maheshwari *et al.*, (2008) and van Zonneveld *et al.*, (2003).

This study found that, the older one gets, the fewer the number of retrieved oocytes and the higher the basal FSH levels, i.e. ovarian capacity or antral follicle count decreased with increasing age in an IVF cycle. Subjects within the age range of 21 – 30 yielded the most follicles and consequentially the most retrieved oocytes with their older counterparts within the age range of 41 – 50 yielding the least of both outcomes (Mean values; 20.92 and 20.31 vs. 8.44 and 7.28). Similar results were found by Hansen *et al.*, (2005) where the number of large follicles developing after stimulation with recombinant FSH was significantly greater in the younger subjects compared to that of the older group.

When basal hormonal markers of study subjects were compared with that of control subjects, only basal FSH showed statistical difference. However, mean basal FSH of control subjects which was 9.5 IU/L is very close to the set upper reference limit of 10 IU/L used by the study site. This gives concern for a bigger study to assess normal ranges of basal hormonal markers in Africa and most specifically the Ghanaian population.

Similar to a study done in Nigeria by Ikechebelu *et al.* in 2003, primary infertility was the most prevalent (80.7%) over 19.3% with secondary infertility. However, our finding was in contrast to some other studies done in Tanzania by Larsen *et al.*, (2006), Olatunji *et al.*, (2003) in Nigeria and by Fiander, (1990) in Ghana where secondary infertility was the predominant.We attribute our findings to the fact that IVF procedure is costly and women who already have children on their own will be quite reluctant to go in for such services unless under some pressing conditions. It is mostly women who have never given birth and under the pressures of society that will gather all their resources to attempt getting pregnant through in-vitro fertilization procedure.

Male only factor was the main cause of infertility in couples and this is in agreement with the study by Ikechebelu et al., (2003) in Nigeria. In Tanzania, Larsen *et al.*, (2006) found contrasting results where only 6.8% of their study populations were attributable to male only factors. Notwithstanding, male factors count a lot in infertility treatment and the focus of treatment should always be on the couple and not just the woman. The exact or varying types of male factors were not in the remit of this study. Unexplained factors were the most predominant of female factors of infertility in our study population followed by anovulatory, uterine, mixed/combined and tubal factors in that order. Predominance of unexplained factors could be attributed again to increasing age of women and for that matter in our study population before attempting pregnancy. Diminished ovarian reserve has been suggested by Miller *et al.*, (1999) as a putative cause of unexplained infertility. Other possible causes of unexplained infertility in older women can be due to decreased coital frequency and other lifestyle factors such as stress.

Subjects who were negative for the β-hCG test had fewer retrieved oocytes than their counterparts with positive β-hCG test. However, by the protocol of transferring at most

three (3) embryos after fertilization (Bancsi *et al.* 2002), there were enough oocytes for fertilization. We are of the opinion that, some other factors like the procedure of embryo transfer whether a stylet is used or not, trauma to the endometrium as a result of the embryo transfer procedure are responsible for achieving pregnancy in the IVF procedure.

Measured outcomes of ovarian stimulation were all better in subjects with normal basal FSH than those with higher FSH. Mean ovarian capacity of normal FSH was 16.71 as against 11.0 follicles by those with high FSH levels with a p-value <0.001. Mean retrieved oocytes was significantly different between subjects with a normal FSH and those with high FSH with a p- value <0.0001 (16.17 vs. 8.82 oocytes). Our results followed same pattern as that of Akande *et al.,* (2002). Similar results were found by Weghofer *et al.* in 2005; higher basal FSH levels lead to fewer eggs. Although subjects with higher basal FSH levels had fewer retrieved oocytes, we agree with suggestion by Wolff *et al.*, (2004) that patients with elevated basal FSH levels should not be excluded from IVF treatment. Although mean number of retrieved oocytes was lower in subjects with high FSH than their counterparts with normal FSH, the numbers of retrieved oocytes were adequate for fertilization and subsequent embryo transfer. At a mean fertilization rate of 50–60% in IVF, ≥ 4 oocytes are necessary to obtain ≥2 embryos as theorized by Bancsi *et al.* (2002), which is the intended number to be transferred in most women. From the study a mean number of 4 embryos will be yielded for transfer from mean oocytes of 8 in subjects with high basal FSH levels.

Again a good number of subjects with high basal FSH recorded a positive pregnancy test; 30.8% as compared to 38.6% pregnancy rate in subjects with normal basal FSH. We agree with Wolff *et al.*, (2004) and Abdalla *et al.*, (2004) that high basal FSH values

should only be used as guidelines in counseling patients but should not necessarily be a strong basis to discourage clients from undergoing a self IVF protocol.

By way of predicting ovarian response, our study reveals that basal FSH levels and FSH/LH ratio better predict response of subjects after ovarian stimulation protocol. There was significant difference ($P<0.05$) in medians between normal and poor responders for basal FSH levels (Median FSH; 9.7 vs. 12.7 respectively). This results corroborates the findings by Yong *et al.* (2003), who asserted that, the concentration of FSH in the early follicular phase showed a highly significant negative correlation with the number of oocytes recovered, but there were no significant correlations with the other basal hormones measured in this phase. The study results were similar to that of Bjercke *et al.* (2005) who found that none of the other basal markers; LH and prolactin showed any significant difference between normal and poor responders. Basal FSH emerged as the most significant contributor to the number of oocytes retrieved. However as mentioned earlier, basal FSH should not serve as a marker to exclude patients from fertility treatment, but should be interpreted according to patient's age and not in absolute terms, even within the generally considered normal range of ≤10IU/L. The study findings agree with findings of Cahill *et al.*, (1994) and Sharif *et al.*, (1998).

The FSH/LH ratio followed a similar pattern as that of basal FSH. Subjects with poor response had significantly higher FSH/LH ratios. Similar results were reported by Weghofer *et al.* (2005) and Liu *et al.* (2008). This suggests that in women with diminished ovarian reserve (defined by basasl FSH levels > 10.0 mIU/ml), high-normal LH levels (i.e. a low FSH/ LH ratio) represented a good prognostic indicator and low-normal LH levels (high FSH/LH ratio) represented a comparatively poor indicator of oocyte yield.

According to our study, none of the basal hormonal markers showed a significant correlation wit a positive β-hCG test. This goes to affirm other studies like that of Creus *et al.*, (2000) which suggests that the various available measures of ovarian reserve are better at predicting response to gonadotrophin stimulation than the chance of pregnancy. That notwithstanding, the measured outcomes of ovarian stimulation; ovarian capacity and number of retrieved oocytes both showed significant correlation to chemical pregnancy. Basal FSH had impact on measured outcomes of stimulation but had no direct significant correlation with pregnancy. Normal FSH level yields enough retrieved oocytes but it is unable to predict pregnancy. This is attributable to the fact that adequate number of oocytes is not the only requirement for a positive pregnancy test in IVF cycles. Other factors like the quality of sperm, number of oocytes that were successfully fertilized by sperm, the quality of the developing embryo and even the embryo transfer technique used could affect outcome of the procedure.

Basal FSH and FSH/LH ratio are the two best parameters to predict response of ovarian stimulation according to our study. They both showed negative correlation to measured outcomes of stimulation; ovarian capacity and number of retrieved oocytes. The other basal markers namely LH and prolactin levels had no significant correlation with the outcome of stimulation. Similarly Cahill *et al.* (1994), Sharif *et al.* (1998) and Ng *et al.*, (2005) all showed a negative correlation between basal FSH levels and number of oocytes retrieved.

Both obesity indices; BMI and WHR increased with age and consequently, a consistent reduction in ovarian capacity/ antral follicle count and number of retrieved oocytes. This is in line with findings of other studies by Wittemer *et al.*, (2000), Wang *et al.*, (2000) and Zaadstra *et al.*, (1993). Body mass and or abdominal obesity works in tandem and in the same direction as age as proposed by Sneed *et al.*, (2008) but when the effect of BMI and

WHR were independently assessed (Table 4.2), increasing values of BMI and WHR were associated with decrease in AFC and number of retrieved oocytes. Unlike the Sneed *et al.*, (2008) assertion that the effect of obesity on outcomes of stimulation was dependent on age, our study indicates that obesity indices are independent of age. Obesity has great impact on outcomes of in-vitro fertilization process because it has a heavy effect on the number of oocytes aspirated. From our study, both obesity indices showed significant negative correlation with outcomes of stimulation. Our findings were consistent with that of Fedorcsak *et al.* (2001) and Wang *et al.* (2000) but contrary to those of Dokras *et al.* (2006), Frattarelli *et al.* (2004) and Spandorfer *et al.* (2004); who found virtually no correlation between obesity indices and ovarian response. This could probably be due to the fact that in obese persons higher doses of ovarian stimulation drugs are perhaps needed to generate a sizable pool of follicles for fertilization.

The effects of regular exercise in reducing obesity have been documented by Homan *et al.*, (2007) and the effect of obesity on reproduction through IVF procedure also documented by Clark *et al.*, (1995); Clark *et al.*, (1998). This study is consistent with these studies since it was found that moderate to high exercise showed a progressive increase in mean follicular count/ovarian capacity from low to moderate to high; 13.07 to 16.55 to 18.25 respectively. Subjects who engaged in regular moderate exercises had the highest mean retrieved oocytes 17.82. The effect of regular exercise for a general health and well-being and for obesity prevention cannot be over-emphasized. From our study, all subjects who had a poor response to the ovarian stimulation protocol were engaged in very minimal or no exercise regimen at all. Rich-Edwards *et al.*, (2002) makes the assertion that exercise is associated with a reduction in risk of ovulatory infertility. Those who exercised regularly and as a result had a normal response of 5 oocytes or more after ovarian stimulation consequently had twice as much chemical pregnancy rate than those who did no or low exercise regimens according to our study.

Chapter 6

CONCLUSIONS

At a relatively younger age of 36years, fecund-ability begins to decline. Increasing age as has been demonstrated by this study is a negative prognosticator of outcomes of controlled ovarian stimulation in IVF cycles and is therefore indicative and stresses the need for child-bearing by Ghanaian women at ages earlier than 36years.

Primary infertility (80.7%) is predominant over secondary infertility (19.3%) in Ghanaian IVF seekers. This finding is attributable to the fact that IVF procedure in Ghana is expensive and women who already have children on their own will be quite reluctant to go in for such services unless under some pressing conditions. It is mostly women who have never given birth and under the pressures of society that will gather all their resources to attempt getting pregnant through in-vitro fertilization procedure. The predominance of primary infertility might not be the case in the general Ghanaian population. A bigger study spanning a wider cross-section of the Ghanaian population is required to ascertain this fact.

In Ghana, women are most often blamed when there are some difficulties with conception. From this study, male factor (39.4%) is the most predominant of infertility causes in the Ghanaian IVF centers. Most often than not in the fertility clinic, the women are the first to report for diagnosis and treatment. From the results of this study, men should be tested first before the women since semen analysis (male test) is less invasive than most diagnostic tests for women and also because male factors are the predominant.

The number of retrieved oocytes impacts positively on the pregnancy outcome in IVF procedures. From the study, a mean of 14 retrieved oocytes was yielded by those classified as normal responders to the controlled ovarian stimulation. The more oocytes there are, the higher the chance of pregnancy. In IVF procedures, the best embryos are selected for transfer. With a wider sample space of embryos to select from, the

embryologist stands a good chance to be able to select quality embryos for transfer whereas if there are only a few, he has no choice than to transfer whatever he has; good quality or not.

From this study higher than normal basal FSH levels leads to poor outcomes of ovarian capacity and retrieved oocytes in IVF cycles.The study also established that basal FSH increases with increasing age and thus a poor prognosticator of ovarian capacity and retrieved oocytes. However, on the basis of pregnancy, having a normal basal FSH does not have any significantly different advantage over those with high basal FSH. We therefore theorize that in the Ghanaian population, higher than normal basal FSH should not be used to deny patients the chance at self IVF cycles but they should be advised on the significant impact that high basal FSH has on measured outcomes such as retrieved oocytes and ovarian capacity.

Among all routine basal blood works, basal FSH and FSH/LH ratio better predicts outcome of controlled ovarian stimulation in IVF cycles but are unable to predict pregnancy. It is clear then that other factors such as the quality of sperm, quality of developing embryos and technique of embryo transfer impacts on whether a pregnancy results or otherwise in an IVF cycles.

The basal FSH of control fertile women was so close to the upper limit of normal, hence we propose for a wider study for the establishment of reference ranges of basal hormonal markers in the Ghanaian women

Regular exercise by women impacts positively on achieving a lean BMI and WHR and consequently on response to controlled ovarian stimulation in IVF cycles; hence, overweight and obese women trying to conceive should exercise regularly to reduce weight and better their chances in an IVF program.

A wider study is again proposed to score the effect of smoking, caffeine and alcohol use on IVF cycles since our study has very little data on subjects who engaged in such activities in the month prior to and in the month of procedure.

RECOMMENDATIONS

1. FSH/LH be used together with basal FSH levels in assessing and predicting response.
2. That subjects whose basal FSH levels are higher than the upper limit of 10IU/L be allowed to try self IVF and not directly prepared into donor IVF cycles since they have shown some success rate
3. Overweight and obese patients seeking assisted conception should be advised to keep regular exercise regimens to lose some weight
4. Other markers like serum estadiol be used together with ultrasound scan in assessing the ovarian pool to find the adequacy of the stimulation protocol. This can help in reducing the cases of OHSS
5. That further study be done in assessing the predictability of other markers like AMH and inhibin B in the Ghanaian population

REFERENCES

(1987) Infections, pregnancies, and infertility: perspectives on prevention. World Health Organization. *Fertil Steril* **47**(6)**:** 964-968.

Abdalla, H, Thum, MY (2004) An elevated basal FSH reflects a quantitative rather than qualitative decline of the ovarian reserve. *Hum Reprod* **19**(4)**:** 893-898.

Ahmed Ebbiary, NA, Lenton, EA, Cooke, ID (1994) Hypothalamic-pituitary ageing: progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women. *Clin Endocrinol (Oxf)* **41**(2)**:** 199-206.

Aitken, RJ (1997) Molecular mechanisms regulating human sperm function. *Mol Hum Reprod* **3**(3)**:** 169-173.

Akande, VA, Fleming, CF, Hunt, LP, Keay, SD, Jenkins, JM (2002) Biological versus chronological ageing of oocytes, distinguishable by raised FSH levels in relation to the success of IVF treatment. *Hum Reprod* **17**(8)**:** 2003-2008.

Astley, SJ, Bailey, D, Talbot, C, Clarren, SK (2000) Fetal alcohol syndrome (FAS) primary prevention through FAS diagnosis: I. Identification of high-risk birth mothers through the diagnosis of their children. *Alcohol Alcohol* **35**(5)**:** 499-508.

ASRM, (2008) Patient guidelines on assisted reproductive technologies.

Augestad, LB, Schei, B, Forsmo, S, Langhammer, A, Flanders, WD (2004) The association between physical activity and forearm bone mineral density in healthy premenopausal women. *J Womens Health (Larchmt)* **13**(3)**:** 301-313.

Augood, C, Duckitt, K, Templeton, AA (1998) Smoking and female infertility: a systematic review and meta-analysis. *Hum Reprod* **13**(6)**:** 1532-1539.

Bancsi, LF, Broekmans, FJ, Eijkemans, MJ, de Jong, FH, Habbema, JD, te Velde, ER (2002) Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertil Steril* **77**(2)**:** 328-336.

Bancsi, LF, Broekmans, FJ, Mol, BW, Habbema, JD, te Velde, ER (2003) Performance of basal follicle-stimulating hormone in the prediction of poor ovarian response and failure to become pregnant after in vitro fertilization: a meta-analysis. *Fertil Steril* **79**(5)**:** 1091-1100.

Baron, JA, La Vecchia, C, Levi, F (1990) The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol* **162**(2)**:** 502-514.

Bayasgalan, G, Naranbat, D, Tsedmaa, B, Tsogmaa, B, Sukhee, D, Amarjargal, O, Lhagvasuren, T, Radnaabazar, J, Rowe, PJ (2004) Clinical patterns and major causes of infertility in Mongolia. *J Obstet Gynaecol Res* **30**(5)**:** 386-393.

Bellver, J, Rossal, LP, Bosch, E, Zuniga, A, Corona, JT, Melendez, F, Gomez, E, Simon, C, Remohi, J, Pellicer, A (2003) Obesity and the risk of spontaneous abortion after oocyte donation. *Fertil Steril* **79**(5)**:** 1136-1140.

Bewley, S, Davies, M, Braude, P (2005) Which career first? *BMJ* **331**(7517)**:** 588-589.

Bjercke, S, Fedorcsak, P, Abyholm, T, Storeng, R, Ertzeid, G, Oldereid, N, Omland, A, Tanbo, T (2005) IVF/ICSI outcome and serum LH concentration on day 1 of ovarian stimulation with recombinant FSH under pituitary suppression. *Hum Reprod* **20**(9)**:** 2441-2447.

Braat, DD, Schoemaker, R, Schoemaker, J (1991) Life table analysis of fecundity in intravenously gonadotropin-releasing hormone-treated patients with normogonadotropic and hypogonadotropic amenorrhea. *Fertil Steril* **55**(2)**:** 266-271.

Broekmans, FJ, Kwee, J, Hendriks, DJ, Mol, BW, Lambalk, CB (2006) A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* **12**(6)**:** 685-718.

Burger, HG, Cahir, N, Robertson, DM, Groome, NP, Dudley, E, Green, A, Dennerstein, L (1998) Serum inhibins A and B fall differentially as FSH rises in perimenopausal women. *Clin Endocrinol (Oxf)* **48**(6)**:** 809-813.

Cahill, DJ, Prosser, CJ, Wardle, PG, Ford, WC, Hull, MG (1994) Relative influence of serum follicle stimulating hormone, age and other factors on ovarian response to gonadotrophin stimulation. *Br J Obstet Gynaecol* **101**(11)**:** 999-1002.

Chang, MY, Chiang, CH, Hsieh, TT, Soong, YK, Hsu, KH (1998) Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil Steril* **69**(3)**:** 505-510.

Chapin, RE, Harris, MW, Davis, BJ, Ward, SM, Wilson, RE, Mauney, MA, Lockhart, AC, Smialowicz, RJ, Moser, VC, Burka, LT, Collins, BJ (1997) The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *Fundam Appl Toxicol* **40**(1)**:** 138-157.

Chapin, RE, Robbins, WA, Schieve, LA, Sweeney, AM, Tabacova, SA, Tomashek, KM (2004) Off to a good start: the influence of pre- and periconceptional exposures, parental fertility, and nutrition on children's health. *Environ Health Perspect* **112**(1)**:** 69-78.

Chaudhuri, JD (2000) An analysis of the teratagenic effects that could possibly be due to alcohol consumption by pregnant mothers. *Indian J Med Sci* **54**(10)**:** 425-431.

Check, JH, Lurie, D, Callan, C, Baker, A, Benfer, K (1994) Comparison of the cumulative probability of pregnancy after in vitro fertilization-embryo transfer by infertility factor and age. *Fertil Steril* **61**(2)**:** 257-261.

Clark, AM, Ledger, W, Galletly, C, Tomlinson, L, Blaney, F, Wang, X, Norman, RJ (1995) Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Hum Reprod* **10**(10)**:** 2705-2712.

Clark, AM, Thornley, B, Tomlinson, L, Galletley, C, Norman, RJ (1998) Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. *Hum Reprod* **13**(6)**:** 1502-1505.

Cohen, MA, Lindheim, SR, Sauer, MV (1999) Donor age is paramount to success in oocyte donation. *Hum Reprod* **14**(11)**:** 2755-2758.

Correa, A, Gray, RH, Cohen, R, Rothman, N, Shah, F, Seacat, H, Corn, M (1996) Ethylene glycol ethers and risks of spontaneous abortion and subfertility. *Am J Epidemiol* **143**(7)**:** 707-717.

Cramer, DW, Barbieri, RL, Fraer, AR, Harlow, BL (2002) Determinants of early follicular phase gonadotrophin and estradiol concentrations in women of late reproductive age. *Hum Reprod* **17**(1)**:** 221-227.

Crellin, NK, Kang, HG, Swan, CL, Chedrese, PJ (2001) Inhibition of basal and stimulated progesterone synthesis by dichlorodiphenyldichloroethylene and methoxychlor in a stable pig granulosa cell line. *Reproduction* **121**(3)**:** 485-492.

Creus, M, Penarrubia, J, Fabregues, F, Vidal, E, Carmona, F, Casamitjana, R, Vanrell, JA, Balasch, J (2000) Day 3 serum inhibin B and FSH and age as predictors of assisted reproduction treatment outcome. *Hum Reprod* **15**(11)**:** 2341-2346.

Crha, I, Hruba, D, Fiala, J, Ventruba, P, Zakova, J, Petrenko, M (2001) The outcome of infertility treatment by in-vitro fertilisation in smoking and non-smoking women. *Cent Eur J Public Health* **9**(2)**:** 64-68.

Crosignani, PG, Ragni, G, Parazzini, F, Wyssling, H, Lombroso, G, Perotti, L (1994) Anthropometric indicators and response to gonadotrophin for ovulation induction. *Hum Reprod* **9**(3)**:** 420-423.

Csemiczky, G, Harlin, J, Fried, G (2002) Predictive power of clomiphene citrate challenge test for failure of in vitro fertilization treatment. *Acta Obstet Gynecol Scand* **81**(10)**:** 954-961.

Damstra, T (2002) Potential effects of certain persistent organic pollutants and endocrine disrupting chemicals on the health of children. *J Toxicol Clin Toxicol* **40**(4)**:** 457-465.

Danforth, DR, Arbogast, LK, Mroueh, J, Kim, MH, Kennard, EA, Seifer, DB, Friedman, CI (1998) Dimeric inhibin: a direct marker of ovarian aging. *Fertil Steril* **70**(1)**:** 119-123.

de Vet, A, Laven, JS, de Jong, FH, Themmen, AP, Fauser, BC (2002) Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* **77**(2)**:** 357-362.

Delbaere, A, Smits, G, Olatunbosun, O, Pierson, R, Vassart, G, Costagliola, S (2004) New insights into the pathophysiology of ovarian hyperstimulation syndrome. What makes the difference between spontaneous and iatrogenic syndrome? *Hum Reprod* **19**(3)**:** 486-489.

Delvigne, A, Rozenberg, S (2001) Preventive attitude of physicians to avoid OHSS in IVF patients. *Hum Reprod* **16**(12)**:** 2491-2495.

Develioglu, OH, Cox, B, Toner, JP, Oehninger, S, Muasher, SJ (1999) The value of basal serum follicle stimulating hormone, luteinizing hormone and oestradiol concentrations following pituitary down-regulation in predicting ovarian response to stimulation with highly purified follicle stimulating hormone. *Hum Reprod* **14**(5)**:** 1168-1174.

Diawara, MM, Chavez, KJ, Hoyer, PB, Williams, DE, Dorsch, J, Kulkosky, P, Franklin, MR (1999) A novel group of ovarian toxicants: the psoralens. *J Biochem Mol Toxicol* **13**(3-4)**:** 195-203.

Dokras, A, Baredziak, L, Blaine, J, Syrop, C, VanVoorhis, BJ, Sparks, A (2006) Obstetric outcomes after in vitro fertilization in obese and morbidly obese women. *Obstet Gynecol* **108**(1)**:** 61-69.

Eggert, J, Theobald, H, Engfeldt, P (2004) Effects of alcohol consumption on female fertility during an 18-year period. *Fertil Steril* **81**(2)**:** 379-383.

El-Toukhy, T, Khalaf, Y, Hart, R, Taylor, A, Braude, P (2002) Young age does not protect against the adverse effects of reduced ovarian reserve--an eight year study. *Hum Reprod* **17**(6)**:** 1519-1524.

Elchalal, U, Schenker, JG (1997) The pathophysiology of ovarian hyperstimulation syndrome--views and ideas. *Hum Reprod* **12**(6)**:** 1129-1137.

Eldar-Geva, T, Ben-Chetrit, A, Spitz, IM, Rabinowitz, R, Markowitz, E, Mimoni, T, Gal, M, Zylber-Haran, E, Margalioth, EJ (2005) Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. *Hum Reprod* **20**(11)**:** 3178-3183.

Elenbogen, A, Lipitz, S, Mashiach, S, Dor, J, Levran, D, Ben-Rafael, Z (1991) The effect of smoking on the outcome of in-vitro fertilization--embryo transfer. *Hum Reprod* **6**(2)**:** 242-244.

Elussein, EA, Magid, YM, Omer, MM, Adam, I (2008) Clinical patterns and major causes of infertility among Sudanese couples. *Trop Doct* **38**(4)**:** 243-244.

Evers, JL, Slaats, P, Land, JA, Dumoulin, JC, Dunselman, GA (1998) Elevated levels of basal estradiol-17beta predict poor response in patients with normal basal levels of follicle-stimulating hormone undergoing in vitro fertilization. *Fertil Steril* **69**(6)**:** 1010-1014.

Faber, BM, Mayer, J, Cox, B, Jones, D, Toner, JP, Oehninger, S, Muasher, SJ (1998) Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. *Fertil Steril* **69**(5)**:** 826-830.

Faber, BM, Mercan, R, Hamacher, P, Muasher, SJ, Toner, JP (1997) The impact of an egg donor's age and her prior fertility on recipient pregnancy outcome. *Fertil Steril* **68**(2)**:** 370-372.

Faddy, MJ, Gosden, RG (1995) A mathematical model of follicle dynamics in the human ovary. *Hum Reprod* **10**(4)**:** 770-775.

Fanchin, R, de Ziegler, D, Olivennes, F, Taieb, J, Dzik, A, Frydman, R (1994) Exogenous follicle stimulating hormone ovarian reserve test (EFORT): a simple and reliable screening test for detecting 'poor responders' in in-vitro fertilization. *Hum Reprod* **9**(9)**:** 1607-1611.

Fanchin, R, Schonauer, LM, Righini, C, Guibourdenche, J, Frydman, R, Taieb, J (2003) Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* **18**(2)**:** 323-327.

Fedorcsak, P, Dale, PO, Storeng, R, Ertzeid, G, Bjercke, S, Oldereid, N, Omland, AK, Abyholm, T, Tanbo, T (2004) Impact of overweight and underweight on assisted reproduction treatment. *Hum Reprod* **19**(11)**:** 2523-2528.

Fedorcsak, P, Dale, PO, Storeng, R, Tanbo, T, Abyholm, T (2001) The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome. *Hum Reprod* **16**(6)**:** 1086-1091.

Ferlitsch, K, Sator, MO, Gruber, DM, Rucklinger, E, Gruber, CJ, Huber, JC (2004) Body mass index, follicle-stimulating hormone and their predictive value in in vitro fertilization. *J Assist Reprod Genet* **21**(12)**:** 431-436.

Fiander, A (1990) Causes of infertility among 1000 patients in Ghana. *Trop Doct* **20**(3)**:** 137-138.

Foster, WG (2003) Do environmental contaminants adversely affect human reproductive physiology? *J Obstet Gynaecol Can* **25**(1)**:** 33-44.

Fraser, LR (1999) New insights into possible causes of male infertility. *Hum Reprod* **14 Suppl 1:** 38-46.

Frattarelli, JL, Kodama, CL (2004) Impact of body mass index on in vitro fertilization outcomes. *J Assist Reprod Genet* **21**(6)**:** 211-215.

Fried, G, Remaeus, K, Harlin, J, Krog, E, Csemiczky, G, Aanesen, A, Tally, M (2003) Inhibin B predicts oocyte number and the ratio IGF-I/IGFBP-1 may indicate oocyte quality during ovarian hyperstimulation for in vitro fertilization. *J Assist Reprod Genet* **20**(5)**:** 167-176.

Fujimoto, VY, Klein, NA, Battaglia, DE, Bremner, WJ, Soules, MR (1996) The anterior pituitary response to a gonadotropin-releasing hormone challenge test in normal older reproductive-age women. *Fertil Steril* **65**(3)**:** 539-544.

Gill, K, Boyle, A, Lake, K, Desaulniers, N (2000) Alcohol-induced locomotor activation in C57BL/6J, A/J, and AXB/BXA recombinant inbred mice: strain distribution patterns and quantitative trait loci analysis. *Psychopharmacology (Berl)* **150**(4)**:** 412-421.

Goransson, M, Magnusson, A, Bergman, H, Rydberg, U, Heilig, M (2003) Fetus at risk: prevalence of alcohol consumption during pregnancy estimated with a simple screening method in Swedish antenatal clinics. *Addiction* **98**(11)**:** 1513-1520.

Gougeon, A (1996) Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* **17**(2)**:** 121-155.

Gougeon, A, Ecochard, R, Thalabard, JC (1994) Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. *Biol Reprod* **50**(3)**:** 653-663.

Gray, LE, Ostby, J, Furr, J, Wolf, CJ, Lambright, C, Parks, L, Veeramachaneni, DN, Wilson, V, Price, M, Hotchkiss, A, Orlando, E, Guillette, L (2001) Effects of environmental antiandrogens on reproductive development in experimental animals. *Hum Reprod Update* **7**(3)**:** 248-264.

Green, BB, Daling, JR, Weiss, NS, Liff, JM, Koepsell, T (1986) Exercise as a risk factor for infertility with ovulatory dysfunction. *Am J Public Health* **76**(12)**:** 1432-1436.

Grodstein, F, Goldman, MB, Cramer, DW (1994) Infertility in women and moderate alcohol use. *Am J Public Health* **84**(9)**:** 1429-1432.

Haadsma, ML, Bukman, A, Groen, H, Roeloffzen, EM, Groenewoud, ER, Heineman, MJ, Hoek, A (2007) The number of small antral follicles (2-6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. *Hum Reprod* **22**(7)**:** 1925-1931.

Hakim, RB, Gray, RH, Zacur, H (1998) Alcohol and caffeine consumption and decreased fertility. *Fertil Steril* **70**(4)**:** 632-637.

Hall, JE, Welt, CK, Cramer, DW (1999) Inhibin A and inhibin B reflect ovarian function in assisted reproduction but are less useful at predicting outcome. *Hum Reprod* **14**(2)**:** 409-415.

Hamilton-Fairley, D, Kiddy, D, Watson, H, Paterson, C, Franks, S (1992) Association of moderate obesity with a poor pregnancy outcome in women with polycystic ovary syndrome treated with low dose gonadotrophin. *Br J Obstet Gynaecol* **99**(2)**:** 128-131.

Hansen, KR, Thyer, AC, Sluss, PM, Bremner, WJ, Soules, MR, Klein, NA (2005) Reproductive ageing and ovarian function: is the early follicular phase FSH rise necessary to maintain adequate secretory function in older ovulatory women? *Hum Reprod* **20**(1)**:** 89-95.

Hansen, LM, Batzer, FR, Gutmann, JN, Corson, SL, Kelly, MP, Gocial, B (1996) Evaluating ovarian reserve: follicle stimulating hormone and oestradiol variability during cycle days 2-5. *Hum Reprod* **11**(3)**:** 486-489.

Harlow, CR, Fahy, UM, Talbot, WM, Wardle, PG, Hull, MG (1996) Stress and stress-related hormones during in-vitro fertilization treatment. *Hum Reprod* **11**(2)**:** 274-279.

Harrison, KL, Breen, TM, Hennessey, JF (1990) The effect of patient smoking habit on the outcome of IVF and GIFT treatment. *Aust N Z J Obstet Gynaecol* **30**(4)**:** 340-342.

Hendriks, DJ, Broekmans, FJ, Bancsi, LF, de Jong, FH, Looman, CW, Te Velde, ER (2005a) Repeated clomiphene citrate challenge testing in the prediction of outcome in IVF: a comparison with basal markers for ovarian reserve. *Hum Reprod* **20**(1)**:** 163-169.

Hendriks, DJ, Broekmans, FJ, Bancsi, LF, Looman, CW, de Jong, FH, te Velde, ER (2005b) Single and repeated GnRH agonist stimulation tests compared with basal markers of ovarian reserve in the prediction of outcome in IVF. *J Assist Reprod Genet* **22**(2)**:** 65-73.

Hendriks, DJ, Broekmans, FJM, Bancsi, LFJMM, de Jong, FH, Looman, CWN, te Velde, ER (2005c) Repeated clomiphene citrate challenge testing in the prediction of outcome in IVF: a comparison with basal markers for ovarian reserve. *Hum. Reprod.* **20**(1)**:** 163-169.

Hollemaert, S, Wautrecht, JC, Capel, P, Abramowicz, MJ, Englert, Y, Delbaere, A (1996) Thrombosis associated with ovarian hyperstimulation syndrome in a carrier of the factor V Leiden mutation. *Thromb Haemost* **76**(2)**:** 275-277.

Homan, GF, Davies, M, Norman, R (2007) The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Hum Reprod Update* **13**(3)**:** 209-223.

Hughes, EG, Brennan, BG (1996) Does cigarette smoking impair natural or assisted fecundity? *Fertil Steril* **66**(5)**:** 679-689.

Hughes, EG, King, C, Wood, EC (1989) A prospective study of prognostic factors in in vitro fertilization and embryo transfer. *Fertil Steril* **51**(5)**:** 838-844.

Huhtaniemi, IT (2000) Polymorphism of gonadotropin action: clinical implications. *Asian J Androl* **2**(4)**:** 241-246.

Hull, MG, Glazener, CM, Kelly, NJ, Conway, DI, Foster, PA, Hinton, RA, Coulson, C, Lambert, PA, Watt, EM, Desai, KM (1985) Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* **291**(6510)**:** 1693-1697.

Ikechebelu, JI, Adinma, JI, Orie, EF, Ikegwuonu, SO (2003) High prevalence of male infertility in southeastern Nigeria. *J Obstet Gynaecol* **23**(6)**:** 657-659.

Jeremias, J, Witkin, SS (1996) Molecular approaches to the diagnosis of male infertility. *Mol Hum Reprod* **2**(3)**:** 195-202.

Jurema, MW, Bracero, NJ, Garcia, JE (2003) Fine tuning cycle day 3 hormonal assessment of ovarian reserve improves in vitro fertilization outcome in gonadotropin-releasing hormone antagonist cycles. *Fertil Steril* **80**(5)**:** 1156-1161.

Keen, CL, Clegg, MS, Hanna, LA, Lanoue, L, Rogers, JM, Daston, GP, Oteiza, P, Uriu-Adams, JY (2003) The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. *J Nutr* **133**(5 Suppl 2)**:** 1597S-1605S.

Kelce, WR, Lambright, CR, Gray, LE, Jr., Roberts, KP (1997) Vinclozolin and p,p'-DDE alter androgen-dependent gene expression: in vivo confirmation of an androgen receptor-mediated mechanism. *Toxicol Appl Pharmacol* **142**(1)**:** 192-200.

Kelce, WR, Stone, CR, Laws, SC, Gray, LE, Kemppainen, JA, Wilson, EM (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. *Nature* **375**(6532)**:** 581-585.

Kim, YK, Wasser, SK, Fujimoto, VY, Klein, NA, Moore, DE, Soules, MR (1997) Utility of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol

and FSH:LH ratio in predicting reproductive age in normal women. *Hum Reprod* **12**(6)**:** 1152-1155.

Klein, NA, Battaglia, DE, Fujimoto, VY, Davis, GS, Bremner, WJ, Soules, MR (1996) Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. *J Clin Endocrinol Metab* **81**(3)**:** 1038-1045.

Klonoff-Cohen, H (2005) Female and male lifestyle habits and IVF: what is known and unknown. *Human Reproduction Update* **11**(2)**:** 180-204.

Klonoff-Cohen, H, Lam-Kruglick, P, Gonzalez, C (2003) Effects of maternal and paternal alcohol consumption on the success rates of in vitro fertilization and gamete intrafallopian transfer. *Fertil Steril* **79**(2)**:** 330-339.

Klonoff-Cohen, H, Natarajan, L, Marrs, R, Yee, B (2001a) Effects of female and male smoking on success rates of IVF and gamete intra-Fallopian transfer. *Hum Reprod* **16**(7)**:** 1382-1390.

Klonoff-Cohen, H, Natarajan, L, Marrs, R, Yee, B (2001b) Effects of female and male smoking on success rates of IVF and gamete intra-Fallopian transfer. *Human Reproduction* **16**(7)**:** 1382-1390.

Krulewitch, CJ (2005) Alcohol consumption during pregnancy. *Annu Rev Nurs Res* **23:** 101-134.

Kumar, S (2004) Occupational exposure associated with reproductive dysfunction. *J Occup Health* **46**(1)**:** 1-19.

Kunzle, R, Mueller, MD, Hanggi, W, Birkhauser, MH, Drescher, H, Bersinger, NA (2003) Semen quality of male smokers and nonsmokers in infertile couples. *Fertil Steril* **79**(2)**:** 287-291.

Kwee, J, Elting, MW, Schats, R, Bezemer, PD, Lambalk, CB, Schoemaker, J (2003) Comparison of endocrine tests with respect to their predictive value on the outcome of ovarian hyperstimulation in IVF treatment: results of a prospective randomized study. *Hum. Reprod.* **18**(7)**:** 1422-1427.

Lahlou, N, Chabbert-Buffet, N, Christin-Maitre, S, Le Nestour, E, Roger, M, Bouchard, P (1999) Main inhibitor of follicle stimulating hormone in the luteal-follicular transition: inhibin A, oestradiol, or inhibin B? *Hum Reprod* **14**(5)**:** 1190-1193.

Larsen, U (2000) Primary and secondary infertility in sub-Saharan Africa. *International Journal of Epidemiology* **29**(2)**:** 285-291.

Larsen, U, Masenga, G, Mlay, J (2006) Infertility in a community and clinic-based sample of couples in Moshi, Northern Tanzania. *East Afr Med J* **83**(1)**:** 10-17.

Lashen, H, Ledger, W, Bernal, AL, Barlow, D (1999) Extremes of body mass do not adversely affect the outcome of superovulation and in-vitro fertilization. *Hum Reprod* **14**(3)**:** 712-715.

Lee, SJ, Lenton, EA, Sexton, L, Cooke, ID (1988) The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. *Hum Reprod* **3**(7)**:** 851-855.

Leke, RJ, Oduma, JA, Bassol-Mayagoitia, S, Bacha, AM, Grigor, KM (1993) Regional and geographical variations in infertility: effects of environmental, cultural, and socioeconomic factors. *Environ Health Perspect* **101 Suppl 2:** 73-80.

Lindenau, A, Fischer, B, Seiler, P, Beier, HM (1994) Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits. *Hum Reprod* **9**(5)**:** 772-780.

Lintsen, AME, Pasker-de Jong, PCM, de Boer, EJ, Burger, CW, Jansen, CAM, Braat, DDM, van Leeuwen, FE, on behalf of the, Opg (2005) Effects of subfertility cause, smoking and body weight on the success rate of IVF. *Human Reproduction* **20**(7)**:** 1867-1875.

Liu, KE, Greenblatt, EM (2008) Elevated day 3 follicle-stimulating hormone/luteinizing hormone ratio >or= 2 is associated with higher rates of cancellation in in vitro fertilization-embryo transfer cycles. *Fertil Steril* **90**(2)**:** 297-301.

Loumaye, E, Billion, JM, Mine, JM, Psalti, I, Pensis, M, Thomas, K (1990) Prediction of individual response to controlled ovarian hyperstimulation by means of a clomiphene citrate challenge test. *Fertil Steril* **53**(2)**:** 295-301.

Lucero, J, Harlow, BL, Barbieri, RL, Sluss, P, Cramer, DW (2001) Early follicular phase hormone levels in relation to patterns of alcohol, tobacco, and coffee use. *Fertil Steril* **76**(4)**:** 723-729.

Luisi, S, Florio, P, Reis, FM, Petraglia, F (2005) Inhibins in female and male reproductive physiology: role in gametogenesis, conception, implantation and early pregnancy. *Human Reproduction Update* **11**(2)**:** 123-135.

Maheshwari, A, Fowler, P, Bhattacharya, S (2006) Assessment of ovarian reserve--should we perform tests of ovarian reserve routinely? *Hum Reprod* **21**(11)**:** 2729-2735.

Maheshwari, A, Hamilton, M, Bhattacharya, S (2008) Effect of female age on the diagnostic categories of infertility. *Hum Reprod* **23**(3)**:** 538-542.

McIlveen, M, Skull, JD, Ledger, WL (2007) Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population. *Hum. Reprod.* **22**(3)**:** 778-785.

Meldrum, DR (1993) Female reproductive aging--ovarian and uterine factors. *Fertil Steril* **59**(1)**:** 1-5.

Molarius, A, Seidell, JC, Sans, S, Tuomilehto, J, Kuulasmaa, K (1999) Waist and hip circumferences, and waist-hip ratio in 19 populations of the WHO MONICA Project. *Int J Obes Relat Metab Disord* **23**(2)**:** 116-125.

Moore, VM, Davies, MJ (2005) Diet during pregnancy, neonatal outcomes and later health. *Reprod Fertil Dev* **17**(3)**:** 341-348.

Mukherjee, T, Copperman, AB, Lapinski, R, Sandler, B, Bustillo, M, Grunfeld, L (1996) An elevated day three follicle-stimulating hormone:luteinizing hormone ratio (FSH:LH) in the presence of a normal day 3 FSH predicts a poor response to controlled ovarian hyperstimulation. *Fertil Steril* **65**(3)**:** 588-593.

Muttukrishna, S, McGarrigle, H, Wakim, R, Khadum, I, Ranieri, DM, Serhal, P (2005) Antral follicle count, anti-mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? *BJOG* **112**(10)**:** 1384-1390.

Navot, D, Bergh, PA, Laufer, N (1992) Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. *Fertil Steril* **58**(2)**:** 249-261.

Navot, D, Drews, MR, Bergh, PA, Guzman, I, Karstaedt, A, Scott, RT, Jr., Garrisi, GJ, Hofmann, GE (1994) Age-related decline in female fertility is not due to diminished capacity of the uterus to sustain embryo implantation. *Fertil Steril* **61**(1)**:** 97-101.

Navot, D, Rosenwaks, Z, Margalioth, EJ (1987) Prognostic assessment of female fecundity. *Lancet* **2**(8560)**:** 645-647.

Neal, MS, Hughes, EG, Holloway, AC, Foster, WG (2005a) Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes. *Hum Reprod* **20**(9)**:** 2531-2535.

Neal, MS, Hughes, EG, Holloway, AC, Foster, WG (2005b) Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes. *Human Reproduction* **20**(9)**:** 2531-2535.

Ng, EHY, Chan, CCW, Tang, OS, Ho, PC (2005) Antral follicle count and FSH concentration after clomiphene citrate challenge test in the prediction of ovarian response during IVF treatment. *Hum. Reprod.* **20**(6)**:** 1647-1654.

Ng, SY, Songra, AK, Bradley, PF (2003) A new approach using intraoperative ultrasound imaging for the localization and removal of multiple foreign bodies in the neck. *Int J Oral Maxillofac Surg* **32**(4)**:** 433-436.

Nichols, JE, Crane, MM, Higdon, HL, Miller, PB, Boone, WR (2003) Extremes of body mass index reduce in vitro fertilization pregnancy rates. *Fertil Steril* **79**(3)**:** 645-647.

Norman, RJ, Noakes, M, Wu, R, Davies, MJ, Moran, L, Wang, JX (2004) Improving reproductive performance in overweight/obese women with effective weight management. *Hum Reprod Update* **10**(3)**:** 267-280.

Olatunji, AO, Sule-Odu, AO (2003) The pattern of infertility cases at a university hospital. *West Afr J Med* **22**(3)**:** 205-207.

Oliva, A, Spira, A, Multigner, L (2001) Contribution of environmental factors to the risk of male infertility. *Hum Reprod* **16**(8)**:** 1768-1776.

Ombelet, W, Cooke, I, Dyer, S, Serour, G, Devroey, P (2008) Infertility and the provision of infertility medical services in developing countries. *Hum Reprod Update* **14**(6)**:** 605-621.

Oosterhuis, GJ, Vermes, I, Michgelsen, HW, Schoemaker, J, Lambalk, CB (2002) Follicle stimulating hormone measured in unextracted urine throughout the menstrual cycle correlates with age and ovarian reserve. *Hum Reprod* **17**(3)**:** 641-646.

Padilla, SL, Bayati, J, Garcia, JE (1990) Prognostic value of the early serum estradiol response to leuprolide acetate in in vitro fertilization. *Fertil Steril* **53**(2)**:** 288-294.

Palermo, R (2007) Differential actions of FSH and LH during folliculogenesis. *Reprod Biomed Online* **15**(3)**:** 326-337.

Parker, L, Pearce, MS, Dickinson, HO, Aitkin, M, Craft, AW (1999) Stillbirths among offspring of male radiation workers at Sellafield nuclear reprocessing plant. *Lancet* **354**(9188)**:** 1407-1414.

Pearlstone, AC, Fournet, N, Gambone, JC, Pang, SC, Buyalos, RP (1992) Ovulation induction in women age 40 and older: the importance of basal follicle-stimulating hormone level and chronological age. *Fertil Steril* **58**(4)**:** 674-679.

Pellicer, A, Lightman, A, Diamond, MP, Russell, JB, DeCherney, AH (1987) Outcome of in vitro fertilization in women with low response to ovarian stimulation. *Fertil Steril* **47**(5)**:** 812-815.

Penarrubia, J, Fabregues, F, Manau, D, Creus, M, Carmona, F, Casamitjana, R, Vanrell, JA, Balasch, J (2005a) Previous cycle cancellation due to poor follicular development as a predictor of ovarian response in cycles stimulated with gonadotrophin-releasing hormone agonist-gonadotrophin treatment. *Hum Reprod* **20**(3)**:** 622-628.

Penarrubia, J, Fabregues, F, Manau, D, Creus, M, Casals, G, Casamitjana, R, Carmona, F, Vanrell, JA, Balasch, J (2005b) Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist--gonadotropin treatment. *Hum Reprod* **20**(4)**:** 915-922.

Randall, CL (1987) Alcohol as a teratogen: a decade of research in review. *Alcohol Alcohol Suppl* **1:** 125-132.

Ranieri, DM, Phophong, P, Khadum, I, Meo, F, Davis, C, Serhal, P (2001) Simultaneous evaluation of basal FSH and oestradiol response to GnRH analogue (F-G-test) allows effective drug regimen selection for IVF. *Hum. Reprod.* **16**(4)**:** 673-675.

Ranieri, DM, Quinn, F, Makhlouf, A, Khadum, I, Ghutmi, W, McGarrigle, H, Davies, M, Serhal, P (1998) Simultaneous evaluation of basal follicle-stimulating hormone and 17 beta-estradiol response to gonadotropin-releasing hormone

analogue stimulation: an improved predictor of ovarian reserve. *Fertil Steril* **70**(2)**:** 227-233.

Rannevik, G, Jeppsson, S, Johnell, O, Bjerre, B, Laurell-Borulf, Y, Svanberg, L (1995) A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. *Maturitas* **21**(2)**:** 103-113.

Rich-Edwards, JW, Spiegelman, D, Garland, M, Hertzmark, E, Hunter, DJ, Colditz, GA, Willett, WC, Wand, H, Manson, JE (2002) Physical activity, body mass index, and ovulatory disorder infertility. *Epidemiology* **13**(2)**:** 184-190.

Roest, J, van Heusden, AM, Mous, H, Zeilmaker, GH, Verhoeff, A (1996) The ovarian response as a predictor for successful in vitro fertilization treatment after the age of 40 years. *Fertil Steril* **66**(6)**:** 969-973.

Roseveare, P, Robinson, D, Allen, K, Sewell, E, Cheal, C, Sikorski, J, Clark, B, Thomas, S, Leap, N (1992) Provision of home births. *BMJ* **304**(6841)**:** 1569.

Roth, D, Grazi, RV, Lobel, SM (2003) Extremes of body mass index do not affect first-trimester pregnancy outcome in patients with infertility. *Am J Obstet Gynecol* **188**(5)**:** 1169-1170.

Ruess, ML, Kline, J, Santos, R, Levin, B, Timor-Tritsch, I (1996) Age and the ovarian follicle pool assessed with transvaginal ultrasonography. *Am J Obstet Gynecol* **174**(2)**:** 624-627.

Sanders, TA (2004) Diet and general health: dietary counselling. *Caries Res* **38 Suppl 1:** 3-8.

Scheffer, GJ, Broekmans, FJ, Dorland, M, Habbema, JD, Looman, CW, te Velde, ER (1999) Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. *Fertil Steril* **72**(5)**:** 845-851.

Scheffer, GJ, Broekmans, FJ, Looman, CW, Blankenstein, M, Fauser, BC, teJong, FH, teVelde, ER (2003) The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. *Hum Reprod* **18**(4)**:** 700-706.

Schieve, LA, Davis, F, Roeske, J, Handler, A, Freels, S, Stinchcomb, T, Keane, A (1997) Evaluation of internal alpha-particle radiation exposure and subsequent fertility among a cohort of women formerly employed in the radium dial industry. *Radiat Res* **147**(2)**:** 236-244.

Schreiber, CA, Sammel, M, Hillier, SL, Barnhart, KT (2009) A little bit pregnant: modeling how the accurate detection of pregnancy can improve HIV prevention trials. *Am J Epidemiol* **169**(4)**:** 515-521.

Scott, RT, Jr. (2004) Diminished ovarian reserve and access to care. *Fertil Steril* **81**(6)**:** 1489-1492; discussion 1496-1487.

Scott, RT, Jr., Illions, EH, Kost, ER, Dellinger, C, Hofmann, GE, Navot, D (1993) Evaluation of the significance of the estradiol response during the clomiphene citrate challenge test. *Fertil Steril* **60**(2)**:** 242-246.

Scott, RT, Opsahl, MS, Leonardi, MR, Neall, GS, Illions, EH, Navot, D (1995) Life table analysis of pregnancy rates in a general infertility population relative to ovarian reserve and patient age. *Hum Reprod* **10**(7)**:** 1706-1710.

Scott, RT, Toner, JP, Muasher, SJ, Oehninger, S, Robinson, S, Rosenwaks, Z (1989) Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. *Fertil Steril* **51**(4)**:** 651-654.

Seifer, DB, Lambert-Messerlian, G, Hogan, JW, Gardiner, AC, Blazar, AS, Berk, CA (1997) Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. *Fertil Steril* **67**(1)**:** 110-114.

Seifer, DB, MacLaughlin, DT, Christian, BP, Feng, B, Shelden, RM (2002) Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril* **77**(3)**:** 468-471.

Seifer, DB, Scott, RT, Jr., Bergh, PA, Abrogast, LK, Friedman, CI, Mack, CK, Danforth, DR (1999) Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. *Fertil Steril* **72**(1)**:** 63-65.

Serri, O, Chik, CL, Ur, E, Ezzat, S (2003) Diagnosis and management of hyperprolactinemia. *CMAJ* **169**(6)**:** 575-581.

Shah, K, Sivapalan, G, Gibbons, N, Tempest, H, Griffin, DK (2003) The genetic basis of infertility. *Reproduction* **126**(1)**:** 13-25.

Sharif, K, Elgendy, M, Lashen, H, Afnan, M (1998) Age and basal follicle stimulating hormone as predictors of in vitro fertilisation outcome. *Br J Obstet Gynaecol* **105**(1)**:** 107-112.

Shiloh, H, Lahav-Baratz, S, Koifman, M, Ishai, D, Bidder, D, Weiner-Meganzi, Z, Dirnfeld, M (2004) The impact of cigarette smoking on zona pellucida thickness of oocytes and embryos prior to transfer into the uterine cavity. *Hum Reprod* **19**(1)**:** 157-159.

Shrim, A, Elizur, SE, Seidman, DS, Rabinovici, J, Wiser, A, Dor, J (2006) Elevated day 3 FSH/LH ratio due to low LH concentrations predicts reduced ovarian response. *Reprod Biomed Online* **12**(4)**:** 418-422.

Simoni, M, Gromoll, J, Nieschlag, E (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr Rev* **18**(6)**:** 739-773.

Smits, G, Olatunbosun, O, Delbaere, A, Pierson, R, Vassart, G, Costagliola, S (2003) Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med* **349**(8)**:** 760-766.

Sneed, ML, Uhler, ML, Grotjan, HE, Rapisarda, JJ, Lederer, KJ, Beltsos, AN (2008) Body mass index: impact on IVF success appears age-related â€ *Human Reproduction* **23**(8)**:** 1835-1839.

Soto, AM, Sonnenschein, C, Chung, KL, Fernandez, MF, Olea, N, Serrano, FO (1995) The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Health Perspect* **103 Suppl 7:** 113-122.

Spandorfer, SD, Kump, L, Goldschlag, D, Brodkin, T, Davis, OK, Rosenwaks, Z (2004) Obesity and in vitro fertilization: negative influences on outcome. *J Reprod Med* **49**(12)**:** 973-977.

Surrey, ES, Bower, J, Hill, DM, Ramsey, J, Surrey, MW (1998) Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertil Steril* **69**(3)**:** 419-424.

Tanbo, T, Dale, PO, Lunde, O, Norman, N, Abyholm, T (1992) Prediction of response to controlled ovarian hyperstimulation: a comparison of basal and clomiphene citrate-stimulated follicle-stimulating hormone levels. *Fertil Steril* **57**(4)**:** 819-824.

Taskinen, H, Kyyronen, P, Hemminki, K, Hoikkala, M, Lajunen, K, Lindbohm, ML (1994) Laboratory work and pregnancy outcome. *J Occup Med* **36**(3)**:** 311-319.

The, ECWG (2006) Nutrition and reproduction in women. *Human Reproduction Update* **12**(3)**:** 193-207.

Tielemans, E, van Kooij, R, Looman, C, Burdorf, A, te Velde, E, Heederik, D (2000) Paternal occupational exposures and embryo implantation rates after IVF. *Fertil Steril* **74**(4)**:** 690-695.

Tinkanen, H, Blauer, M, Laippala, P, Tuohimaa, P, Kujansuu, E (2001) Correlation between serum inhibin B and other indicators of the ovarian function. *Eur J Obstet Gynecol Reprod Biol* **94**(1)**:** 109-113.

Toner, JP (2003) Age = egg quality, FSH level = egg quantity. *Fertil Steril* **79**(3)**:** 491.

Toner, JP (2004) Modest follicle-stimulating hormone elevations in younger women: warn but don't disqualify. *Fertil Steril* **81**(6)**:** 1493-1495; discussion 1496-1497.

Toner, JP (1993) The significance of elevated FSH for reproductive function. *Baillieres Clin Obstet Gynaecol* **7**(2)**:** 283-295.

Toner, JP, Philput, CB, Jones, GS, Muasher, SJ (1991) Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. *Fertil Steril* **55**(4)**:** 784-791.

Trapp, M, Kemeter, P, Feichtinger, W (1986) Smoking and in-vitro fertilization. *Hum Reprod* **1**(6)**:** 357-358.

Uehara, S, Hashiyada, M, Sato, K, Sato, Y, Fujimori, K, Okamura, K (2001) Preferential X-chromosome inactivation in women with idiopathic recurrent pregnancy loss. *Fertil Steril* **76**(5)**:** 908-914.

Umezulike, AC, Efetie, ER (2004) The psychological trauma of infertility in Nigeria. *Int J Gynaecol Obstet* **84**(2)**:** 178-180.

van Montfrans, JM, van Hooff, MHA, Huirne, JA, Tanahatoe, SJ, Sadrezadeh, S, Martens, F, van Vugt, JMG, Lambalk, CB (2004) Basal FSH concentrations as a marker of ovarian ageing are not related to pregnancy outcome in a general population of women over 30 years. *Hum. Reprod.* **19**(2)**:** 430-434.

van Noord-Zaadstra, BM, Looman, CW, Alsbach, H, Habbema, JD, te Velde, ER, Karbaat, J (1991) Delaying childbearing: effect of age on fecundity and outcome of pregnancy. *BMJ* **302**(6789)**:** 1361-1365.

van Rooij, IA, Bancsi, LF, Broekmans, FJ, Looman, CW, Habbema, JD, te Velde, ER (2003) Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in in vitro fertilization. *Fertil Steril* **79**(3)**:** 482-488.

van Swieten, EC, van der Leeuw-Harmsen, L, Badings, EA, van der Linden, PJ (2005) Obesity and Clomiphene Challenge Test as predictors of outcome of in vitro fertilization and intracytoplasmic sperm injection. *Gynecol Obstet Invest* **59**(4)**:** 220-224.

Van Voorhis, BJ, Dawson, JD, Stovall, DW, Sparks, AE, Syrop, CH (1996) The effects of smoking on ovarian function and fertility during assisted reproduction cycles. *Obstet Gynecol* **88**(5)**:** 785-791.

van Zonneveld, P, Scheffer, GJ, Broekmans, FJ, Blankenstein, MA, de Jong, FH, Looman, CW, Habbema, JD, te Velde, ER (2003) Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. *Hum Reprod* **18**(3)**:** 495-501.

Wang, JX, Davies, M, Norman, RJ (2000) Body mass and probability of pregnancy during assisted reproduction treatment: retrospective study. *BMJ* **321**(7272)**:** 1320-1321.

Wang, JX, Davies, MJ, Norman, RJ (2001) Polycystic ovarian syndrome and the risk of spontaneous abortion following assisted reproductive technology treatment. *Hum Reprod* **16**(12)**:** 2606-2609.

Weghofer, A, Gleicher, N (2009) Ovarian function: a theory of relativity. *Hum Reprod* **24**(1)**:** 17-19.

Weghofer, A, Margreiter, M, Fauster, Y, Schaetz, T, Brandstetter, A, Boehm, D, Feichtinger, W (2005) Age-specific FSH levels as a tool for appropriate patient counselling in assisted reproduction. *Hum Reprod* **20**(9)**:** 2448-2452.

Weigert, M, Hofstetter, G, Kaipl, D, Gottlich, H, Krischker, U, Bichler, K, Poehl, M, Feichtinger, W (1999) The effect of smoking on oocyte quality and hormonal parameters of patients undergoing in vitro fertilization-embryo transfer. *J Assist Reprod Genet* **16**(6)**:** 287-293.

Wennborg, H, Bodin, L, Vainio, H, Axelsson, G (2000) Pregnancy outcome of personnel in Swedish biomedical research laboratories. *J Occup Environ Med* **42**(4)**:** 438-446.

Windham, GC, Fenster, L, Swan, SH (1992) Moderate maternal and paternal alcohol consumption and the risk of spontaneous abortion. *Epidemiology* **3**(4)**:** 364-370.

Winslow, KL, Toner, JP, Brzyski, RG, Oehninger, SC, Acosta, AA, Muasher, SJ (1991) The gonadotropin-releasing hormone agonist stimulation test--a sensitive predictor of performance in the flare-up in vitro fertilization cycle. *Fertil Steril* **56**(4)**:** 711-717.

Wittemer, C, Ohl, J, Bettahar-Lebugle, K, Viville, S, Nisand, I (2000) A quantitative and morphological analysis of oocytes collected during 438 IVF cycles. *J Assist Reprod Genet* **17**(1)**:** 44-50.

Wolff, EF, Taylor, HS (2004) Value of the day 3 follicle-stimulating hormone measurement. *Fertil Steril* **81**(6)**:** 1486-1488; discussion 1496-1487.

Wong, WY, Zielhuis, GA, Thomas, CM, Merkus, HM, Steegers-Theunissen, RP (2003) New evidence of the influence of exogenous and endogenous factors on sperm count in man. *Eur J Obstet Gynecol Reprod Biol* **110**(1)**:** 49-54.

Wright, KP, Trimarchi, JR, Allsworth, J, Keefe, D (2006) The effect of female tobacco smoking on IVF outcomes. *Human Reproduction* **21**(11)**:** 2930-2934.

Yanushpolsky, EH, Hurwitz, S, Tikh, E, Racowsky, C (2003) Predictive usefulness of cycle day 10 follicle-stimulating hormone level in a clomiphene citrate challenge test for in vitro fertilization outcome in women younger than 40 years of age. *Fertil Steril* **80**(1)**:** 111-115.

Yong, PY, Baird, DT, Thong, KJ, McNeilly, AS, Anderson, RA (2003) Prospective analysis of the relationships between the ovarian follicle cohort and basal FSH concentration, the inhibin response to exogenous FSH and ovarian follicle number at different stages of the normal menstrual cycle and after pituitary down-regulation. *Hum Reprod* **18**(1)**:** 35-44.

You, L, Sar, M, Bartolucci, E, Ploch, S, Whitt, M (2001) Induction of hepatic aromatase by p,p'-DDE in adult male rats. *Mol Cell Endocrinol* **178**(1-2)**:** 207-214.

Younglai, EV, Foster, WG, Hughes, EG, Trim, K, Jarrell, JF (2002) Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. *Arch Environ Contam Toxicol* **43**(1)**:** 121-126.

Younglai, EV, Holloway, AC, Foster, WG (2005a) Environmental and occupational factors affecting fertility and IVF success. *Hum Reprod Update* **11**(1)**:** 43-57.

Younglai, EV, Holloway, AC, Foster, WG (2005b) Environmental and occupational factors affecting fertility and IVF success. *Human Reproduction Update* **11**(1)**:** 43-57.

Zaadstra, BM, Looman, CW, te Velde, ER, Habbema, JD, Karbaat, J (1994) Moderate drinking: no impact on female fecundity. *Fertil Steril* **62**(5)**:** 948-954.

Zaadstra, BM, Seidell, JC, Van Noord, PA, te Velde, ER, Habbema, JD, Vrieswijk, B, Karbaat, J (1993) Fat and female fecundity: prospective study of effect of body fat distribution on conception rates. *BMJ* **306**(6876)**:** 484-487.

Zenzes, MT (2000) Smoking and reproduction: gene damage to human gametes and embryos. *Hum Reprod Update* **6**(2)**:** 122-131.

Zenzes, MT, Bielecki, R, Reed, TE (1999) Detection of benzo(a)pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. *Fertil Steril* **72**(2)**:** 330-335.

Zitzmann, M, Rolf, C, Nordhoff, V, Schrader, G, Rickert-Fohring, M, Gassner, P, Behre, HM, Greb, RR, Kiesel, L, Nieschlag, E (2003) Male smokers have a decreased success rate for in vitro fertilization and intracytoplasmic sperm injection. *Fertil Steril* **79 Suppl 3:** 1550-1554.