KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

IMMUNOLOGICAL FACTORS IN FEMALE INFERTILITY: ANTITHYROID AND ANTIPHOSPHOLIPID ANTIBODIES

A THESIS SUBMITTED TO THE DEPARTMENT OF MOLECULAR MEDICINE,

SCHOOL OF MEDICAL SCIENCES

IN

FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF

MASTER OF PHILOSOPHY

IN

IMMUNOLOGY

BY

ISAAC ACHEAMPONG

PG1175613

NOVEMBER, 2016

DECLARATION

All the experimental work described in this thesis was done at the Department of Molecular Medicine, KNUST and the University Hospital, KNUST. This thesis has not been submitted for any other degree in this Institution or elsewhere.

…….. ……

Isaac Acheampong Date

(Student)

…… ……

Prof. (Mrs) M. T Frempong Date

(Supervisor)

…… ……

Prof. F. A Yeboah Date

(Head of Department)

ABSTRACT

Background: Mixed findings regarding the association between the presence of autoantibodies (TPO-abs, TG-abs and APL-abs) and female infertility besides spontaneous abortion have been reported in most studies in developed countries. To screen for these autoantibodies still remains divisive. Additionally, very little is known about their relationship with menstrual abnormalities, body mass index (BMI) and haematological parameters in infertile women in developing countries.

Aim: The aim of this study was to investigate the relationship between a panel of autoantibodies (TPO-abs, TG-abs and APL-abs) and infertility, spontaneous abortion, menstrual abnormalities, body mass index and haematological parameters.

Methods: A case-control study comprising 52 infertile cases and 38 fertile controls in three major hospitals in the Ashanti region of Ghana was undertaken. A standard questionnaire was administered, blood pressure (BP) measured and BMI computed from the weight and height. Blood specimen was collected for haematological (NLR, PLR, #NEUT and #Lymphocyte) and biochemical analysis (TPO-abs, TG-abs and APL-abs). TPO-abs (IgG) >100IU/mL and TG-abs (IgG) >100IU/mL were regarded as positives for the thyroid autoantibodies while APL-abs (IgG/IgM) ≥10U/mL was also regarded as positive for the antiphospholipid antibodies.

Results: Infertility was found to be significantly associated with the presence of thyroperoxidase antibody (TPO-abs: $\chi 2$ (1) = 7.047, p=0.011) and antiphospholipid antibody positivity (APL-abs: $\chi 2$ (1) = 5.55, p=0.02) but not thyroglobulin antibody positivity ($\chi 2$ (1) = 0.907, p=0.357). Neither of the three autoantibodies (TPO-abs, TG-abs and APL-abs) was found to be associated with a history of spontaneous abortion (p>0.05). The odds of APL and both (TPO+TG-abs) positivity were found to be 1.71 and 1.45 times greater respectively among infertile women with menstrual abnormalities compared to those without menstrual abnormalities [APL-abs: (OR=1.71, (95% CI): 0.18-15.95; p=0.636) ; TPO + TG-abs: (OR =1.45, (95% CI): 0.27-7.82; p=0.667)], however both associations were not significant. BMI was comparable between infertile cases and fertile controls and did not correlate significantly with any of the autoantibodies. Neutrophil-lymphocyte ratio (NLR), Platelet-lymphocyte ratio (PLR), absolute neutrophils (#NEUT) and absolute lymphocyte count (#Lymphocyte) were significantly lower in infertile women compared to fertile controls (p<0.05). These haematological parameters however did not correlate significantly with any of the autoantibodies in either infertile cases or controls.

Conclusion: Infertility among Ghanaian women is associated with TPO-abs and APL-abs positivity therefore these antibodies may be screened as part of routine checks for appropriate intervention. However, these autoantibodies may not be directly associated with a history of spontaneous abortion. BMI, NLR, PLR, #NEUT, and #Lymphocyte do not correlate with the presence of autoantibodies in Ghanaian infertile women.

DEDICATION

This thesis is dedicated firstly to the Almighty God for his abundant supply of grace to finish this project and secondly to my supervisor, Prof. (Mrs) M. T Frempong for her motherly love, patience, mentorship and support throughout this project.

ACKNOWLEDGEMENT

I wish to acknowledge the immense support of the following individuals who helped in diverse ways to make this project a success:

Prof. (Mrs) M. T Frempong (my supervisor and Lecturer at the Department of Molecular Medicine) for graciously supervising my work and ensuring the completion of my study.

Prof. C. A Turpin (Head, Department Of Obstetrics And Gynaecology, KATH) for allowing me to select some participants from his department for my study.

Prof. A. T Odoi (CEO, Trust Care Specialist Clinic) for making his fertility clinic available for me to carry out my study and aiding me to select some participants from his clinic.

Dr A. Tawiah (Senior Resident, Department of Obstetrics and Gynaecology, KATH) for his unending support throughout this project.

Dr S. S Asamoah (Lecturer at the Department of Molecular Medicine) for availing his laboratory to me to analyse my samples.

Mr M. Dompreh (Head, Department of Serology) for helping me store my samples at the requisite temperature in his department prior to the analysis.

Mr S. Kwofie (Biochemistry practical instructor, Department of Molecular Medicine), for assisting me with the laboratory analysis of my samples.

Mr Enoch Odame (Student, Department of Molecular Medicine) for assisting me with my laboratory work.

All my friends and family who helped one way or the other to make this project successful, I say God richly bless you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 CHAPTER ONE

1.1. INTRODUCTION

Infertility (clinically) is defined as the inability to achieve clinical pregnancy after twelve months of regular sexual intercourse without contraception (ESHRE Capri Workshop Group, 2002; ASRM, 2008; Zegers-Hochschild et al., 2009). It has important implications for couples in the socio-cultural settings of every society and it is therefore a problem of public health concern globally (Ombelet et al., 2008; WHO, 2015). However, attention to reproductive health is low in developing countries (Leke et al., 2004; WHO, 2015).

Globally, about 48.5 million couples suffer from infertility with approximately 10 million of them from developing countries (Boivin et al., 2007; Mascarenhas et al., 2012b). Couples with infertility go through some degree of stigmatisation that may result in depression especially among infertile females in most socio-cultural settings in Sub-Saharan Africa (Ombelet et al., 2008). The prevalence of depression among infertile females in Ghana, according to a study carried out in Tamale, stands at 62% (Alhassan et al., 2014). This is further aggravated by the high financial burden associated with assessing modern techniques of reproductive care (Bushnik et al., 2012; Cui, 2010). The prevalence of infertility in sub-Saharan Africa differs from one country to the other because of socio-cultural diversities, with Ghana recording a female infertility prevalence of about 11.8% (Geelhoed et al., 2002). The prevalence of immunologically associated female infertility is unknown but about 10% of the causes of infertility generally have been associated with it (Beer et al., 1996). The world-wide prevalence of infertility among nulliparous women of child bearing age in 2010 was 1.9% and 10.5% for women with one previous birth (Mascarenhas et al., 2012b).

Females have been reported to be more prone to autoimmune diseases compared to their male counterparts and this has been ascribed to the effects of oestrogens on the

normal immunity (ESHRE Capri Workshop Group, 2002). Infertility in females may be divided into primary and secondary. Primary infertility involves the inability of a woman of reproductive age with no previous birth to conceive, in spite of regular sexual intercourse without contraception over one year. Secondary infertility involves the inability of a woman of reproductive age with previous birth(s) to conceive, though exposed to regular sexual intercourse over one year without contraception (Mascarenhas et al., 2012b). The duration for classification as infertility is one year for the clinical definition and two years for the epidemiological definition (WHO, 2006; Zegers-Hochschild et al., 2009).

Various immunological factors have been associated with both primary and secondary infertility and notable amongst them are antiphospholipid antibodies (APL-abs) and the thyroid autoantibodies [thyroglobulin (TG-abs) and thyroperoxidase antibodies (TPO-abs)] both of which have been associated with spontaneous abortion (Li et al., 1998; Balucan et al., 2013). APL-abs have been reported to be the possible culprit that initiates the inflammatory response accountable for the eventual foetal loss in recurrent abortion through complement activation and neutrophil infiltration (Shetty and Ghosh, 2009; Meroni et al., 2010). They result in the antiphospholipid antibody syndrome which may be characterized by multiple thrombosis, thrombocytopenia, recurrent foetal loss, pregnancy induced hypertension, intrauterine growth retardation and prematurity (Aron et al., 1995; Shetty and Ghosh, 2009).

Thyroid autoantibodies (TPO-abs and TG-abs) have also been reported to be known culprits whose presence results in adverse effects distressing both mother and child in pregnancy (Poppe et al., 2008; Gaberščček and Zaletel, 2011). It has been associated with reproductive failure in females of reproductive age. Spontaneous abortion has also been reported to be significantly higher in such women positive for the thyroid autoantibodies as compared to controls (Glinoer et al., 1991; Zhong et al., 2012). Furthermore, thyroid autoimmune disease such as

Hashimoto's thyroiditis, which is as a result of the effect of thyroid autoantibodies have been identified as a major cause of subclinical or overt hypothyroidism and it is associated with menstrual abnormalities in females of reproductive age (Poppe et al., 2008).

Spontaneous abortion or miscarriage is defined as the loss of clinical pregnancy spontaneously before the 20th week of gestation (Zegers-Hochschild et al., 2009). The prevalence of spontaneous abortion in Ghana varies from 5% (WHA, 2011) to about 10% (Oliveras et al., 2008) and is sometimes associated with maternal death which stands at 350 per 100,000 births currently, aside the psychological trauma it brings to infertile couples (Rominski and Lori, 2014). Spontaneous abortion as a result of the effect of the presence of non-organ specific antiphospholipid autoantibodies as well as organ specific thyroid autoantibodies therefore needs to be further investigated since most studies have been inconclusive about their association. (Thangaratinam et al., 2011; Petrikova et al., 2014).

1.2. PROBLEM STATEMENT

The prevalence of infertility in sub-Saharan Africa ranges between 11-20% (Leke et al., 2002) however, 5-15% is estimated as the practical range of prevalence in both developed and developing countries (Boivin et al., 2007). Infertility is a contributory factor for most marital problems leading to divorce, the effect of which cannot be overlooked in an African society like Ghana where most couples (especially the women) go through a high degree of stigmatisation from family members (Ericksen and Brunette, 1996; Ombelet et al., 2008; Fledderjohann, 2012). As a result, some couples would seek reproductive health care to understand the cause of their problem and initiate appropriate intervention. Unfortunately, 1020% of the cause of infertility still remains unknown though most of these have been associated with some immunological factors (ESHRE Capri Workshop Group, 2002). Several studies have given mixed reports about the association between female infertility and the presence of autoantibodies (APL-abs, TG-abs

and TPO-abs) in the serum (Petrikova et al., 2014; Thangaratinam et al., 2011; Kutteh et al., 1999a; Geva et al., 1997).

1.3. JUSTIFICATION

Thyroid autoantibodies (TPO-abs, TG-abs) and antiphospholipid antibodies (APL-abs) have been reported to have some association with spontaneous abortion but its association with other aspects of infertility among females remains unclear (Poppe et al., 2008; Meroni et al., 2010). The evaluation of autoantibody abnormalities in all cases of reproductive failure remains relevant in most jurisdictions (Petrikova et al., 2014). Most of the studies carried out on autoantibodies with respect to reproductive failure were done in Caucasians while very little is known among African women presenting with infertility. There is scarcity of information regarding autoantibody associated female infertility in Ghana. The findings from this study may therefore help clinicians to extend the screening of infertile women to include these autoantibodies in order to expedite appropriate treatment. It will also help scientist to know the impact of these autoantibodies on infertility in the Ghanaian female population.

1.4. ALTERNATE HYPOTHESIS

- Autoantibodies (TPO-abs, TG-abs and APL-abs) are associated with infertility, haematological parameters and BMI among Ghanaian women.
- The presence of autoantibodies (TPO-abs, TG-abs and APL-abs) in infertile women are associated with a history of spontaneous abortion and menstrual abnormalities.

1.5. AIM

The aim of this study is to investigate the relationship between a panel of autoantibodies (TPO-abs, TG-abs and APL-abs) and infertility, spontaneous abortion, menstrual abnormalities, haematological parameters and body mass index among infertile women in Ghana.

1.6. SPECIFIC OBJECTIVES

- To characterize the relationship between autoantibodies (TPO-abs, TG-abs and APL-abs) and infertility, body mass index and haematological parameters in the female study participants.
- To describe the association between autoantibody (TPO-abs, TG-abs and APL-abs) positivity and a history of spontaneous abortion among infertile women.
- To describe the relationship between the presence of thyroid autoantibodies (TPO-abs, TG-abs) and menstrual abnormalities among infertile women.

2.0 CHAPTER TWO

2.1. INFERTILITY (DEFINITIONS)

Infertility is a condition that affects married couples worldwide, the cause of which has been associated with both male and female factors. It has two main definitions; namely clinical and epidemiological definition (Mascarenhas et al., 2012b). However, demographers have modified the epidemiological definition into a third; the demographic definition (Larsen, 2000).

The clinical definition defines infertility as a disease of the reproductive system caused by the failure to achieve a clinical pregnancy after one year of sexual intercourse without contraception according to the World Health Organisation (Zegers-Hochschild et al., 2009). The American Society for Reproductive Medicine defines infertility as 12 months or more of trying to achieve a successful pregnancy with regular sexual intercourse without contraception (ASRM, 2008). The epidemiological definition defines infertility as the inability of a female of child bearing age, exposed to continuous risk of pregnancy, to achieve gravidness after two years of trying (WHO, 2006). The reason for the difference in duration stems from the fact that whereas it is of uttermost importance for early diagnosis and initiation of appropriate treatment in clinical practice as per the clinical definition, the epidemiological definition is tailored to reduce the capturing of false negative cases as infertility (Larsen, 2005). The demographic definition differs from the two definitions stated above only in relation to live births instead of clinical pregnancy and spans a period of one to seven years in contraceptive-naïve couples in most studies (Gurunath et al., 2011).

The variation in epidemiological and demographic definitions of infertility has affected the estimation of the prevalence of infertility resulting in a lack of dependable estimates (Mascarenhas et al., 2012a). It is bequeathed with differences in mainly outcome of pregnancy, duration of exposure to risk of pregnancy, type of exposure and age ranges (Gurunath et al., 2011).

Infertility can be further classified as primary and secondary based on the history of previous conception or live births (WHO, 1975). The first type of infertility is described as primary if the female has never conceived. Those with previous conceptions or live births but with current inability to conceive after 12 months are considered clinically as having secondary infertility. Therefore, desiring females with histories of spontaneous abortions or still births with current inability to conceive are described as having secondary infertility (Rowe et al., 1993; Mascarenhas et al., 2012a). Other terms associated with infertility which have often been interchanged and have also affected estimates includes subfertility and subfecundity. Subfertility refers to any form or grade of temporary inability of desiring couples to conceive (Gnoth et al., 2005). It is sometimes interchanged with infertility because current modern assisted reproductive technology (ARTs) aids most couple to conceive who hitherto would have been difficult without intervention (Gnoth et al., 2005; Zegers-Hochschild et al., 2009). Subfecundity refers to the decreased inability to deliver (Karmaus et al., 1999).

2.2. PREVALENCE OF INFERTILITY

Healthy couples who are within their active reproductive age have a 20-25% probability of achieving conception per reproductive cycle with a cumulative probability of 60% within the first 6 months of continuous coitus and 84% within the first twelve months (Esteves et al., 2011). Despite these expected outcomes, some couples are presented with a problem where conception becomes unattainable despite continuous sexual intercourse over 12 months, hence their classification as infertile clinically. Demographic and house-hold data form 277 surveys across countries revealed a worldwide prevalence of infertility of 48.5 million in 2010 (Mascarenhas et al., 2012b). Boivin et al. (2007) in estimating the prevalence of infertility from population surveys pegs infertility at affecting 72.4 million women within the ages of 20 and 44 who have been trying over 12 months

without contraception to deliver. Out of this number, he estimates that 40.5 million had access to some medical interventions. 5-15% is estimated as practical range of prevalence in both developed and developing countries (Boivin et al., 2007).

Very few studies have reported on the prevalence of infertility in developing countries especially in Africa (Ombelet et al., 2008). As a result, varying figures are reported from one geographical location to the other with some from demographic and Health survey data and others from community survey and house-hold data published over a decade ago. Although the general prevalence in developing countries is pegged at 6.9% to about 9.3% (Boivin et al., 2007), individual countries have varying prevalence with some as high as 20%. Nigeria is reported as having a prevalence of 20-30% (Okonofua, 1996). A recent study by Sule et al. (2008) places their primary infertility prevalence at 22.5%. Gambia is reported with a prevalence of 9% (Sundby et al., 1998). According to Geelhoed et al. (2002) in a community-based survey in a rural district in Ghana, the prevalence of infertility among women is estimated at 11.8% and 15.8% for men. Aside the fact that this study was done among rural community dwellers, extrapolation to urban centres may achieve varying figures because of the differences in the standards of living, and the availability of accessible fertility treatment centres or artificial reproductive technology in the urban communities.

The worldwide prevalence of infertility in nulliparous women in 2010 was estimated to range from 0.6% to 3.4% (primary infertility) while that in primiparous and multiparous women ranged from 8.7%-32.6% (secondary infertility) (Mascarenhas et al., 2012a). In Africa primary infertility is estimated to have declined from 2.7% in 1990 to 1.9% in 2010. The reduction was attributed to a reported low prevalence among countries in Sub-Saharan Africa, which ranged between 1.0% to 1.1% (Mascarenhas et al., 2012b). Again, the prevalence of secondary infertility in sub-Saharan Africa was also reported to have declined

from 13.5% to 11.6% in 1990 and 2010 respectively, relative to rise in some parts of northern Africa which reported as high as 18.0% (Mascarenhas et al., 2012b). The decline in the prevalence of primary infertility and secondary infertility could be attributed to a reduction in infections including sexually transmitted diseases over the past 20 years which in earlier studies by the WHO task force in 1987 revealed that 85% of women from Africa had an infertility diagnosis attributable to infection (WHO, 1987).

2.3. CAUSES OF INFERTILITY

The aetiology of infertility is multifactorial ranging from male factors to female factors. These factors may be diseases or defects associated with the reproductive tract, neuroendocrine system and immune system resulting in reproductive failure (Haller-Kikkatalo et al., 2011).

It is reported that a viable foetus is achieved in less than half (30%) of all female conceptions while 50% is lost before the first missed menstrual period. Approximately 25% of embryos are also reabsorbed within a 7-14 day range after implantation in the endometrium. Also, 15% of implanted embryos are lost during maturation before the 20th week of pregnancy (Choudhury and Knapp, 2000).

2.3.1. *Role of immunological factors*

Immunological factors have been identified to contribute to infertility, pregnancy loss, intrauterine foetal demise and pregnancy complications such as pregnancy induced hypertension in recent years resulting in reproductive failure (Geva et al., 1997; Gleicher, 2002). Several causes have been reported to be associated with the development of autoimmune disease including aging, infectious agents, genetic, endocrine and environmental triggers. In the endocrine system, hormones play an important role in autoimmune infertility especially in females. Females have been reported to be more prone to autoimmune conditions compared to males

because of the increased oestrogen levels in females which is known to influence the normal immunity (ESHRE Capri Workshop Group, 2002).

The immune system of the human body is designed to distinguish between local antigens produced by itself (self) and foreign agents (non-self) (Bourke et al., 2013). This mechanism is present in order to recognise and dismiss invasions by infections and cancer cells. In an instance where a local agent produced by itself is recognised as foreign, and the body elicits a strong immune response, an autoimmune disease is said to have resulted which may lead to an inflammation (Bourke et al., 2013). The foetus in pregnancy is a foreign body (with male chromosomal components) implanted in the uterus and the body's immunological network must adjust to accommodate its invasion of the uterine lining during implantation by recognising it as 'self' in order to prevent elimination; so the foetus can flourish (Mor et al., 2011). Pregnancy therefore can be described as a state of immunological tolerance (Weiss et al., 2009). Two types of immune responses may act individually in response to the presence of a foreign molecule: Th-1 or Th-2 response. They are mediated by various lymphokines or cytokines based on the invading agent. While a Th-1 response will involve IL-2, TNF-β, interferon-γ, a Th-2 response involves IL-4, IL-5, and IL-10. Both responses are characterized by IL-3, IL-6, IL-1, IL-13, TNF-α, GM-CSF, and encephalin (Gleicher, 2002). In pregnancy an interplay of both Th-1 and Th-2 occurs. Some studies have postulated that, the first trimester and third trimester of pregnancy usually involves a Th-1 response whiles the second trimester involves a Th-2 response (Choudhury and Knapp, 2000). Clinical conditions associated with a Th1 response includes cytotoxic reactions, inflammatory reactions, delayed-type hypersensitivity and tissue injury. Th-2 reaction primarily involves antibody production, IgE responses and eosinophil proliferation. Gleicher (2002), postulates that the presence of which ever autoantibodies, ranging from nonorgan

specific antiphospholipid to organ specific thyroid autoantibodies in reproductive failure may be simply as a result of a Th-1 dominance in pregnancy.

2.3.2. Thyroid Autoimmunity

Described as the commonest cause of hypothyroidism and thyroid dysfunction in general, especially in women of child bearing age, thyroid autoimmunity is characterized by the presence of thyroid autoantibodies (Vissenberg et al., 2015). Other causes of thyroid dysfunction may include iodine deficiency, drug induced and post-partum thyroiditis. It is established that thyroid hormones have an effect on the normal ovarian function by directly affecting the granulosa cells, luteal cells and the oocytes (Ajmani et al., 2015). Therefore, thyroid dysfunction is associated with ovarian dysfunction, menstrual irregularities, subfertility and recurrent miscarriages (Krassas et al., 2010; Thangaratinam et al., 2011). Three antibodies have been isolated as culprits in thyroid autoimmunity. These include thyrotropin (TSH) receptor antibody, thyroperoxidase antibody (formerly called thyroid microsomal antibody), and thyroglobulin antibody. The effect of the thyrotropin receptor can either be stimulatory or inhibitory and it's been associated with Graves' disease when it is stimulatory (Kamath et al., 2012; Vissenberg et al., 2015).

2.3.3. Thyroid Hormone Production and Thyroid Autoantibodies

Thyroid hormone production

Thyroglobulin (TG) was first described to provoke an immune response in 1925 by Hektoen and Schulhof (Hektoen and Schulhof, 1925). Thyroglobulin antibodies mostly of the IgG class, with very low levels of the IgA class, have also been associated with both Graves' and Hashimoto's diseases. Thyroperoxidase antibodies (TPO-abs), mostly of the IgG class are produced against the thyroperoxidase enzyme. The presence of TPO-abs have been commonly associated with the autoimmune disease Hashimoto thyroiditis, which is often characterised by subclinical hypothyroidism and first described by Hakaru

Hashimoto (Chan and Boelaert, 2015). Thyroglobulin and thyroperoxidase are required in the synthesis of thyroid hormones and its secretion. In the synthesis of thyroid hormones, thyroglobulin homodimers are iodinated to form monoiodotyrosine and di-iodotyrosine. This happens after its synthesis and glycosylation in the rough endoplasmic reticulum and it is exocytosed through the apical membrane of the thyroid follicular cells (Yen, 2001). Thyroperoxidase enzyme is responsible for oxidising the iodine into iodide, organifying the iodide to the tyrosine residue and to thyroglobulin forming mono- and di-iodotyrosine and finally coupling the formed units into either thyroxine (T_4) or triiodothyronine (T_3) (Balucan et al., 2013). The production of T_3 and T_4 is stimulated by thyroid stimulating hormone (TSH).

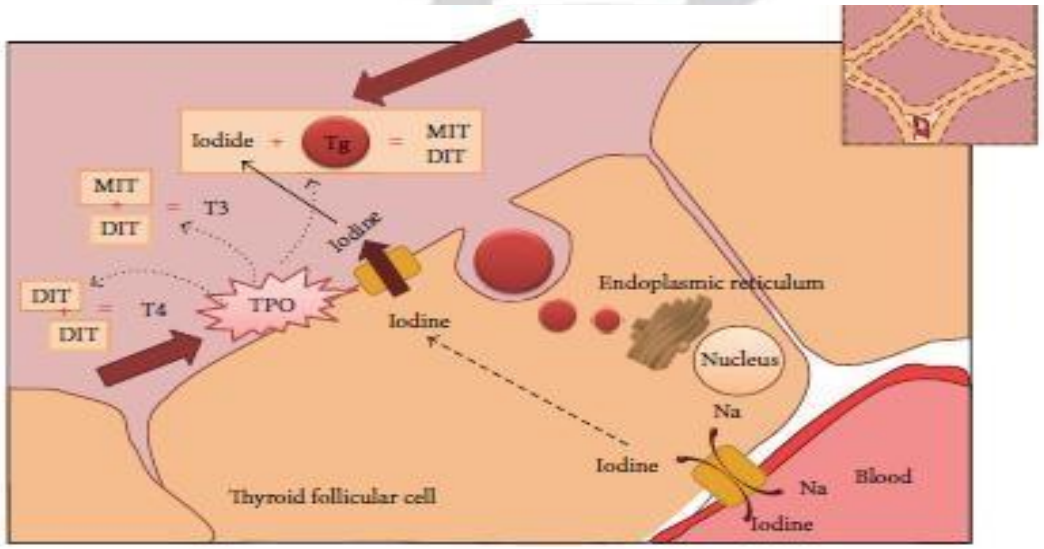

Figure 2.1. Thyroglobulin and thyroperoxidase in thyroid hormone synthesis (source Balucan et al., 2013)

T_3 and T_4 are responsible for physiological processes in the human body including metabolism, temperature, growth and development (Yen, 2001).

The effect of the TPO-abs destroy the thyroperoxidase enzyme affecting oxidation and organification of iodide and the coupling of the mono-and di-iodothyrosine units. Thyroglobulin homodimers are also destroyed by the thyroglobulin

antibodies. Therefore in autoimmune thyroiditis, despite the stimulus from thyroid stimulating hormone (TSH) secreted by the pituitary gland, insufficient amounts of T_3 and T_4 are produced leading to subclinical or overt hypothyroidism.

In pregnancy, slight changes in the thyroid enzymes affect foetal development (Chan and Boelaert, 2015). Contrary to the action of TPO-abs and TG-abs, thyroidstimulating immunoglobulin can mimic TSH and cause overproduction of thyroid hormones resulting in hyperthyroidism which is characterized in pregnancy by congestive heart failure, pre-eclampsia, miscarriage, pre-term and low weight births (Gaberšček and Zaletel, 2011).

2.3.4. Thyroid Hormones in Pregnancy and Reproductive Failure

Pregnancy presents with increased thyroid hormone requirements as a result of elevated thyroid-binding globulin concentrations induced by oestrogen, increased volume of distribution of thyroid hormones and increased transport and degradation of thyroxine by the placenta (Chan and Boelaert, 2015). Placental levels of human chorionic gonadotrophic hormone (βHCG) rises after fertilisation and peaks at 10 weeks gestation, then decrease to lower levels (Glinoer, 1997). The increased in βHCG causes an increase in maternal oestrogen levels which stimulates the liver to produce thyroxine-binding globulin (TBG). TBG has a high affinity for thyroid hormones and serves as their transport protein in blood (Balucan et al., 2013). The thyroid hormones play pivotal role in foetal development. T3 and T4 levels affect foetal metabolism and the development of

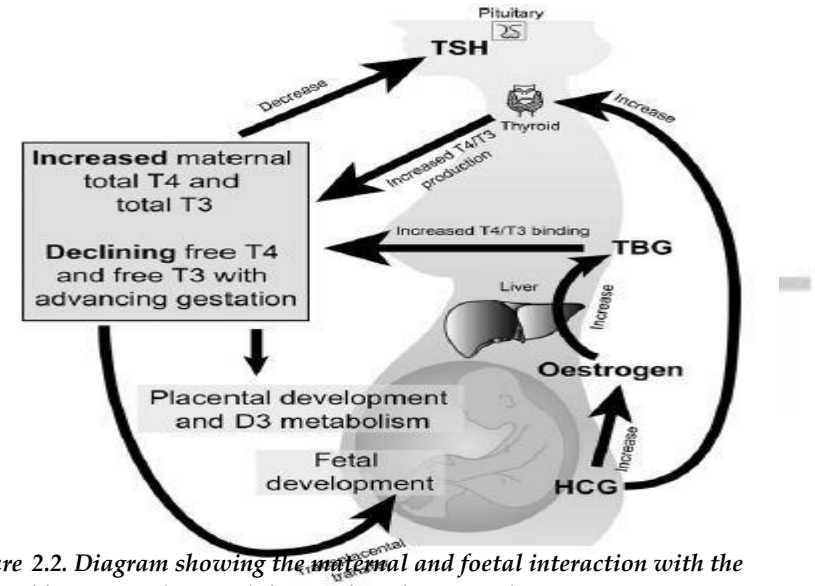

Figure 2.2. Diagram showing the maternal and foetal interaction with the thyroid hormones. (Source (Chan and Boelaert, 2015)

the brain and nervous system. The foetus is dependent on maternal thyroid hormones during the first trimester until its own thyroid gland begins to function at around 12 weeks (Leung, 2012). Even at this stage excessively varying maternal levels may still affects the foetal development (Glinoer, 1997).

2.3.5. Thyroid Autoimmunity as it Relates With Infertility, Menstrual Abnormalities and Spontaneous Abortion

As already reported, thyroid autoimmune disease, especially as it relates to hypothyroidism as a result of the presence of TPO-abs and TG-abs, affects gonadal function in both men and women (Krassas et al., 2010). In women it affects the granulosa cells, luteal cells and the oocytes leading to menstrual abnormalities (especially oligomenorrhea, infertility and reproductive failure in general). Altered levels of thyroid hormones in pregnant women is associated with an increased risk of obstetric complications such as intrauterine foetal demise, gestational hypertension placental abruption and poor perinatal outcome. Autoimmune hypothyroidism early in pregnancy leads to an increased risk of spontaneous miscarriage and post-partum thyroiditis (Glinoer, 1997). StagnaroGreen et al., 1990 and Glinoer et al., 1991 were the first to establish a strong relationship between hypothyroidism due to autoimmune thyroid dysfunction and spontaneous abortion. Afterwards, there has been several studies

that have given mixed reports about the relationship between the presence of thyroid autoantibodies and spontaneous abortion especially in recurrent miscarriage (Kutteh et al., 1999b; Zhong et al., 2012). While some reports have reported a two-
to four-fold greater risk of spontaneous miscarriage among women with autoimmune thyroid disease (Bakimer et al., 1994), others have found no
association (Ashrafi et al., 2007).

2.3.6. Antiphospholipid Antibodies Associated Infertility and Spontaneous Abortion

During copulation, the spermatozoa from the male is injected into the female and climbs up the uterus to fertilize the ovum that forms the zygote. The implantation of the fertilized ovum is achieved approximately six days after fertilisation and the blastocyst become completely covered by maternal epithelium within 9-10 days (Sebire et al., 2002). There are interactions between the maternal decidua and the trophoblast of the placenta, developed from the outer cells mass of the blastocyst after differentiation. Early in pregnancy, the inhibition of normal endovascular trophoblast invasion as a result of interaction of circulating maternal antibodies from the endothelium in maternal decidua vessels may be responsible for early pregnancy loss (Sebire et al., 2002).

The antiphospholipid antibodies have been reported to be a very strong risk factor in spontaneous abortion and present in as high as 40% of women with recurrent abortion (Shetty and Ghosh, 2009). APL-abs is made of three autoantibodies namely, anticardiolipin antibodies (aCL-abs), β2glycoprotein-1 (anti-β2GP1) antibodies and lupus anticoagulant. In antiphospholipid syndrome (APS), antiβ2GP1 antibodies have been considered the main antibodies effecting its clinical symptoms (Meroni et al., 2010). Antiphospholipid antibodies are acquired antibodies, IgG, IgM and or IgA that elicit their effect through a thrombotic, inflammatory and an immune-modulatory mechanism. Despite these elucidated, the pathological mechanism of APL-abs still remains unclear. APL-abs can act

directly by binding anionic phospholipids or plasma lipids bound to anionic phospholipids. In the implanted foetal unit, phospholipids are responsible for the holding of the diving cells of the syncytioblastocyte together. This aids the growth and nourishment of the foeto-plancental unit through its interaction with the uterine wall of the mother and helps in waste removal (Evain-Brion et al., 2009). The mechanism of action of APL-abs have been proposed in some studies to bind to monocytes, platelets and endothelial cells which induce a proinflammatory and a prothrombotic state on the cytotrophoblastic cells (CT) of the placenta (Giannakopoulos et al., 2007). It is these CT cells that differentiate into the syncytiotrophoblast, which acts as a barrier of protection between the mother and the foetus, through a fusion of the villous trophoblast (Evain-Brion et al., 2009). The extra villous trophoblast also invade and colonises the endometrium of the mother progressively. The presence of APL-abs is suspected to compromise the holding ability of the phospholipids and cause infarction through an activation of the complement cascade. Therefore, they cause a placental thrombosis and invade trophoblast by binding to the trophoblastic membrane through both a β2 –GP1 dependent and independent APL-abs binding, resulting in the infarction of the placenta with resultant embryo toxicity (Holers et al., 2002; Simone et al., 2010). The resultant effect is the antiphospholipid syndrome (APS). Another mechanism elucidated to play a role in APS is the immunomodulation by toll-like receptors (TLR) of the innate immune response. TLR-2 and TLR-4 have been reported to activate monocytes, endothelial cells and platelets in thrombotic APS models (Giannakopoulos et al., 2007). TLR-4 activates HTR-8 cell line which is an extra villous trophoblastic cell line in the presence of APL-abs leading to uncontrolled inflammation and apoptosis (Jovanović et al., 2010).

The APS is characterized by multiple thrombosis, thrombocytopenia, pregnancy induced hypertension, intrauterine growth retardation, prematurity and

recurrent foetal loss (Levy et al., 2010; Meroni et al., 2010). APS can therefore simply be described as an autoimmune disorder associated with antibodies that attack the cell-membrane phospholipids of the dividing special cells of the foeto-placental unit provoking blood clots through complement activation and eventually leading to infarction of the blood vessels in the placenta (Akhlaghi et al., 2013). It is diagnosed using clinical history and laboratory measurement of autoantibodies when one clinical and one laboratory criteria are met. The classification is reported to hold between 12 weeks to 5years of diagnosis of APL-abs and Clinical symptoms (Miyakis et al., 2006). The criteria is as follows:

- When in any tissue or organ, at least one episode of arterial, venous or small vessel thrombosis clinically diagnosed.
- Pregnancy morbidity:
  - ✓ At least one unexplained demise of clinically confirmed (ultrasound or direct examination) morphologically normal foetus before the two and a half months (10th week) in the first trimester or
  - ✓ At least one preterm delivery of a neonate before the 34th week, who is morphologically normal, either through eclampsia, preeclampsia or identifiable features of placental insufficiency or
  - ✓ At least three consecutive inexplicable spontaneous abortions in the first trimester before the 10th week of gestation, where maternal and paternal chromosomal causes are excluded as well as maternal anatomic or hormonal abnormalities (Marchetti et al., 2013).
- Laboratory diagnosis of lupus anticoagulant (LA), IgG and IgM antibodies of anticardiolipin (aCL) and IgG and IgM of anti-β_2 glycoprotein-1 in serum or plasma on at least two occasions in a minimum of 12weeks apart by standard methods. aCL must be >40 GPL or MPL, LA must be according to

the guidelines of the international society on thrombosis and haemostasis (Miyakis et al., 2006).

APL-abs have been reported to be associated with recurrent abortions, foetal demise, foetal growth retardation and early pre-eclampsia in APL-abs positive women (McNeil et al., 1991). APL-abs target and bind to beta-2-glycoprotein 1 (β2-GPI) and prothrombin (Giannakopoulos et al., 2007). Prothrombin, also called factor II, is a proenzyme that is converted to thrombin upon cleavage by prothrombinase. It plays a role in the clotting cascade (Blank et al., 1991; Ağar et al., 2010). β2 -GPI, formerly known as Apo lipoprotein H is a 43-kDa multifunctional highly glycosylated protein present in the plasma that binds anionic phospholipid containing moieties and membrane phospholipids. It consists of a 5 short consensus repeat domains of 326 amino acids. Its effect is seen in coagulation and apoptosis. It serves as the auto-antigen that binds to APL-abs in APL-abs positive patients (Ağar et al., 2010). It exists in two conformations; an activated open conformation and a circular closed plasma conformation. Among the 5 domains made up of domain I to V, domain V has been reported as the culprit that binds the anionic phospholipids (Blank et al., 1991) .

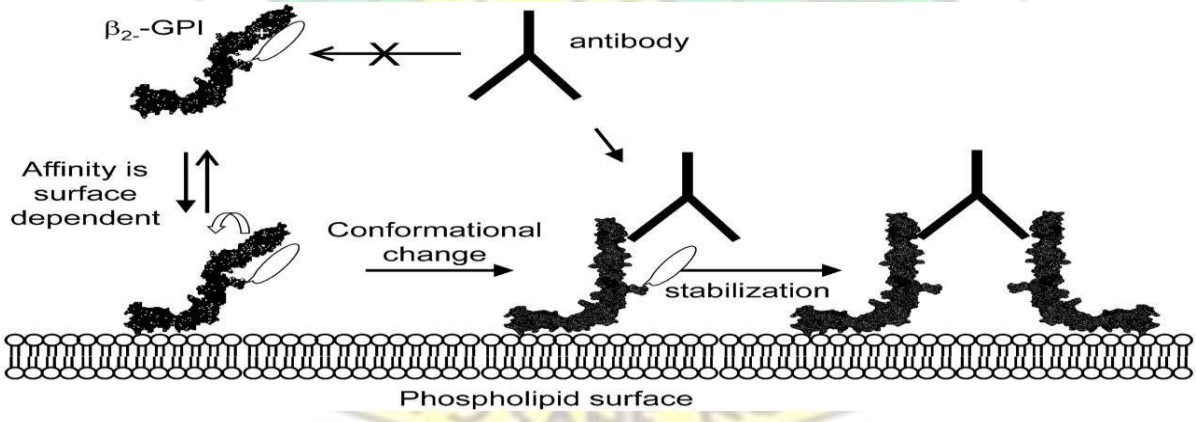

Figure 2.3. Diagram showing binding of β2-GPI to antibody and phospholipid antibody. (Source: Google images at www.google.com)

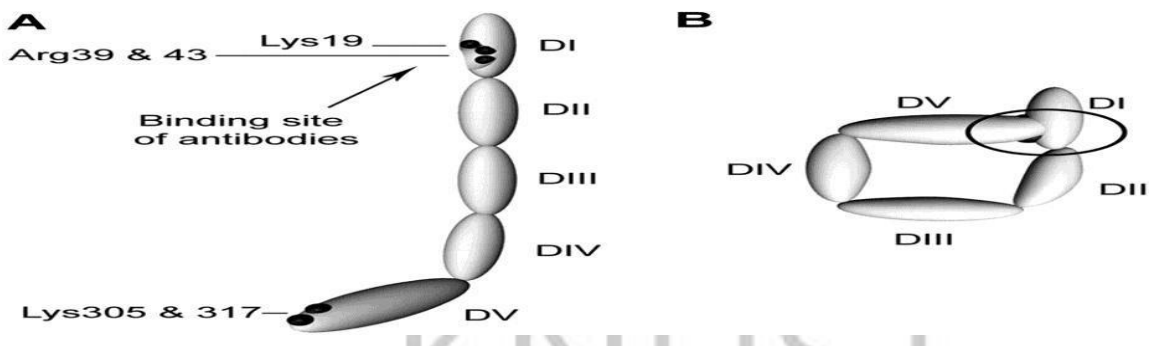

Figure 2.4. Diagram showing the two conformational structures of β2-GPI. (Source: Ağar et al., 2010)

The circulation of β2 –GPI in the blood is in the circular conformation and opens up when activated following its exposure to anionic phospholipids. This open conformation remains open for a much longer time and is able to form complexes with other plasma proteins in the presence of autoantibodies and expresses new epitopes (De Groot and Urbanus, 2012).

2.4. Male Factor in Infertility

Male infertility factor has been reported to be accountable for up to 50% of infertility problems among couples (Jungwirth et al., 2015). The male factor depends primarily on two things:

- The ability to make healthy spermatozoa and
- The ability to have and sustain an erection and ejaculate into the female so spermatozoa can reach the ova alive.

Making abnormal and insufficient sperms is accountable for majority of male factor problems resulting in infertility. In most cases the spermatozoa may be few (oligozoospermia) or may not be produced at all (azoospermia) (Esteves et al., 2011). In other cases, the spermatozoa may be abnormal, ranging from immature forms, poor motility, and abnormal cephalics, middle piece or tails. The root cause of these outcomes may range from hormonal problems (neuroendocrine abnormalities), inflammatory diseases, anatomical defects, genetic defects,

immunological factors such as producing anti-sperm antibodies and environmental problems including infection and lifestyle factors such as alcohol abuse. Inability to ejaculate is mostly due to neurological problems that may require psychotherapy (Manetti and Honig, 2010; Esteves et al., 2011; Vissenberg et al., 2015).

2.5. OBESITY AND INFERTILITY

The prevalence of obesity among women of child bearing age is increasing world over and comparable between males and females in developing countries, but in Ghana, where weight gain is seen as a sign of good living, it is 5.5% higher in females than males (Biritwum et al., 2005). While some studies have related obesity with lower pregnancy rates in sub-fertile women (Steeg et al., 2008), others have associated obesity with only some signs of poor reproductive function like menstrual abnormalities, greater risk of miscarriage, and complications in pregnancy (Balen et al., 2007). They therefore describe its association with reproductive function as complex and difficult to establish a linear relationship (Jungheim et al., 2012). Recently, measures such as skin fold thickness, waist circumference, waist-to-hip ratio, bio-impedance and even adiposity have been reported to be relatively better assessments of obesity compared to BMI, however, BMI still remains the most widely used and easily accessible measuring tool for obesity (Balen et al., 2007; Jungheim et al., 2012).

The mechanism by which obesity causes reproductive failure has been linked to signalling molecules which are produced based on the amount of adipose tissue mass in the body. These signalling molecules are called adipokines and include adiponectin, free fatty acids, leptin, tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) (Gosman et al., 2006). They are reported to have a direct effect on the hypothalamic pituitary adrenal axis. The adipokines affect normal oocyte recruitment and ovulation, especially TNF-α and leptins. They act as signalling

molecules, whose signals may affect implantation in pregnant obese women (Jungheim et al., 2012).

Most of these aforementioned players in obesity have been isolated as culprits in immune modulation. The adipokines have been reported to play a role in the pathogenesis of autoimmune diseases that contribute to infertility such as Hashimoto's thyroiditis (Biondi, 2010). Leptins affect immune self-tolerance by controlling the responsiveness and function of Treg cells (Procaccini et al., 2011). In a study by Marzullo et al. (2010), he found that autoimmune thyroid disease patients positive for the TPO-abs were mostly severe compared to negative controls. Other studies have also reported that the relationship between autoimmune thyroid disease and obesity is not a one way relationship and other associations must be investigated (Rotondi et al., 2011). Tamer et al. (2011), found that the thyroid autoantibodies correlated well with obesity and hyperlipidaemia.

2.6. HAEMATOLOGICAL PARAMETERS AND INFERTILITY

Platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR) have been reported as non-specific markers of inflammation in the peripheral blood (Cakmak et al., 2015). Pregnancy, Spontaneous abortion and some aspects of infertility including menstrual abnormalities such as endometriosis are also considered as inflammatory processes. Antiphospholipid syndrome (APS) which is associated with APL positive women is characterized by thrombocytopenia and recurrent abortion and the culprits for the inflammatory process in women with APS (Aron et al., 1995; Shetty and Ghosh, 2009; Meroni et al., 2010). Based on these, we hypothesized that infertility may be associated with changes in peripheral blood haematological parameters such as NLR, PLR, platelet count, absolute lymphocyte count and absolute neutrophil count.

3.0 CHAPTER THREE

3.1. METHODOLOGY

3.1.1. Study Site

The study was conducted at the Obstetric and Gynaecological Unit of the Komfo Anokye Teaching Hospital (KATH), the Maternity unit of the Kumasi South Hospital and the Fertility Clinic of the Trust Care Specialist Hospital in the Ashanti region of Ghana. The Ashanti region lies in the middle belt of the country and has a population of 4,780,280 making up 19.4% of the total national population according to the 2010 population and housing census. The Kumasi metropolis, where this study was sited has the largest share of the region's total population, making up 42.6% (Ghana Statistical Service, 2013).

The Komfo Anokye Teaching Hospital is a 1200-bed tertiary teaching hospital that trains medical students in collaboration with the Kwame Nkrumah University of Science and Technology. The hospitals receive referrals from other hospitals spanning the whole middle belt of Ghana as well as the northern and upper regions. This study was cited in the Obstetric and Gynaecological Unit of the hospital. Majority of the study participants were resident in the Ashanti region.

The Kumasi South Hospital is a primary care facility that serves as the regional hospital under the strata of the Ghana Health Service in the Ashanti region. As a result, it serves as the first point of care for most indigenes around the Chirapatre Agogo area as well as others from the whole Kumasi Metropolis. This current study was cited at the Maternity unit of the hospital.

The Trust Care Specialist Hospital is a 30-bed private health facility that runs specialisations in internal medicine, general and paediatrics/child health and obstetrics/gynaecology. It serves as a preferred centre for both nationals and internationals who choose to seek healthcare in a private setting within the region.

This current study was sited at the obstetrics and gynaecology unit (specifically the fertility clinic) of the hospital.

3.1.2. Study Design

The study design was a non-randomized case-control study. Infertile women visiting the Obstetric and Gynaecologist unit of KATH, the Fertility Clinic of the Trust Care Specialist Hospital and the Maternity Clinic of the Kumasi South Hospital who fit the inclusion criteria were included in the study as cases. As well, fertile women visiting those same clinics who fit the inclusion criteria were included in the study as controls.

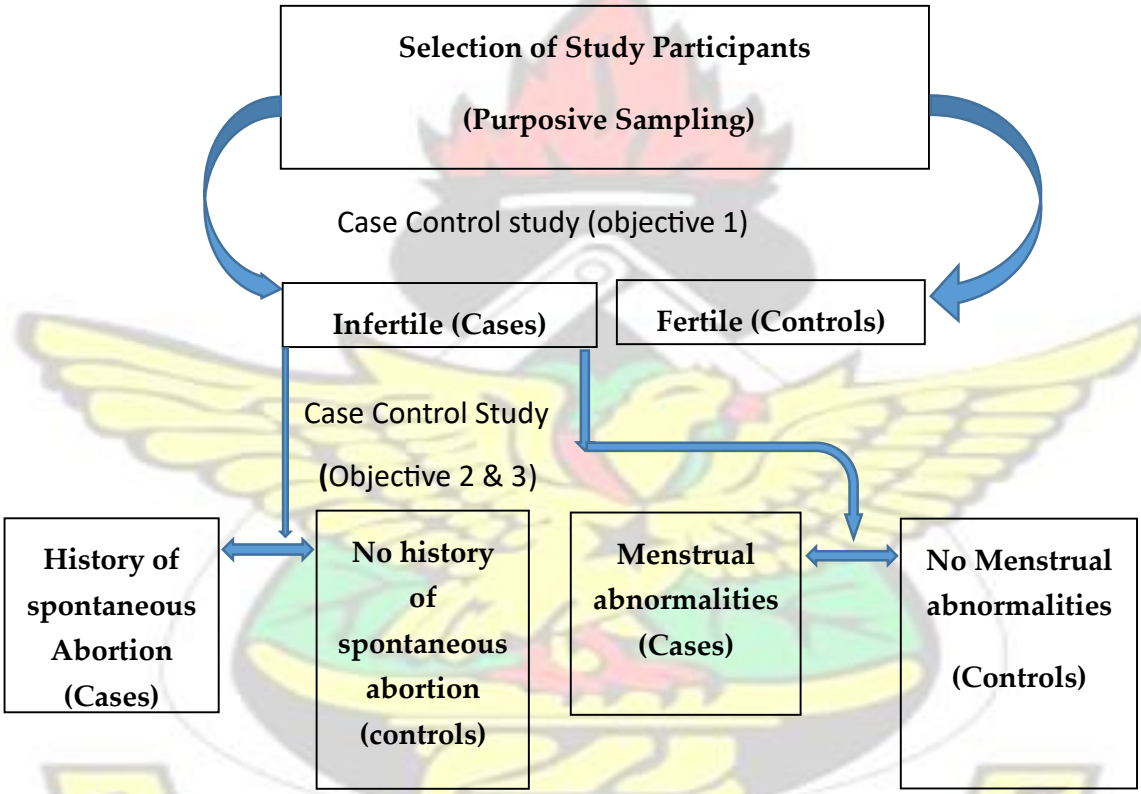

Figure 3.1 Schematic representation of study design

3.1.3. Sample Size

The sample size was calculated from the equation below;

$$\text{Sample size} = \frac{Z^2 (P)(1-P)}{E^2}$$

Where:

Z = the number relating to the degree of confidence you wish to have in the result. The standard score for the confidence interval of 95% is 1.96.

P = a practical estimate of infertility both in developed and developing countries was estimated as 5-15% (Boivin et al., 2007). We used 6.5% in this study.

E = the proportion of error we are prepared to accept. Using 5% allowable limits.

Therefore:

$$\text{Sample size} = \frac{(1.96)^2\,(0.065)\,(1-0.065)}{(0.05)^2}$$

$$= 87 \approx 90$$

Ratio of controls to cases for the study: 1:1.5

Therefore cases=52, and controls =38

3.1.4. Sampling Method

A purposive sampling method was used to select the study participants from the three clinics who met the inclusion and exclusion criteria among a population of infertile and fertile women visiting the respective Obstetric and Gynaecologist units. 30 infertile cases and 20 fertile controls from KATH, 15 infertile cases and 10 fertile controls from the Trust Care Specialist Hospital and 7 infertile cases 8 fertile controls from Kumasi south hospital.

3.1.5. Study Population

The total study population comprised 90 female participants between the ages of 17years and 36 years. Out of the 90 participants, 52 were infertile women and 38 were fertile women. The Clinical definition of infertility defined as the inability to achieve clinical pregnancy after 12 months or more of trying with regular sexual intercourse without contraception from the World Health Organisation was used

to select the cases (Zegers-Hochschild et al., 2009). Fertility was defined as a woman who had recently given birth (less than 6 months) and had never reported infertility. Only participants who consented to the study were included and information received were treated as confidential for the purpose of the study alone.

3.2. ETHICAL CONSIDERATION

Ethical clearance was sought and approved by the Committee on Human Research, Publications and Ethics (CHRPE), Komfo Anokye Teaching Hospital and School of Medical Sciences, KNUST.

3.3. INCLUSION CRITERIA

- Infertile females aged 17 to 36 years with desire to get pregnant and exposed to regular sexual intercourse without contraception for more than one year at the time of the study were included as cases.
- Fertile women aged 17 to 36 years with one live birth over the past one year were included in the study as controls. These had not used contraceptives for less than one year

3.4. EXCLUSION CRITERIA

- Infertile Females outside the ages of 17 to 36 years were excluded from the study.
- Fertile women outside the ages of 17 to 36 years were excluded from the study.
- Infertile women who had used contraceptives within the past one year were excluded from the study.
- Fertile women who lost their current baby at birth were excluded from the study.
- Infertile women whose husbands had abnormal semen analysis or whose semen analysis were unknown were excluded from the study.

- Infertile women and fertile women with known syphilis infection were excluded from the study.

3.5. STUDY METHODS

3.5.1. Data Collection

The purpose of the research was explained to the participants to decide voluntarily to take part in the research. Those who consented were recruited for the study. Questionnaires were then administered in the form of interviews to obtain demographic data and other patient history. Participant folders were also reviewed to confirm the histories obtained from the direct questionnaire interviews.

3.5.2. Questionnaires

Questionnaires were completed by the interviewer directly after sourcing the information from the participants and this included demographics and obstetric history such as menstrual abnormalities, spontaneous abortions, ectopic pregnancies, still births, etc.

3.5.3. Physical Measurements

The physical measurements included weight, height and blood pressure. The weight and height were determined using a calibrated Camry weight balance (Zhongshan Camry Electronic co. Ltd, Guangdong, China) and a stadiometer respectively. All standard operating procedures were adhered strictly such as removal of shoes and other heavy materials. The participants stood such that the Frankfort plane was horizontal (orbitale- tragion horizontal line). Weight was taken in kilograms (kg) while height was in centimetres (cm), but converted to metres (m).

Systolic and diastolic blood pressure was determined using a standard mercury sphygmomanometer and a statoscope by a qualified Nursing officer after patient

had rested for a minimum of 10 minutes. Patient was carefully seated according to the American heart society recommendation where forearm was supported at the heart level on a straight and smooth table and slightly flexed (Kirkendall et al., 1967). Systolic and diastolic arterial blood pressure were recorded in millimetres of mercury (mmHg). Two duplicates were taken at intervals of five minutes and the average determined as the blood pressure of the patient.

3.5.4. Specimen Collection

Venous blood specimen were collected from the median-cubital vein from all subjects and controls. A rubber tourniquet was applied for less than one minute and the site to be punctured disinfected with 70% methylated spirit. 5mls of Whole blood specimen was then collected from each patient using a 5ml and a 21 gauge syringe and needle respectively by standard method of phlebotomy. 3mls was transferred into a BD serum separator Gel tube (SST tubes) while the remaining 2mls was transferred into a Tri-potassium ethylene diamine tetra-acetic acid (K_3EDTA) tube and used to assess the full blood count of the subjects. The clotted samples in the BD SST gel tubes was centrifuged at 3,000*g* for 10 minutes. The serum obtained was divided into three screw-cap polypropylene cryo-tubes and two transported in cold boxes to be stored immediately at –80°C at the serology department of KATH. These were used for the measurement of antiphospholipid, thyroglobulin and thyroperoxidase antibodies. The last tube was used to screen for *Treponema pallidum* antibodies using a rapid diagnostic Syphilis test, which works by immunochromatography, within an hour of specimen collection.

3.6. LABORATORY ANALYSIS

3.6.1. Full Blood Count

Full blood count was estimated using a Sysmex KX 21N (Germany) automated haematology analyser within one hour of specimen collection. The parameters measured and obtained from the analyser included haemoglobin estimation (Hb),

Total leukocyte count (WBC), Platelet count, percentage haematocrit (HCT %), absolute lymphocyte count and absolute neutrophil count. Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) were both computed as ratios from the neutrophils, platelets and lymphocytes.

Principle of test

The Sysmex KX 21N is an automated multi-parameter blood cell counter (18-parameter) that works by a light deflection method. Platelets and WBC's are determined by a direct current (DC) detection method while Hb is determined by a non-cyanide method. HCT% is determined by a cumulative pulse height detection method. The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block. The RBC count and platelets are taken by the RBC detector block. The Hb detector block measures the haemoglobin concentration using the non-cyanide haemoglobin method.

Direct Current (DC) detection method

The DC detection method is able to determine the size of blood cells by assessing the changes in the direct-current resistance as blood components passes through the detector chamber after pre-dilution into the required ratio. Concurrently it detects blood cell interior by changes in the radio-frequency resistance. The detector chamber has a tint hole with electrodes on both sides that pass the direct current and radio-frequency current. The changing direct-current resistance and radio-frequency resistance as the blood cell components pass through the aperture is used to tell the size of the blood cell and the blood cell interior (size of the nucleus) respectively. These are recorded as electrical pulses and based on the size of the pulses, a two dimensional distribution (scatter graph) of the blood-cell size

and internal density can be drawn. This is used to obtain all the cell information.

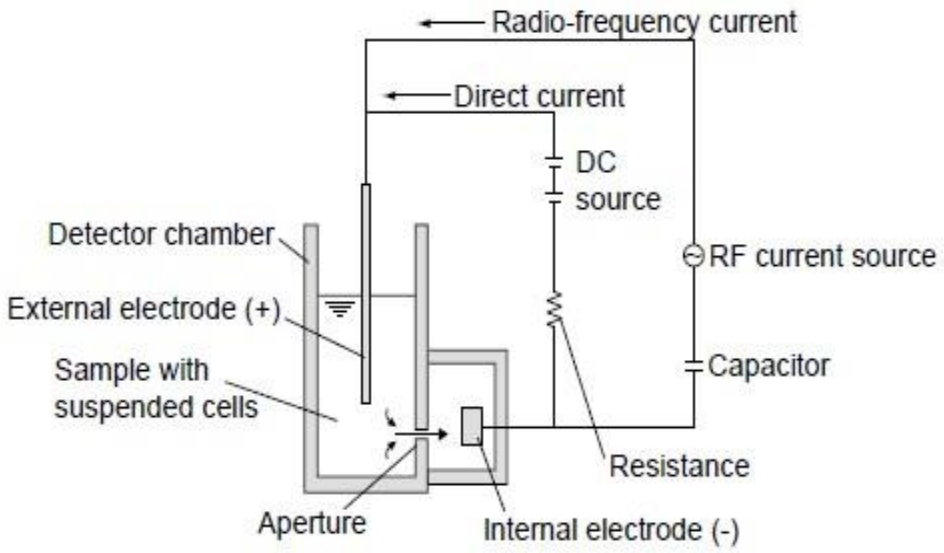

Fig. 3.1 diagram showing DC detection method source (Sysmex corporation, 2007)

Test Procedure

A well-mixed whole blood sample in the 2ml K_3EDTA tube was placed in the sample slot section of the KX21N Sysmex haematology analyser until the aspirator needle touched the bottom of the tube. When the aspirate button is pressed, 50ul of specimen is drawn into the analyser for analysis. Results are printed out by a peripheral printer.

3.6.2. Enzyme linked Immunosorbent Assay (ELISA)

Levels of thyroperoxidase, thyroglobulin and antiphospholipid antibodies in the Serum of participants were determined by Indirect ELISA using ELISA kits from Green Stone Swiss Co. Limited and read with a micro well plate reader from Iqaba Biotec, Japan Corporation.

Principle of Test

Thyroperoxidase antibody levels were determined using a human anti-thyroperoxidase (TPO-Abs) Elisa kit from Green Stone Swiss Co. Limited. This kit is an in-vitro enzyme-linked immunosorbent assay for the quantitative measurement of autoantibodies to thyroperoxidase in human serum. Serum was

added to pre-coated micro plate wells containing recombinant thyroperoxidase (TPO) to bind corresponding antibodies of the specimen by incubation for 30 minutes at 37° Celsius. After washing the wells to remove all unbound sample material, an enzyme conjugate of horseradish peroxidase (HRP) labelled antihuman IgG antibody (secondary enzyme-labelled antibody) was then added and re-incubated at 37° Celsius. This conjugate bound to the captured TPO-specific antibodies. A third washing was done to rid unbound conjugate. The immune complex formed by the bound conjugate was then visualised by adding Tetramethylbenzidine substrate which gave a blue reaction product after 10 minutes incubation at 37° Celsius. Sulphuric acid was added to stop the reaction and this produced a yellow endpoint product. The amount of TPO specific IgG was proportional to the intensity of the colour change. Absorbance at 450nm was then read using the ELISA micro well plate reader spectrophotometrically within 15 minutes. The lowest detectable limit of the assay was 5 U/mL.

The thyroglobulin antibody ELISA kit is an in-vitro kit intended for the quantitative measurement of autoantibodies to thyroglobulin in human serum. The micro-plate wells had been pre-coated with purified thyroglobulin antigen which acts as the solid phase from the manufacturer. Serum test sample was added to the pre-coated wells which was followed by washing to get rid of unbound antibodies after incubation for 30 minutes at 37° Celsius. An enzyme conjugate of horseradish peroxidase (HRP) labelled anti-human IgG antibody (secondary enzyme-labelled antibody) was added and re-incubated at 37° Celsius for 30minutes. This forms an antigen-antibody-secondary antibody-enzyme complex. Another washing is done before adding to rid the unbound conjugate and visualise the bound by adding the Tetramethylbenzidine substrate which gave a blue reaction product after 10 minutes incubation at 37° Celsius. Sulphuric acid was added to stop the reaction and the absorbance read within 15 minutes at 450nm with the plate reader. The lowest detectable limit for the assay was 4 U/mL

TPO-abs/TG-abs >100 U/mL was considered as positive. This concentration is the most widely used threshold of thyroperoxidase and thyroglobulin antibodies regarded as positive (Bussen et al., 2000; Thangaratinam et al., 2011).

A combined APL IgG/IgM ELISA kit found to be highly specific compared to other individual kits for the antiphospholipid antibodies was used for this study (Suh-Lailam et al., 2012). The antiphospholipid antibody kit is a combined APL IgG/IgM ELISA kit for the quantitative in-vitro assay for the determination of antiphospholipid antibody concentrations in human serum. The kit uses a precoated wells coated with purified phospholipid antigens which forms the solid phase. Serum test sample was added to the wells and incubated at 37° Celsius for 30 minutes. Afterwards, the wells were washed and an enzyme conjugate of horseradish peroxidase (HRP) labelled anti-human IgG/IgM antibody (secondary enzyme-labelled antibody) was added and re-incubated at 37° Celsius for 30 minutes to form an antigen-antibody-enzyme-antibody complex. Another washing is done before adding to rid the unbound conjugate and visualise the bound by adding the Tetramethylbenzidine substrate which gave a blue reaction product after 10 minutes incubation at 37° Celsius. Sulphuric acid was added to stop the reaction and the absorbance read within 15 minutes at 450nm with the plate reader. The lowest detectable limit for the assay was 0.5 U/mL. APL-abs ≥10 U/mL was considered as positive.

3.7. STATISTICAL ANALYSIS

Statistical difference between groups (two) were computed using independent T-test and Mann-Whitney U test for parametric and non-parametric data respectively. ANOVA and Kruskal Wallis test were also used to compare more than three groups and appropriate post –Hoc test and between group comparisons done, with appropriately adjusted alpha value. Chi-square analysis was used to compare proportions. Statistical significance was achieved at a p-value < 0.05.

4.0 CHAPTER FOUR

4.1. RESULTS

4.1.1. Socio-demographic Characteristics of Study Population

Table 4.1 displays the distribution of socio-demographic parameters among infertile and fertile study participants. The study comprised a total of 52 infertile women and 38 fertile women age between 17 and 36 years old. In reference to age distribution, 1.9% of the infertile women and 26.3% of the fertile women were aged between 17 and 23 years. 71.2% of the infertile women and 44.7% of the fertile women were aged between 24 and 30 years. 26.9% of the infertile women and 28.9% of the fertile women were aged between 31 and 36 years. There was significantly association between age and infertility, $\chi 2$ (2) = 14.308, p=0.001.

Educational status was defined as the maximum educational level of the participant at the time of the study. 9.6% of the infertile women and 15.8% of the fertile women were illiterates with no formal education. 13.5% of the infertile women and 10.5% of the fertile women had primary education. It was clear that, majority of the participants had secondary education making up 48.1% of the infertile women and 50% of the fertile women. 28.8% of the infertile women had tertiary education while 23.7% of the fertile women had the same. Educational status was comparable between infertile and fertile study participants, p=0.784.

With respect to occupation, 1.9% of the infertile women were unemployed while 13.2% of the fertile women were unemployed. The remaining employed participants were classified either as self-employed or publicly employed. Selfemployed participants comprised traders, seamstresses, Hairdressers, caterers, farmers, fish-mongers, pastors, waste managers, a cosmetologist, cleaner and a missionary. It was clear that most of the participants were self-employed comprising 71.2% of the infertile and 71.1% of the fertile women. Police women, teachers, nurses and accountants were classified as publicly employed and made

up 26.9% of the infertile women and 15.8% of the fertile women. Occupation was comparable between infertile and fertile women, p=0.061.

Most of the participants married between the ages of 18 and 25 years making up 59.6% of the infertile women and 68.4% of the fertile women. 3.8% of the infertile women married before 18 years while 10.5% of the fertile women also married before 18 years. Few women married after 30 years comprising 11.5% of infertile women and 2.6% of fertile women. The rest of the women married between 26 and 30 years. These were composed of 25% of the infertile women and 18.4% of the fertile women. The age at which the infertile women got married was comparable to that of the fertile women, p=0.193.

Years in marriage was defined as the time from continuous intimate cohabitation with a man till the time of study. 71.2% of the infertile women had been married for 1-5years while 57.9% of the fertile women had been married within that same time spun. 19.2% of infertile women and 36.8% of fertile women had been married for 6-10years. The remaining 9.6% of infertile women and 5.3% of the fertile women had been married for more than 10years. Years of marriage was not significantly associated with infertility, p=0.16.

Table 4.1. Distribution of Socio-demographic Parameters among Study Participants

Parameter	Infertile Women N=52	Fertile Women N=38	Likelihood Ratio (df)	P-value
Age (Years)			**14.308(2)**	**0.001****
17-23	**1 (1.9%)**	**10 (26.3%)**		
24-30	**37 (71.2%)**	**17 (44.7%)**		
31-36	**14 (26.9%)**	**11 (28.9%)**		
Educational Status			**1.070(3)**	**0.784**
Illiterate	**5 (9.6%)**	**6 (15.8%)**		
Primary	**7 (13.5%)**	**4 (10.5%)**		

Secondary	**25 (48.1%)**	**19 (50.0%)**		
Tertiary	**15 (28.8%)**	**9 (23.7%)**		
Occupation				
Unemployed	**1 (1.9%)**	**5 (13.2%)**	**5.585(2)**	**0.061**
Self-Employed	**37 (71.2%)**	**27 (71.1%)**		
Publicly Employed	**14 (26.9%)**	**6 (15.8%)**		
Age Got Married (Years)			**4.726(3)**	**0.193**
<18	**2 (3.8%)**	**4 (10.5%)**		
18-25	**31 (59.6%)**	**26 (68.4%)**		
26-30	**13 (25.0%)**	**7 (18.4%)**		
>30	**6 (11.5%)**	**1 (2.6%)**		
Years In Marriage (Years)			**3.667(2)**	**0.16**
***1-5**	**37 (71.2%)**	**22 (57.9%)**		
***6-10**	**10 (19.2%)**	**14 (36.8%)**		
>10	**5 (9.6%)**	**2 (5.3%)**		

Results are expressed as: actual values (percentages)

4.1.2. Obstetric Characteristics and BMI of Study Population

Table 4.2 displays the distribution of obstetric parameters and BMI categories among the study participants. 69% of the infertile women were nulliparous and therefore had primary infertility. The rest were primiparous and multiparous making up 27% and 4% respectively. 21% of the fertile women were primiparous and 79% were multiparous. There was a highly significant association between parity and infertility, $\chi 2$ (2) = 61.45, p=0.0001.

A little over half (58%) of the infertile participants had no history of spontaneous abortion compared to 63% of the fertile women. Spontaneous abortion (SA) was seen in 32% of the infertile women and 16% of the fertile women. The rest of the women comprising 10% of the infertile women and 21% of the fertile women had a history of recurrent abortion (RA). Recurrent abortion was defined as two or

more losses of pregnancy before 20 week gestation. Among the fertile women who had RA (21%), none had lost more than two pregnancies while all those infertile women with RA (10%) had lost three or more pregnancies at the time of the study. It is clear that there was no relationship between a history of spontaneous abortion (RA or SA) and infertility, p=0.103.

Still Birth was defined as a baby born dead on or after 28 weeks of gestation. 90% and 95% of the infertile and fertile women had no history of still birth respectively. The remaining participants had a history of one or more still births. There was no association between still birth and infertility, p=0.446.

79% of the infertile women reported menstrual abnormalities compared to 37% of the fertile women with no such history. The abnormalities included 13% with dysmenorrhea, 21% with amenorrhea, 21% with oligomenorrhea and 8% with menorrhagia among the infertile women. 11% dysmenorrhea, 0% amenorrhea, 26% oligomenorrhea and 0% menorrhagia was found among fertile women. There was a highly significant association between type of menstrual abnormality and infertility, $\chi 2$ (5) = 27.17, p=0.0001.

25% of the infertile women had a history of hormonal contraceptive use. Out of this, 21% had used it for 3-5 years before the study and 4% had used it between 12 years. None had used it greater than five years or less than or equal to one year. The remaining 75% had no history of hormonal contraceptive use. Among the fertile women, 26% had a history of hormonal contraceptive use with 11% between 3-5 years and 16% between 1-2 years. There was no significant association between a history of hormonal contraceptive use and infertility, p=0.081.

BMI (kg/m^2) was calculated as weight in kilograms divided by a square of the height in meters. It was categorized into four groups as follows: underweight (BMI<18kg/m^2), normal weight (BMI= 18-24.9kg/m^2), overweight (BMI=2529.9kg/m^2) and Obese (BMI>30kg/m^2). 3.8% of the infertile women were

obese, 28.8% were overweight, 65.4% were normal weight and 1.9% were underweight. These were comparable to the BMIs of the fertile women in the study comprising 10.5% obese, 21.1% overweight, 65.8% normal weight and 2.6% underweight ($\chi 2$ (4) =2.04, p=0.56).

Table 4.2. Distribution of Obstetric Parameters and BMI categories among Study Participants.

		Infertile Women N=52	Fertile Women N=38	Pearson's $\chi 2$ (df)	Fisher's Exact (2 sided)P-Value
Parity				**61.445(2)**	**0.0001*** **
	Nulliparous	**36 (69%)**	**0(0%)**		
	Primiparous	**14 (27%)**	**8 (21%)**		
	Multiparous	**2 (4%)**	**30 (79%)**		
Abortus				**4.552(2)**	**0.103**
	None	**30 (58%)**	**24 (63%)**		
	SA	**17 (32%)**	**6 (16%)**		
	RA	**5 (10%)**	**8 (21%)**		
Still Birth				**0.58(1)**	**0.446**
	None	**47 (90%)**	**36 (95%)**		
	1 Or More	**5 (10%)**	**2 (5%)**		
Menstrual History				**16.30(1)**	**0.0001*** **
	Normal Menses	**11 (21%)**	**24 (63%)**		
	Abnormal Menses	**41 (79%)**	**14 (37%)**		
Menstrual Abnormality Type				**27.174(5)**	**0.0001*** **
	None	**11 (21%)**	**24 (63%)**		

	Dysmenorrhea	7 (13%)	4 (11%)		
	Amenorrhea	11 (21%)	0 (0%)		
	Oligomenorrhea	11 (21%)	10 (26%)		
	Menorrhagia	4 (8%)	0 (0%)		
	> 1 Abnormality	8 (15%)	0 (0%)		
History Of HCU				0.20(1)	0.888
	None	39 (75%)	28 (74%)		
	Yes	13 (25%)	10 (26%)		
Years After HCU				5.016(2)	0.081
	None	39 (75%)	28 (74%)		
	1-2years	2 (4%)	6 (16%)		
	3-5years	11 (21%)	4 (11%)		
	>5years	0 (0%)	0 (0%)		
Age At HCU Initiation				3.37(4)	0.497
	None	39 (75%)	28 (74%)		
	15-20	5 (10%)	1 (3%)		
	21-25	4 (8%)	4 (11%)		
	26-30	4 (8%)	4 (11%)		
	31-35	0 (0%)	1 (3%)		
BMI Categories				2.04 (4)	0.56
	Underweight	1 (1.9%)	1 (2.6%)		
	Normal weight	34 (65.4%)	25 (65.8%)		
	Overweight	15 (28.8%)	8 (21.1%)		
	Obese	2 (3.8%)	4 (10.5%)		

Chi-Square Test: Results are expressed as: actual values (percentages), SA= Spontaneous abortion, RA= Recurrent abortion, HCU= Hormonal contraceptive use, P-Value= Statistical significance of infertile women compared to fertile women, *P-Value= Degree of significance.

4.1.3. Age, BMI and Diastolic Blood Pressure Distribution of Study

Participants

Table 4.3 shows the distribution of Age, body mass index, diastolic blood pressures, and haematological parameters of the infertile women compared to the fertile women. The mean age of the infertile women was 28.73±3.35 years and that of the fertile women was 28.29±4.06 years. There was no significant difference between the ages of our participants (p=0.574). Body mass index, haemoglobin levels, platelet count and percentage haematocrit did not differ significantly between infertile and fertile women (p=0.851). Diastolic blood pressure however was slightly but significantly higher in the infertile women (78.44±13.92 mmHg) compared to the fertile women (72.89±9.84mmHg), p=0.038. The mean plateletlymphocyte ratio (PLR) in the fertile group (133.20 ± 44.85) was moderately but significantly higher than that of the infertile group (104.23±36.02), p=0.01.

Table 4.3. Age, BMI, DBP, and Haematological Parameters Compared between Fertile and Infertile Women.

Parameter	Infertile women N = 52	Fertile women N= 38	P-value P<0.05
Age	28.73±3.35	28.29±4.06	0.574
BMI	24.36±3.19	24.50±3.78	0.851
DBP	78.44±13.92	72.89±9.84	0.038*
HB (g/dI)	11.99±1.85	11.84±1.40	0.67
PLT (x10^3/μl)	251±73.22	274±96.91	0.212
PLR	104.23±36.02	133.20±44.85	0.01*
HCT (%)	35.37±4.77	34.37±4.16	0.306

Independent-Samples T test: N=Sample size; Data=Mean±Standard Deviation, BMI= Body Mass Index, DBP=Diastolic Blood Pressure, HB=Haemoglobin, PLR= platelet lymphocyte ratio, HCT%= Percentage Haematocrit, P-Value= Statistical significance of infertile women compared to fertile women, *P-Value= Degree of significance.

4.1.4. Distribution of SBP, Autoantibodies and Haematological Parameters of

Study Participants

Table 4.4 shows the distribution of systolic blood pressure, autoantibodies and haematological parameters among infertile women compared to fertile women. The Systolic blood pressure of the infertile women (Median=120mmHg) were significantly higher compared to the fertile women (Median=110mmHg), p=0.012. The distribution of thyroperoxidase antibodies, thyroglobulin antibodies and antiphospholipid antibodies levels were comparable between infertile and fertile women (p>0.05). The distribution of Leukocytes, absolute neutrophil count and neutrophil-lymphocyte ratio (NLR) were also significantly higher in the fertile women compared to the infertile women (p=0.017, p=0.0001 and p=0.0001 respectively). Absolute lymphocyte count on the other hand was moderately but significantly higher in the infertile group compared to the fertile group (P=0.002).

Table 4.4. SBP, Autoantibodies and Haematological Parameters Compared between Infertile and Fertile Women.

	Infertile women	Fertile women		
Parameter	N=52 Median	N=38 Median	Percentiles Median (25th -75th)	P-value P<0.05
SBP	120	110	118 (107.8-122.8)	0.012*
TPO-abs	28.3	34.7	33.3 (6.1-70.0)	0.676
TG-abs	23	66.4	37.1 (2.7-123.2)	0.067
APL-abs	1.9	1.6	1.7 (0.4-4.0)	0.167
WBC (X10^3/µl	5.7	6.7	5.9 (4.9-7.1)	0.017*
#LYMPH (X10^3/µl	2.5	2.1	2.3 (1.9-2.6)	0.002**
# NEUT (x10^3/µl	2.6	3.8	2.9 (2.2-4.0)	0.0001**
NLR	1.1	1.8	1.2 (0.9-1.8)	0.0001***

Mann-Whitney U test: N=Sample size; Data=Median, Median (Interquartile Ranges), SBP=Systolic Blood, TPO-abs= Thyroperoxidase Antibodies, TG-Abs= Thyroglobulin Antibodies, APL-Abs= Antiphospholipid Antibodies, WBC= Leukocytes, #LYMPH= Absolute lymphocyte count, #Neutrophil= Absolute Neutrophil Count, NLR= Neutrophil Lymphocyte Ratio, P-Value= Statistical significance of infertile women compared to fertile women, *PValue= Degree of significance.

4.1.5. Association between Autoantibodies and Infertility

Table 4.5 shows the association between women positive for the thyroid autoantibodies, antiphospholipid antibodies and infertility. Infertility was found to significantly associated with thyroperoxidase antibody positivity (TPO-abs > 100 U/mL) in this study; $\chi 2$ (1) = 7.047, p=0.011. Thyroglobulin antibody positivity (TG-abs >100 U/mL) had no association with infertility and did not differ significantly between cases and controls; p=0.357. Antiphospholipid antibody positivity (APL-abs ≥10 U/mL) was also found to be significantly associated with infertility, $\chi 2$ (1) = 5.55, p=0.02.

Table 4.5. Association between Autoantibodies and Infertility

Parameter		Infertile Women Frequency (%)	Fertile Women Frequency (%)	Pearson χ^2 Value	Fisher's Exact (2-sided) P-value
TPO-abs				7.047	0.011*
	Positive	14 (26.9%)	2 (5.3%)		
	Negative	38 (73.1%)	36 (94.7%)		
TG-abs				0.907	0.357
	Positive	13 (25.0%)	13 (28.9%)		
	Negative	39 (75.0%)	25 (71.1%)		
APL-abs				5.55	0.02*
	Positive	7 (13.5%)	0 (0.0%)		
	Negative	45 (86.5%)	38 (100%)		

Chi square test: Data= Observed count (percentages), TPO-abs= Thyroperoxiase antibodies, TGabs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid Antibodies, Degree of freedom= 1, at P<0.05, P-Value= Statistical significance of infertile women compared to fertile women, *PValue= Degree of significance.

4.1.6. Distribution of BMI, DBP and Haematological Parameters of Infertile

Women with a History of Spontaneous Abortion

Table 4.6 shows the distribution of body mass index, diastolic blood pressure and haematological parameters of infertile women with a history of spontaneous abortion compared to those with no history. Body mass index, diastolic blood pressure and all haematological parameters (PLT, HB, PLR, HCT %) did not differ significantly between the infertile women with no history of spontaneous abortion and those with a history of one or more abortions.

Table 4.6. Distribution of BMI, DBP, and Haematological Parameters of Women with History of Spontaneous Abortion Compared With Those without Any History in the Infertile Group

	Infertile Women		
Parameter	No abortion History (N=30) Mean±SD	History of one or more abortions (N=22) Mean±SD	P- value
BMI(Kg/m2)	23.92±2.98	24.96±3.43	0.247
DBP	78.03±16.65	79 ±9.33	0.807
PLT (x10^3/μl)	238.33±66.82	270.09±79.02	0.123
HB (g/dl)	12.15±2.07	11.78±1.53	0.479
PLR	104.17±35.19	104.31±37.97	0.989
HCT%	35.83±5.37	34.73±3.84	0.414

Independent-Samples T Test: N=Sample size; Data=Mean±Standard Deviation, BMI= Body Mass Index, DBP=Diastolic Blood Pressure, HB=Haemoglobin, PLR= platelet lymphocyte ratio, HCT= Haematocrit.

4.1.7. Distribution of SBP, Autoantibodies and Haematological Parameters of Infertile Women with a History of Spontaneous Abortion

Table 4.7 shows the distribution of systolic blood pressure, autoantibodies and haematological parameters of infertile women with a history of spontaneous abortion compared to those with no history. The distribution of the thyroid autoantibodies (TPO-abs and TG-abs) as well as the haematological parameters

(WBC, #LYMPH, #NEUT, NLR) was comparable between infertile women with no history of spontaneous abortion and those with a history of one or more abortions (p>0.05). The antiphospholipid antibody levels however was significantly higher in the women with no history of abortion (Median=2.99 U/ml) compared to those with a history (Median=1.14 U/ml); p=0.035.

Table 4.7. SBP, Autoantibodies and Haematological Parameters Compared between Women with a History of Spontaneous Abortion and Those without any History in the Infertile Group

	Infertile women			
	History of one No abortion or more abortions		N=30 N=22	
Percentiles value Parameter	history Median	Median	Median (25th -75th)	P-value P<0.05
SBP				0.622
120.5 120	120 (110.0-128.5)			
TPO-abs	39.07	23.16	28.3 (4.3-148.7)	0.167
TG-abs	31.02	5.05	23.0 (0.0-109.2)	0.089
APL-abs	2.99	1.14	1.9 (0.6-5.0)	0.035*
WBC ($X10^3/\mu l$)	5.45	5.9	5.7 (4.6-6.6)	0.282
#LYMPH ($X10^3/\mu l$)	2.4	2.55	2.5 (2.0-3.1)	0.153
# NEUT ($x10^3/\mu l$)	2.6	2.7	2.6 (2.0-3.1)	0.604
NLR	1.09	1.12	1.1 (0.8-1.3)	0.774

Mann-Whitney U test: N=Sample size; Data=Medians, Median (Interquartile Ranges), SBP=Systolic Blood Pressure, TPO-abs= Thyroperoxidase Antibodies, TG-abs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid Antibodies, WBC= Leukocytes, #LYMPH= Absolute lymphocyte count, #Neutrophil= Absolute Neutrophil Count, NLR= Neutrophil-Lymphocyte Ratio, P-Value= Statistical significance of spontaneously aborting women compared with those without such a history, *P-Value= Degree of Significance.

4.1.8. Distribution of BMI, DBP and Haematological Parameters of Infertile Women with SA, RA and None

Table 4.8 shows the distribution of body mass index, diastolic blood pressure and haematological parameters (PLT, HB, PLR, HCT %) of infertile women with no

history of spontaneous abortion (None), spontaneous abortion (SA) and recurrent abortion (RA). Thrombocyte count (PLT) was found to significantly differ between the groups; F (2, 49) =3.591 p=0.035. A Tukey Post-hoc test revealed that the platelet count was moderately significantly higher in infertile women with recurrent abortion (RA) (328.60± 78.93 $x10^3/\mu l$) compared to infertile women with no history (238.33±66.82$x10^3/\mu l$), p=0.026, eta^2=0.128. Body mass index, haemoglobin level, platelet-lymphocyte ratio and percentage haematocrit were all comparable between groups (p>0.05).

Table 4.8. Distribution of BMI, DBP, and Haematological Parameters of Infertile Women with No History of Spontaneous Abortion Compared With Spontaneous Abortion and Recurrent Abortion

	Infertile Women					
	None	SA	RA			
Parameter	(N=30)	(N=17)	(N=5)		P-value	Significant
	Mean±SD	Mean±SD	Mean±SD	F value	P<0.05	Pairs
BMI(Kg/m^2)	23.92±2.98	24.82±3.90	25.46±0.72	0.751	0.477	Nil
DBP	78.03±16.65	79.88±10.15	76.00±5.48	0.175	0.84	Nil
PLT ($x10^3/\mu l$)	238.33±66.82	252.88±72.4	328.60±78.93	3.591	0.035*	None vs. RA
HB (g/dl)	12.15±2.07	11.58±1.61	12.46±1.12	0.687	0.508	Nil
PLR	104.17±35.19	101.62±40.3	113.44±30.62	0.202	0.818	Nil
HCT%	35.83±5.37	34.61±4.05	35.37±4.77	0.354	0.704	Nil

One-Way ANOVA: N=Sample size; Data=Mean±Standard Deviation, None= No history of abortion, SA= spontaneous abortion, RA=Recurrent abortion, BMI= Body Mass Index, DBP=Diastolic Blood Pressure, HB=Haemoglobin, PLR= platelet lymphocyte ratio, HCT= Haematocrit, degrees of freedom (between, within groups) = (2, 49), Post-Hoc test of Tukey HSD was significant at p<0.05, Eta^2 for PLT=0.128.

4.1.9. Distribution of SBP, Autoantibodies and Haematological Parameters of

Infertile Women with No Abortion (None), SA and RA

Table 4.9 shows the distribution of systolic blood pressure, autoantibodies and haematological indices among infertile women with no history of abortion (None), spontaneous abortion (SA) and recurrent abortion (RA). Thyroperoxidase antibodies were comparable between the three groups ($p>0.05$). Thyroglobulin antibody levels however were found to be significantly higher among patients with no history of abortion (Median= 31.02 U/mL) as compared to those with spontaneous abortion (2.43 U/mL); H (2) =6.922, p=0.031. Also, antiphospholipid antibodies were found to be significantly higher among infertile women with no history of spontaneous abortion compared to those with spontaneous abortion; H (2) =6.912, p=0.032). Systolic blood pressure and the haematological parameters (WBC, #LYMPH, #NEUT, and NLR) on the other hand, did not differ significantly between groups.

Table 4.9. SBP, Autoantibodies and Haematological Parameters Compared Between Women with No History of Spontaneous Abortion, Spontaneous Abortion and Recurrent Abortion

	Infertile women						
	None	SA	RA			Pvalue	Significant
	N=30	N=17	N=5	Percentiles			
Parameter	Median	Median	Median	50th (25th -75th)	$\chi 2$	P<0.05	Pairs
SBP	120.5	120	112	120.0 (110.0-128.5)	4.064	0.131	Nil
TPO-abs	39.07	12.47	34.03	28.3 (4.3-148.7)	2.858	0.24	Nil
TG-abs	31.02	2.43	58.9	23.0 (0.0-109.2)	6.922	0.031*	SA vs. None
APL-abs	2.99	0.98	7.09	1.9 (0.6-5.0)	6.912	0.032*	SA vs. None
WBC ($x10^3/\mu l$)	5.45	5.9	7.8	5.7 (4.6-6.6)	1.825	0.402	Nil
#LYMPH($x10^3/\mu l$)	2.4	2.5	3.1	2.5 (2.0-3.1)	3.098	0.212	Nil
# NEUT($x10^3/\mu l$)	2.6	2.5	3.9	2.6 (2.0-3.1)	1.437	0.488	Nil
NLR	1.09	1.07	1.26	1.1 (0.8-1.3)	0.274	0.872	Nil

Kruskal-Wallis H test: N=Sample size; Data=Median, Median (Interquartile Ranges), SBP=Systolic Blood Pressure, TPO-abs= Thyroperoxidase Antibodies, TG-abs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid Antibodies, WBC= Leukocytes, #LYMPH= Absolute lymphocyte count, NLR= Neutrophil-Lymphocyte Ratio, #Neutrophil= Absolute Neutrophil Count, P-Value= Statistical significance of spontaneously aborting women compared with those without such a history, degree of freedom (df) =2, Bonferroni correction (α=0.016).

4.1.10. Association between Autoantibody Positivity and a History of Spontaneous Abortion

Table 4.10 shows an association between autoantibody positivity and a history of spontaneous abortion. Out of the 22 infertile women with a history of spontaneous abortion, 18.2% were positive for the thyroperoxidase antibody, 18.2% were positive for thyroglobulin antibody and only 4.5% were positive for antiphospholipid antibody. There was no significant association between antibody positivity and spontaneous abortion ($p>0.05$) among infertile women.

Table 4.10. Association between Autoantibody Positivity and History of Spontaneous Abortion among Infertile Women

	Infertile women			
	No Abortion (N=30)	Abortion (N=22)	Pearson	Fisher's Exact Test
Parameter	Frequency (%)	Frequency (%)	$\chi 2$ Value	Sig.(2-sided)
TPO-abs			1.481	0.344
Positive	10 (33.3%)	4 (18.2%)		
Negative	20 (66.7%)	18 (81.8%)		
TG-abs			0.945	0.518
Positive	9 (30.0%)	4 (18.2%)		
Negative	21 (70.0%)	18 (81.8%)		
APL-abs			2.602	0.216
Positive	6 (20.0%)	1 (4.5%)		
Negative	24 (80.0%)	21 (95.5%)		

Chi square Test: Data= Observed count (percentages), TPO-abs= Thyroperoxiase antibodies, TG-abs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid Antibodies, degree of freedom= 1, at P<0.05

4.1.11. Odds Ratio of Autoantibody Positive Women's Association with

Spontaneous Abortion

Table 4.11 shows the odds of thyroid autoantibodies (TPO-abs, TG-abs) positive women or antiphospholipid antibody positive women's likelihood to have a history of spontaneous abortion. BOTH refers to women positive for the two specified antibodies at the same time. ALL refers to women positive for all three antibodies. The thyroid autoantibodies and the antiphospholipid antibodies positive infertile women were found not to be significantly associated with a history of spontaneous abortion ($p>0.05$). Those positive for two or more of the autoantibodies were also not associated with any significant odds.

Table 4.11. Odds of Autoantibody Positive Women's association with a History of Spontaneous Abortion.

Parameter	OR (95% CI)	P-value
TPO-abs	0.44 (0.12-1.67)	0.23
TG-abs	0.52 (0.14-1.97)	0.335
APL-abs	0.19 (0.21-1.71)	0.139
BOTH (TPO+TG)	0.61 (0.16-2.4)	0.475
BOTH (TPO+APL-abs)	0.19 (0.21-1.71)	0.139
ALL (TPO+APL+TG-abs)	0.14 (0.21-1.71)	0.139
BOTH (TG+APL-abs)	0.19 (0.02-1.71)	0.139

Odds Ratio: TPO-abs= Thyroperoxidase antibodies, TG-abs= Thyroglobulin Antibodies, APLabs= Antiphospholipid Antibodies, OR (95%CI) = Odds ratio (95% confidence interval) with degree of freedom= 1, at $P<0.05$.

4.1.12. Distribution of Autoantibody Levels of Infertile Women with Menstrual Abnormalities

Table 4.12 shows the distribution of thyroperoxidase, thyroglobulin and antiphospholipid antibody levels of infertile women with menstrual abnormalities compared with those without any abnormality. The

thyroperoxidase antibody, thyroglobulin antibody and antiphospholipid antibody levels were found to be comparable between women with no menstrual abnormality and those with one or more menstrual abnormality among infertile group (p>0.05).

Table 4.12. Autoantibodies Compared Between Infertile Women with One or More Menstrual Abnormalities and Those without Any Menstrual Abnormality.

	Infertile women			
	No Menstrual abnormality (N=11)	Have menstrual abnormality (N=41)	Percentiles	P-value
Parameter	Median	Median	Median (25th -75th)	P<0.05
TPO-abs	30.83	28.13	28.3(4.3-148.7)	0.902
TG-abs	45.58	22.9	23.0(0.0-109.2	0.244
APL-abs	2.19	1.87	1.9(0.6-5.0)	0.973

Mann-Whitney U test: N=Sample size; Data=Medians, Median (Interquartile Ranges), TPOabs= Thyroperoxidase antibodies, TG-abs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid Antibodies, P-Value=Statistical significance of women with one or more menstrual abnormalities compared with those without any abnormality.

4.1.13. Association between Autoantibodies and Menstrual Abnormalities in Infertile Women

Table 4.13 shows the association between autoantibodies and menstrual abnormalities among the infertile women. Out of 41 women who had menstrual abnormalities, 26.8% were positive for the thyroperoxidase antibody, 24.4% were positive for the thyroglobulin antibody, 14.6% were positive for antiphospholipid antibodies and 24.4% positive for both thyroid autoantibodies. The thyroperoxidase antibody positives and negatives were comparable between the two groups; infertile women with menstrual abnormalities and those without any

menstrual abnormalities ($\chi 2$ (1) =0.001, p=1). The thyroglobulin antibody positives and negatives were also comparable between the two groups; infertile women with menstrual abnormalities versus those without any menstrual abnormalities ($\chi 2$ (1) =0.038, p=1). Both (TPO+TG-abs) positives were comparable between the two groups ($\chi 2$ (1) =0.188, p=1). Antiphospholipid antibody positives and negatives were also comparable between the two groups ($\chi 2$ (1) =0.229, p=1).

Table 4.13. Association between Autoantibodies and Menstrual Abnormalities among Infertile Women

		Infertile Women			
Parameter		No Menstrual abnormality Frequency (%)	Menstrual abnormality Frequency (%)	Pearson's $\chi 2$	Fisher's Exact Test (2-sided)
TPO-abs				0.001	1
	Positive	3 (27.3%)	11 (26.8%)		
	Negative	8 (72.7%)	30 (73.1%)		
TG-abs				0.038	1
	Positive	3 (27.3%)	10 (24.4%)		
	Negative	8 (72.8%)	31 (75.6%)		
APL-abs				0.229	1
	Positive	1 (9.1%)	6 (14.6%)		
	Negative	10 (90.9%)	35 (85.4%)		
BOTH (TPO+TG-abs)				0.188	1
	Positive	2 (18.2%)	10 (24.4%)		
	Negative	9 (81.8%)	31 (75.6%)		

Chi square Test: Data= Observed count (percentages), TPO-abs= Thyroperoxidase antibodies, TG-abs= Thyroglobulin Antibodies, BOTH (TPO+TG-abs) = women positive for both thyroid autoantibodies, degree of freedom= 1, at P<0.05.

4.1.14. Odds Ratio of Menstrual abnormality's association with Autoantibody Positivity among Infertile Women.

Table 4.14 shows the odds of an infertile woman with menstrual abnormalities' likelihood to be autoantibody positive. Menstrual abnormalities among infertile women were not found to be significantly associated with Thyroperoxidase positive (OR (95% CI) =0.98, p=0.977) and thyroglobulin positivity (OR (95% CI) =0.86, p=0.84). The odds of being APL-abs positive was 1.71 times greater among infertile women with menstrual abnormalities compared to those without menstrual abnormalities, but the association was not significant (OR (95%CI) =1.71, p=0.636). Both (TPO + TG-abs) was defined as women positive for both thyroperoxidase antibody and the thyroglobulin antibody at the same time. The odds of being both (TPO+TG-abs) positive was also 1.45 times greater in infertile women with menstrual abnormalities compared to those without menstrual abnormalities though the difference was not significant (OR (95% CI) =1.45, p=0.667).

Table 4.14. Odds Ratio of Autoantibody Positive Infertile Women's association with Menstrual Abnormality.

Parameter	OR (95% CI)	P-value
TPO-abs	**0.98 (0.22-4.36)**	**0.977**
TG-abs	**0.86 (0.19-3.88)**	**0.845**
APL-abs	***1.71 (0.18-15.95)**	**0.636**
Both(TPO+TG-abs)	***1.45 (0.27-7.87)**	**0.667**

Odds Ratio: TPO-abs= Thyroperoxidase antibodies, TG-abs= Thyroglobulin Antibodies, APLabs= Antiphospholipid Antibodies, OR (95%CI) = Odds ratio (95% confidence interval) with degree of freedom= 1, at P<0.05.

4.1.15. Correlation of Continuous Data Parameters among Infertile Women Table 4.15 shows a Pearson correlation of age, body mass index, systolic blood pressure, diastolic blood pressure, thyroperoxidase, thyroglobulin and antiphospholipid

antibody concentration among infertile women. Age inversely correlated weakly but significantly with thyroperoxidase and thyroglobulin antibody levels (r= -0.314, and r= -0.275, $p<0.05$ respectively). Age also weakly but significantly correlated directly with leukocyte count (r= 0.285, $p<0.05$) and absolute neutrophil count (r= 0.313, $p<0.05$). Body mass index did not correlate significantly with any of the measured parameters among infertile women. Systolic blood pressure strongly correlated directly and significantly with diastolic blood pressure (r= 0.639, $p<0.01$). Thyroperoxidase antibodies strongly correlated directly and significantly with thyroglobulin (r= 0.897, $p<0.01$) and antiphospholipid antibodies (r=0.676, $p<0.01$). Thyroglobulin antibodies also directly correlated strongly and significantly with antiphospholipid antibodies (r=0.707, $p<0.01$). Haemoglobin levels strongly correlated directly and significantly with absolute lymphocyte count (r= 0.359 $p<0.01$) and the percentage haematocrit (r= 0.954, $p<0.01$) but inversely and significantly with the neutrophillymphocyte ratio (r= -0.457, $p<0.01$). Platelet count correlated directly but weakly with the total leukocyte count (r=0.339, $p<0.05$) and absolute neutrophil count (r=0.333, $p<0.05$) but strongly with the platelet-lymphocyte ratio (r=0.644, $p<0.01$). Total leukocyte count correlated strongly with absolute lymphocyte count (r=0.778, $p<0.01$) and absolute neutrophil count (r=0.846, $p< 0.01$). Absolute lymphocyte count weakly correlated significantly with absolute neutrophil count (r=0.348, $p<0.05$) and percentage haematocrit (r=0.313, $p<0.05$). Obviously absolute lymphocyte count also strongly correlated inversely with the neutrophil lymphocyte ratio (r= -0.478, $p<0.01$) and the platelet lymphocyte ratio (r= -0.519, $p<0.01$). Additionally, the absolute lymphocyte count strongly correlated directly with the neutrophil-lymphocyte ratio (r=0.592, $p<0.01$). The neutrophil

lymphocyte ratio strongly correlated directly with the platelet-lymphocyte ratio ($r=0.492$, $p<0.01$) and inversely with the percentage haematocrit ($r= -0.473$, $p<0.01$) among infertile women.

Table 4.15. Pearson Correlation among Age, Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure, Autoantibodies and Haematological Parameters of the Infertile Women

	AGE	BMI	SBP	DBP	TPO-abs	TG-abs	APL-abs	HB	PLT	WBC	# LYMPH.	# NEUT.	NLR	PLR	HCT%
AGE		0.005	0.027	0.011	**-.314***	**-.275***	-0.129	-0.256	0.195	**.285***	0.115	**.313***	0.211	0.037	-0.242
BMI			-0.186	-0.098	0.171	0.138	0.133	0.122	0.251	0.02	0.078	-0.039	-0.079	0.125	0.202
SBP				**.639****	-0.061	-0.07	-0.011	-0.22	-0.122	0.065	-0.018	0.13	0.178	-0.065	-0.189
DBP					-0.089	-0.112	-0.245	-0.13	-0.087	0.108	0.013	0.162	0.162	-0.085	-0.097
TPO-abs						**.897****	**.676****	0.01	-0.024	-0.013	-0.037	-0.013	-0.035	-0.014	0.022
TG-abs							**.707****	0.109	-0.054	-0.042	-0.062	-0.023	-0.022	0.003	0.13
APL-abs								0.07	0.122	0.005	-0.057	0.062	0.053	0.119	0.064
HB									0.129	0.163	**.359****	-0.038	**-.457****	-0.135	**.954****
PLT										**.339***	0.238	**.333***	0.088	**.644****	0.136
WBC											**.778****	**.846****	0.122	-0.267	0.098
# LYMPH												**.348***	**-.478****	**-.519****	**.313***
# NEUT													**.592****	0.051	-0.099
NLR														**.492****	**-.473****
PLR															-0.078
HCT%															

SBP=Systolic Blood Pressure, TPO-abs= Thyroperoxidase antibodies, TG-abs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid, Antibodies, WBC=Leukocytes, #LYMPH= count, #Neutrophil= Absolute Neutrophil Count, NLR= Neutrophil-Lymphocyte Ratio, Correlation was level * significant at the 0.05; ** correlation was significant at the 0.01 level.

4.1.16. Correlation of Continuous Data Parameters of Fertile Women

Table 4.16 displays the Pearson correlation of age, body mass index, systolic blood pressure, diastolic blood pressure, thyroperoxidase, thyroglobulin and antiphospholipid antibody concentration among fertile women.

Age significantly correlated directly but weakly with thyroglobulin antibodies among the fertile women ($r=0.366$, $p<0.05$). Age also inversely correlated weakly but significantly with total leukocyte count ($R= -0.374$, $P<0.05$) and absolute neutrophil count ($r= -0.374$, $p<0.05$). Body mass index did not correlate significantly with any of the measured parameters among the fertile women. Systolic blood pressure strongly correlated directly and significantly with diastolic blood pressure ($r= 0.639$, $p<0.01$), thyroglobulin antibodies ($r=0.454$, $p<0.01$) and antiphospholipid antibodies ($r=0.489$, $p<0.01$). Diastolic blood pressure also strongly correlated directly and significantly with thyroglobulin antibodies ($r=0.417$, $p<0.01$) and antiphospholipid antibodies ($r=0.547$, $p<0.01$).

Thyroperoxidase antibodies strongly correlated directly and significantly with thyroglobulin antibodies ($r= 0.496$, $p<0.01$) but weakly with antiphospholipid antibodies ($r=0.362$, $p<0.05$). Thyroglobulin antibodies on the other hand did not correlate significantly with antiphospholipid antibodies among fertile women.

Haemoglobin levels weakly correlated inversely and significantly with absolute neutrophil count ($r= -0.326$ $p<0.05$) and the neutrophil lymphocyte ratio ($r= -0.395$, $p<0.05$) while it strongly correlated directly and significantly with the percentage haematocrit ($r= 0.974$, $p<0.01$). Platelet count correlated directly but weakly with the absolute lymphocyte count ($r=0.368$, $p<0.05$) but strongly with the plateletlymphocyte ratio ($r=0.751$, $p<0.01$). Total leukocyte count correlated weakly with absolute lymphocyte count ($r=0.397$, $p<0.05$) but strongly and significantly with the absolute neutrophil count ($r=0.931$, $p< 0.01$) and the neutrophil lymphocyte ratio ($r=0.698$, $p<0.01$). Absolute lymphocyte count weakly correlated inversely

but significantly with the neutrophil lymphocyte ratio (r= -0.326, p<0.05).

Obviously, the absolute neutrophil count strongly correlated directly with the neutrophil-lymphocyte ratio (r=0.887, p<0.01) but weakly and inversely with the percentage haematocrit (r= -0.360, p<0.05). The neutrophil lymphocyte ratio strongly correlated inversely with the percentage haematocrit (r= -0.469, p<0.01) among the fertile women.

Table 4.16. Pearson Correlation among Age, Body Mass Index, Systolic Blood Pressure, Diastolic Blood Pressure, Autoantibodies and Haematological Parameters of the Fertile Women.

	AGE	BMI	SBP	DBP	TPO-abs	TG-abs	APLabs	HB	PLT	WBC	# LYMPH	# NEUT	NLR	PLR	HCT%
AGE		0.179	0.187	0.269	0.232	**.366***	0.237	0.186	-0.209	**-.374***	-0.279	**-.374***	-0.203	-0.044	0.219
BMI			0.049	-0.013	0.192	0.159	-0.065	0.02	0.034	0.157	0.242	0.098	0.028	-0.102	0.036
SBP				**.785****	0.254	**.454****	**.489****	0.053	-0.238	0.179	0.011	0.052	0.082	-0.22	0.087
DBP					0.248	**.417****	**.547****	0.102	-0.114	0.202	-0.112	0.153	0.228	-0.005	0.091
TPO-abs						**.496****	**.362***	0.091	0.029	0.101	-0.073	0.111	0.144	0.095	0.063
TG-abs							0.299	0.236	-0.13	0.037	0.065	-0.036	-0.055	-0.16	0.229
APL-abs								0.12	-0.08	0.185	0.001	0.209	0.156	-0.085	0.086
HB									0.091	-0.273	0.172	**-.326***	**-.395***	-0.037	**.974****
PLT										0.216	**.368***	0.164	0.029	**.751****	0.07
WBC											**.397***	**.931****	**.698****	-0.045	-0.269
# LYMPH												0.122	**-.326***	-0.307	0.236
# NEUT													**.887****	0.078	**-.360***

NLR	0.256	**-.469****
PLR		-0.111
HCT%		

SBP=Systolic Blood Pressure, TPO-abs= Thyroperoxidase antibodies, TG-abs= Thyroglobulin Antibodies, APL-abs= Antiphospholipid, Antibodies, WBC=Leukocytes, #LYMPH= count, #Neutrophil= Absolute Neutrophil Count, NLR= Neutrophil- Lymphocyte Ratio, Correlation was level * significant at the 0.05; ** correlation was significant at the 0.01 level.

5.0 CHAPTER FIVE

5.1. DISCUSSION

5.1.1. Relationship between Thyroid Autoantibodies, Infertility and a History of Spontaneous abortion.

The association between the presence of thyroid autoantibodies and female infertility, especially as it relates to spontaneous abortion, have been established in most studies in recent times (Thangaratinam et al., 2011). Though their presence have been reported in women of reproductive age to range between 5-20%, increasing levels are reported in women with miscarriage and infertility ranging between 14-33% (Poppe et al., 2008). In this current study, Infertile women were significantly more likely to be TPO-abs positive (TPO-abs >100 U/mL) as compared to their fertile counterparts and the prevalence of TPO-abs positivity was 26.9% among infertile women and 18.2% among infertile women with a history of spontaneous abortion, consistent with Poppe et al. (2008) (Table 4.5 and Table 4.10). Despite this significant association with infertility, women with a history of spontaneous abortion were not significantly associated with the presence of TPO-abs (Table 4.7 and Table 4.10), neither was the presence of TPOabs associated with a history of recurrent abortion (RA) (Table 4.9). This outcome in the present study was also consistent with studies by Ashrafi et al. (2007) who also found no significant association between the presence of TPO-abs and RA. It is reported that TPO-abs positive women are unable to mount the required immune response to sustain the implanted foetus and this may lead to pregnancy loss (Thangaratinam et al., 2011) however, the pathogenesis of RA is multifactorial and up to 50% of the aetiology could be attributable to some genetic, uterine and hormonal factors (Ashrafi et al., 2007).

Thyroglobulin antibody positivity (TG-abs >100U/mL) was not associated with infertility, nor a history of spontaneous abortion in the present study (Table 4.5 and Table 4.10). Significantly higher levels of TG-abs were found in infertile women with no history of spontaneous abortion compared to those with spontaneous abortion (SA) (Table 4.9). This study confirms previous reports that the presence of thyroglobulin antibodies may not be associated with recurrent abortion (Kutteh et al., 1999a). Rushworth et al. (2000) in a study of non-pregnant women with a history of more than two pregnancy losses reported TG-abs to be present in only 5% and found no association between spontaneous abortion and the presence of thyroid autoantibodies. In the present study among infertile women with a history of spontaneous abortion, TG-abs positives were found in 18.2% of them. The differences in TG-abs prevalence in their study however may be attributable to the fact that their study population were women with a history of spontaneous abortion and not necessarily infertile women as well as differences in sample size. It has been noted that in women with spontaneous abortion, the presence of thyroid autoantibodies may not necessarily be as a result of thyroid dysfunction but could be as a result of other abnormal autoimmune activation. Contrary to the findings of our present study and that of Kutteh et al. (1999a) and Rushworth et al. (2000), a recent study found that thyroid autoantibody positive women (TPO-abs and/or TG-abs) had lower rates of fertilization, implantation, pregnancy and high abortion (Zhong et al., 2012). The difference in the outcome of their study and this current one may be due to the differences in study design. Their study involved in-vitro fertilisation and embryo transfer outcome and conditions that regulate these processes in-utero will certainly vary.

5.1.2. Relationship between Antiphospholipid Antibodies (APL-Abs), Infertility and Spontaneous Abortion.

APL-abs have been identified to cause the antiphospholipid syndrome (APS) which is characterized by vascular thrombosis, pregnancy morbidity,

thrombocytopenia, recurrent foetal loss, pregnancy induced hypertension, intrauterine growth retardation and prematurity (Shetty and Ghosh, 2009). In the present study we found that infertile women were significantly more likely to be positive for the APL-abs (APL-abs≥10U/mL) antibodies compared to their fertile counterparts (Table 4.5). The prevalence of APL-abs positives among infertile women was 13.5%. APL positivity however was not associated with a history of spontaneous abortion (Table 4.10). The median concentration of APL-abs however was much higher among infertile women with no abortion as compared to those with SA (Table 4.9).

The 13.5% prevalence finding in this study was consistent with the findings of Birdsall et al. (1996) who reported a prevalence of 15% among infertile women undergoing IVF. Similarly in that study, APL-abs positives was not significantly associated with a history of spontaneous abortion. Buckingham and Chamley (2009) observed that there was no evidence that APL-abs are associated with implantation failure and hence spontaneous abortion despite their high levels reported among infertile population as compared to the fertile population. However recent reviews have elucidated possible mechanisms by which the APLabs affect the foeto-placental unit (Marchetti et al., 2013). Antiphospholipids bind mainly the β2GP1 which is a free cationic protein in the plasma to illicit their effect

on the trophoblastic cell surface either by thrombosis, inflammation, immunomodulation and defective placentation. Other mechanisms on the endometrial cells have also been elucidated (Marchetti et al., 2013). In the current study, infertile patients with a history of spontaneous abortion were not classified as having antiphospholipid syndrome and that could have accounted for the outcome.

5.1.3. Relationship between BMI, Infertility and Menstrual Abnormalities Steeg et al. (2008) related obesity with lower pregnancy rates in sub-fertile women while

Balen et al. (2007) associated obesity with only some signs of poor reproductive function like menstrual abnormalities and greater risk of miscarriage. Jungheim et al. (2012) described the association with reproductive function as complex and difficult to establish a linear relationship. In the present study, the prevalence of overweight among infertile women (BMI: 25-29.9 kg/m^2) was 28.8% (Table 4.2). This was consistent with a study conducted by Shanthakumari et al. (2014), and Zeidan (2015) who found a 26.7% and 24% prevalence of overweight respectively. The prevalence of obesity in this present study was 3.8% among infertile women. These differed from 8.3% and 65% found by Shanthakumari et al. (2014) and Zeidan (2015) respectively. The differences might be due to genetic or environmental variance between the individual study populations. While the current study was carried out in Ghana, those of Shanthakumari et al. (2014) and Zeidan (2015) were undertaken in India and Bagdad respectively. The mean BMI in the present study was comparable between fertile (24.50±3.78 kg/m^2) and infertile women (24.36±3.19 kg/m^2) (Table 4.3). On the contrary, Wise et al. (2013) found that obesity as measured by BMI was associated with delayed conception and thus subfertility. The relationship between obesity and infertility is complex and as such a simple linear relationship may be difficult to establish (Jungheim et al., 2012).

In the present study BMI was comparable between infertile women with a history of spontaneous abortion and those without spontaneous abortion (Table 4.6). BMI also did not differ between infertile women with no abortion, SA and RA (Table 4.5). This finding was not consistent with Bellver et al. (2003) who found that obesity as measured by BMI increases the risk of miscarriage. The differences in outcome may be due to differences in study population and genetic or environmental factors. Bellver's population involved women recipients of ovum donation and the outcome of the implanted ovum, which may be affected by

many factors including the quality of the ovum and allograft rejection. Further his study population was in Spain and such a population differs in genetic and environmental factors in comparison to the infertile Ghanaian population in the current study.

5.1.4. Relationship between Haematological Parameters, Systolic and Diastolic Blood Pressure and Infertility

Platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR) have been reported as non-specific markers of inflammation in the peripheral blood (Cakmak et al., 2015). Infertility due to autoimmune thyroiditis or the antiphospholipid syndrome has been described as an inflammatory process (Giannakopoulos and Krilis, 2013; Marchetti et al., 2013; Vissenberg et al., 2015). We therefore hypothesized that infertility, as a result of these may be associated with changes in peripheral blood haematological parameters such as NLR, PLR, platelet count, absolute lymphocyte count and absolute neutrophil count. In the present study, we observed that the mean PLR and median NLR, total leukocytes and absolute neutrophil count in the peripheral blood was significantly higher in fertile women compared to the infertile women (Table 4.3 and Table 4.4). However, absolute lymphocyte count was significantly higher in infertile women as compared to the fertile women. Platelet count was comparable between the two groups. The high difference in NLR, PLR, total leukocytes and absolute neutrophils in our fertile group may be due the fact that most (92.1%) of our fertile controls had just freshly delivered (less than 2 weeks). Leucocytosis has been reported to prevail in the pregnant state primarily due to the physiological stress during pregnancy and the stress of delivery and it takes approximately four weeks or greater for this state to be restored (Chandra et al., 2012). Therefore, the relative differences in these parameters (NLR, PLR, total leukocytes and absolute neutrophils) between our cases (infertile women) and controls (fertile women) may not be of clinical relevance. In response to pregnancy, the modulation of the

immune response (change and interchange between TH1 to TH2) to accommodate the allograft may have accounted for the relatively low absolute lymphocyte count in our fertile group and normal physiological levels is only restored after approximately four weeks postpartum ((Mor et al., 2011; Chandra et al., 2012).

Some studies have reported the prevalence of pulmonary arterial hypertension to be higher in patients with autoimmune thyroid disease (Chu et al., 2002). In the present study, Systolic and diastolic blood pressure were significantly higher among infertile women as compared to the fertile women (Table 4.3 and Table 4.4). This finding was not consistent with Farland et al., 2015 who found that infertile women were at no greater risk of hypertension compared to controls, but rather those with tubal disease were at a higher risk. The differences in outcome of his study compared to the present study might be due to the differences in sample size and study population. While the controls in the present study were comprised of mostly fertile women with birth not more than three months, the controls in his study were females who had never reported infertility. The outcome in the present study is expected because high blood pressure in pregnancy is mostly associated with pre-eclampsia and none of the controls had any history of pre-eclampsia. Their normal blood physiological pressure in pregnancy remains a few days to a week postpartum. Infertile women may have

some psychological stress that could induce an effect on their systolic and diastolic blood pressures as a result of societal and family pressure on them to conceive. Again, the present study however did not find any association between blood pressure and spontaneous abortion (Table 4.6, Table 4.7, Table 4.8 and Table 4.9).

5.1.5. Relationship between Autoantibodies and Menstrual Abnormalities Many women who present with a thyroid dysfunction experience some form of menstrual abnormalities and thyroid autoimmunity is described as the

commonest cause of hypothyroidism (Hollowell et al., 2002). Ghoraishian et al. (2006) found that among individuals with high thyroid stimulating hormone

(TSH) levels, 64.45% had significantly high TPO-abs levels (>100U/mL), the occurrence of which has been correlated to be of prognostic significance in overt hypothyroidism when TSH is above 2mIU/L (Vanderpump et al., 1995). Approximately 23% of hypothyroid women present with menstrual abnormalities because severe hypothyroidism is associated with ovulatory dysfunction (Poppe and Glinoer, 2003). In the present study, the prevalence of menstrual abnormality among infertile women was 79% and significantly higher than that of fertile women (37%). The odds of being positive for both (TO+TG-abs) was 1.45 times greater in those infertile women having menstrual abnormalities compared to those without menstrual abnormalities (Table 4.14). This outcome was similar for women who tested positive for only APL-abs (OR (95% CI) =1.71, p=0.67). Thyroid autoantibodies was present in 24% to 27% of infertile women with menstrual abnormalities (Table 4.13). This finding is similar to that of Ajmani et al. (2015) who also found that autoimmune thyroid antibodies were present in 30% of women with menstrual abnormalities. There is no reported established relationship between antiphospholipid antibodies and menstrual abnormalities.

5.1.6. Correlation among Autoantibodies and Haematological Parameters In the present study, we found that age inversely correlated weakly but significantly with thyroperoxidase and thyroglobulin antibody levels among infertile women (Table 4.15). On the other hand among fertile women, age correlation positively with only thyroglobulin antibodies. Antiphospholipid antibodies did not correlate with age. This was consistent with Ruiz et al. (1996). Ashrafi et al. (2007) found no correlation between age and thyroid autoantibodies. Although it is expected that autoimmune antibodies increase with increasing age because of the association of age with infertility, age alone cannot account for the levels of autoantibodies among fertile women (Kutteh et al., 1999b).

Systolic and diastolic blood pressures correlated directly and strongly with TGabs and APL-abs among the fertile women (Table 4.16). Antiphospholipid syndrome

which is characterized by the presence of APL-abs presents with pregnancy induced hypertension (Shetty and Ghosh, 2009) and most of our fertile participants had freshly delivered (less than two weeks). Chu et al., 2002, reported a significant association between thyroid autoimmunity and pulmonary arterial hypertension.

TPO-abs correlated directly and strongly with both TG-abs and APL-abs among infertile women (Table 4.15). Again among fertile women, TPO-abs correlated directly and strongly with TG-abs and moderately with APL-abs. This outcome is expected because these autoantibodies are all associated with infertility and in most studies also associated with miscarriage (Thangaratinam et al., 2011; Zhong et al., 2012). Therefore, they are all associated with the same outcome.

6.0 CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

- Among Ghanaians, infertile women are more likely to be TPO-abs positive and APL-abs positive but these association does not relate directly with a history of spontaneous abortion.
- Although infertile women with menstrual abnormalities have a higher odds of being positive for both of the thyroid autoantibodies, the association is not significant.
- TG-abs are not directly associated with infertility, neither is it associated with spontaneous abortion among infertile women.
- BMI does not differ between fertile and infertile women. BMI is comparable among infertile women with no history of spontaneous abortion, SA's and RA's.
- NLR, PLR, #NEUT and #Lymph do not correlate with the presence of autoantibodies (TPO-abs, TG-abs, APL-abs) in infertile women.

6.2. RECOMMENDATIONS

- Autoantibody screening should be carried out as part of a routine check for all infertile women seeking fertility care for appropriate intervention.
- Prospective studies should be carried out to elucidate the effects of the presence of TPO-abs and APL-abs in infertile women apart from spontaneous abortion using a larger sample size.

7.0 REFERENCES

Ağar, Ç., Os, G.M.A. van, Mörgelin, M., Sprenger, R.R., Marquart, J.A., Urbanus, R.T., et al. (2010). β2-Glycoprotein I can exist in 2 conformations: implications for our understanding of the antiphospholipid syndrome. Blood *116*: 1336–1343.

Ajmani, N.S., Sarbhai, V., Yadav, N., Paul, M., Ahmad, A., and Ajmani, A.K. (2015). Role of Thyroid Dysfunction in Patients with Menstrual Disorders in Tertiary Care Center of Walled City of Delhi. J. Obstet. Gynecol. India *66*: 115–119.

Akhlaghi, F., Keramati, M.R., and Tafazoli, M. (2013). Study on Antiphospholipid/Anticardiolipin Antibodies in Women With Recurrent Abortion. Iran. Red Crescent Med. J. *15*: 718–722.

Alhassan, A., Ziblim, A.R., and Muntaka, S. (2014). A survey on depression among infertile women in Ghana. BMC Womens Health *14*: 42.

Aron, A.L., Gharavi, A.E., and Shoenfeld, Y. (1995). Mechanisms of action of antiphospholipid antibodies in the antiphospholipid syndrome. Int. Arch. Allergy Immunol. *106*: 8–12.

Ashrafi, M., Yazdi, R.S., Madani, T., and Bazrafshan, A. (2007). Anti-thyroid peroxidase and risk of recurrent spontaneous abortion. Iran. J. Fertil. Steril. *1*: 113–116.

ASRM (2008). Definitions of infertility and recurrent pregnancy loss. Fertil. Steril. *90*: S60.

Bakimer, R., Cohen, J.R., and Shoenfeld, Y. (1994). What really happens to fecundity in autoimmune diseases? Immunol. Allergy Clin. North Am. *14*: 701–723.

Balen, A.H., Anderson, R.A., and Policy & Practice Committee of the BFS (2007). Impact of obesity on female reproductive health: British Fertility Society, Policy and Practice Guidelines. Hum. Fertil. Camb. Engl. *10*: 195–206.

Balucan, F.S., Morshed, S.A., and Davies, T.F. (2013). Thyroid Autoantibodies in Pregnancy: Their Role, Regulation and Clinical Relevance. J. Thyroid Res. *2013*: 1–15.

Beer, A.E., Kwak, J.Y.H., and Ruiz, J.E. (1996). Immunophenotypic Profiles of Peripheral Blood Lymphocytes in Women With Recurrent Pregnancy Losses and in Infertile Women With Multiple Failed In-Vitro Fertilization Cycles. Am. J. Reprod. Immunol. *35*: 376–382.

Bellver, J., Rossal, L.P., Bosch, E., Zúñiga, A., Corona, J.T., Meléndez, F., et al. (2003). Obesity and the risk of spontaneous abortion after oocyte donation. Fertil. Steril. *79*: 1136–1140.

Biondi, B. (2010). Thyroid and Obesity: An Intriguing Relationship. J. Clin. Endocrinol. Metab. *95*: 3614–3617.

Birdsall, M.A., Lockwood, G.M., Ledger, W.L., Johnson, P.M., and Chamley, L.W. (1996). Antiphospholipid antibodies in women having in-vitro fertilization. Hum. Reprod. *11*: 1185–1189.

Biritwum, R., Gyapong, J., and Mensah, G. (2005). The Epidemiology of Obesity in Ghana. Ghana Med. J. *39*: 82–85.

Blank, M., Cohen, J., Toder, V., and Shoenfeld, Y. (1991). Induction of antiphospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti-cardiolipin antibodies. Proc. Natl. Acad. Sci. *88*: 3069–3073.

Boivin, J., Bunting, L., Collins, J.A., and Nygren, K.G. (2007). International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum. Reprod. *22*: 1506–1512.

Bourke, C.D., Nausch, N., Rujeni, N., Appleby, L.J., Mitchell, K.M., Midzi, N., et al. (2013). Integrated Analysis of Innate, Th1, Th2, Th17, and Regulatory Cytokines Identifies Changes in Immune Polarisation Following Treatment of Human Schistosomiasis. J. Infect. Dis. *208*: 159–169.

Buckingham, K.L., and Chamley, L.W. (2009). A critical assessment of the role of antiphospholipid antibodies in infertility. J. Reprod. Immunol. *80*: 132–145.

Bushnik, T., Cook, J.L., Yuzpe, A.A., Tough, S., and Collins, J. (2012). Estimating the prevalence of infertility in Canada. Hum. Reprod. *27*: 738–746.

Bussen, S., Steck, T., and Dietl, J. (2000). Increased prevalence of thyroid antibodies in euthyroid women with a history of recurrent in-vitro fertilization failure. Hum. Reprod. *15*: 545–548.

Cakmak, B., Gulucu, S., Aliyev, N., Ozsoy, Z., Nacar, M., and Koseoglu, D. (2015). Neutrophil-lymphocyte and platelet-lymphocyte ratios in endometrial hyperplasia. Obstet. Gynecol. Sci. *58*: 157–161.

Chandra, S., Tripathi, A.K., Mishra, S., Amzarul, M., and Vaish, A.K. (2012). Physiological Changes in Hematological Parameters During Pregnancy. Indian J. Hematol. Blood Transfus. *28*: 144–146.

Chan, S., and Boelaert, K. (2015). Optimal management of hypothyroidism, hypothyroxinaemia and euthyroid TPO antibody positivity preconception and in pregnancy. Clin. Endocrinol. (Oxf.) *82*: 313–326.

Choudhury, S.R., and Knapp, L.A. (2000). Human reproductive failure 1: immunological factors. Hum. Reprod. Update *7*: 113–134.

Chu, J.W., Kao, P.N., Faul, J.L., and Doyle, R.L. (2002). High prevalence of autoimmune thyroid disease in pulmonary arterial hypertension. Chest *122*: 1668–1673.

Cui, W. (2010). Mother or nothing: the agony of infertility. Bull. World Health Organ. *88*: 881–882.

De Groot, P., and Urbanus, R. (2012). The Future of Antiphospholipid Antibody Testing. Semin. Thromb. Hemost. *38*: 412–420.

Ericksen, K., and Brunette, T. (1996). Patterns and predictors of infertility among African women: a cross-national survey of twenty-seven nations. Soc. Sci. Med. *42*: 209–220.

ESHRE Capri Workshop Group, T.E.C.W. (2002). Physiopathological determinants of human infertility. Hum. Reprod. Update *8*: 435–447.

Esteves, S.C., Miyaoka, R., and Agarwal, A. (2011). An update on the clinical assessment of the infertile male. Clinics *66*: 691–700.

Evain-Brion, D., Guibourdenche, J., Tsatrsaris, V., and Fournier, T. (2009). [Human trophoblast differentiation]. Bull. Acad. Natl. Med. *193*: 1017–25; discussion 1025–7, 1067–8.

Farland, L.V., Grodstein, F., Srouji, S.S., Forman, J.P., Rich-Edwards, J., Chavarro, J.E., et al. (2015). Infertility, fertility treatment, and risk of hypertension. Fertil. Steril. *104*: 391–397.

Fledderjohann, J.J. (2012). 'Zero is not good for me': implications of infertility in Ghana. Hum. Reprod. *27*: 1383–1390.

Gaberščček, S., and Zaletel, K. (2011). Thyroid physiology and autoimmunity in pregnancy and after delivery. Expert Rev. Clin. Immunol. *7*: 697–707.

Geelhoed, D.W., Nayembil, D., Asare, K., Schagen van Leeuwen, J.H., and Roosmalen, J. van (2002). Infertility in rural Ghana. Int. J. Gynaecol. Obstet. Off. Organ Int. Fed. Gynaecol. Obstet. *79*: 137–142.

Geva, E., Amit, A., Lerner-Geva, L., and Lessing, J.B. (1997). Autoimmunity and reproduction. Fertil. Steril. *67*: 599–611.

Ghana Statistical Service (2013). The 2010 Population and Housing Census: Regional Analytical Report: Ashanti region.pdf.

Ghoraishian, S.M., Moghaddam, S.H.H., and Ardekani, M.A. (2006). Relationship between anti-thyroid peroxidase antibody and thyroid function test. Iran J Immunol *3*: 146.

Giannakopoulos, B., and Krilis, S.A. (2013). The Pathogenesis of the Antiphospholipid Syndrome. N. Engl. J. Med. *368*: 1033–1044.

Giannakopoulos, B., Passam, F., Rahgozar, S., and Krilis, S.A. (2007). Current concepts on the pathogenesis of the antiphospholipid syndrome. Blood *109*: 422–430.

Gleicher, N. (2002). Some thoughts on reproductive auto-immune failure syndrone TH1 vrs TH2. Am. J. Reprod. Immunol. 252–254.

Glinoer, D. (1997). The Regulation of Thyroid Function in Pregnancy: Pathways of Endocrine Adaptation from Physiology to Pathology. Endocr. Rev. *18*: 404–433.

Glinoer, D., Soto, M.F., Bourdoux, P., Lejeune, B., Delange, F., Lemone, M., et al. (1991). Pregnancy in patients with mild thyroid abnormalities: maternal and neonatal repercussions. J. Clin. Endocrinol. Metab. *73*: 421–427.

Gnoth, C., Godehardt, E., Frank-Herrmann, P., Friol, K., Tigges, J., and Freundl, G. (2005). Definition and prevalence of subfertility and infertility. Hum. Reprod. *20*: 1144–1147.

Gosman, G.G., Katcher, H.I., and Legro, R.S. (2006). Obesity and the role of gut and adipose hormones in female reproduction. Hum. Reprod. Update *12*: 585–601.

Gurunath, S., Pandian, Z., Anderson, R.A., and Bhattacharya, S. (2011). Defining infertility—a systematic review of prevalence studies. Hum. Reprod. Update *17*: 575–588.

Haller-Kikkatalo, K., Salumets, A., Uibo, R., Haller-Kikkatalo, K., Salumets, A., and Uibo, R. (2011). Review on Autoimmune Reactions in Female Infertility: Antibodies to Follicle Stimulating Hormone, Review on Autoimmune Reactions in Female Infertility: Antibodies to Follicle Stimulating Hormone. J. Immunol. Res. J. Immunol. Res. *2012, 2012*: e762541.

Hektoen, L., and Schulhof, K. (1925). The Precipitin Reaction of Thyroglobulin Specificness; Presence of Thyroglobulin in Human Thyroid Veins; Production by Rabbit of Precipitin for Rabbit Thyroglobulin; Thyroglobulin in the Foetal Thyroid and in Exophthalmic Goiter. Proc. Natl. Acad. Sci. *11*: 481–484.

Holers, V.M., Girardi, G., Mo, L., Guthridge, J.M., Molina, H., Pierangeli, S.S., et al. (2002). Complement C3 Activation Is Required for Antiphospholipid Antibody-induced Fetal Loss. J. Exp. Med. *195*: 211–220.

Hollowell, J.G., Staehling, N.W., Flanders, W.D., Hannon, W.H., Gunter, E.W., Spencer, C.A., et al. (2002). Serum TSH, T4, and Thyroid Antibodies in the United States Population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J. Clin. Endocrinol. Metab. *87*: 489–499.

Jovanović, M., Božić, M., Kovačević, T., Radojčić, L., Petronijević, M., and Vićovac, L. (2010). Effects of anti-phospholipid antibodies on a human trophoblast cell line (HTR-8/SVneo). Acta Histochem. *112*: 34–41.

Jungheim, E.S., Travieso, J.L., Carson, K.R., and Moley, K.H. (2012). Obesity and Reproductive Function. Obstet. Gynecol. Clin. North Am. *39*: 479–493.

Jungwirth, A., Diemer, T., Dohle, G.R., Giwercman, A., Kopa, Z., Krausz, C., et al. (2015). Guidelines on male infertility.pdf.

Kamath, C., Adlan, M.A., Premawardhana, L.D., Kamath, C., Adlan, M.A., and Premawardhana, L.D. (2012). The Role of Thyrotrophin Receptor Antibody Assays in Graves' Disease. J. Thyroid Res. J. Thyroid Res. *2012, 2012*: e525936.

Karmaus, W., Juul, S., and others (1999). Infertility and subfecundity in population-based samples from Denmark, Germany, Italy, Poland and Spain. Eur. J. Public Health *9*: 229–235.

Kirkendall, W.M., Burton, A.C., Epstein, F.H., and Freis, E.D. (1967). Recommendations for Human Blood Pressure Determination by Sphygmomanometers. Circulation *36*: 980–988.

Krassas, G.E., Poppe, K., and Glinoer, D. (2010). Thyroid Function and Human Reproductive Health. Endocr. Rev. *31*: 702–755.

Kutteh, W.H., Schoolcraft, W.B., and Scott, R.T. (1999a). Antithyroid antibodies do not affect pregnancy outcome in women undergoing assisted reproduction. Hum. Reprod. *14*: 2886–2890.

Kutteh, W.H., Yetman, D.L., Carr, A.C., Beck, L.A., and Scott, R.T. (1999b). Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction. Fertil. Steril. *71*: 843–848.

Larsen, U. (2000). Primary and secondary infertility in sub-Saharan Africa. Int. J. Epidemiol. 285–291.

Larsen, U. (2005). Research on infertility: Which definition should we use? Fertil. Steril. *83*: 846–852.

Leke, R.J., Goyaux, N., Matsuda, T., and Thonneau, P.F. (2004). Ectopic pregnancy in Africa: a population-based study. Obstet. Gynecol. *103*: 692–697.

Leke, R.J., Kigondu, C. ekadde-, Chikamata, D., and Franken, D. (2002). The prevalence of infertility and its preventive measures in sub-Saharan Africa. In Management of Infertility in AFRO & EMRO Countries. Geneva, Switzerland: World Health Organization Proceedings of a Workshop, pp 79–91.

Leung, A.M. (2012). Thyroid function in pregnancy. J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS *26*: 137–140.

Levy, R.A., Jesús, G.R.R., and Jesús, N.R. (2010). Obstetric antiphospholipid syndrome: still a challenge. Lupus *19*: 457–459.

Li, Q., Li, H., and Tian, X (1998). The study of autoantibodies in women with habitual abortion of unknown etiology. Zhonghua Fu Chan Ke Za Zhi *33*: 13–16.

Manetti, G.J., and Honig, S.C. (2010). Update on Male Hormonal Contraception: Is the Vasectomy in Jeopardy? Int. J. Impot. Res. *22*: 159–170.

Marchetti, T., Cohen, M., and Moerloose, P. de (2013). Obstetrical Antiphospholipid Syndrome: From the Pathogenesis to the Clinical and Therapeutic Implications. Clin. Dev. Immunol. *2013*: 1–9.

Marzullo, P., Minocci, A., Tagliaferri, M.A., Guzzaloni, G., Di Blasio, A., De Medici, C., et al. (2010). Investigations of Thyroid Hormones and Antibodies in Obesity: Leptin Levels Are Associated with Thyroid Autoimmunity Independent of Bioanthropometric, Hormonal, and Weight-Related Determinants. J. Clin. Endocrinol. Metab. *95*: 3965–3972.

Mascarenhas, M.N., Cheung, H., Mathers, C.D., and Stevens, G.A. (2012a). Measuring infertility in populations: constructing a standard definition for use with demographic and reproductive health surveys. Popul. Health Metr. *10*: 17.

Mascarenhas, M.N., Flaxman, S.R., Boerma, T., Vanderpoel, S., and Stevens, G.A. (2012b). National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Med. *9*: e1001356.

McNeil, H.P., Chesterman, C.N., and Krilis, S.A. (1991). Immunology and clinical importance of antiphospholipid antibodies. Adv. Immunol. *49*: 193–280.

Meroni, P.L., Tedesco, F., Locati, M., Vecchi, A., Di Simone, N., Acaia, B., et al. (2010). Anti-phospholipid antibody mediated fetal loss: still an open question from a pathogenic point of view. Lupus *19*: 453–456.

Miyakis, S., Lockshin, M.D., Atsumi, T., Branch, D.W., Brey, R.L., Cervera, R., et al. (2006). International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J. Thromb. Haemost. *4*: 295–306.

Mor, G., Cardenas, I., Abrahams, V., and Guller, S. (2011). Inflammation and pregnancy: the role of the immune system at the implantation site. Ann. N. Y. Acad. Sci. *1221*: 80–87.

Okonofua, F.E. (1996). The case against new reproductive technologies in developing countries. BJOG Int. J. Obstet. Gynaecol. *103*: 957–962.

Oliveras, E., Ahiadeke, C., Adanu, R.M., and Hill, A.G. (2008). Clinic-based Surveillance of Adverse Pregnancy Outcomes to Identify Induced Abortions in Accra, Ghana. Stud. Fam. Plann. *39*: 133–140.

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

Petrikova, J., Lazurova, I., Dravecka, I., Vrbikova, J., Kozakova, D., Figurova, J., et al. (2014). The prevalence of non organ specific and thyroid autoimmunity in patients with polycystic ovary syndrome. Biomed. Pap.

Poppe, K., and Glinoer, D. (2003). Thyroid autoimmunity and hypothyroidism before and during pregnancy. Hum. Reprod. Update *9*: 149–161.

Poppe, K., Velkeniers, B., and Glinoer, D. (2008). The role of thyroid autoimmunity in fertility and pregnancy. Nat. Clin. Pract. Endocrinol. 38 Metab. *4*: 394–405.

Procaccini, C., Carbone, F., Galgani, M., La Rocca, C., De Rosa, V., Cassano, S., et al. (2011). Obesity and susceptibility to autoimmune diseases. Expert Rev. Clin. Immunol. *7*: 287–294.

Rominski, S.D., and Lori, J.R. (2014). Abortion care in Ghana: a critical review of the literature. Afr. J. Reprod. Health *18*: 17–35.

Rotondi, M., Magri, F., and Chiovato, L. (2011). Thyroid and Obesity: Not a OneWay Interaction. J. Clin. Endocrinol. Metab. *96*: 344–346.

Rowe, P., Comhaire, F., Hargreave, T., and Mellows, H. (1993). WHO manual for the standardized investigation and diagnosis of infertile couple. Camb. Univ. Press.

Ruiz, A.M., Kwak, J.Y., Kwak, F.M., and Beer, A.E. (1996). Impact of age on reproductive outcome in women with recurrent spontaneous abortions and infertility of immune etiology. Am. J. Reprod. Immunol. N. Y. N 1989 *35*: 408–414.

Rushworth, F.H., Backos, M., Rai, R., Chilcott, I.T., Baxter, N., and Regan, L. (2000). Prospective pregnancy outcome in untreated recurrent miscarriers with thyroid autoantibodies. Hum. Reprod. *15*: 1637–1639.

Sebire, N.J., Regan, L., and Rai, R. (2002). Biology and pathology of the placenta in relation to antiphospholipid antibody-associated pregnancy failure. Lupus *11*: 641–643.

Shanthakumari, K., Frank, R.W., and Tamrakar, A. (2014). Prevalence of Obesity among Infertile Women Visiting Selected Infer tility Clinic at Mangalore with a View to Develop an Informational Pamphlet. IOSR J. Nurs. Health Sci. *3*: 7–10.

Shetty, S., and Ghosh, K. (2009). Anti-phospholipid antibodies and other immunological causes of recurrent foetal loss--a review of literature of various therapeutic protocols. Am. J. Reprod. Immunol. N. Y. N 1989 *62*: 9–24.

Simone, N.D., Nicuolo, F.D., D'Ippolito, S., Castellani, R., Tersigni, C., Caruso, A., et al. (2010). Antiphospholipid Antibodies Affect Human Endometrial Angiogenesis. Biol. Reprod. *83*: 212–219.

Stagnaro-Green, Roman SH, Cobin RH, El-Harazy E, Alvarez-Marfany M, and Davies TF (1990). Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies. JAMA *264*: 1422–1425.

Steeg, J.W. van der, Steures, P., Eijkemans, M.J.C., Habbema, J.D.F., Hompes, P.G.A., Burggraaff, J.M., et al. (2008). Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. Hum. Reprod. *23*: 324–328.

Suh-Lailam, B.B., Cromar, A., Davis, K.W., and Tebo, A.E. (2012). APhL antibody ELISA as an alternative to anticardiolipin test for the diagnosis of antiphospholipid syndrome. Int. J. Clin. Exp. Pathol. *5*: 210.

Sule, J.O., Erigbali, P., and Eruom, L. (2008). Prevalence of infertility in women in a southwestern Nigerian community. Afr. J. Biomed. Res. *11.*:

Sundby, J., Mboge, R., and Sonko, S. (1998). Infertility in the Gambia: frequency and health care seeking. Soc. Sci. Med. 1982 *46*: 891–899.

Sysmex corporation (2007). Sysmex XE-2100 operators manual: Detection principle (Sysmex corporation).

Tamer, G., Mert, M., Tamer, I., Mesci, B., Kilic, D., and Arik, S. (2011). Effects of thyroid autoimmunity on abdominal obesity and hyperlipidaemia. Endokrynol Pol *62*: 421–8.

Thangaratinam, S., Tan, A., Knox, E., Kilby, M.D., Franklyn, J., and Coomarasamy, A. (2011). Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. BMJ *342*: d2616.

Vanderpump, M.P., Tunbridge, W.M., French, J.M., Appleton, D., Bates, D., Clark, F., et al. (1995). The incidence of thyroid disorders in the community: a twentyyear follow-up of the Whickham Survey. Clin. Endocrinol. (Oxf.) *43*: 55–68.

Vissenberg, R., Manders, V.D., Mastenbroek, S., Fliers, E., Afink, G.B., RisStalpers, C., et al. (2015). Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum. Reprod. Update *21*: 378–387.

Weiss, G., Goldsmith, L.T., Taylor, R.N., Bellet, D., and Taylor, H.S. (2009). Inflammation in Reproductive Disorders. Reprod. Sci. *16*: 216–229.

WHA (2011). Women's Health Study of Accra wave II (WHSA-II). Institue Stat. Soc. Econ. Res. Univ. Ghana Legon Accra Ghana 2011 *Technical Publication No. 91.*:

WHO (1975). The epidemiology of infertility. A report of the WHO scientific group (Geneva: World Health Organization).

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

WHO (2006). Reproductive health indicators: guidelines for their generation, interpretation and analysis for global monitoring (Geneva: World Health Organization).

WHO (2015). WHO | Infertility is a global public health issue.

Wise, L.A., Palmer, J.R., and Rosenberg, L. (2013). Body size and time-topregnancy in black women. Hum. Reprod. *28*: 2856–2864.

Yen, P.M. (2001). Physiological and Molecular Basis of Thyroid Hormone Action. Physiol. Rev. *81*: 1097–1142.

Zegers-Hochschild, F., Adamson, G.D., Mouzon, J. de, Ishihara, O., Mansour, R., Nygren, K., et al. (2009). International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009∗. Fertil. Steril. *92*: 1520–1524.

Zeidan, M.A.K. (2015). Body Mass Index Finding among a Sample of Women with Infertility in Baghdad City. Kufa J. Nurs. Sci. *5*: 1–7.

Zhong, Y., Ying, Y., Wu, H., Zhou, C., Xu, Y., Wang, Q., et al. (2012). Relationship between Antithyroid Antibody and Pregnancy Outcome following in Vitro Fertilization and Embryo Transfer. Int. J. Med. Sci. *9*: 121–125.

8.0 APPENDIX

8.1. ETHICAL APPROVAL

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/143/15

22nd April, 2015.

Mr. Isaac Acheampong
Department of Molecular Medicine
School of Medical Sciences
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title: *"Immunological Factors Associated with Female Infertility in Ghana: Antiphospholipid Antithyroglobulin and Antithyroperoxidase Antibodies.*

Proposed Site: *Komfo Anokye Teaching Hospital, Kumasi South Hospital and Trust Care Specialist Hospital.*

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 12th March, 2015 from the Komfo Anokye Teaching Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A notification letter of 18th March, 2015 from the Kumasi South Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A notification letter of 13th March, 2015 from the Trust Care Specialist Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Osomfuor Prof. Sir J. W. Acheampong MD, FWACP
Chairman

8.2. QUESTIONAIRE

PROJECT TOPIC: Immunological factors associated with female infertility in Ghana: antithyroid and antiphospholipid antibodies.

*Any information obtained from you shall be used ONLY for the purpose of this study and CANNOT BE TRACEABLE TO YOU. You are at liberty to decide not to answer any question you deem unfit. (**(Please answer/thick appropriately).***

STUDY ID: …….. **DATE:** ……
AGE: ….....

EDUCATIONAL LEVEL:
……
……

OCCUPATION:
……
……..

Clinical/Personal Information

1. How many children do you have? (Parity) ……
2. How long have you been married? a.[ ] 1-5years b. [ ] 6-10years c. [ ] >10years
3. How old were you when you got married? a.[ ] <18years b. [ ]18-25years c.[ ]25-30years d.[ ] >30years
4. When was the last time you gave birth? a. [ ] 1-5yrs b. [ ] >5yrs c. [ ] Never given birth
5. How long (years) have you desired to get pregnant without contraception?
   a. [ ] <1year b. [ ] 1-2years c. [ ] 3- 5years d. [ ] >5years
6. Have you ever lost a pregnancy before? a. [ ] Yes b. [ ] No. If yes how many times? a. [ ] once b. [ ] twice c. [ ] 3-5 times d. [ ] >5times
7. If yes, how long did you carry the pregnancy before losing your last pregnancy?
   a. [ ] < 1 month b. [ ] 1-3 months c. [ ] 4-6 months d. [ ] 7-9 months e. [ ] N/A

8. If yes did you have any of these adverse effects in that pregnancy? (Please tick)

   a. [ ] none b. [ ] gestational diabetes c. [ ] anaemia d. [ ] fainting spells

   e. [ ] pre-eclampsia f. [ ] others (please specify)……

9. Do you know of any female family member who could not deliver after one year of trying?

   a. Yes [ ] b. No [ ]. If yes, did she also lose any pregnancy? ..... If yes how many times? a. [ ] once b. [ ] twice c. [ ] 3-5 times d. [ ] >5times

10. Have you ever suffered still birth? a. [ ] Yes b. [ ] No. If yes how many times?

    a. [ ] once b. [ ] twice c. [ ] thrice d. [ ] >3times

11. Do you know what kind of problem is causing the infertility condition? a. [ ] Yes b. [ ] No. If yes what is the name of the problem?

    .....

12. Which date of the month did you have your last menses? Please state

    …….....

13. Do you have irregular menstruation? a. [ ] Yes b. [ ] No. If yes what type of disorder is it? a. [ ] oligomenorrhea b. [ ] amenorrhea c. [ ] dysmenorrhea

    d. [ ] endometriosis e. [ ] other (please state)……..

14. Do you have any of the following abnormal symptoms during menstruation?

a. [ ] Severe cramps b. [ ] fatigue c. [ ] fainting spells d.

Others……..

15. Are you taking any drug for the abnormal menses? a. [ ] Yes b. [ ] No

    If yes what is the name of the drug, if you know?

    .....

16. Are you on any drug for the infertility condition? a. [ ] Yes b. [ ] No

If yes, what is the name of the drug? .....

17. Have you ever been on any family planning contraceptive? a. [ ] Yes b. [ ] No If yes what was the name of the contraceptive? ……

18. What type of family planning contraceptive does it belong to? if you know

a. [ ] Never used any contraceptive b. [ ] IUD b. [ ] Implant c. [ ] injectable d. [ ] the Pill (combined oral contraceptive) e. [ ] progestin only Pill f. [ ] patch g. [ ] hormonal vaginal contraceptive ring h. [ ] emergency contraception i. [ ] Female/ Male condom

19. How long has it been since you stopped using the family planning contraceptive?

a. [ ] <1year b. [ ] 1-2years c. [ ] 3- 5years d. [ ] >5years e. [ ] N/A

20. How old were you when you first used birth control? …(Age in years)

21. Has your husband ever done a semen analysis test before? a. [ ] Yes b. [ ] No. If yes what was the outcome of the test? a. [ ] Normal Semen test b. [ ] low sperm count c. [ ] poor sperm motility d. [ ] antisperm antibodies e. [ ] I have no idea

22. Have you been diagnosed of any of the following infections in the past one year?

a. [ ] UTI

b. [ ] syphilis c. [ ] candidiasis d. [ ] gonorrhoea f. [ ] none g. [ ] other ……

23. Approximately how many hours do you spend at work? a. [ ] 6-8 hours b. [ ] 9-12 hours

c. [ ] >12 hours d. [ ] unemployed

24. How many days of the week are you present at work? a.[ ] <5days b. [ ] 5days

c. [ ] 6days d. [ ] 7days e. [ ] unemployed

25. Is your work stressful? a. [ ] Yes b. [ ] No If yes, how will you grade yourself on a scale of 1-10 with reference to your stress level? (Personal stress assessment, 0=no stress, 10= extremely stressful) ……

8.3. PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title of the Research: Immunological factors associated with female infertility in Ghana: antithyroid and antiphospholipid antibodies.

Names and Affiliations of Researchers:

This research is being conducted by Mr Isaac Acheampong, Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Prof. (Mrs.) Margret T. Frempong, Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science, Prof. A. T. Odoi, Department of Obstetrics and Gynaecology, Komfo Anokye Teaching Hospital and Trust Care Specialist Hospital and Prof. C. A Turpin, Department of Obstetrics and Gynaecology, Komfo Anokye Teaching Hospital.

Background:

This research is about autoantibodies in infertility. Infertility is defined as absence of conception after twelve months of sexual intercourse without contraception. About 10% of the cause of infertility is unknown; however, most have been associated with some autoantibodies. Several studies have given mixed reports about the association between female infertility and the presence of autoantibodies in the serum. Since Ghana has a high infertility prevalence of 11.8%, the findings from this study may therefore help clinicians to extend the screening of infertile women to include these immunological culprits in order to expedite appropriate treatment. It will also help scientist to know the impact of autoantibodies on infertility in the Ghanaian female population.

Purpose of the Research:

The purpose of this study is to assess the association between a panel of autoantibodies and infertile women in Ghana.

Procedure of the Research:

To accomplish the purpose of this research, about one and a half teaspoon (approximately 5mls) of blood will be drawn from your arm using a syringe and needle for laboratory investigations. You will also be required to fill a questionnaire for the study. One hundred and sixty (160) participants would be recruited for this research.

Risk:

The blood sample taking can be inconvenient to you. You may feel slight pinching sensation at the point on your arm where the blood will be drawn since a syringe and needle would be used.

Benefit:

You would get to know if autoantibodies contribute to your infertility problem. The outcome of this research in Ghana would also help clinicians to extend the screening of infertile women to include these autoantibodies in order to expedite appropriate treatment. It will also help scientist to know the impact of autoantibodies on infertility in the Ghanaian female population.

Confidentiality:

All information which will be collected from you in this research will be given code numbers. Data collected will not be linked to you in anyway. No name or identifier will be used in any publication or reports from this research. However, as part of our responsibility to conduct this research properly, we may allow officials from the ethics committee to have access to your records.

Voluntariness:

Taking part in this research should be out of your own free will. You are not under any obligation to take part in this research. This research is entirely voluntary.

Alternatives to Participation:

If you choose not to participate in this research, your treatment in this hospital will not be affected in any way. **Withdrawal from the Research:**

You may choose to withdraw from the research at anytime without having to explain yourself to the researchers. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to you if you choose to withdraw from this research. Please note however that, some of the information that may have been obtained from you, before you chose to withdraw, may have been modified or used in analysis of reports and publications. These cannot be removed anymore. We do promise to comply with your wishes as much as practicable.

Contacts:

If you have any question concerning this research, please do not hesitate to contact Mr. Isaac Acheampong (0248 273 152), Prof. (Mrs.) Margret T. Frempong (0208 186 136), or Prof. A.T Odoi (0208181056).

Further, if you have any concern about the conduct of this research, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman Committee on Human Research and Publication Ethics Kumasi Tel: 0322 063 248 or 0205 453 785

CONSENT FORM

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the research, including that on procedures, risks and benefits, to enable the prospective participant to make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research to decide that I want to take part in it.

I understand that I may freely stop being part of this research at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.
NAME: _____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to _____ (Name of Participant), in the local language, is a true reflection of what I have read from the research Participant Information Leaflet, attached.

WITNESS' SIGNATURE: _____

Contacts: Please, if you have any question concerning this study, do not hesitate to contact the researchers at the Department of Molecular Medicine, KNUST

1. MR ISAAC ACHEAMPONG

2. PROF. (MRS) M.T FREMPONG

If you have any concern regarding this study and would like to contact someone at the blind side of the researchers, you are encouraged to contact,

The Head of Department

Department of Molecular Medicine

School of Medical Sciences

KNUST

Kumasi.