LABORATORY EVALUATION OF GHANAIAN WOMEN ATTENDING FERTILITY CLINIC AT LISTER HOSPITAL AND FERTILITY CENTRE, ACCRA-GHANA

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DECLARATION

The experimental work described in this thesis was carried out at the laboratory Department of Lister Hospital and fertility Center, Accra. This work has not been submitted for any other degree.

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ABSTRACT

Over 70 million couples suffer from infertility worldwide and the majority of these couples can be found in developing countries. In comparison to the Western world the negative consequences of childlessness are experienced to a greater degree in developing countries. Sexually transmitted diseases and hormonal imbalances are the most common cause of infertility in developing countries, and this can be treated with assisted reproductive technologies (ART). The unavailability or the expensiveness of new reproductive technologies in developing countries is a problem. This study aims to determine if there is any significant difference in the haematological and hormonal profiles of infertile and fertile women and also the prevalence of sexually transmitted infections, toxoplasmosis and rubella immunity in infertile women. Three hundred and forty coagulated and non coagulated blood and high vaginal swab samples were collected from women within the age range of 24yrs to 58yrs attending infertility (300) and post natal (40) clinics at Lister Hospital and Fertility centre, Accra in Ghana. The enzyme-linked immune-sorbent assay (ELISA) method was used in analyzing the fertility hormones using the NoviWell™ assay kits (HySkill Diagnostics, Bahlingen, Germany) and the serological tests using the Orgenics® assay kits (Inverness Medical Innovations Group, Yavne, Israel).The haematological parameters were done using the QBC® Autoread Plus haematological analyzer (QBC Diagnostic Incorporated, Philipsburg, Pennsylvania, USA) and the gonorrhoea infection test using the Gram's staining technique in Medical Laboratory Manual for Tropical Countries (Tropical Health Technology, Doddington, United Kingdom). The hormonal profile in the infertile women were higher: testosterone (1.86 ± 0.24) , follicle stimulating hormone (11.51 ± 0.84) and prolactin (22.36 ± 0.67) than the fertile subjects: testosterone (0.48 ± 0.04) , follicle stimulating hormone (6.99 ± 0.27) and prolactin (12.05 ± 0.62). From this study, the prevalence of toxoplasma gondii infection was similar in both fertile (27.5%) and infertile (20.0%) women and this increased with age. There was no significant difference in the prevalence of non-immunity to rubella among infertile (26%) and fertile (30%) subjects in this study. Though the prevalence of sexually transmitted infections in the infertile women was higher, it did not reach statistically significant level. The hormonal profile and the prevalence of Toxoplasma gondii in infertile women were higher comparatively to their fertile counterpart.

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Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

There is no standard definition of normal fertility. Biologically, inability to procreate can be classified as infertility (the inability of a couple to conceive) or infecundity (the inability of a couple to produce a live birth) (Healy et al., 1994). The word "barren" conveys the trying, potent emotional toll that failure to produce children exerts on a couple (Lukse and Vacc, 1999). In a study by Ombelet et al., (2008) they gave a background that more than seventy million couples worldwide suffer from infertility, the majority being residents of developing countries. Also negative consequences of childlessness are experienced to a greater degree in developing countries when compared with Western societies. Bilateral tubal occlusion due to sexually transmitted diseases and pregnancy-related infections is the most common cause of infertility in developing countries, a condition that is potentially treatable with assisted reproductive technologies (ART) (Ombelet et al., 2008). New reproductive technologies are either unavailable or very costly in developing countries (Ombelet et al., 2008). The importance of male infertility should not be minimized but this paper, however, focuses on the medical importance of infertility treatments among women.

During their reproductive lives, 10% to 15% of couples are unable to achieve conception and deliver a living child after one year of unprotected coitus (Healy et al., 1994; Speroff et al., 1999). The proportion of women unable to bear children increases with age (Rosene-Montella et al., 2000). Among the Hutterite Brethren of North America, contraception is discouraged and there is no incentive to limit family size. Although the fecundity of Hutterite women is legendary, Teitze, (1957) found that 11% bore no children after 34 years of age, 33% bore no children after 40 years of age, and 87% of women were infertile by the time they reached 45 years of age. Approximately one third of women who defer pregnancy until their middle to

late 30 years and half of women who defer pregnancy until after 40 years of age will be unable to conceive (Gilbert *et al.*, 1999). Advancing maternal age is associated with a higher risk for maternal illness, which may in turn be associated with infertility or increased obstetric risk (Rosene-Montella *et al.*, 2000).

Public health officials originally introduced the term sexually transmitted infection (STI), which clinicians are increasingly using alongside the term sexually transmitted disease (STD) in order to distinguish it from the former. The most frequently asked question is "are sexually transmitted infections (STIs) different from sexually transmitted diseases (STDs)?, In general, an STI is an infection that has a negligible probability of transmission by means other than sexual contact, but has a realistic means of transmission by sexual contact (more sophisticated means eg. blood transfusion, sharing of hypodermic needles are not taken into account). Thus, one may presume that, if a person is infected with an STI, e.g., Human Immunodeficiency Virus (HIV), gonorrhoea, Hepatitis B Virus (HBV), it was transmitted to him/her by means of sexual contact.

Specifically, the term STD refers only to infections that are causing symptoms. Sometimes the terms STI and STD are used interchangeably. This can be confusing and not always accurate, so it helps first to understand the difference between infection and disease. Infection simply means that a germ (virus, bacteria, or parasite) that can cause disease or sickness is present inside a person's body. An infected person does not necessarily have any symptoms or signs that the virus or bacteria is actually hurting his or her body; they do not necessarily feel sick. A disease means that the infection is actually causing the infected person to feel sick, or to notice something is wrong. For this reason, the term STI which refers to infection with any germ that can cause an STD, even if the infected person has no symptoms is a much broader term than STD.

Hormonal dysfunction is the inability of a hormone to exert its expected impact due to its insufficiency or overproduction. This may lead to a lot of adverse

medical conditions eg, infertility and this situation may be reversed if the underlying cause is corrected. In this study the focus is on the fertility hormones and infertility could be caused by hormonal insufficiency (hypogonadotrophic hypogonadism-secondary) or hormonal overproduction (hypergonadotrophic hypogonadism-primary). Hormonal disorders of the female reproductive system include an assortment of problems resulting from aberrant operation of the hypothalamic-pituitary-ovarian axis (Scott et al., 1989).These relatively common disorders often lead to infertility and are now more readily understood, diagnosed, and treated (Burger and Baker, 1987; DeCherney et al., 1988). Measurements of peptide and steroid hormones in serum play a key role in both the investigation and the treatment of female infertility and reproductive problems (Nanji, 1984). Appropriate testing varies widely according to the clinical presentation, physical findings, and results of other diagnostic procedures. In general, the most important hormones measured are protein hormones such as pituitary LH, FSH, prolactin and a variety of steroid hormones, including oestrogen and testosterone.

1.2 JUSTIFICATION

One of the most important and underappreciated reproductive health problems in developing countries is the high rate of infertility and childlessness (Bergstrom, 1992). Infertility may have far-reaching consequences for the individual or couple, the health system, family planning programmes, sexual behavior and the spread of HIV/AIDS (Favot *et al.*, 1997). Several anthropological and demographic studies have reported that unions with fertility problems have more extra-marital partners (Buzzard, 1982) and are more likely to be short-lived and lead to divorce (Nabaitu *et al.*, 1994). Marital instability, in turn, has been shown to be associated with higher risks of HIV infection (Carael, 1994). Childless women are frequently stigmatized, resulting in isolation, neglect, domestic violence and polygamy (Wiersema *et al.*, 2006). There is paucity of information on the correlation between infertility and sexually transmitted infections, toxoplasmosis and rubella immunity in the Ghanaian populace.

1.3 AIMS AND OBJECTIVES

1) To determine if there is any significant difference in the haematological and hormonal profiles of infertile and fertile women

2) To determine the prevalence of sexually transmitted infections, toxoplasmosis and rubella immunity in infertile women

Chapter 2

LITERATURE REVIEW

2.1 INFERTILITY

Medical and surgical services for infertility increased dramatically in number and sophistication during the last quarter of the 20th century (Wilcox and Mosher, 1993). Although good documentation of the prevalence of infertility is lacking, it is generally believed that more than 70 million couples suffer from infertility worldwide (Fathalla, 1992; Boivin et al., 2007). The goals of an infertility evaluation are threefold: to discover the aetiology of past infertility, to provide a prognosis for future fertility and to obtain a successful pregnancy for both mother and fetus (Balasch et al., 1992).

Apart from patterns in incidence and causes, some studies have examined patterns in people's health seeking behaviour for infertility, that is, the actions taken to solve a fertility problem. They show that people in Africa frequently seek help for infertility. Barden-O'Fallon, (2005) and Walraven *et al.,* (2001) found that slightly less than 60% sought biomedical or indigenous treatment for fertility problems. Walraven *et al.,* (2001) found in their study of reproductive illness in the Gambia that this percentage is higher than for any other reproductive health problem. Cates *et al.,* (1985) found that, like incidence and causes, also patterns of health seeking behaviour are different in Africa than in other regions of the world. Women in Africa appear to use infertility services when they are relatively young: 42% of the women in the study by Cates *et al.,* (1985) who sought biomedical care in Africa were below 24 years, compared to 25% in the developed countries. At the same time, Cates *et al.,* (1985) found that women in Africa had waited significantly longer before seeking biomedical health services than women in the developed world. In Africa, 33% had waited for more than 2.5 years, whereas in the developed world, 50% had waited less than 2 years (Cates *et al.*, 1985). Hence, it appears that for women in Africa infertility problems become apparent at a relative

young age. Cates *et al.,* (1985) found that, unlike women, men in Africa who sought biomedical care tended to be older than men in other parts of the world. Barden-O'Fallon, (2005) found that in Malawi, women consult biomedical practitioners more often than men. These findings seem to indicate that men are less eager than women to seek biomedical services, especially when they are younger. Several studies indicate that people consult indigenous healers more frequently than biomedical practitioners (Favot *et al.*, 1997; Walraven *et al.*, 2001; Barden-O'Fallon, 2005). Barden-O'Fallon, (2005) for example found that in Malawi, 74.7% of all women in their survey who had made use of some form of health care, had consulted indigenous healers, and 80% of all men who had sought help from health services. It appears that when people consult healers, they do so frequently: in the study by Favot *et al.,* (1997), infertile women reported on average 5.9 visits to indigenous healers, and 1.7 visits to western health services. Regarding demographic factors associated with use of health services, Barden-O'Fallon, (2005) found a significant positive association between being educated and seeking biomedical or indigenous treatment in Malawian women with fertility impairments. However, this association was not found in men: for them having no education was related to seeking help. Thus, like the association between education and infertility, the relation between education and use of health services is unclear.

2.1.1 INCIDENCE OF INFERTILITY

Normally, a distinction is made between primary infertility, when a woman has never conceived or given birth to a live child, and secondary infertility, when a woman has given birth at least once, and subsequently becomes infertile (WHO, 1975).Epidemiological and demographic studies demonstrate that infertility rates are considerable in sub Saharan Africa. Ericksen and Brunette, (1996) calculated the percentages of women, between 20 and 41 years, who have been exposed to conception but have not given birth for a period of at least 5 to 7 years. Excluded were women who use contraception, or had never had sex, or had not been menstruating for 5 years.

As the Table A below shows, Ericksen and Brunette, (1996) found that overall, primary infertility rates are low across Africa: most rates are below 4%. According to Ericksen and Brunette, (1996), Ghana has a primary infertility rate of 1.6%.

Table 2.1 Selection of infertility rates in African nations, from Ericksen and Brunette (1996)

Nations	Infertility Range (%)	Midpoint (%)	Primary infertility (%)
Burundi	8.6-11.5	10 .5	1.3
Ghana	**10.1-13.5**	**11.8**	**1.6**
Sudan	10.6-14.0	12.3	3.1
Nigeria	10.5-14.6	12.6	4.0
Cote d'Ivoire	11.5-14.8	13.2	5.0
Malawi	12.2-15.0	13.6	1.1
Kenya	13.7-16.7	15.2	2.7
Zambia	13.8-17.5	15.7	1.4
Botswana	14.9-21.0	18.0	3.6
Zimbabwe	16.8-22.4	19.6	2.8
Lesotho	17.1-21.5	19.3	4.0
Sub-Saharan Average	5-16.0	14.5	

However, secondary infertility is much more common than primary infertility in Africa, unlike in other parts of the world where primary infertility rates appear higher (Cates *et al.*, 1985). According to Ericksen and Brunette, (1996), combined primary and secondary infertility rates vary between African nations from 8.6% to 22.4 % of women between 20 and 41 years old. They found that Southern Africa has the highest incidence of infertility, although others argue that they are highest in Central Africa, also called 'the infertility belt' (Collet *et al.*, 1988).

In Malawi, Ericksen and Brunette, (1996) found an incidence rate between 12.2% and 15%, with a midpoint of 13.6%, whilst Larsen, (2000) reports a somewhat higher secondary infertility rate of 17%. Ericksen and Brunette, (1996) point out that the total number of women suffering from infertility during some period in their lives, is higher than these infertility rates suggest. Their survey points out that a significant proportion of currently fertile women had infertile, childless intervals for a period of 5 to 7 years in previous periods in their lives. In Malawi, this was the case for 15.8% of currently fertile women.

2.1.2 CAUSES OF INFERTILITY

Demographic factors which have been found to correlate with infertility are being in a polygamous marriage (Barden-O'Fallon, 2005), being or having been divorced (Larsen, 1995; Ericksen and Brunette, 1996; Favot *et al.*, 1997) and urban, rather than rural residence (Larsen, 1995; Ericksen and Brunette, 1996). In addition, Ericksen and Brunette, (1996) found a relationship between ethnicity and infertility in various African nations, with some ethnic groups within the same nation having higher infertility rates than others. The relationship between socio-economic class and infertility has been examined as well, but findings are equivocal. Larsen, (1995) reports that in Cameroon and Nigeria, socio-economic class, education and occupation are not significantly related to infertility. By contrast, Barden-O'Fallon, (Barden-O'Fallon, 2005) found that in Malawi, being in the highest income group is significantly associated with infertility, but only in men. It is worth noting that correlational data do not allow determination of the direction of the associations between infertility and the various related factors. Thus, STD and HIV can both be the causes of infertility, and the result of an infertile status, for example because people with fertility problems may engage in more 'unsafe' sexual relationships, in an attempt to bear children. In addition, being sexually active from a young age, having more sexual relationships, polygamy and divorce can be seen as risk factors of STDs and HIV/AIDS (Ericksen and Brunette, 1996), and may therefore increase the chance of becoming infertile. However, with the exception of being sexually

active at a young age, these characteristics can also be a result of an infertile condition.

Bilateral tubal occlusion due to sexually transmitted diseases and pregnancy-related infections is the most common cause of infertility in developing countries, a condition that is potentially treatable with assisted reproductive technologies (ART) (Ombelet et al., 2008). Unfortunately, a large majority of the population cannot afford infertility treatment since new reproductive technologies are either unavailable or very costly (Nachtigall, 2006). When reflecting on the possible role of ART in developing countries, many concerns and barriers become apparent (Vayena et al., 2002). Central to these is the question whether expensive techniques, which have a low success rate (live birth rate, 25% per cycle), can be justified in countries, where poverty is still an important issue. Social and scientific trends (such as delayed childbearing), increases in sexually transmitted diseases that can cause fallopian tube dysfunction, new drugs and techniques for treating infertile women and men, and an expanding cohort of physicians specializing in reproductive assistance have been the driving forces behind this remarkable extension of reproductive opportunities (Speroff et al., 1999). Internists and family physicians may be faced with questions and decisions about infertility evaluation and pregnancy management in older patients, patients with serious medical illness, and patients with heritable conditions that affect reproduction (Adashi and Hennebold, 1999).

The number of childless women over the age of 35 years is increasing (Maroulis, 1991). In a population of women not using contraceptives, relative fertility falls with age by about 40% at age 40 years (Brewis, 1993), irrespective of the characteristics of the male partner, as is proven by statistics on donor insemination (Maroulis, 1991). In the case of female fertility, the most important age-related factor is ovarian age (decrease in number of oocytes); the uterine state, although a relatively minor factor, seems also to affect the implantation rate (Abdalla et al., 1993). Age is the single most important factor involved in unexplained infertility,

and the age effect on prognosis is generally underestimated (ESHRE, 1997). Women seeking infertility treatment are often older than 35 years of age (Rosene-Montella et al., 2000). Other studies of conception and gestation in achieved pregnancies indicate that age has a greater effect on the gamete than on the capacity of a healthy uterus to support pregnancy (Gilbert et al., 1999). Collins et al., (1983) found that female age was significantly related to the cumulative conception rate when all couples were considered (P = 0.05), but not to the 'treatment-independent' conception rate (P = 0.44). In contrast, Lenton et al., (1977) found no significant difference in the distribution of female age between the conception and non-conception groups of couples who had 'unexplained' infertility.

2.2 HORMONAL DYSFUNCTION AND INFERTILITY

Female reproductive function is reliant on pituitary-derived FSH and LH. FSH controls cyclic recruitment of small growing follicles, supports follicle development to preovulatory stage, and confers sensitivity to LH-stimulated ovulation and luteinization (McGee and Hsueh, 2000). Increasing basal levels of circulating FSH throughout the menstrual cycle, especially in the early follicular stage, is one of the earliest signs of human reproductive aging was observed by Sherman and Korenman, (1975) preceding any changes in serum LH or estradiol before menopause (Lee et al., 1988). Klein et al., (1996) observed that elevated FSH is associated with shorter follicular phase and Reame et al., (1998) with cycle length in aging women in their late 30s to mid 40s before the menopause transition, coinciding with the marked decline in fecundity (Menken et al., 1986). Rising levels of serum FSH with age is at least in part due to declining inhibin B secretion (Menken et al., 1986). Inhibin B is known to suppress pituitary FSH secretion, so reduced inhibin production by a decreasing growing follicle population (as ovarian reserve is depleted with age) may elevate FSH secretion (Menken et al., 1986). Whether or not rising FSH represents a passive reflection or a direct effector

of age-related changes in human follicle dynamics, ovulatory cycles, and fecundity remains conjectural.

Understanding the contributing factors to age-related declining female fecundity has important clinical relevance considering greater proportions of women in developed countries delay childbearing until at least 30 yr of age (te Velde and Pearson, 2002). Advancing our knowledge of FSH effects may have profound implications for human female fertility, including ovarian follicle recruitment and depletion with age (van Zonneveld et al., 2003). Increased FSH levels may accelerate female reproductive aging by increasing recruitment of small growing follicles and thereby accelerating final depletion of the diminishing follicle reserve (Richardson and Nelson, 1990). Baird et al., (2005) found out that an exponential decline in female fertility begins several years before menopause, raising the possibility that rising FSH may directly influence ovarian function or female reproductive aging before exhaustion of ovarian reserve. Premature ovarian failure resulting from the early depletion of ovarian follicles is defined as primary or secondary amenorrhea associated with elevated gonadotropin levels and infertility in women younger than 40 years of age (Rosene-Montella et al., 2000). It accounts for 10% to 28% of primary amenorrhea and 4% to 18% of secondary amenorrhea (Anasti, 1998). Although intermittent ovulation may occur, many women with this condition require oocyte donation to conceive. Lee et al., (1988) suggested that there is no significant alteration in gonadotrophin secretion in regularly cycling women until they reach their fourth decade, suggesting that ovarian endocrine function is retained. Mahadevan et al., (1983) found that a female age of up to 44 years did not significantly influence the success of in-vitro fertilization and this suggests that gametes from such women retain their ability to be fertilized. Therefore the mechanisms by which advancing female age adversely influence fecundity remains to be elucidated.

Rosene-Montella et al., (2000) in a review made the observation that the disruption of the hypothalamic–pituitary axis (hypogonadotropic hypogonadism) may be

secondary to physical injury to the hypothalamus or pituitary gland (neoplasm, ischemia, infiltration, or granulomatous) or disturbed regulation (anorexia nervosa, long-term oral contraceptive use, excessive exercise, chronic renal failure, cirrhosis, and hyperprolactinemia). The resulting deficiency in levels of gonadotropin-releasing hormone or decrease in luteinizing hormone or follicle-stimulating hormone levels necessitates assisted reproduction for fertility (Rosene-Montella et al., 2000). Hyperprolactinemia and adrenocorticotropic hormone deficiency are all commonly associated with pituitary infertility (Rosene-Montella et al., 2000). In a review by Rosene-Montella et al., (2000) they noted that there may also be continued overproduction from a secreting pituitary neoplasm (for example, prolactinoma), which may increase in size with pregnancy or oral contraceptive use. To treat hypopituitarism, the deficient hormones must be replaced.

In a research done by (Lee *et al.*, 1988), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and oestradiol (E2) concentrations in plasma were obtained daily throughout the menstrual cycles of ninety-four (94) regularly cycling women, aged between twenty-four (24) and fifty (50) years. Although mean LH concentrations changed little with advancing age, mean FSH concentrations were significantly elevated from the age of thirty-nine (39) years. FSH concentrations in the oldest women studied (48–50 years) were approximately 3-fold greater than in the younger controls (women aged 23–35 years). LH concentrations rose slightly during the last five (5) years only. The increase in FSH concentration was not, however, uniform across the cycle, but was confined predominantly to the mid-follicular and post-ovulatory phases (i.e. those times in the normal menstrual cycle when circulating inhibin concentrations appear to be minimal). Despite the clear increases in FSH concentration, there was little alteration in the mean steroid profiles which remained within the normal fertile range throughout the last decade of reproductive life. The only exception to this was a small, transient, but significant decrease in pre-ovulatory oestradiol

concentration between the ages of thirty-six (36) and thirty-eight (38) years, which was followed by a transient increase in oestradiol concentration between thirty-nine (39) and forty-four (44) years.

2.3 SEXUALLY TRANSMITTED INFECTIONS AND INFERTILITY

There is a long history of sexually transmitted infections (STI) in human societies (Swinton et al., 1992). Epidemiological studies show that the most common cause of infertility in Africa appears to be infections, leading to pelvic inflammatory disease (PID) (Cates et al., 1985; Larsen, 1995; Sundby, 1997). PID can cause infertility when it results in blocked fallopian tubes, pelvic adhesions (Cates et al., 1985) or ectopic pregnancies (Bhatti and Fikree, 2002). Cates et al., (1985) report that in their study, in over 85% of infertility cases in Africa, the diagnosis was related to infections, in comparison to 36% in the developed world. The infections responsible for PID can be related to unsafe, unhygienic pregnancies and abortions (Cates et al., 1985; Larsen, 1995; Ericksen and Brunette, 1996; Sundby, 1997) and STDs (Cates et al., 1985; Collet et al., 1988; Samucidine et al., 1999), especially HIV/Aids (Favot et al., 1997), and gonorrhoea and chlamydia (Ericksen and Brunette, 1996). Syphilis can lead to infertility as well, by causing miscarriages or stillbirths (Cates et al., 1985; Larsen, 1995).

It should be noted that findings regarding the relationship between STDs and infertility are somewhat ambiguous. According to Ericksen and Brunette, (1996), 70% of PID cases in Africa is due to STDs, especially gonorrhoea and chlamydia. However, Barden-O'Fallon, (2005) and Favot et al., (1997) found that STDs were not significantly associated with infertility, in respectively Malawi and Tanzania. In addition, although Barden-O'Fallon, (2005) found an association between self-reported infertility and HIV positivity in Malawi, the relation was not significant. However, they point out that a rather large percentage of participants did not want to be tested for HIV (20% of women, 33% of men). As this group was included in the group with negative HIV test results, which was compared with those who tested positively, this may have diluted the findings. Despite some ambiguity in

the findings of epidemiological studies, STDs including HIV are normally seen as one of the main causes of infertility in the developing world. Other factors which are thought to lead to infertility in Africa, although mentioned less often, are malaria, as it can cause miscarriage (Larsen, 1995), malnutrition (Sundby, 1997), female circumcision (Larsen, 1995; Gerrits, 1997; Sundby, 1997), the insertion of intrauterine devices (IUD) (Gerrits et al., 1999) and cervical cancer (Gerrits et al., 1999).

Most sexually transmitted diseases have a detrimental effect on female fertility or male virility in some fraction of infected individuals, and as such they have the potential to induce demographic changes if they are prevalent within a given community (Swinton et al., 1992). Health factors that can be assessed by a survey and that are known to cause a large proportion of fertility problems in Africa are related to reproductive tract infections, especially those caused by sexually transmitted infections (STIs), including gonorrhea and syphilis (Mayaud, 2001). Human immunodeficiency virus (HIV) has also been found to be associated with infertility and subfertility among women and men in Africa (Ikechebelu et al., 2002).

2.3.1 SYPHILIS

Syphilis is known, on the basis of the skeletal changes it induces, to predate Columbus in the Americas. Indeed, Swinton et al., (1992) stated that the first well documented major epidemic of syphilis in Europe followed the return of Columbus in 1493. During this epidemic the connection with sexual intercourse was not made; the disease was viewed as a scourge for godlessness and academically trained doctors refused to handle the disease (Swinton et al., 1992). Once the mode of transmission was clearly established, however, issues of control were inevitably linked with preaching on sexual and marital morality (Swinton et al., 1992). Some sufferers, no doubt particularly women, were considered sinners and criminals (Quetel, 1990).

A serological test for syphilis has been traditionally included as routine preliminary laboratory examination for infertile women (Behrman and Kistner, 1975), in spite of the low prevalence of sero-positive patients reported. Leader et al., (1984) found a 0.23% prevalence of syphilitic infection among 1283 cases. This figure is similar to the 0.28% reported in a study by Balasch et al., (1992). On the basis of their results, Leader et al., (1984) suggested that syphilitic screening should be removed from the investigative protocol for infertility. However, infertile patients are women actively seeking pregnancy and recent work has documented that *Treponema pallidum* can cross the placenta and invade the fetus as early as 8 weeks of gestation (Sweet and Gibbs, 1990). It has been an accepted practice to screen women undergoing infertility investigations for syphilis, both because of its role in infertility and because of its known effects on the fetus (Behrman and Kistner, 1975). The total number of cases of syphilis in the industrialized world progressively decreased from the 1940s until the late fifties when the trend reversed and the incidence has been steadily increasing since then; however, much of this increase occurred in the male homosexual population (Sweet and Gibbs, 1990). Thus, in the opinion of Balasch et al., (1992) serological tests for syphilis should be routinely performed in infertility units.

2.3.2 GONORRHOEA

The earliest records of gonococcal infections are believed to go back to several thousand years before Christ (BC).Hare, (1988) focused in particular on Neisseria gonorrhoeae, the aetiological agent of the disease gonorrhea as a possible cause of infertility. This is not the only sexually transmitted infection that causes infertility, but in comparison with other diseases such as chlamydia and syphilis (Hare, 1988), a significant body of epidemiological data exists for gonococcal infection in both developing and developed countries (Hethcote and Yorke, 1984) which permits the estimation of many of the major parameters that determine transmission success. Sexually transmitted infections such as gonorrhoea are a significant cause of infertility in women when the infection is untreated (Swinton et al., 1992). They have the potential to alter human population growth rates in many developing

countries where sexually transmitted diseases are prevalent due to limited public health facilities for diagnosis and treatment (Swinton et al., 1992). Swinton et al., (1992) in a research analysis observed the significant influence gonococcal infection may have in reducing population growth rate in some communities. For example, the simple model predicts that a prevalence of 20% in sexually active adults results in a 50% reduction in the population growth rate.

2.3.3 HUMAN IMMUNODEFICIENCY VIRUS (H.I.V)

While it has been accepted practice to screen women undergoing infertility evaluation for syphilis, there are few data in the literature regarding the seroprevalence of human immunodeficiency virus (HIV) infection in infertile patients despite the increasing number of HIV-positive women (Balasch et al., 1992). In a study by Balasch et al., (1992), six out of 2137 infertility patients were seropositive for syphilis (0.28%) and four out of 791 were HIV positive (0.5%). All four women with HIV antibodies had negative tests for syphilis and none of them related any risk factor for HIV infection on their initial visit. The 0.5% sero-positivity rate found in this study warrants routine HIV testing in infertile patients (Balasch et al., 1992).Despite its initial confinement to high-risk groups (principally homosexual males), acquired immunodeficiency syndrome (AIDS) has spread to involve all sectors of the community (Balasch et al., 1992). Balasch et al., (1992) reported that infected individuals include those who are heterosexual, drug abusers and recipients of infected blood or tissue. The number of AIDS cases in women is showing a steady increase each year in most countries (Bannon and Quirk, 1989), as well as in the autonomous community of Catalonia, Spain, with six million inhabitants and where the first case of AIDS was reported in 1982 (Casabona et al., 1989). Furthermore greater than 80% of these women are in their child-bearing years, and they are the major source of human immunodeficiency virus (HIV) infection in children (Casabona et al., 1989).

The increasing prevalence of AIDS cases in women represents only a fraction of women infected with HIV. It has been documented that for each patient with

AIDS, several others have HIV-related disease and a still larger group carry the virus asymptomatically (Bannon and Quirk, 1989). A better understanding of HIV infection in child-bearing women is one key to developing and implementing effective prevention strategies. Prevention of perinatal transmission of HIV requires identification of HIV infected women prior to conception and advising those who give a positive test result to avoid pregnancy (Hearst and Hulley, 1988). Patients attending an infertility clinic are a population of women actively seeking pregnancy. However, there are only two recent reports in the literature regarding the seroprevalence of HIV infection in infertile patients (Bray et al., 1991). In Gabon HIV prevalence was highest among women with primary infertility (9.3%), followed by secondary infertility (2.1%) and fertile women (0.7%) (Schrijvers et al., 1991). Also in Zimbabwe, women admitted to a hospital with infertility were compared with controls from the maternity ward (De Muylder et al., 1990). HIV infection was more common among infertile women (9/227:3.9%) than the 104 fertile women (none had HIV infection). Numbers however were small (a total of nine seropositive women).

In contrast to syphilis, there are no data regarding the value of routine HIV testing in infertility evaluation, despite the growing number of women with AIDS or with serological evidence of exposure to HIV (Balasch et al., 1992). Furthermore, women seeking infertility evaluation provide a unique opportunity for pre-conception screening and possible prevention of perinatal spread of HIV infection. Bray et al., (1991) reported a 0.6% HIV sero-positivity rate among 182 infertile women belonging to a demographic area with a higher risk for HIV infection. However, in that blind study there were 122 women registered in the infertility clinic who were not screened for HIV antibody. Thus, although blind testing reduces selection bias, leaving out part of the infertile clinic population could partially invalidate the results. In non-blind testing, participants can be informed of their test results and counseled appropriately, which is an important goal in infertility. Studies using non-blind testing however, may be subject to bias because participation is

voluntary (persons who know themselves to be infected or perceive themselves at risk may elect not to participate). This was not the case in our study where all patients gave informed consent. Also, sero-positive patients were appropriately counseled.

Evidence of HIV infection in a sizeable proportion of an infertile population is a matter of serious concern (Bray et al., 1991). It has been suggested that decisions about the need for counseling and testing programmes in a community should be based on the best available estimates of the prevalence of HIV infection and the demographic variables of infection (Selik et al., 1989). Furthermore, it has been emphasized that in populations with a sufficiently high prevalence of HIV infection (e.g. 0.5%), strategies for preventing transmission that would otherwise be reserved for women in high-risk groups may be appropriate for all sexually active women (Selik et al., 1989). Thus, the 0.5% sero-positivity rate found in the study by Balasch et al., (1992) may justify routine HIV testing in all infertile patients. This is further emphasized by the problem of effectively determining risk status on a prospective basis. Thus, social and sexual histories as recorded in medical charts are a poor guide for identification of those women who are at increased risk for HIV infection (Bayer, 1989). Clearly, many women do not know that they have been placed at risk or they are unwilling to reveal pertinent information when interviewed. This is well exemplified by the four sero-positive cases observed by Balasch et al., (1992). Thus, a recent Editorial on prenatal screening for HIV infection concludes that the benefits of universal voluntary screening outweigh the potential disadvantages (Duff, 1989). This reinforces the conclusion by Balasch et al., (1992) that routine HIV testing in infertility is warranted.

2.3.4 HEPATITIS B VIRUS (H.B.V)

As previously stated, to obtain a successful pregnancy is a basic goal in an infertility evaluation. Prenatal care for the normal obstetric patient implies routine laboratory baseline studies including screening for hepatitis B virus (Kochenour,

1990). Routine prenatal hepatitis B screening has been recommended by the immunization Practices Advisory Committee (1988). This change from previous policy (which advocated screening of only those individuals with recognized epidemiological risk factors) is a very costly strategy to use in an effort to prevent the perinatal spread of hepatitis B in the 'no-risk-factor' population (Koretz, 1989). Conversely, selective prenatal screening for HBV infection, as originally recommended by the Centres for Disease Control, does not appear to be an appropriate policy because 40-70% of women do not recognize or acknowledge that they are at risk for acquiring HBV infection (Lindsay et al., 1989).

2.4 TOXOPLASMOSIS AND INFERTILITY

Toxoplasmosis is a zoonotic disease caused by a protozoan parasite called *Toxoplasma gondii* which infect all mammals and birds species throughout the world (Ghoneim et al., 2009). Most of the toxoplasma infections are asymptomatic; the diagnosis relies mainly on the results of serological tests. Routine serologic diagnosis of toxoplasmosis provides high sensitivity, but specificity varies depending on the test used (Liesenfeld et al., 1996). Pelloux et al., (1998) stated that diagnosis of primary and late infection with *Toxoplasma gondii* in pregnancy can be improved by determination of Toxoplasma DNA. The clinical implications of toxoplasma infection in pregnant patients are manifold. Such patients may have spontaneous abortions, still births or premature delivery in addition to various fetal anomalies (Jani and Dave, 1994). Ideally every woman should know her toxoplasma status before conception (Ghoneim et al., 2009). Toxoplasma antibodies may persist in the serum of asymptomatic people for years at higher titres (Yaneza and Kumari, 1994). Economical losses of toxoplasmosis have medical and veterinary importance which in humans are due to abortion and fetal abnormalities (Buxton, (1998) as well as morbidity and mortality in congenitally infected and immunocompromised individuals (Dunn et al., 1999; Petersen et al., 2001). Avelino et al., (2004) have indicated that pregnant women living under unfavourable environmental conditions had an approximately two times increased

risk of being infected for each risk factor (contact with host animals and presence of vehicles of oocysts transmission). Previous pregnancy is the risk factor that had the strongest influence on acquiring toxoplasmosis (Ghoneim et al., 2009). Han et al., (2008) stated that Toxoplasma gondii infection in Korea is positively correlated with eating raw meat, but is not associated with the consumption of unwashed vegetables, drinking untreated water, history of raising a cat, or blood transfusion. While, infection of healthy adult humans is usually mild, serious disease can result in utero or when the host is immunocompromised. The foetus is only at risk of congenital disease when acute infection occurs during pregnancy. Congenital infection has also been reported from a chronically infected immunocompromised mother with a reactivation of toxoplasmosis (Marcinek et al., 2008).

2.5 RUBELLA AND INFERTILITY

German measles or rubella is a mild illness in terms of its symptoms. However, it can be dangerous for a pregnant woman because the Rubella virus could cause severe abnormalities in the baby. If a woman is susceptible to rubella and contracts the infection in pregnancy, that could cause damage to the baby, especially in the first twelve weeks of pregnancy. Women who are concerned about their fertility should be offered rubella (German measles) susceptibility screening (blood test) so that those who are susceptible to rubella can be offered rubella vaccination and advised not to become pregnant for at least 1 month following vaccination.

Chapter 3

MATERIALS AND METHODS

3.1 SUBJECTS' SELECTION

The study was conducted at Lister Hospital and Fertility Centre in Accra, Southern Ghana from January, 2007 to November, 2008. Women presenting with infertility problems at the out-patient infertility clinic were interviewed using a standardized questionnaire on obstetrics, sexually transmitted infection (STI) history and other known behavioural risk factors for infertility. The interview was followed by clinical examination, including a vaginal swab, and drawing of blood to test for the various laboratory tests. For the purpose of this study infertility was defined as not having had a child during the past 5 years whilst having coitus without contraception among women who presented themselves to the infertility out-patient clinic. The control group were women who came to deliver in the hospital. These women were only included as control if they had a previous birth during the past 4 years at the Lister hospital and Fertility Centre.

Based on the subjects' selection criteria, a total of three hundred (300) Ghanaian women between the ages of twenty four (24) and fifty-eight (58) years attending infertility clinic at Lister Hospital and Fertility Centre, Accra were randomly selected for the survey. Another forty (40) fertile Ghanaian women within a similar age group were studied as control. The control samples were treated similarly as the test samples.

3.2 SPECIMEN COLLECTION

Rubber tourniquet was applied around the arm above the elbow for less than one minute and the ante cubital fossa was cleaned with 70% methylated spirit. Phlebotomy was performed using 19G needles fixed on 10 ml syringes and 10 ml of subject's blood was taken from the ante cubital vein. An amount of 3.5 ml of blood was collected in a vial containing 2.5 μg of ethylene diamine tetraacetic acid

(EDTA) as an anticoagulant and the rest of the blood was collected into Vacutainer® plain tubes.

The blood in the Vacutainer® plain tube was allowed to clot and centrifuged at 500 g for 5 min and serum was collected and stored at – 80^0C until assayed, whilst the EDTA anticoagulated blood was mixed to prevent clotting using a mechanical mixer. The EDTA anticoagulated blood samples were used for measuring the haematological parameters using the Quantitative Blood Count (QBC®) Autoread Plus haematological automatic analyzer (QBC Diagnostic Incorporated, State College, Pennsylvania, USA). The sera were used for the fertility hormonal analysis and serological testing for Sexually Transmitted Infection (STIs), Toxoplasma IgM and Rubella IgG antibodies using the Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA).

3.3 ESTIMATION OF FERTILITY HORMONES

3.3.1 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 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, 25 µl aliquots of standards and samples were dispensed into their respective wells in ready-to-use microtitre plates pre-coated with anti-hormone IgG antibodies. After the addition of 100 µl of (1:100 dilution) anti-hormone-HRP conjugate to each well, the plates were incubated for 30 minutes at room temperature in the dark. The contents of the wells were then decanted and the wells washed twice with 300 µl of distilled water.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/hydrogen peroxide system) into each well. The microtitre plates were then incubated for 10 minutes at room temperature. The reaction was stopped by the addition of 100 µl of 0.15 M Sulphuric acid (H_2SO4). 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 303 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.

3.3.2 Enzyme-linked immunosorbent assay competitive method for oestradiol and testosterone

Serum Oestradiol (E_2) and Testosterone (TESTO) were determined using the competitive enzyme-linked immunosorbent assay (ELISA) method produced by NoviWell™ assay kits (HySkill Diagnostics, Bahlingen, Germany). The manufacturer's instructions for the performance of the assays were meticulously followed. The principle underlying the assay is the competition for anti-hormone binding sites on a pre-coated well between the hormone in serum and a horseradish peroxidase labeled hormone conjugate. Briefly, 25 µl aliquots of standards and samples were dispensed into their respective wells in ready-to-use microtitre plates pre-coated with anti-hormone IgG antibodies. After the addition of 100 µl of HRP labeled hormone conjugate to each well, the plates were incubated at 37^{o}C for 60 minutes and 120 minutes for Testosterone and Oestradiol respectively. The contents of the well were then decanted and the wells washed twice with 300 µl of distilled water.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/hydrogen peroxide system) into each well. The microtitre plates were then incubated for 15 minutes and 30 minutes in the dark at room

temperature for Testosterone and Oestradiol respectively. The reaction was stopped by the addition of 100 µl of 0.15 M H_2SO_4. The end-point colour developed is inversely proportional to the amount of hormone. Absorbances were measured at 450 nm and the concentrations calculated from standard curves generated using respective standards of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). Within-assays coefficients of variations were 6.2% for Testosterone and 5.4% for Oestradiol. The analytic sensitivities of the assays were 0.1 ng/ml for Testosterone and 5.0 pg/ml for Oestradiol as indicated by the manufacturer.

3.4 SEXUALLY TRANSMITTED INFECTIONS

3.4.1 Enzyme-linked immunosorbent assay sandwich method for hepatitis B surface antigen

The Orgenics® assay kits (Inverness Medical Innovations Group, Yavne, Israel) were used in determining the presence of Hepatitis B surface Antigen (HBsAg) in the sera using the sandwich enzyme-linked immunosorbent assay (ELISA) method. The manufacturer's instructions for the performance of the assays were strictly followed. The principle underlying the assay is the simultaneous binding of antigen to two monoclonal antibodies; a solid phase one on a microplate and the other a soluble one conjugated with horseradish peroxidase (HRP). The wells were pre-hydrated and then 150 µl aliquots of negative control, positive control and samples were dispensed into their respective wells in ready-to-use microtitre plates pre-coated with anti-Hepatitis B surface Antigen (anti-HBsAg). After the addition of 100 µl of (1:20 dilution) anti-HBsAg-HRP conjugate to each well, the plates were incubated for 120 minutes at 37^{o}C temperature in the dark. The contents of the wells were then decanted and the wells washed 3-4 times with 350 µl of 10 mM phosphate buffer solution.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/hydrogen peroxide system) into each well. The microtitre plates were then incubated for 30 minutes at room temperature. The reaction was

stopped by the addition of 100 µl of 0.3 M H_2SO_4. The end-point colour developed is directly proportional to the amount of HBsAg. Absorbances were measured at 450 nm in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA).

The test results were interpreted as a ratio of sample absorbance at 450nm and cut-off value. The cut-off value = Negative control absorbance + 0.050. Ratios less than 0.9 is Negative, 0.9 – 1.1 is equivocal and greater than 1.1 is Positive

3.4.2 Enzyme-linked immunosorbent assay sandwich method for hepatitis B surface antibody, hepatitis C virus antibody and Treponema pallidum antibody (Syphilis)

The presence of Hepatitis B surface Antibody, Hepatitis C virus antibody and Treponema pallidum antibody in the sera were determined by the sandwich enzyme-linked immunosorbent assay (ELISA) method using Orgenics® assay kits (Inverness Medical Innovations Group, Yavne, Israel). The principle underlying the assay is the binding of antibody to two highly purified antigens; an immobilized one on a microplate and the other a soluble one conjugated with horseradish peroxidase (HRP). Briefly, 100 µl aliquots of calibrators and samples were dispensed into their respective wells after 50 µl of sample diluent has been added to ready-to-use microtitre plates pre-coated with Hepatitis B surface Antigen, Hepatitis C antigen and Treponema pallidum (TP) recombinant antigen respectively and incubated at 37^{o}C for 60 minutes. The contents of the wells were then decanted and the wells washed 3-4 times with 350 µl of 10 mM phosphate buffer solution. After the addition of 100 µl of (1:100 dilution) HBsAg-HRP conjugate, HCV-HRP conjugate and TP-HRP conjugate to each respective well, the plates were incubated for 60 minutes at 37^{o}C. The contents of the wells were then decanted and the wells washed thrice with 350 µl of 10 mM phosphate buffer solution.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/hydrogen peroxide system) into each well. The microtitre plates were then incubated for 20 minutes at room temperature. The reaction was stopped by the addition of 100 µl of 0.15 M H_2SO_4. The end-point colour developed is directly proportional to the amount of antibody. Absorbances were measured at 450 nm and the concentrations calculated from standard curve generated using respective calibrators of known concentrations in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). The test results were interpreted as antibodies' values less than 10 mIU/ml is Negative and values greater than 10mIU/ml is Positive.

3.4.3 Enzyme-linked immunosorbent assay sandwich method for human immunodeficiency virus (HIV) 1+2 antibodies

The presence of HIV (1+2) antibodies (anti-HIV) in the sera were determined by the sandwich enzyme-linked immunosorbent assay (ELISA) method using Orgenics® assay kits (Inverness Medical Innovations Group, Yavne, Israel). The principle underlying the assay is the binding of anti-HIV to two recombinant HIV antigens; an immobilized one on a microplate and the other a soluble one conjugated with horseradish peroxidase (HRP). 100 µl of sample diluent was added to ready-to-use microtitre plates pre-coated with recombinant HIV antigen, this was followed by the addition of 50 µl of negative control, positive control and samples into their respective wells and mixed very well on a vibrating mixer. This was then incubated at 37^{o}C for 60 minutes. The contents of the wells were then decanted and the wells washed 3-4 times with 350 µl of 10 mM phosphate buffer solution. After the addition of 100 µl of (1:100 dilution) HIV-HRP conjugate to each respective well, the plates were incubated for 30 minutes at 37^{o}C. The contents of the wells were then decanted and the wells washed thrice with 350 µl of 10 mM phosphate buffer solution.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/citrate-phosphate/hydrogen peroxide) into each well. The

microtitre plates were then incubated for 30 minutes at room temperature. The reaction was stopped by the addition of 100 µl of 1N H_2SO_4. The end-point colour developed is directly proportional to the amount of antibody. Absorbances were measured at 450 nm in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA). The test results were interpreted using the mean of the negative controls absorbance to calculate a cut-off value. The cut-off value = Negative control mean absorbance + 0.300. Absorbance less than cut-off value is Negative and greater than or equal to cut-off value is Positive

3.4.4 Gram staining technique for the detection of Neisseria gonorrhea

The vaginal swab was used to prepare an evenly distributed smear of diameter 15-20 mm on a glass slide. It was air dried and fixed with methanol. This was then stained using the Gram's staining technique in Medical Laboratory Manual for Tropical Countries (Tropical Health Technology, Doddington, United Kingdom). The principle underlying this technique is based on the differences in the cell wall structure of microorganisms. Thus the organisms that are able to withstand the decolorization stage of the Gram's technique and therefore do not pick up the neutral red counterstain are termed Gram positive and those that are decolorized and therefore pick up the neutral red counterstain are termed Gram negative. The fixed smear was covered with Crystal violet stain for 30-60 seconds and the stain rapidly washed off with clean water. The water was tipped off and the smear was flooded with Lugol's iodine for 30-60 seconds and the stain washed off with clean water. The smear was then decolorized rapidly for some few seconds with acetone-alcohol and immediately washed with clean water. The smear was covered with neutral red for 2 minutes and the stain washed with clean water. The back of the slide was wiped clean and placed in a draining rack for the stained smear to air-dry. The air dried stained smear was examined microscopically with X100 oil

immersion objective for Gram negative intracellular diplococcic (GNID) which appear as dark or pale-red round organism paired within the cells.

3.5 ENZYME-LINKED IMMUNOSORBENT ASSAY SANDWICH METHOD FOR TOXOPLASMA IGM AND RUBELLA IGG

The presence of Toxoplasma IgM and Rubella IgG antibodies in the sera were determined by the sandwich enzyme-linked immunosorbent assay (ELISA) method using Orgenics® assay kits (Inverness Medical Innovations Group, Yavne, Israel). The principle underlying the assay is the binding of antibody to two highly purified antigens; an immobilized one on a microplate and the other a soluble one conjugated with horseradish peroxidase (HRP). Briefly, 100 µl aliquots of negative control, positive control and samples were dispensed into their respective wells after 50 µl of sample diluent has been added to ready-to-use microtitre plates pre-coated with *Toxoplasma gondii* antigen (TOXO) and Rubella antigen respectively and incubated at 37^{o}C for 60 minutes. The contents of the wells were then decanted and the wells washed 3-4 times with 300 µl of 10 mM phosphate buffer solution. After the addition of 100 µl of (1:100 dilution) TOXO-HRP conjugate and RUBELLA-HRP conjugate to each respective well, the plates were incubated for 60 minutes at 37^{o}C. The contents of the wells were then decanted and the wells washed thrice with 350 µl of 10 mM phosphate buffer solution.

The enzyme reaction was started by the addition of 100 µl chromogen (tetramethylbenzidine/hydrogen peroxide system) into each well. The microtitre plates were then incubated for 20 minutes at room temperature. The reaction was stopped by the addition of 100 µl of 0.15 M H_2SO_4. The end-point colour developed is directly proportional to the amount of antibody. Absorbances were measured at 450 nm in a Stat Fax 303 Plus Microplate Reader (Awareness Technology Incorporated, Palm City, Florida, USA).

3.6 HAEMATOLOGICAL PARAMETERS

The QBC® Autoread Plus haematological analyzer (QBC Diagnostic Incorporated, Philipsburg, Pennsylvania, USA) was used to measure the haematological parameters at a room temperature of 20-32^{0}C. The principle underlying this method is the utilization of electro-optical linear measurements of the discrete layers of packed blood cells in a microhaematocrit type tube. A precision-bore glass QBC™ microhaematocrit tube pre-coated with potassium oxalate, acridine orange fluorochrome stain and an agglutinating agent was filled with an EDTA anticoagulated blood up to a pre-marked portion on the tube. This was carefully mixed and span at 12,000 +/- 80 revolutions per minute (rpm) for 5 minutes in a QBC™ microhaematocrit centrifuge (QBC Diagnostic Incorporated, State College, Pennsylvania, USA)

The spun QBC™ tube was inserted into the QBC® Autoread Plus haematological analyzer (QBC Diagnostic Incorporated, State College, Pennsylvania, USA) where it is automatically scanned and fluorescence and absorbance readings are made to identify the expanded layers of differentiated cells. Volumes of these packed cell layers are then computed to obtain quantitative values of haemoglobin (Hb), white blood cells (WBC) and platelets (PLT).

Chapter 4

RESULTS

4.1 HORMONAL AND HAEMATOLOGICAL PARAMETERS

Even though subjects and control group of similar age were selected, the mean age of the infertile subjects (34.89 ± 0.40) showed significant difference ($p<0.0001$) from that of the control group (i.e. 30.40 ± 0.57). Table 4.1 presents the general characteristics of the 300 infertile and 40 fertile women. From this table, apart from WBC, LH and E2 which did not show any significant difference when the infertile women were compared to the fertile women, Hb and Plt decreased significantly when the two groups were compared. However, FSH, PRL and TESTO increased significantly when the infertile group was compared to the fertile group.

Table 4.1 General characteristic of the haematological and hormonal profile among the infertile and fertile group.

Parameters	Fertile	Infertile	P Value
Age (yrs)	30.40 ± 0.57	34.89 ± 0.40	<0.0001
Hb (g/dl)	13.13 ± 0.10	12.23 ± 0.08	0.0002
WBC (x 10^9/l)	5.76 ± 0.15	6.19 ± 0.11	0.1798
PLT (x 10^9/l)	272.10 ± 12.27	238.10 ± 3.99	0.0043
LH (mIU/ml)	8.12 ± 0.77	7.59 ± 0.20	0.3955
FSH (mIU/ml)	6.99 ± 0.27	11.51 ± 0.84	0.0412
PRL (mIU/ml)	12.05 ± 0.62	22.36 ± 0.67	<0.0001
TESTO (ng/ml)	0.48 ± 0.04	1.86 ± 0.24	0.0391
E_2 (pg/ml)	57.60 ± 2.51	61.77 ± 2.59	0.5609

Hb= Haemoglobin, WBC= White blood cell count, PLT= Platelet, LH= Luteinizing hormone, FSH= Follicle stimulating hormone, PRL= Prolactin, TESTO= Testosterone, E_2= Oestradiol. Results are presented as means ± SEM. The infertile group was compared to fertile group using unpaired t-test.

4.2 PREVALENCE OF STI AND OTHER PARAMETERS

The prevalence of STI, Toxoplasma IgM, Rubella IgG and G-6-PD among the fertile and infertile group is as shown in table 4.2. Apart from Hepatitis B surface antigen ($p=0.0354$) and antibody ($p<0.0001$) which showed significant increase when the infertile group was compared to the fertile group using Fischer's exact test, all the other parameters did not show any significant difference.

Even though, the prevalence of HIV and G-6-PD in infertile group was higher than that of the fertile group and the prevalence of gonorrhoea in the fertile group was higher than that of the infertile, they all did not reach significant level (Table 4.2). Though the specificity of using STI, Toxoplasma IgM, Rubella IgG and G-6-PD as a marker of infertility from this study was high, their sensitivity was very poor and as such they could not be used as a marker of infertility (Table 4.2).

Table 4.2 The prevalence of STI, Toxoplasma IgM, Rubella IgG and G-6-PD among the fertile and infertile group

Parameters	Fertile	Infertile	P value	RR	OR	Sens. (%)	Spec. (%)
Toxoplasma gondii IgM	11(27.5%)	60(20.0%)	0.3006	1.44	1.52	27.50	80.00
No Rubella IgG immunity	12(30.0%)	80(26.7%)	0.7053	1.16	1.18	30.00	73.33
Treponema pallidum	0(0.0%)	0(0.0%)	1.0000	---	---	---	---
Gonorrhoea	1(2.5%)	0(0.0%)	0.1176	8.69	22.82	2.50	100.00
HIV	0(0.0%)	2(0.7%)	1.0000	0.00	1.47	0.00	99.33
HbsAg	0(0.0%)	28(9.3%)	0.0354	0.00	0.11	0.00	89.67
HbsAb	0(0.0%)	86(28.7%)	<0.0001	0.00	0.03	0.00	71.33
HCV	0(0.0%)	0(0.0%)	1.0000	---	---	---	---
G6PD (Full+Partial defect)	0(0.0%)	10(3.3%)	0.6138	0.00	0.34	0.00	96.67

STI= Sexually transmitted infection, IgM=Immunoglobulin M, IgG=Immunoglobulin G, G-6-PD= Glucose-6-phosphate dehydrogenase deficiency, HIV= Human immunodeficiency virus, HBsAg= Hepatitis B surface antigen, HBsAb = Hepatits B surface antibody, HCV= Hepatitis C virus.

4.3 AGE STRATIFICATION OF SEXUALLY TRANSMITTED INFECTIONS, TOXOPLASMA IGM, RUBELLA IGG AND G-6-PD

From the age stratification in Table 4.3, gonorrhoea, syphilis, HCV, HIV and G-6-PD deficiency do not appear to change with age among the infertile group. The number as well as the percentage prevalence of the infertile women that have been exposed to toxoplasma gondii increases with age up to the 40-49 year. However, the number as well as the percentage prevalence of the infertile women that have no immunity against rubella decreases with age up to the > 50 year group (Table 4.3).

Generally, the number as well as the percentage prevalence of the infertile women that have HBsAg as well as HBsAb also increases with age. However, the prevalence of HBsAb was higher than the corresponding HBsAg. Unexpectedly, at the > 50 years group the prevalence of HBsAg was 100% with 0.00% corresponding HBsAb (Table 4.3).

Table 4.3 The prevalence of STI, Toxoplasma IgM, Rubella IgG and G-6-PD among the fertile and infertile group stratified by age

Parameters	Age Stratification (years)			
	20-29 (74)	30-39 (152)	40-49 (68)	≥50 (6)
Toxoplasma gondii IgM	8(10.8%)	26(17.1%)	26(38.2%)	0(0.0%)
No Rubella IgG immunity	40(54.1%)	30(19.8%)	10(14.7%)	0(0.0%)
Treponema pallidum	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Gonorrhoea	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
HIV	0(0.0%)	0(0.0%)	2(2.9%)	0(0.0%)
HbsAg	2(2.7%)	16(10.5%)	4(5.9%)	6(100.0%)
HbsAb	18(24.3%)	34(22.4%)	34(50.0%)	0(0.0%)
HCV	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
G6PD (Full+Partial defect)	0(0.0%)	10(6.6%)	0(0.0%)	0(0.0%)

STD=Sexually transmitted disease, IgM= Immunoglobulin M, IgG= Immunoglobulin G, G-6-PD= Glucose-6-phosphate dehydrogenase deficiency, HIV= Human immunodeficiency virus, HBsAg= Hepatitis B surface antigen, HBsAb= Hepatits B surface antibody, HCV= Hepatitis C virus.

4.4 AGE STRATIFICATION OF THE HAEMATOLOGICAL PARAMETERS

When age stratification of the infertile group was compared to the fertile group using one way analysis of variance, there was a significant difference ($F_{4,335}$= 7.920; P<0.0001) between the infertile group and the control group with respect to their haemoglobin (Figure 4.1A). The mean Hb for the infertile group decreased from 12.48, 12.40, to 11.62 and then increased to 12.00 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 13.13 for the fertile group (Figure 4.1A).

White blood cell count also showed a significant difference ($F_{4,335}$ = 8.813; P<0.0001) when these groups were compared. The mean WBC for the infertile group decreased from 7.07, 6.02, 5.86 to 3.50 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 5.76 for the fertile group (Figure 4.1B).

Platelet showed a significant decrease ($F_{4,335}$=9.924; P<0.0001) when the age stratification of the infertile group was compared to the fertile group. The mean PLT for the infertile group decreased from 246.60, 230.10, 258.00 to 110.00 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 272.10 for the fertile group (Figure 4.1C).

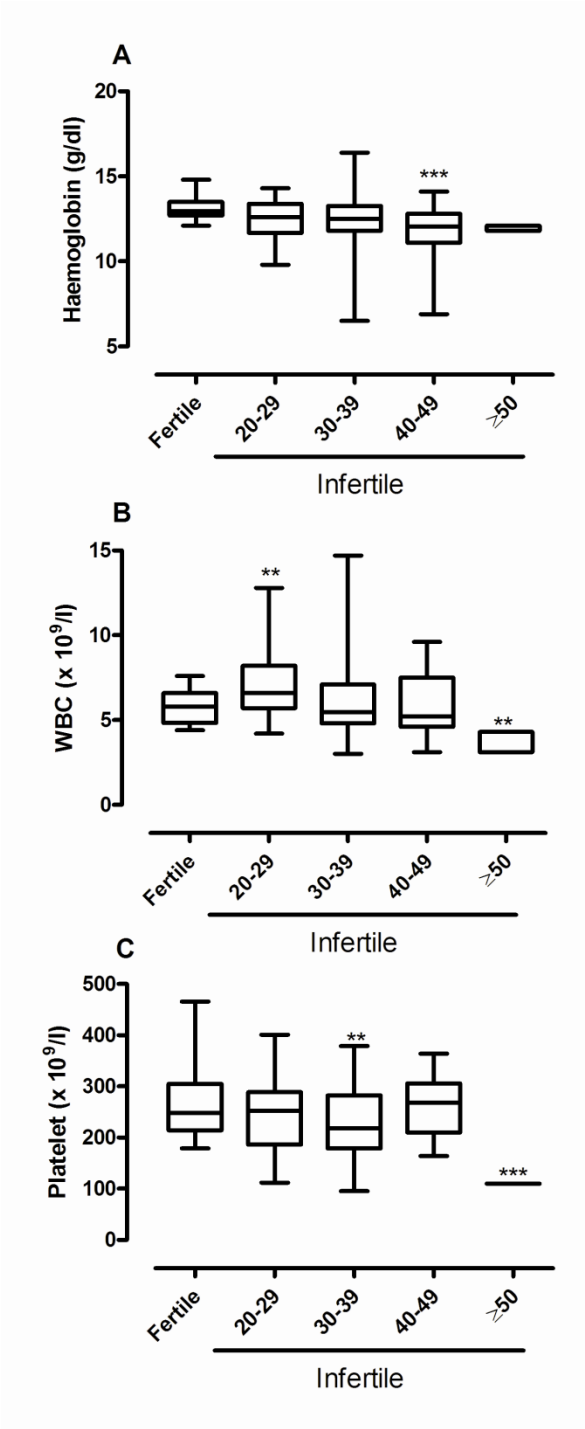

Figure 4.1 Haematological profile of fertile and infertile women stratified by age. A = Haemoglobin, B= WBC and C= Platelet. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. All *P*-values: * <0.05, ** <0.001, * <0.0001 using one way analysis of variance.**

4.5 AGE STRATIFICATION OF THE HORMONAL PROFILE

Figure 4.2 represents the age stratification of hormonal profile among the infertile group as compared to the fertile group. From this study, the level of LH significantly decreases ($F_{4,327}$= 4.291; P=0.0021) when the age stratification of infertile women was compared to the fertile women (Figure 4.2A). The mean LH of 6.85 and 6.90 for the infertile age groups of 20-29 and > 50 respectively showed a decrease from the mean LH of 7.23 for the control group. Whereas the mean LH of 7.01 and 8.42 for the infertile age groups of 30-39 and 40-49 respectively showed an increment from that of the control group (Figure 4.2A). The level FSH significantly increases ($F_{4,331}$=72.35; P<0.0001) when the age stratification of infertile women was compared to the fertile women (Figure 4.2B). The mean FSH for the infertile group ranged from 9.29, 8.52, 9.91 to 43.25 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 7.00 for the fertile group (Figure 4.2B).

From the one way ANOVA, the level of prolactin also significantly increases ($F_{4,323}$=26.14; P<0.0001) when the age stratification of infertile women was compared to the fertile women (Figure 4.2C). The mean PRL for the infertile group decreased with age from 22.96, 22.43, 17.89 to 12.20 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 12.05 for the fertile group (Figure 4.2C). Testosterone also showed significant increase ($F_{4,305}$=5.337; P=0.0004) in value when the age stratification of infertile women was compared to the fertile women (Figure 4.2D). The mean TESTO for the infertile group increased with age from 0.76, 1.01, 1.18 to 1.15 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 0.49 for the fertile group (Figure 4.2D).

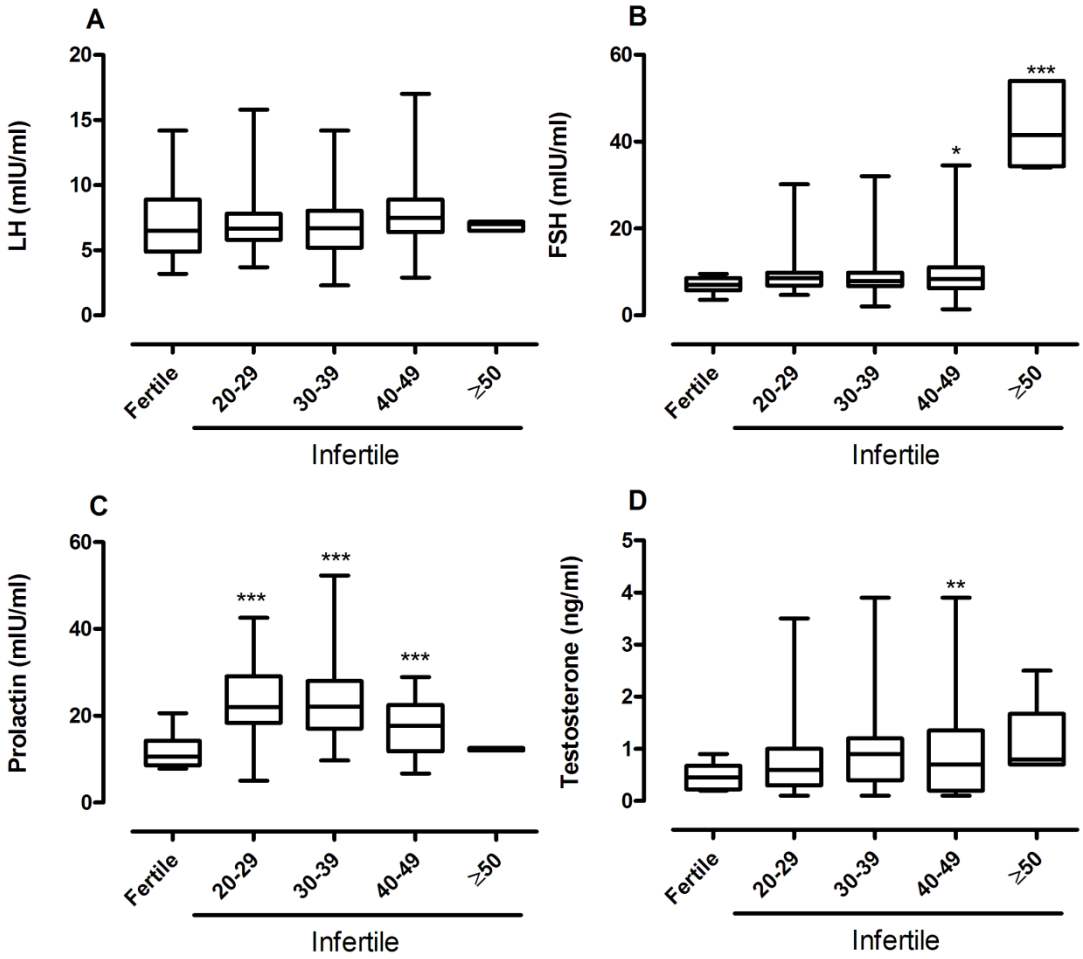

Figure 4.2 Age stratification of hormonal profile among the infertile group as compared to fertile group. Graph A= LH, B= FSH, C= Prolactin and D= Testosterone. LH= Luteinizing hormone, FSH= Follicle stimulating hormone. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box. All *P*-values: * <0.05, ** <0.001, * <0.0001 using one way analysis of variance.**

The level of oestradiol however did not show any significant difference ($F_{4,302}$=1.147; P=0.3343) when the age stratification of infertile women was compared to the fertile women (Figure 4.3). Though the mean E_2 for the infertile group increased with age from 59.41, 64.51, 66.29 to 78.70 for 20-29, 30-39, 40-49 and > 50 years age group respectively as compared to 57.60 for the fertile group, this difference did not reach a significant level (Figure 4.3).

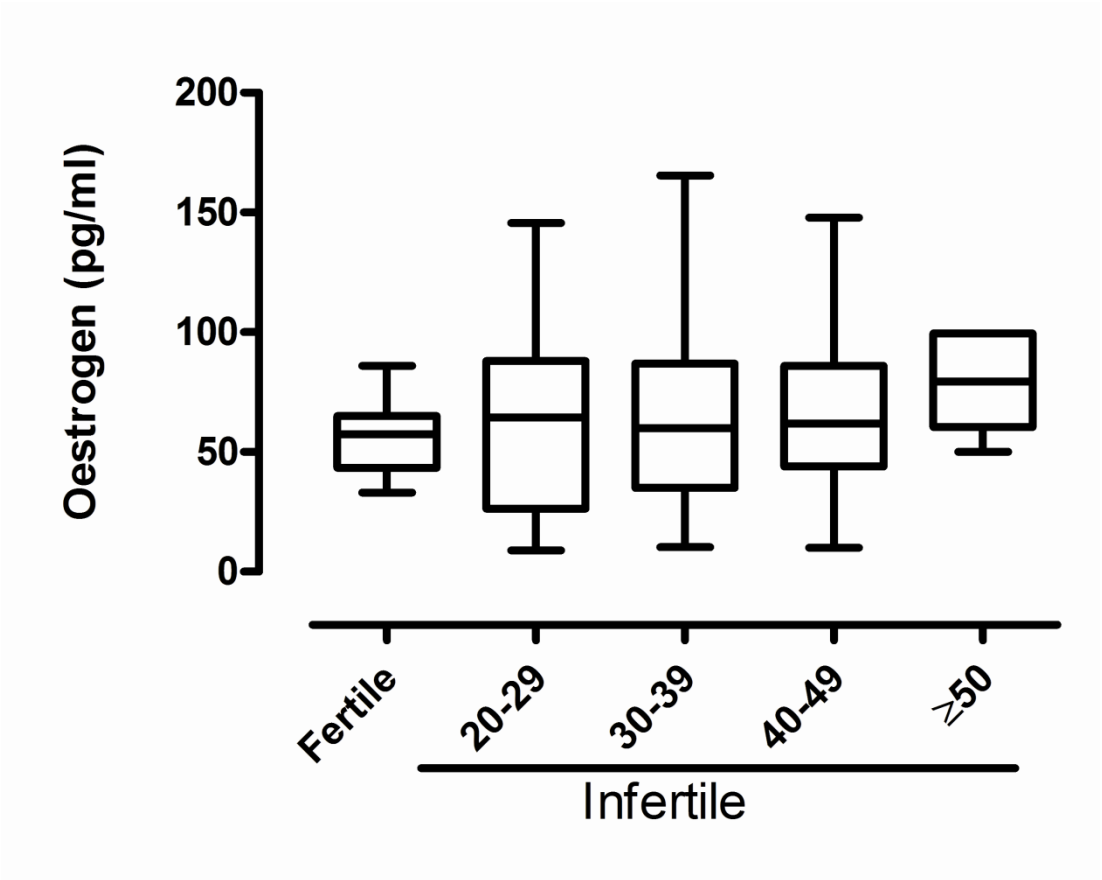

Figure 4.3 Age stratification of oestradiol levels among the infertile group as compared to fertile group. The lower and upper margins of the box represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles, respectively. The median is shown as the horizontal line within the box.

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4.6 CORRELATION BETWEEN THE VARIOUS PARAMETERS

Among the infertile group in this study, FSH showed a significant positive correlation with age and LH but a significantly negative correlation with the haematological parameters (Hb, WBC and PLT). Also testosterone, showed a significant negative correlation with the haematological parameters (WBC and PLT) but a positive correlation with prolactin. However, prolactin showed a positive correlation with the haematological parameters (WBC and PLT) but a negative correlation with FSH. E_2 like FSH also showed a significant positive correlation with LH. As the infertile subjects aged, their haemoglobin and WBC reduced. Platelet gave an inverse relationship with haemoglobin but a direct relationship with WBC (Table 4.4).

Among the fertile control group, testosterone showed a significant negative correlation with age, FSH and PRL. As the fertile group also aged, their level of platelet, FSH and prolactin rises. Just as in the infertile group, the haemoglobin gave a significant negative correlation with FSH (Table 4.4).

Table 4.4 Pearson correlation coefficients haematological variables and hormonal profile for infertile group (upper right-hand side) and fertile group (lower left-hand side)

	Age	Hb	WBC	PLT	LH	FSH	PRL	TEST	E 2
Age		-0.19**	-0.30***	-0.05	-0.02	0.39***	-0.03	-0.04	0.09
Hb	-0.16		0.13*	-0.20***	0.09	-0.21***	0.07	0.01	-0.03
WBC	-0.09	-0.13		0.20***	0.01	-0.22***	0.17**	-0.12*	-0.01
PLT	0.57***	0.08	-0.19		0.01	-0.15*	0.22***	-0.14*	0.03
LH	0.16	0.25	-0.19	-0.07		0.26***	0.04	-0.10	0.14*
FSH	0.59***	-0.34*	-0.07	0.25	0.08		-0.16**	-0.01	0.05
PRL	0.32*	0.00	-0.16	0.16	0.05	-0.12		0.12*	-0.07
TEST	-0.40*	0.12	-0.21	-0.23	0.09	-0.35*	-0.36*		-0.10
E 2	-0.13	-0.11	0.18	0.11	-0.17	0.22	0.06	-0.18	

.Correlation is significant at the 0.05 level (2-tailed), **.Correlation is significant at the 0.001 level (2-tailed), ***.Correlation is significant at the 0.0001 level (2-tailed). Hb= Haemoglobin, WBC= White blood cell count, PLT= Platelet, LH= Luteinizing hormone, FSH= Follicle stimulating hormone, PRL= Prolactin, TESTO= Testosterone, E2= Oestradiol.

Chapter 5

DISCUSSION

Infertility is common in Africa, but virtually no data exists on HIV prevalence among infertile women. Only a few studies have examined the association between infertility and HIV prevalence. Mainly anthropological studies in Africa have shown that infertile women have higher risks of marital instability and possibly also have more sexual partners than fertile women. Despite much recent work on the transmission and control of sexually transmitted infections, little attention has been given to the demographic impact of such infections on human population growth. Most sexually transmitted infections have a detrimental effect on female fertility or male virility in some fraction of infected individuals, and as such they have the potential to induce demographic changes if they are prevalent within a given community. Some, such as syphilis, may induce mortality. The interplay between the epidemiology of disease transmission, the course of infection within an individual, and the demography of the human host population is complex.

This study examined whether sexually transmitted infections, were more common among women with fertility problems than among women without fertility problems at Lister hospital population in Ghana. In this study women attending Lister hospital with infertility problems were compared with controls from the women attending the post-natal clinic. It was found that HIV infection was more common among infertile women (2/300:0.7%) than the forty (40) fertile women (none had HIV infection). A total of two (2) seropositive infertile women was however very small. This result however conforms to earlier studies done in Gabon and Zimbabwe where the prevalence of HIV among infertile women were higher than that of fertile women (De Muylder *et al.*, 1990).

On the one hand, population size, structure, and social organization influence the net rate of transmission within a community. On the other hand, the

infection itself may influence, via infertility or mortality, population size, structure and organization. With the emergence of the human immunodeficiency viruses (HIV-1 and HIV-2), the aetiological agents of the lethal acquired immune deficiency syndrome (AIDS), epidemiologists have recently begun to formulate theories concerning the transmission of infection and the growth of human populations in order to assess the likely demographic impacts of AIDS (Anderson *et al.*, 1991). As yet, however, aside from AIDS, little attention has been given to the demographic effects of other sexually transmitted infections whose influence is more on female fertility than on male and female mortality. It is clear that these infections may have important effects since transmission is largely restricted to females and males in the peak reproductive age classes (Swinton *et al.*, 1992). The magnitude of the impact of sexually transmitted infections on the net fertility of a given population will depend on both the prevalence of infection in these reproductive age classes and the likelihood that infection induces infertility.

From this study, it was observed that the infertile women were averagely older. This finding is not different from earlier works done on the correlation between advanced maternal age and infertility. This is aptly captured by the findings of Brewis, (1993) that in a population of women who do not use contraceptives, relative fertility falls with age by about 40% at age 40, irrespective of the characteristics of the male partner, as is proven by statistics on donor insemination (Maroulis, 1991). In female fertility, the most important age-related factor is ovarian age (decrease in number of oocytes); the uterine state, although a relatively minor factor, seems also to affect the implantation rate (Abdalla *et al.*, 1993). The ovarian reserve is the dominant factor in the relative infertility of women >40 years (Pearlstone *et al.*, 1992). The ovulatory reserve decreases with age even in women with normal ovarian reserve (that is, those whose FSH is normal and who respond to clomiphene; Scott *et al.*, (1995). If the basal FSH is >25 mIU and the age is > 41 years, pregnancy is highly unlikely (Pearlstone *et al.*, 1992).

The mean FSH for the infertile women in this study was significantly higher than that of the fertile women .This finding conforms to the observation made in a study by Lee *et al.,* (1988). This is so because most of the infertile women were averagely older and due to the decrease in the number of oocytes as the women aged, there is a corresponding increment in FSH levels (Abdalla *et al.*, 1993). This is also corroborated by the fact that Inhibin B is known to suppress pituitary FSH secretion, so reduced inhibin production by a decreasing growing follicle population (as ovarian reserve is depleted with age) may elevate FSH secretion (Klein *et al.*, 1996).

Most of the infertile women in this study had hyperprolactinaemia as compared to the fertile women. This finding is in conformity with the review by Rosene-Montella *et al.,* (2000). Idrisa *et al.,* (2003) in a study conducted in North Eastern region of Nigeria showed an increase in the trend of hyperprolactinaemia amongst infertile females. This can be attributed to the fact that hyperprolactinaemia has the potential to adversely affect fertility by impairing gonadotrophin releasing hormone (GnRH) pulsatility and thereby ovarian function (Zollner *et al.*, 2001; Poppe and Velkeniers, 2003).

High testosterone values were found in the infertile women as compared to the fertile ones in this study. This finding is corroborated by a study done by Zaidi *et al.,* (2004) where they found that in some cases there was an extraordinary increase in the levels of testosterone in infertile women, which might be the cause of amenorrhea. It has been reported earlier by Kundi, (1991) that androgen production by the ovary may be markedly increased in some abnormal states, usually associated with amenorrhea. However other observations by Roh *et al.,* (1999) are in contrast with this finding since they observed that there was no significant difference in the testosterone values for the fertile and infertile women in their study.

Upon the basis of the findings generated from this study (i.e. high level of testosterone in infertile women as compared to their fertile counterpart and the significant increase in testosterone with age), it can be safely concluded that the elevated testosterone levels may significantly contribute towards the development of infertility among this study group. However, the observed increase in testosterone with age in this study is contrary to the finding by Zaidi *et al.,* (2004) where testosterone elevation was not found to be age dependent. Since testosterone elevation always has severe indirect effects, identifying the underlying cause of this increase in testosterone is recommended in further study.

From this study, the prevalence of toxoplasma gondii was similar in both fertile (27.5%) and infertile (20.0%) women. The relevance of toxoplasma gondii infection to female fertility was not established from this study. However, observed prevalences were slightly higher than what was observed by Ghoneim *et al.,* (2009) among pregnant women (20.45%) and non-pregnant women (7.95%). This slightly higher seroprevalence among the fertile women compared to the infertile women found in this study may be due to alterations in the immune mechanisms during pregnancy leading to an increase of the invasion of this parasite (Crouch *et al.*, 1995).

The direct relationship between the percentage prevalence of the Toxoplasma gondii infection with age is in agreement with previous studies. Liesenfeld *et al.*, (1996) mentioned that the prevalence of the infection with Toxoplasma gondii increases with age and there are considerable geographic differences in prevalence rates. Hung *et al.,* (2007) mentioned that older age group of 35 years or more had a significantly higher seroprevalence than that of the younger age group of 15-25 years. Also, Fallah *et al.,* (2008) reported that age was statistically significantly associated with higher infection rates. This study showed that the prevalence of no immunity to rubella in fertile women and their infertile counterparts are similar.

Chapter 6

CONCLUSION

6.1 GENERAL CONCLUSION

The observation made from this study indicates that the hormonal profile of infertile women differs from that of their fertile counterpart. In summary this study established a significant increase in testosterone, FSH and prolactin levels amongst the infertile women. However the relevance of the increase in testosterone level to the genesis or aggravation of fertility problem in these women remains to be determined.

Though there were significant decreases in the haemoglobin level and platelets count in the infertile women, the levels were within the reference range.

It can be concluded from this study that the prevalence of sexually transmitted infections were generally higher in the infertile subjects than their fertile counterparts.

From this study, the prevalence of T. gondii is slightly lower among the infertile women (20.0%) as compared to the fertile women (27.5%). Age poses as a risk factor from this study since the prevalence among the infertile women increases as the subject aged.

Though the percentage prevalence of those without Rubella IgG immunity was similar between the fertile and infertile women, there was an improvement in immunity as the women aged. However, the relevance of this finding to the cause of infertility would require further study.

6.2 RECOMMENDATION

Further studies with a larger sample size and probably long follow-up are necessary to validate the variation in the hormonal level as well as other parameters measured in this study to clarify the etiology of the various findings for better management of infertility cases.

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