AUTO- ANTIBODIES AND IMMUNOLOGICAL CORRELATES AS INDICATORS OF INFERTILITY IN SOME EUTHYROID NIGERIAN WOMEN

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CERTIFICATION

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DEDICATION

This work is dedicated to the continuing work of the Loving Father who after the sixth day work, rested and on the seventh, made man in His Own image and likeness, then He *blessed man saying "be fruitful and multiply and replenish the Earth, have dominion....."*

This work is dedicated to the active Word of God who says "ask and you shall receive," He is the Living master, my Lord and Saviour, who says *"none shall be barren in His house..."*

This work is dedicated to the true counselor and teacher who *knows the mind of God towards man. that it is good and not evil to give the hope and the future.....*

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ABSTRACT

This study was carried out to determine the serum auto-antibody levels as primary markers and immunological correlates as indicators of infertility in some euthyroid Nigerian women. Some biochemical indices such as serum hormone levels and the levels of immune enhancers (ie vitamins E and C and the micro-nutrients iron and zinc) were evaluated, as secondary markers in the investigation of auto- antibody as being implicated in infertility in women.

A total of two hundred and ninety (290) screened euthyroid female volunteers were used (following the Lagos State Health Management Board Ethics Committee recommendations). Blood samples were collected from non-pregnant (after a week of their menstrual period) and pregnant women in each of the groups. The control group women were made up of forty six (46) nulligravida, fifty eight (58) multiparous, as well as sixty (60) pregnant women (in their first and second trimester). The test group women were made up of thirty four (34) primary infertile, forty six (46) secondary infertile and forty six (46) recurrent spontaneous aborter (RSA).

The serum anti-thyroglobulin (Tg-Ab) and anti-microsomal (anti-thyroperoxidase i.e TPO-Ab) auto-antibodies determined (using the diagnostic agglutination and automation ELISA – enzyme-linked immunosorbent assay) showed the following results: The serum anti-thyroglobulin (Tg-Ab) level was significantly higher ($P < 0.05$) in the women in the secondary infertile (809.65$\pm$ 3.23 U/ml) and primary infertile (539.59$\pm$3.79 U/ml) groups as well as the recurrent spontaneous aborter (490.00$\pm$3.20 U/ml) group, compared with the control women in the nulligravida (42.48$\pm$3.16 U/ml), multiparous (32.02$\pm$ 2.82 U/ml) and the pregnant (31.90$\pm$ 2.77 U/ml) groups. The anti-thyroperoxidase (TPO-Ab) mean titer of the study group was equally higher and significant ($P < 0.05$) compared to the women in the control group.

Natural Killer Cells' (NK) functional activities determined with the cytotoxicity assay against the cell line K562, in a flow cytometric technique, showed that the CD4 level of

55.11 ± 1.11% for the primary infertile group and 54.71 ± 1.22% for the RSA were significantly higher when compared with, 43.58 ± 1.36% of the control nulligravida ($P < 0.05$). Whereas the CD8 level of 27.03 ± 1.11% of the nulligravida control women were significantly higher than the primary infertile group level of 13.36 ± 0.90% and that of the recurrent spontaneous aborter group with 13.84 ± 0.99% ($P < 0.05$). Conversely the NK cell's value of CD 56 + 16 obtained in the multiparous group (7.24 ± 0.57%) was significantly lower, compared with the primary infertile group with the value of 14.92 ± 0.70% and with the RSA value of 14.57 ± 0.77% ($P < 0.05$). The CD56 value of 6.21 ± 0.51% in nulligravida and 6.67 ± 0.34% in the multiparous was significantly higher compared with the 2.70 ± 0.42% in primary infertility group and 2.60 ±0.46% in RSA ($P < 0.05$).

The serum hormonal assay of progesterone, testosterone, estradiol and prolactin determined using diagnostic automation ELISA kits, showed that the mean titer value of prolactin (183.90 ± 11.23ng/ml) and testosterone (304.43 ± 4.11ng/ml) obtained in the primary infertile women with anti-microsomal antibodies were significantly higher ($P<0.05$), compared with the women in the control group. The progesterone level of the women in primary infertile group was 2.93 ± 1.76 ng/ml and that of the secondary infertile group was 0.29 ± 0.24 ng/ml. these were significantly lower than the RSA group with 16.88 ± 0.83 ng/ml. However the progesterone levels in the nulligravida of 6.66 ±0.83 ng/ml and 0.47 ± 0.15 ng/ml obtained for the multiparous group were significantly lower than that of the pregnant group value of 40.25 ± 1.28 ng/ml ($P < 0.05$). The estradiol levels in the control group showed that, the women of nulligravida group had 19.08 ± 2.44 pg/ml, while that of the multiparous group was 6.71 ± 0.43 pg/ml, these were significantly lower than the 197.12 ± 3.78 pg/ml in the pregnant group ($P<0.05$). The estradiol level in the primary infertile group was 0.22 ± 0.05 pg/ml, that of the secondary infertile group was 0.41 ± 0.07 pg/ml and the RSA group was 11.94± 2.44 pg/ml. These values in the test groups were significantly lower at $P < 0.05$, when compared with the nulligravida group and the pregnant group of the control.

The serum vitamin E level obtained in the women within the nulligravida control group was 12.38 ± 0.50 µg/dl, in the multiparous women it was 8.95 ± 0.89 µg/dl and in the pregnant

women it was 7.05 ± 0.78 μg/dl. In the test group on the other hand, the value in the primary infertile women was 7.38 ± 1.07 μg/dl, the secondary infertile women was 10.49 ± 1.49 μg/dl and RSA women was 8.82 ± 0.50 μg/dl. The result showed that the value for the nulligravida was significantly higher ($P < 0.05$), when compared with all the other groups. The serum vitamin C level obtained in the control nulligravida women was 2.83 ± 0.21 mg/dl, in the multiparous women was 2.32 ± 0.38 mg/dl and in the pregnant women was 2.24 ± 0.33 mg/dl. Whereas, in the test groups, namely primary infertile women it was 1.72 ± 0.45 mg/dl, in the secondary infertile women it was 2.11 ± 0.63 mg/dl and in the RSA women it was 0.75 ± 0.21 mg/dl, which were significantly lower than the control groups at $P < 0.05$.

The serum zinc level obtained in the women within the control group that is, in the nulligravida was 0.61 ± 0.02 μg/dl, multiparous, 0.37 ± 0.04 μg/dl and pregnant, 0.47 ± 0.04 μg/dl. In the test group on the other hand, the value in the primary infertile women was 0.55 ± 0.05 μg/dl, the secondary infertile, 0.50 ± 0.07 μg/dl and RSA, 0.40 ± 0.02 μg/dl. The result showed that the value for the nulligravida was significantly higher ($P < 0.05$), when compared with all the other groups. The serum iron level obtained in the control nulligravida group was 0.86 ± 0.04 μg/dl, in the multiparous group was 0.54 ± 0.06 μg/dl and in the pregnant group was 0.99 ± 0.05 μg/dl, in the test group, namely primary infertile group it was 0.92 ± 0.07 μg/dl and in the RSA it was 0.92 ± 0.04 μg/dl. Whereas, in the secondary infertile group, the serum iron level obtained was 0.67 ± 0.10 μg/dl which was significantly lower than the pregnant control groups at $P < 0.05$. The levels of the serum antioxidant element, iron was significantly higher in the test groups when compared to the control multiparous group at $P < 0.05$.

This study has established the significant presence of anti-thyroglobulin and anti-thyroperoxidase as immune species marker in the serum of some euthyroid Nigerian women experiencing reproductive failure. Moreover, a correlation has also been established in the level of some reproductive female hormones namely progesterone, estradiol and antioxidants levels with some corresponding changes in immune species along T-cell clusters of differentiation in the test groups when compared to the control multiparous group.

CHAPTER ONE

1.0 INTRODUCTION AND BACKGROUND OF STUDY

Infertility in women is the inability of women of child bearing age (16-45 years) to get pregnant after about 12 months of regular unprotected sexual intercourse (Yayima et al., 2002). Infertility can be classified into three types namely; Primary infertility which describes a woman that has never been able to conceive (pregnancy) after a minimum of one year of unprotected intercourse without any known cause of infertility condition as an impediment i.e. unexplained infertility (Yayima et al., 2002; De-Carolis et al., 2004). Secondary infertility is used to describe a woman who has been previously pregnant and later desires to have a child but finds it extremely difficult to get pregnant again (Geva et al., 1997a).While recurrent spontaneous abortion (RSA) is used to describe a woman who has had two or more consecutive spontaneous abortion before 20 weeks of gestation with no intercalated pregnancy resulting in life birth (Bussen, and Steck, 1995; Geva et al., 1997a).

Immunological implication in pregnancy losses is gaining more attention in diagnosis for some infertility conditions in women. Recurrent pregnancy loss (i.e. recurrent spontaneous abortion) was the first clinical aspect of infertility to be associated with abnormal autoimmune function. Since then it has been suggested that autoimmune abnormalities may be closely related to reproductive failure (Gleicher et al., 1993; Beer, 2003; Bustos et al., 2006). Proposals for possible mechanisms of developing an immune response to auto-antigens have not been fully understood, but they are being investigated as influence of the expression of certain autoimmune diseases. It is known that the hypothalamus, thyroid and adrenal glands affect the homeostasis of the lymphoid system and respond to antigens by as yet uncharacterized mechanisms (Gleicher et al., 1993).

Reproductive immunology has been proposed as an important key to diagnosing pregnancy loss at least in the last decade. In this area of research (reproductive immunology), it has been suggested that as many as 80 percent of "unexplained" pregnancy loss may be attributed to immunological factors (Coulam, 1992; Coulam and Roussev, 2002; 2003a).

Previously, there was little a couple could do if they suffered from recurrent pregnancy losses. Miscarriages that could not be attributed to chromosomal defects, hormonal problems or abnormalities of the uterus were labeled "unexplained" and couples could get pregnant, only to suffer time and time again as they lose their potential babies in abortion. Human reproductive failure may be a consequence of aberrant expression of immunological factors during pregnancy. Studies in human subjects suggest that reproductive failure may be influenced by immunological factors or by gene encoding immunological factors and regulating mechanisms controlling immunological expression (Coulam and Roussev, 2002). The relative importance of immunological factors in human reproduction remains controversial. It has been further suggested that human leukocyte antigen (HLA), anti-sperm antibodies, integrins, the leukemia inhibitory factor (LIF), cytokines, anti-phospholipid antibodies, endometrial adhesion factors, mucins and uterine natural killer cells may contribute to reproductive failure (Coulam and Roussev, 2003a). In contrast, fewer data support the roles of anti-trophoblast antibodies, anti-endometrial antibodies, peripheral natural killers, anti-HLA antibodies, and suppressor cell in reproductive failure (Coulam and Roussev, 2003b).

Maternal and fetal immunology is also difficult to investigate in humans, thus other parameters would serve as valuable alternatives to investigate how the immune system affects reproductive outcome (Coulam., 1992; Coulam. et al., 2006). An essential feature of a successful pregnancy is very much dependent on the immunological defense system. Maternal immune recognition of the fetus is thought to play an important role in fetal acceptance (Glinoer et al., 1991; 1994). Inability to discriminate between "self" and "non-self" could lead to the synthesis of antibodies against specific components of the body of the individual called auto-antibody. Pratt et al. (1993a) suggested that the detection of thyroid auto-antibodies before conception carried an increased risk for pregnancy loss. This often led to very early rejection of the embryo, even before the pregnancy could be detected by the most sensitive regular medical tests (Roussev et al., 1995; 1996). Throughout the course of a pregnancy, a mother can develop autoimmune problem that can lead to reproductive failure. Several autoimmune abnormalities exist such as anti-phospholipid antibodies, anti-nuclear antibodies, anti-thyroid antibodies and CD19+5 cells (Sher et al., 2000).

The presence of thyroid antibodies, specifically thyroglobulin (Tg Ab) and thyroid peroxidase (TPO Ab) antibodies, may play a significant role in reproductive failure. Thyroid antibody levels may be associated with a high risk of miscarriage i.e. spontaneous abortion (Gleicher et al., 1989; Rushworth et al., 2000). These auto-antibodies can persist in patients who have suffered from hyperthyroidism or hypothyroidism and even after normalization of their thyroid functions by appropriate pharmacological treatment. Furthermore, anti-thyroid auto-antibodies can occur in asymptomatic euthyroid women who have never suffered from a thyroid disease (Gleicher et al., 1994; Rosen et al., 1995). Thyroid peroxidase is an enzyme responsible for the ionization of thyroid residues along with coupling of iodinated residues to form thyroid hormones. The biological role of thyroid stimulating hormone (TSH), as the primary trophic hormone of the thyroid, is for thyroid growth and development.

1.1 STATEMENTS OF PROBLEM

Despite extensive gynecological investigative tests about 10% of married couples do not know why they cannot conceive nor bear their own biological children. The psychological trauma of a woman who has been classified as having an unexplained reproductive failure but had to go through extensive medical treatment with the attendant over-stretched economic implications, has been a source of concern (Kwak-kim et al., 2005).

The fact that auto-anti-thyroperoxidase had been implicated as having suspected consequential roles in reproductive failure without defined mechanism makes the subject open to further research. The study of reproductive immunology would help to find a solution to the hopeless situation of women suffering from reproductive failure. Moreover, some of the daring but willing subjects had continued to have a closer look into the problem and subjecting themselves for direct assessment in order to establish that failure to conceive may be due to immunological problems (Rote et al., 1992; Rote, 1995; Coulam et al., 1996).

Previous research efforts were geared towards establishing a relationship between auto-anti-phospholipids with reproductive processes. The lack of established scientific report on

study. Thyroid auto-antibody measurement can be used as a marker of auto-immune thyroid disease-related activation of B- cells. It has been hypothesized that this may serve as peripheral marker for abnormal T- cell function that may be responsible for pregnancy loss (Stagnaro-Green et al., 1990; O'Connor and Davis, 1990). It is therefore important to have a set of tests to clarify the diagnosis of reproductive failure so that the appropriate therapy can be instituted.

1.2 AIMS AND OBJECTIVES

To identify and study Nigerian women whose infertility diagnosis showed no established cause of reproductive disorder and are euthyroid (i.e. no thyroid disorder).

To determine the immune – phenotypic auto-antibodies in the categories of women classified as primary infertile and spontaneous aborter.

To quantify the serum levels of blood glucose, cholesterol, triglycerides, albumin and some enzymes as biochemical indices, so as to evaluate the physiological state of the various categories of women in the study.

To determine the levels of auto anti-thyroglobulin and anti-thyroperoxidase in the peripheral blood samples in the test subjects compared to the control women.

To evaluate the species of clusters of differentiation on the lymphocytes, so as to characterize the immune reactions and the cell types involved in the reproductive failure of the women.

To determine the serum levels of some hormones such as testosterone, prolactin, progesterone and estradiol with the aim of establishing a correlation between the hormones as biochemical indices and reproductive ability.

To estimate the levels of some anti-oxidants (i.e. iron (Fe) and zinc (Zn), vitamin C and Vitamin E) as immuno-sensitive parameters.

1.3 SIGNIFICANCE OF STUDY.

From the foregoing statement of the problems, the significance of the study therefore will include the following:

- To establish the incidence of this immunological related reproductive problems in the Nigerian women population.

- To propose a possible correlation of the levels of auto-antibodies with some biochemical indices as relevant secondary markers.

- To document findings so as to contribute to the search for the treatment and reversal of the possible immunological implications in reproduction failures and in spontaneous abortions.

- Attempt to provide some explanations of the etiology of reproductive failure that has previously been referred to as "unexplained infertility" in the class of primary infertile Nigerian women.

1.4 OPERATIONAL DEFINITION OF TERMS

A

Antigen. The chemical that is introduced into a person that starts and completes an immune response. When one is vaccinated (measles), the measles virus is the antigen that results in the antibody that protects one from developing measles.

APA (Antiphospholipid Antibody). These are antibodies in the blood that attach to phospholipids that are structures on the surfaces of all cells. A positive APA test indicates that the woman's blood clots too fast cutting off blood flow to the baby. These antibodies can also cause the placenta to attach too weakly to the uterus. The usual treatment is baby aspirin and heparin (a blood thinner). Heparin is given as an injection. Both medications are started before pregnancy during the cycle of conception; these are the least controversial of all the treatments for infertility or recurrent pregnancy losses.

Autoimmunity. A condition characterized by a specific antibody (antiphospholipid antibody or antibodies to DNA) or cells (such as Natural Killer Cells) which react with molecules or constituents of the body's own tissue and cause disease such as Rheumatoid arthritis and lupus.

B

Blocking Antibodies. Blocking antibodies are the antibodies produced during pregnancy that protect the placenta from rejection and cause the placenta cells to grow.

C

CD (Cluster of differentiation). This is a designation used to catalogue surface molecules (antennae) on lymphocytes (white blood cells). It tells what the cell is and how activated it is. For example, Natural Killer cells have CD 56+. B-lymphocytes express CD 19+ and produce antibodies that sometimes can damage a pregnancy.

CD4 T Helper Cell. This is a T lymphocyte that helps activate CD3 cells in an immune response by producing cytokines that cause proliferation, differentiation and growth of other cells necessary for completing the immune reaction. These are usually higher than normal in women with infertility and recurrent pregnancy losses.

Cell mediated Immunity. This is immune destruction caused by killer cells, not antibodies. Natural Killer Cells can become activated in infertile women or women who lose pregnancies through miscarriage. These activated cells produce Tumor Necrosis Factor that kills placental cells.

Cytokine. These are hormones produced by white blood cells (lymphocytes) that communicate with other cells of the immune system, recruiting them to perform more aggressive roles in the fighting off of infection or in the rejection of something from the body such as a baby, a kidney or a bone marrow transplant.

Cytotrophoblast. This is a specialized cell of the placenta (also a trophoblast) whose function is to attach the baby to the mother's uterus. It provides the anchor. It grows deeply into the lining of the uterus (deciduas) and firmly attaches the placenta for the duration of pregnancy.

E

ELISA (enzyme-linked immunosorbent assay). This is a laboratory test method to analyze blood for the presence and the amount of antibodies in the woman to DNA, phospholipids, and other things. The test tubes are coated with the substance such as the phospholipids. Then the serum to be tested is added. If there are antibodies in the serum against the phospholipids, they attach and in the process of attachment a color (dye) is released that turns the fluid in the test tube a different color. The intensity of the color tells you how high the immunity is (how much antibody is present).

Etiology .Study of causes, the philosophical investigation of causes and origins; the set of factors that contributes to the occurrence of a disease.

Estradiol. The female hormone that is produced by cells that live in the follicle (nest) around the developing egg. There are three estrogens Estrone E-1, Estradiol E-2 and Estriol E-3.

Estrogen. These are a family of three hormones produced by the ovaries, which are responsible for the development of the female sex characteristics. Estrogens are responsible along with progesterone for preparing the uterine lining (endometrium) to thicken for pregnancy. Estrogens are also important for healthy bones, sexual drive, overall health and

well being. A small amount of estrogens are also produced in the male. These do not result in the development of the female sex characteristics for the male hormone testosterone counteracts these effects.

Euthyroid . Women with no any established thyroid disorder.

H

HLA (Human Leukocyte Antigen). These are molecules on the surface of human cells that determine a person's white blood cell type. Ten numbers make up this system. There are two A numbers, two B numbers, two C numbers, two DR numbers and two DQ numbers. One number at each locus (A, B, C, DR and DQ) comes from the mother the other one comes from the father. Couples with infertility and recurrent pregnancy losses share too many DR and DQ HLA factors. They are too compatible. This is referred to as the unlucky match for a successful pregnancy to occur.

I

IgG (Immunoglobulin G). This is a protein in the blood produced by B lymphocytes (CD19+). It is an antibody that is present primarily in the lymph system. It is produced by IgM (Immunoglobulin) that resides in the blood. One IgM produces five IgG antibody molecules in a balanced system. It is a Y shaped molecule consisting of two heavy and two light chains, each having variable and constant regions. The variable regions determine the fit with the antigen that initiated the response like a key- in -lock situation.

K

Killer Cells. These are a family of cells including Natural Killer Cells that destroy target cells (like placental cells and embryos) in a "kiss of death" type encounter.

L

Lymphocyte. This is a white blood cell that is in charge of starting antigen specific immunity responses. There are many types of lymphocytes in the immune family. These consist of T cells, B cells, granulocytes. basophiles, mast cells, eosiniophils, Natural Killer Cells, etc. Each cell type has a CD designation.

P

Path physiology. Changes caused by disease, the disturbance of function that a disease causes in an organ, as distinct from any changes in structure that might be caused.

Post partum. Depression following birth, a psychiatric disorder consisting of severe depressions that can affect women soon after giving birth to a baby.

Progesterone. A female hormone secreted by the ovary after ovulation during the second half of the menstrual cycle (luteal phase). The cells producing the hormone are called luteal cells. The body producing the progesterone in the ovary is called the corpus luteum. Adequate progesterone production is essential for the success of all pregnancies.

R

Reproductive Immunophenotype. This check for the presence of Natural Killer Cells. In most cases, Natural Killer Cells are good because they reduce the likelihood of developing cancer. These tests measure the following CD (Cell Designation) levels: high numbers in this category interfere with the reproductive hormones necessary for pregnancy.

S

Syncytiotrophoblast. This is a specialized cell of the placenta (also a trophoblast), which acts like a dialysis membrane between the mother's blood and the baby's blood, feeding the baby as well as removing waste products from the baby's blood.

T

Testosterone. Do the testicles produce primarily a male hormone, which is responsible for the development and the release of sperm, male physical characteristics and sexual drive. Small amounts of testosterone are also produced in women by the ovaries and the adrenal glands.

ABREVIATION

Clusters of differentiation (CD)

Major histocompatibility complex (MHC)

Antithyroid antibodies (ATA)

Recurrent spontaneous abortion (RSA)

Thyroglobulin (Tg)

Antithyroglobulin auto antibodies (TgAb)

Thyroid Microsomal (thyroid peroxidase) (TPO)

Thyroid Peroxidase auto antibodies (TPOAb)

Enzyme linked immunoasorbent assay (ELISA)

Thyroid stimulating Hormone (TSH)

Antinuclear Antibody (ATA)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE IMMUNE SYSTEM

The immune system defends the body from attack by invaders recognized as foreign. This is an extraordinarily complex system that relies on an elaborate and dynamic communication network that exists among the many different kinds of immune system cells that patrol the body (Amino et al., 1976). At the heart of the system is the ability to recognise and respond to substances called antigens whether they are infectious agents or, in some cases, part of the body (self antigens). The immune system is a remarkable defence mechanism found in its most advanced form in higher vertebrates. It provides the means to make rapid, highly specific and very protective response against the myriad of potentially pathogenic antigens (microorganisms) that inhabit the world in which we live (Ogasawara et al., 1999). The immune system is one of the most intricate and complex systems in the body, which functions as first line of defence against disease. It works by identifying a macro-molecule as either self or foreign. The immune response to a foreign protein is to neutralise or destroy it as an antigen (Abramson and Stagnaro-Green, 2001).

Immune responses are initiated by the encounter of an individual with a foreign antigenic substance, generally an infectious microorganism (Ogasawara et al., 1999). Therefore a logical place to look for immunological marker is within the complex system of cells that make up the immune system. An antigen is the recognition protein marker on the surface of a cell that identifies the cell as "self" or "non self". Antigens stimulate the production of antibodies, which are complex compounds, made by the white blood cells (WBCs) that combine with specific antigens to destroy or control foreign invasion. As bacteria enter the body, white blood cells produce antibodies to provide protection against the antigen (Coulam, 1999).

2.1.1 FEATURES OF THE IMMUNE SYSTEM

Humoral and cellular immune responses are interconnected in the sense that almost all B-cell responses require the help of T-cell. The T -cell also helps in the response system to

determine the presence of foreign antigens (Roberts et al., 1996). The humoral immunity, is the antibody response in the form of secreted immunoglobulins (Table 1), carried through the body via the blood circulation and tissue fluids from its site of production to where the antigen are located (Roberts et al., 1996). Both B and T -lymphocytes show the hallmark of specificity as they present with specific receptors for the antigens. The B-cells is the first line of mature antibody cells produced after antigen stimulation. The antibody response to most foreign antigens is dependent on the help of T -cells that can respond to determinants the carried as the antigenic molecules (Geva et al., 1994). Although he receptor of T-cell was very poorly understood, the T-cells cannot recognise foreign antigens and bind them directly in the way that B -cells can. Nevertheless T-cells are very specific as shown by the ability of delayed hypersensitivity responses that is able to discriminate between small changes in the amino acid sequence of synthetic polypeptides (Ruiz et al., 1996; Coulam et al., 2006).

2.1.2 CELLULAR AND HUMORAL IMMUNITY COORDINATION

The serum immunoglobulins represent the sum of individual collective B-cell responses to the total antigenic stimuli of the environment, such that different antibodies are directed towards different antigens (Roberts et al., 1996; Jablonowska et al., 2002). The B -cells express specific immunoglobulins, on their surface. When these interact with the homologous antigen, the B-cells are triggered to differentiate into plasma cells. This comprises the humoral component of the immune system (with such properties shown on table 2a and 2b). The immunoglobulins produced by the B -cells attack foreign antigens by marking them for destruction, (Ruiz et al., 1996; Ng et al., 2002). The T-cell is the second major class of immune cells, which matures in the thymus. The T-cells have two major roles of immune defense. Regulatory T-cells are essential for orchestrating the response of an elaborate immune system. The helper T-cells with different clusters of differentiation (CD) alert B -cells on the making of antibodies; they can also activate other T-cells and immune system scavenger cells called macrophages (Ribbing et al., 1988; Ntrivalas et al., 2001; 2005). T-cells are critical to immunity, in that they help to destroy infected cells and coordinate the overall immune response. The T-cell has on its surface the T-cell receptor. This receptor interacts with molecules called major histocompatibility complex (MHC),

which help T-cells recognize antigen fragments displaced on the surface of antigen-presenting cells (Ribbing et al., 1988; Farrell et al., 2001).

2.1.3 FUNCTION OF IMMUNOGLOBULINS

B -lymphocytes initiate the formation of immunoglobulin, while T -lymphocyte is instrumental in cellular immunity (for example manifestation of delayed skin reaction in homograft rejection). T-cell may also assist B-cells in the formation of immunoglobulin. T-cells and B-cells are both involved in antigen recognition. The subsequent immunoglobulin direct reaction with a foreign substance hastens its removal from the body (Birkenfeld et al., 1994). The main immunoglobulin class on the surface of the peripheral blood B -cells is IgM, see Table 1. This is the membrane bound immunoglobulin carried by B-cells that are stained with immunofluorescent anti-immunoglobulin in the serum.

Table 1: **Immunoglobulins and their major functions**

Immunoglobulin	Major functions
IgG	Main antibody in the secondary response, opsonizes bacteria, making them easier to phagocytocytose bacteria. It crosses the placenta.
IgA	Secretory IgA prevents attachment of bacteria and viruses to mucous membrane, it does not fix complement.
IgM	Produced in the primary response to an antigen; fixes complement and does not cross the placenta.
IgD	Uncertain, found on the surface of many B-cells as well as in serum.
IgE	Mediates immediate hypersensitivity reactions by causing release of pharmacological mediators from mast cells and basophils upon exposure to antigen (allergens). Defends against worm infection by causing release of enzymes from eosinophils. does not complement main host defense against infections.

Adapted from: Leon et al., (1994

Table 2a: PROPERTIES OF HUMAN IMMUNOGLOBULINS

Property	IgG	IgA	IgM	IgD	IgE
Immunoglobulin in serum (%)	75	15	9	0.2	0.004
Serum concentration (mg/dl)	1000	200	120	3	0.05
Sedimentation coefficient	7S	7Sor11S	19S	7S	8S
Molecular weight (x1000)	150	170/400	900	180	190
Subunits	Monomer	Dimer	Pentamer	Monomer	Monomer
Chain symbols	γ	α	μ	δ	∈
Complement fixation	+	-	+	-	-
Transplacental passage	+	-	-	?	-
Mediation of allergy	-	-	-	-	+
Opsonisation (secretion)	+	+	-	-	-
Antigen receptor on B- cell	-	-	+	?	-
Polymeric form	-	+	+	-	-
Half life (days)	23	5.5	5.1	2.8	2.8

Adapted from Speroff et al., (1994). (key: + is present, - is absent)

Table 2b: PROPERTIES OF HUMAN IgG SUBCLASS

Property	IgG_1	IgG_2	IgG_3	IgG_4
Disulfide linkage	2	4	5-15	2
Molecular wt (kd)	146	146	165	146
% Total serum IgG	34-87	0.5-56	0.5-12	7-12
Ave. adult free serum (mg/ml)	59±2.6	3±2.5	0.06±0.55	0.9±0.25
Macrophage binding FC	+	-	+	+
Placental transfer	+	++	+	++
Classical complement	+++	++	++++	+
Alternative complement	+	+	+	+++

Adapted from Speroff et al., (1994).

2.2.0 REPRODUCTIVE IMMUNOLOGY AND AUTOIMMUNITY

The word "auto" is the Greek word for self. The immune system is a complicated network of cells and cell components that normally work to defend the body and eliminate infections caused by bacteria, viruses and other invading microbes (Pratt et al., 1998). When a person has an autoimmune disease, it means the immune system had mistaken self- cells as target for attack in tissues and organs of the person's own body. This leads to collection of immune system cells and molecules at the target site, largely resulting in inflammation (Ju et al., 2000).

Autoimmunity occurs when the immune system mistakes self-tissue for non-self and mounts an attack to destroy it, resulting in an autoimmune disorder. Autoimmune antibodies are those antibodies that either interfere with the normal function of tissues or initiate the destruction of the tissue. There are various forms of auto-antibodies, among such are those associated with reproductive failure which include; anti-thyroid, anti-nuclear, anti-phospholipid and anti-thyroglobulin and many others (Coulam, 1992; Coulam et al., 2006).

2.2.1 WHO IS AFFECTED BY AUTO IMMUNE DISEASE?

Autoimmune disease affects millions of blacks in America. Most autoimmune diseases strike women more often than men; in particular they affect women of working age and during their childbearing years (Finberg et al., 1992; Eckler et al., 1993).

Many of the autoimmune diseases are rare, some autoimmune diseases occur more frequently in certain minority populations. For example, lupus is more common in Black-American and in Hispanic women than in Caucasian women of European ancestry (Coulam, 1991). Rheumatoid arthritis and scleroderma affect a higher percentage of residents in some native American countries than in the general U.S population (Coulam, 1991). Thus the socio-economic, and health impact from autoimmune disease is far reaching and extends not only to family but also to employers, co-workers and friends (Harris et al., 1994).

2.2.2 EXAMPLES OF AUTO IMMUNE DISEASE

No autoimmune disease has ever been shown to be contagious or "catching". Autoimmune disease highlighted on Table 3, does not spread to other people like infections. They are not related to AIDS, nor are they a type of cancer (Fukui et al., 1999). The genes people inherit contribute to their susceptibility for developing an autoimmune disease. Certain diseases such as psoriasis can occur among several members of the same family (Fukui et al., 1999). This suggests that a specific gene or set of genes predisposes a family member to psoriasis. In addition, individual family members with autoimmune disease may inherit and share a set of abnormal genes, although they may develop different autoimmune diseases. For example, one first cousin may have lupus, another may have dermatomyositis, and one of their mothers may have rheumatoid arthritis (El-Roeiy et al., 1987; El-Roeiy and Gleicher, 1988; Fukui et al., 1999).

Table: 3 **EXAMPLES OF AUTO - IMMUNE DISEASES**

NERVOUS SYSTEM

Multiple sclerosis

Myasthenia gravis

Autoimmune neuropathies

GASTRO INTESTINAL SYSTEM

(Including the musculoskeletal system).

Ulcerative colitis

Primary biliary cirrhosis.

Autoimmune hepatitis.

ENDOCRINE ORGANS.

Grave's disease.

Hashimoto's-thyroiditis

Autoimmune nephritis and orchitis

Autoimmune disease of the adrenal gland.

-BLOOD

–Autoimmune hemolytic anemia

– Pernicious anemia

– Autoimmune. Guillaine - Baire

- MULTIPLE ORGANS

-Rheumatoid arthritis

-Systemic lupus erythematosus.

--SKIN -Scleroderma.

Adapted from Geva et al.(1997a)

2.2.3 AUTOIMMUNE FACTORS

There are four different autoimmune problems that can cause recurrent spontaneous abortion (RSA) (Coulam and Roussev, 2003a; 2003b). A woman may have one or more of these underlying problems; anti-phospholipid antibodies, anti-thyroid antibodies, anti-nuclear antibodies, and lupus like coagulant. Thirty percent of women with "unexplained" RSA will test positive for an autoimmune problem (Roussev et al., 1995; 1996; Geva et al., 1997a).

2.2.3.1 ANTI-PHOSPHOLIPID-ANTIBODIES.

In pregnancy, phospholipids act like a sort of glue that holds the dividing cells together, and are necessary for growth of the placenta into the walls of the uterus. Phospholipids also filter nourishment from the mother's blood to the baby, and in turn, filter the baby's waste back through the placenta (Coulam and Stern, 1992; Rote, 1995).

If a woman tests positive to any one variety of anti-phospholipid antibodies (APA), it indicates the presence of an underlying process that can cause recurrent pregnancy loss. The antibodies themselves do not cause miscarriage, but their presence indicate that an abnormal autoimmune process will likely interrupt the ability of the phospholipid to do their jobs, thus putting the woman at risk of miscarriage of second trimester loss, intrauterine growth retardation (IUGR) and pre-eclampsia (Coulam, 1992;Roubey and Hoffman, 1997).

Testing for anti-cardiolipins (cardiolipins are a kind of phospholipid) is a standard procedure in some infertility clinics, however this test alone cannot identify the presence of all underlying autoimmune processes that cause RSA. A series of tests for antibodies to six additional phospholipids is recommended to determine the presence of APA. Testing positive for one or more kinds of anti-phospholipid antibodies indicates that the woman has immune response that can cause RSA (Harris et al., 1994). The markers that test for each of seven phospholipids include IgG and IgA. These are circulating immunoglobulins. In some patients, various markers can identify elevation of immunoglobulins to unknown protein and signal, which can trigger RSA by some yet unidentified process which exists (Coulam 1992; Denis et al., 1997). The life birth rate of a patient with untreated APA ranges from

11% to 20%. Individuals with recurrent pregnancy loss and /or implantation failure, venous or arterial thrombosis, thrombocytopenia and elevated or a circulating lupus- like anti-coagulant, are among those at risk for development of APA. Also at risk may be a woman experiencing infertility associated with endometriosis, premature ovarian failures, multiple failures of in vitro fertilization and unexplained infertility. With treatment, the live birth rate for women with APA increases to 70-80 percent (Coulam, et al., 1994; Coulam and Beaman, 1995; Coulam, 1999).

2.2.3.2 ANTI-NUCLEAR ANTIBODIES

Anti-nuclear antibodies (ANA) react against normal components of the cell nucleus. They can be present in a number of immunologic diseases, including: systemic lupus erythematosus (SLE) or lupus, progressive systemic sclerosis, Sjogren's syndrome, scleroderma polymyositis, dermatomyositis and in persons taking hydralazine and procainamide or isoniazid (Coulam et al., 1995; Kwak-kim et al., 2005). In addition, ANA is present in some normal individuals or those who have collagen vascular diseases. The presence of ANA indicates that there may be an underlying autoimmune process that affects the development of the placenta and can lead to early pregnancy loss (Coulam, 1992).

2.2.3.3 ALLO-IMMUNE FACTORS

There are two possible reasons why women with allo-immune problems, lose their pregnancies in miscarriage; either her immune system does not recognise the pregnancy, or she develops an abnormal immunologic response to the pregnancy (Coulam, 1986).

Successful pregnancy has been associated with the presence of circulatory "blocking antibodies". These are antibodies that are formed by a woman's immune system when she is pregnant, and they "mask" or disguise the pregnancy so it is not recognized as "foreign" (Ribbing et al., 1988). Pregnancy that ends with RSA has been associated with the absence of blocking antibodies. An antigen known as R80K has been identified on the surface of syncytiotrophoblasts. It is a kind of protein marker to which the blocking antibodies respond during a successful pregnancy at the cell's outer layer covering of the chorionis villi of the placenta. These cells are in contact with maternal blood (Coulam, 1986). The antibodies to this antigen react in a specific way to the antigens from the father's genetic

material in the developing embryo; and thus create the protective. blocking antibodies (Geva et al., 1994).

2.2.3.4 NATURAL KILLER CELLS

These are white blood cells (WBC) that belong to the group of cells that kill anything perceived as foreign. These kill abnormal invaders including virally infected cells. Some types of natural killer cells (NK) produce a substance called tumor necrosis factor (TNF). This might be described as the body's version of chemotherapy, and is toxic to a developing fetus. Patients who have high levels of these cells are at risk of implantation failure and miscarriages (Kaider et al., 1996; 1999a).

The proportion of NK cells is determined by a reproductive immuno-phenotype [RIP] test, which looks for cells that have the CD56+ marker. An NK [CD56+] cell range above 12 percent is abnormal, patients with high NK cell activity will respond very well to intravenous immunoglobulin G (ivig) therapy (Ntrivalas et al., 2001; 2006). In fact, the live birth rate with preconception treatment is more than 90 percent, compared to 20 percent without treatment (Kaider et al., 1996; Kwak et al., 1996; Kwak-Kim et al., 2003).

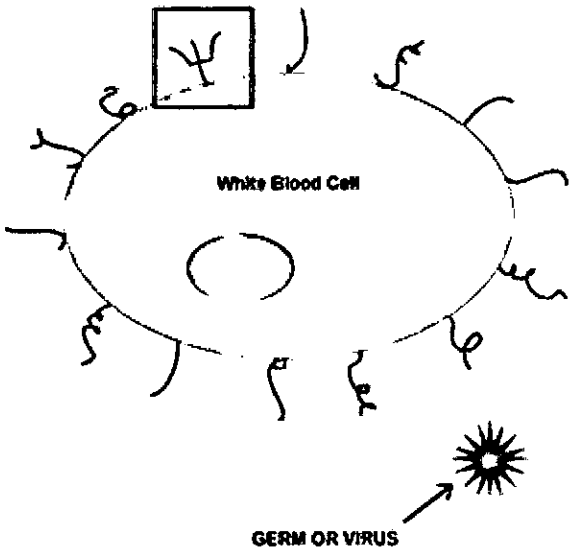
2.2.3.5 EMBRYO TOXICITY

Cells produce proteins called cytokines. Different cytokines do different things; some, stimulate growth of cells while some inhibit growth. The pro-inflammatory cytokines stimulate inflammatory response, while others inhibit inflammatory response of cells. The embryo toxicity assay (ETA) is used to detect cytokines that kill embryos (Kaider et al., 1999b). Embryo toxic factors have been identified in as many as 60 percent of women with recurrent (unexplained) miscarriage, and also reported among women with endometriosis associated infertility (Birdsall et al., 1996).

2.2.4. LEUKOCYTES AND IMMUNO- REPRODUCTIVITY

Granulocytes otherwise called polymorphonuclear leukocytes form the numerous white blood cells (see figure 1) of about 4000-11000 cells per microlitre of blood are basically

grouped into neutrophils (neutrophilic granules), eosinophils, (granules that stain with acidic dyes) and basophils, (Lea et al., 1999). These cytoplasmic granules (granulocytes) are the primary body defence mechanism, which contain biologically active substances mediating inflammatory and allergic reactions, such as histamine and other inflammatory mediators. These are activated by a histamine releasing factor secreted by T-lymphocytes and are essential for immediate hypersensitivity reactions, (Concato et al., 2000).



HLA Antigens: serve as antennae to recognize foreign germs or viruses entering the body. They Communicate this information to the white blood cells to initiate an immune response.

Adapted from: Beer et al., (1996)

Figure 1 : HLA antigens on white blood cells ensure immunity

The neutrophils detect, ingest and kill bacteria, thus acting as the first line of body defence mechanism against antigens, by adhesion molecule (integrin family), as they are attracted to the neutrophils (Bhat et al., 1995). The bacterial product interacting with the plasma factors produces agents such as leukotriene which is the chemotactic agent that attracts the neutrophils. This usually initiates the chemotactic phenomenon, as a component of the complement system. The stimulatory effector of C5 in the chemotactic activity is enhanced by immunoglobulin G, whereas eosinophils attack parasites that are too large to be engulfed by phagocytosis. Leukotriene C4 when produced is involved in allergic reaction.

2.2.4.1. CYTOKINES

Cytokines are hormone-like chemical messengers secreted by cells such as endothelial and lymphocyte cells, named initially after their action on B-cell differentiation or B-cell stimulating factors (Lin et al., 1993; Clark and Croitoru, 2001). Cytokines are regulatory glycoproteins that can affect virtually every cell type in the body and have regulatory effects on hematopoietic, endocrine, nervous and immune systems. Some cytokines are involved in cell differentiation, some stimulate growth of cells and some inhibit growth. The pro-inflammatory cytokines stimulate inflammatory response (Kwak et al., 1992a; Clifford et al., 1999).

2.2.4.2 RECOGNITION OF SELF ANTIGEN

The recognition of self antigen mechanism, explains why T- and B- cells do not form antibodies against and destroy self cells and organs. The cell in which they develop form suppressor cells which show inhibition mechanism, called the suppressor T- cells. This is the same reason why the mother does not reject her foetus being a foreign graft, even though they are genetically distinct individuals (Mosman and Coffman, 1989; Berner et al., 2000). Effectively, this foetus is like a tolerated transplant as against the expected rejection reaction characteristic of foreign tissue transplant. It appears that one reason that fetal graft is not rejected is that the placental trophoblast which separates maternal and fetal tissues does not express the polymorphic class I and II MHC genes. Instead, HLA – G (Human leukocyte Antigen- Gene) a non-polymorphic gene is expressed. To a large extent antibodies against the fetal proteins do not develop. Moreover, there is a decrease in maternal antibody production during pregnancy (Dokras et al., 1993).

2.3.0 PREGNANCY AND MATERNAL ALLOIMMUNITY.

A successful pregnancy requires a state of maternal immune tolerance to accommodate antigens expressed by the product of conception, the conceptus. Implantation of the conceptus is a process that is not easily achieved, 78% of fertilized oocytes do not proceed to birth and 62% of conceptions are lost before the 12^{th} week of pregnancy. The conceptus is a semi-allogeneic organism that contains self (maternal) and non-self (paternal) DNA, yet

it escapes immunological actions that normally would quickly respond in the presence of foreign material (Billingtoon, 1989; Horimoto et al., 1992).

Scientists have hypothesized that prolonged maternal exposure to the fetal tissues may result in acquired immunological tolerance and / or that the immune response to the paternal allogeneic material, sperm, is actively suppressed (Beer et al., 1996). Failure of this mechanism to accommodate and protect the fetus can result in pregnancy loss. In addition, during pregnancy, the mother may develop an autoimmune response that will hinder her ability to carry the pregnancy to term. The production of auto-antibodies reactive to self-antigens may interfere with normal fetal growth and development (Billingham and Head, 1981).

Allo-immunity is an immune reaction against non-self material. The usual mechanism for immunological rejection of any allograft involves the processing and presentation of foreign antigens by macrophages, the proliferation of effector T -cells, and the production of antibodies and cytotoxic T-lymphocytes that cause the destruction of the foreign graft, (Abramson and Stagnaro-Green,2001).

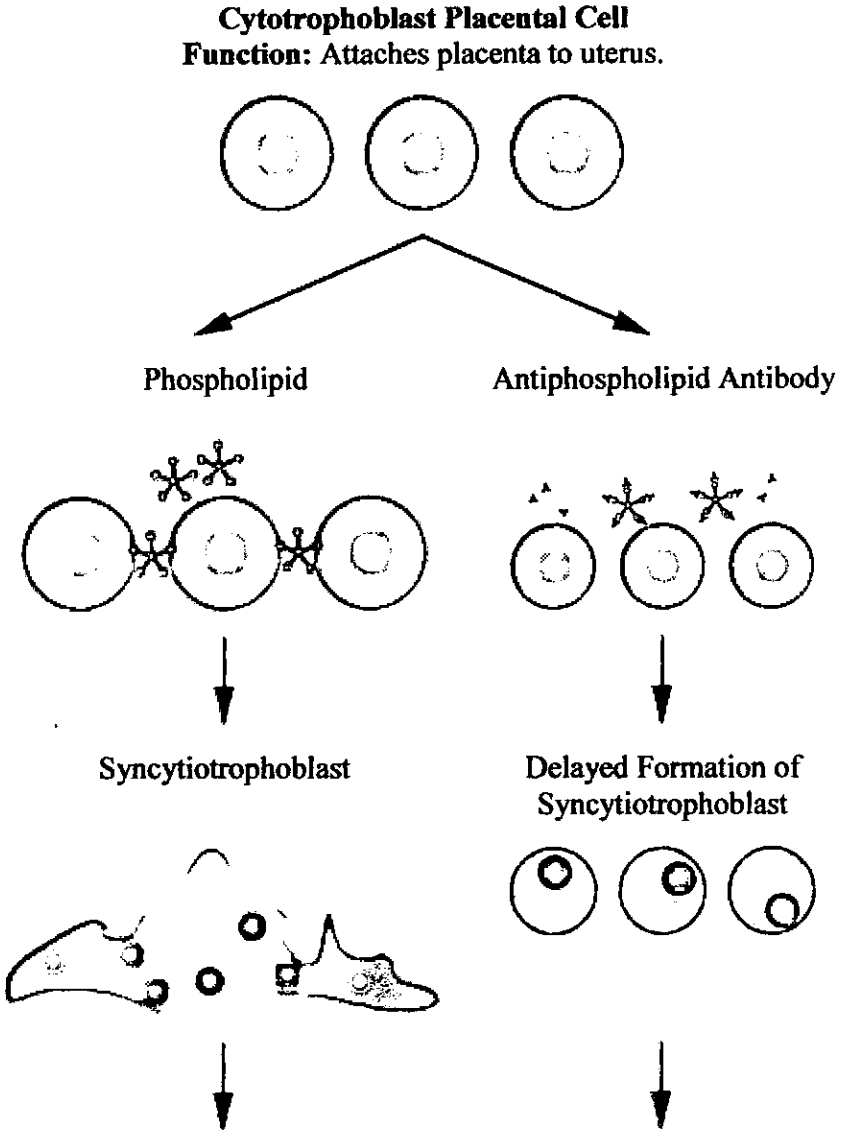
During pregnancy however, even though the fetus is a semi-allograft, it should be the target of maternal immunologic attack. It has been widely hypothesized that the semi-allogeneic conceptus, formed by the fertilization of an allogeneic sperm and an egg, may regulate its expression of HLA that eventually leads to the development of maternal tolerance. In the 1950s British medical researcher and scientist, Sir Peter Medawar, postulated that the foetus does not trigger immune responses because the uterus is an immunologically privileged site (Bodensky et al., 1984). The privileged site, according to Medawar was isolated behind blood tissue-barriers and lacked lymphatic drainage. It later became clear that foreign tissues or allograft placed in the privileged site or uterus could eventually evoke an immune response (Branch. 1990). Understanding the mechanism that allows implantation and growth of the conceptus into fetus is very limited. A successful pregnancy requires a state of maternal immune tolerance to accommodate the conceptus (Blumenfield et al., 1993).

2.3.1 HISTOCOMPATIBILITY.

The immune system has mechanisms capable of identifying and destroying "non-self" or foreign cells. Key players in these recognition mechanisms are human leukocyte antigens (HLA) and the human major histocompatibility complex (MHC). HLA are membrane-bound glycoproteins encoded by the human major histocompatibility complex located in chromosome 6 (Hutter and Dohr, 1998). All cells of the body have HLA on their surface. When the mother becomes pregnant she carries a fetus that expresses one HLA haplotype of paternal origin. Although the paternal HLA class I and II molecules expressed by the fetus are alloantigens against the mother's immune system, the fetus does not provoke an immune response during pregnancy (Kwak et al., 1995a; 1995b ; Thum et al., 2004; 2005). T–cells serve the purpose of recognizing class 1 HLA on the surface of antigen presenting cells, as expressed in figure 1, if the HLA presented to the T-cell is not consistent with "self HLA, the T-cell begins the process of destroying this foreign entry (Beer et al., 1981; Shimada et al., 2004).

2.3.2. TROPHOBLAST

The trophoblast is an epithelial layer of fetal origin present in placenta, separating fetal and maternal blood and lymphatic system. This fetal cell type is the only one exposed to the maternal uterine deciduas and maternal tissue surrounding the embryo and blood. Owing to its location. it was suggested that trophoblasts are thought to shield the fetus from rejection by serving as a barrier to maternal effector cells (Beer et al., 1998). When the trophoblast fails to express either MHC class I or class II molecules, the maternal T -cells bearing the B and T-cell receptor mount a classic cytotoxic attack against the foetal paternal allo-antigens as expressed in figure 2 (Beer et al., 1998).



Adapted from: (Beer et al., 1981)

Figure 2 : Syncytiotrophoblast function ensures placenta implantation

2.3.3. THE ROLE OF HLA -G.

The role of the highly polymorphic classical class I molecules HLA, A, B and C, are expressed by almost all somatic cells, and is stimulated by the induction of a specific immune response to the antigen. The non classical HLA class I molecule, HLA – G is thought to be involved in the induction of immune tolerance by acting as a ligand for inhibitory receptors present on natural killer cells and macrophages (Gleicher et al., 1987, Clark et al., 1991). HLA - G is characterized by a unique expression mainly in the human placenta, specifically on the extravillious cytotrophoblast (as expressed in figure 2, the cell layer that arises from the trophoblast).This is important in preventing fetal rejection which indicates that HLA - G expressed on the placenta is likely to prevent viral antigens with the aide of maternal suppressor CD8 + T–cells. However, HLA – G is proposed to interact with human killer immunoglobulin – like receptor that inhibits the lysis by the natural killer cell on trophoblast (Gleicher et al., 1992; Raghupathy et al., 1999).

In addition it has been observed that at the site of implantation, only a few CD8 cells are present. Soluble HLA – G (sHLA-G) have the ability to induce apoptosis of activated CD8+ T cells (Rezaei and Dabbagh, 2002; Michou et al., 2003). Therefore sHLA G molecules synthesized by placental cell lineages located at the materno-fetal interfaces might contribute to the elimination of cytotoxic T-cells that can potentially terminate pregnancy. The sHLA-G antigens, similar to membrane bound HLA-G molecules have been seen to mediate inhibitory effects on cytotoxic activities by binding to CD8+ lymphocytes and natural killer cells (Beer et al., 1981; Gleicher et al., 1992)

2.4.0 ANTI-THYROGLOBULIN AUTO-ANTIBODIES

Anti-thyroglobulin auto-antibody (TgAb) was the first auto-antibody implicated to be involved in patients with autoimmune thyroid disease (AITD). Beever et al. (1989) used enzyme-linked immunosorbent assay (ELISA) as an improved detection method over the agglutination technique to evaluate the auto-antibody (Kowalik et al., 1997). Anti-thyroglobulin auto-antibodies are primarily of the IgG class with poor activations of the complement cascade (Clark et al., 1991). The inability of TgAb to fix complement could

not be explained by the skewness of their subclass distribution: there seems to be neither defined biological action nor pathogenetic importance attached to TgAb (Kutteh et al., 1999).

Cowchock et al. (1992) opined that auto-antibodies may affect the maternal thyroid gland. The auto-antibodies crossing the placenta may affect the foetus, as seen in 40% of neonates born to mothers with elevated auto-antibody levels. At birth, a newborn ought to have normal globulin and thyroid stimulating hormone (TSH) to the fifth day of life (Kwak et al., 1992a; 1992b). The potential importance of the presence of elevated antigen passively transmitted from the mother remains to be clarified (Glinoer et al., 1991). However the gestational factors like anti-phospholipids may be affected, which causes desidual vascularity and placental insufficiency. And those thyroid auto-antibodies may be the result rather than the cause of recurrent pregnancy loss (Gilman-Sachs et al., 1989; Weetman and McGregor, 1994). However, an interaction exists between thyrotropin-like hormones produced by human placenta, human chorionic gonadotrophin and the thyroid auto-antibodies resulting in early pregnancy loss (Singh et al., 1995).

2.4.1. THYROID PHYSIOLOGY AND FUNCTIONS IN PREGNANCY

Pregnancy has profound effects on thyroid function and particularly on the causes of thyroid disease. Serum microsomal and thyroglobulin antibodies may cross the placenta but they are not cytotoxic to the fetal thyroid cells (Kutteh et al., 1997; Liewellyn -Jones, 1998).

Autoimmune thyroid disease is especially common in women and has a high prevalence during the childbearing period in women. It is important to understand precisely the expected changes in thyroid function that are associated with normal pregnancy. This condition may affect pre-existing thyroiditis, hypothyroidism and graves disease in these women (Liewellyn-Jones, 1984; Dmowski et al., 1995). The antigen for thyroid and microsomal antibodies, the peroxidase enzyme, may be detected by haemagglutination techniques. The relationship of autoimmune thyroid disease to pregnancy has been the object of consideration with the recognition of the post partum thyroid disease syndrome (Burrow, 1993).

Thyroglobulin (Tg) is a large glycoprotein antigen dimer with 330kd subunit that stimulates the production of TgAb. Tg is synthesized by thyroid peroxidase and secreted by the cell membrane as the iodinated tyrosine residue (Beever et al., 1989; Pratt et al., 1993a). The basal production of thyroid hormone results from pinocytosis of iodinated Tg and hydrolysis by microsomal enzymes. The post-translational modifications, with the degree of iodination of Tg are important determinants of the immunogenicity of the Tg molecule in the thyroid colloid (Pratt et al., 1993b).

Thyroid peroxidase (TPO) belongs to the family of glycoproteins with molecular weight of 100 – 700 KD present on the thyroid cell surface and is the important antigenic target in autoimmune thyroid disease. It is responsible for the iodination of tyrosine residues on thyroglobulin and the intermolecular coupling reaction of iodinated tyrosine leading to the formation of thyroxin (T_4) and triiodiothyronine (T_3) (Glinoer et al., 1990; 1991; Lejeune et al., 1993a; 1993c).

Esplin et al. (1998) suggested that the serum level of Tg is higher than the microsomal level. Also a relationship between high incidence of thyroid antibodies to elevated globulin was suggested, thereby establishing an association between autoimmunity and recurrent miscarriage in reproduction failures. Branch (1990), opined that auto-antibodies may affect the maternal thyroid gland. The auto-antibodies crossing the placenta may affect the fetus, as seen in 40% of neonates born to mothers with elevated auto-antibody levels (Gleicher et al., 1989; 1994; Ho et al., 1991; Lejeune et al., 1993b; 1993c; Hunt et al., 1997).

Thyroid peroxidase auto-antibodies (TPO) were first recognized by complement fixation and indirect immunofluorescent staining of fixed section of thyroid tissue and later by using heamaglutination assay technique (Kutteh et al., 1997). Regardless of the technique used, TPO Ab is present in the serum of almost all patients with thyroid disorder cases and in patients with thyroid autoimmune disease (Cayzer et al., 1978; Carreres and Mooney, 1992). Thyroid peroxidase antibodies in most serum samples react with both linear and conformational epitopes on enzyme antigen TPO, which is recognized by microsomal

antibodies. It seems likely that the TPO Ab arose as a consequence of thyroid damage with limited biological importance, but is an excellent marker of the underlying autoimmunity (Khoury et al., 1984; Gleicher and El-Roeiy, 1988).

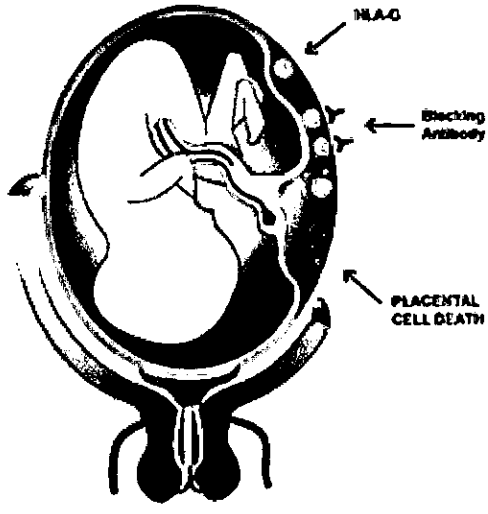
Women with anti-thyroid antibodies (ATA), face double risk of miscarriage as against women without them (Geva et al., 1997a; 1997b). Increased thyroglobulin and thyroid-microsomal (thyroid peroxidase) auto-antibody levels show relationship to an increased miscarriage rate. About 31 percent of women experiencing recurrent spontaneous abortion (RSA) are positive to one or both antibodies (Coulam, 1992). The chance of a loss in the first trimester pregnancy increases to 20 percent, and there is also an increased risk of post partum thyroid dysfunction (Gleicher et al., 1987; 1992).

2.4.2. INITIATION AND PROGRESSION OF PREGNANCY IN THE HUMAN

Pregnancy is initiated when a male sperm is introduced into the female genital organ and the sperm fertilizes a female ovum (egg). The fertilized ovum then travels down the fallopian tube and gets implanted in the linings of the uterus. The critical step of pregnancy initiation is the step when a single spermatozoon penetrates the zona pellucida of the ovum. The sperm's tail is stuck in the shell and drops off leaving the sperm head free in the egg. Numerous maturational events occur in the zygote, which freely floats for 3 days before implantation and the blastocyst normally occurs in 6 days following ovulation in the female. The blastocyst must be prepared to draw nutrients from the endometrium upon its arrival in the uterine cavity: hence endometrial preparation is essential for pregnancy to progress successfully. The embryonic development initiated through the process of fertilization is presented by the fusion of two haploid cells, each bearing 22 autosomes and 1 sex chromosome. This intermingling of the maternal and paternal chromosomes occurs at the metaphase of the meiotic division. (WHO, 1975).

2.4.3. GESTATIONAL PERIOD AND GROWTH.

The period of gestation (pregnancy) is about 38 weeks from conception (40 weeks from the end of the last menstrual period). The first few days of human development after fertilization are spent in the fallopian tube, with the zygote cleaving itself into the eight cell stages. The entire gestational period is commonly divided into 3 parts of trimesters each consisting of 3 calendar months, the first trimester is a critical period of development because of the great sensitivity of the fetus to teratogens, agents that can cause malformations. By the end of the first trimester, all of the major organ systems are developed. At 8th week of pregnancy the growing embryo is called fetus, see figure 3. The rest of the fetal period is concerned with the further growth and differentiation of the organs (Liewellyn-Jones, 1998).



Adapted from: Beer et al.(1981)

Figure 3: An embryo in pregnancy progression

2.5.0 SUGGESTED CAUSES OF PREGNANCY WASTAGE

From the broad outline of the various suggested causes of female infertility and pregnancy wastage, female factors may account for 40 to 70 percent of causes of infertility in the general population. Female causes may in particular be over-estimated, as less is known

about impairment of the male reproductive system, than that of the female. Many cases of infertility may also be due to a combination of factors. Furthermore, the relative contribution of these factors appears to differ among population groups. For example on Table 4, tubal abnormalities reportedly represent the chief cause of infertility in many developing countries, whereas, ovulation disorders, male factors and tubal obstruction are generally listed as the most common causes of infertility in developed countries (Liewellyn-Jones, 1998).

Table 4: Suggested causes of pregnancy wastage and female infertility

Pregnancy Wastage	Positive	strongly positive
Maternal infections (Other illnesses)	+	+++
Endocrine Thyroid dysfunction	+	
Uterine malfunctions	+	++
Uterine adhesions (Fibroids)	+	+++
Cervical incompetence	+	
Immunologic factors	+	
Female Infertility:		
Endometriosis	+	++++
Tubal obstruction or Defect	+	+++
Polycystic ovarian syndrome	+	++
Pituitary disease or dysfunction or failure	+	
Sexual malfunction	+	++
Psychological stress	+	++
Ovulation disorders	+	+++
Thyroid, adrenal disorders	+	
Severe nutritional disturbances	+	++
Significant weight loss	+	
Cervicitis, Abnormal cervica mucus	+	++
Exposure to chemical and physical agents	+	++
Immunologic factors	+	

Adapted from Liewellyn Jones, (1998)

2.5.1. FACTORS ADVERSELY AFFECTING CONCEPTION RATE

Suggested causes of female infertility listed in table 4, represents a broad summary of some of those factors that adversely affect conception rate, however, the causes of miscarriage are not clearly established. Abnormal development of the embryo or of the placental tissue, which links the embryo to the mother, or both, is found in about half the cases (Benson and Hartz, 2000). These abnormalities may be due to inherent faults in the germ cells (egg or sperm cells) or may be secondary to faulty implantation of the developing fertilized egg or to other characteristics of the maternal environment.

Severe vitamin deficiencies have been shown to play a role in miscarriages in experimental animals (Christiansen et al., 1992). Hormone deficiencies also have been found in women who are subject to recurrent miscarriage. Other factors include maternal abnormalities such as acute infectious diseases, systemic diseases such as nephritis (kidney disease), diabetes and severe trauma. Uterine malformations, including tumors, are responsible in some instances, and extreme anxiety and other psychological disturbances may contribute to the premature expulsion of the foetus (Benson and Hartz, 2000). The most common symptom of threatened miscarriage is vaginal bleeding, with or without intermittent pain. About one-fourth of all pregnant women bleed at some time during early pregnancy, however up to 50 percent of these women carry the fetus for the full term (Christiansen et al., 1992; Coulam, 1992).

2.5.2. EFFECT OF ANTI-THYROID ANTIBODIES IN PREGNANT AND NON-PREGNANT WOMEN.

Anti-thyroid antibodies are known to be found in an apparently healthy population and are observed more frequently in women during their reproductive years. The occurrence of organ specific thyroid antibodies parallel to non-organ-specific thyroid antibodies is not uncommon. Anti-thyroid antibodies (ATA) were found in up to 45% of women with systemic lupus erythetomatus. At least one third of women with induced autoimmune disease demonstrated non-organ specific antibodies such as anti-thyroid antibodies (Sher et al., 2000).

Stagnaro – Green et al. (1990), were the first to describe the association between anti-thyroid antibodies and pregnancy loss, independent of the presence of non-organ specific auto-antibodies. In that study they randomly screened a chosen obstetric population without prior history of increased pregnancy wastage for the presence of thyroid antibodies and found that these antibodies were connected significantly with an increased rate of miscarriage. Anti-microsomal antibodies in 19% of the patients with failed pregnancy were compared with 10% in the control group. Stagnaro -Green et al. (1992), were also the first to point out that anti-thyroid antibody may serve as peripheral marker for abnormal T-cell function that in turn may be responsible for pregnancy loss.

Glinoer et al. (1991), also reported a specific association between anti-thyroid auto-antibodies and spontaneous abortion. Pratt et al., (1993a) showed an increase in anti-cardiolipin antibodies in patients with recurrent pregnancy loss and anti-thyroid antibodies. Therefore they concluded that anti-thyroid auto-antibodies may serve as independent markers for the risk or repeated pregnancy loss (Sergent and Dokras, 1996: Scott, 1987).

2.5.3. FACTORS THAT REDUCE CHANCES OF SPONTANEOUS ABORTION

Treatment for threatened miscarriage usually consists of bed rest. Almost continuous bed rest throughout pregnancy is required in some cases of repeated miscarriage; vitamin and hormone therapy also may be given (Rothchid, 1983; Sher et al., 1998a: 1998b). Surgical correction of uterine abnormalities may be needed in certain of these cases (Michel et al., 1989; McIntyre et al., 1989; Coulam, 1991).

Diethylstilbestrol, or DES, synthetic estrogen, a sex hormone was once widely used by pregnant women to prevent miscarriages. It was first administered in large doses to many women in the United States; however, researchers discovered that when women used the drug before the 18th week of pregnancy, their female offspring were at risk of developing vaginal cancer; this therefore limited the use of the hormone. DES remains in use for various gynecological disorders and some forms of cancer in both women and men, (Llewellyn-Jones, 1998).

Autoimmune treatments such as, the use of heparin (a natural anticoagulant) and aspirin (prostaglandin inhibitor), have been developed to try to lower the risk of pregnancy loss and recurrent abortion (Rai et al., 1997). This further reinforced the suspicion of auto-antibody as playing key role in reproductive failure. The levels of auto-antibody are lowered by appropriate dosage of the drug. It has been observed that aspirin alone was not better than placebo or usual care, but heparin plus aspirin resulted in lower rates of pregnancy loss (Sher et al., 1994; 1998a; Bussen et al., 2000).

2.6.0. HORMONES AND IMMUNO-REPRODUCTIVITY

2.6.1 FEMALE HORMONE

Sex hormones are of two classes which are protein and steroid hormones. The steroid hormones have a molecular weight of about 300 daltons, and because of their size and lipo solubility, they can easily diffuse through target cells and bind to intracellular receptors (Muller et al., 1999; Kaider and Coulam, 2000). The protein hormones which weigh more than 5,000 daltons are not fat soluble and cannot penetrate the cells; they therefore require membrane receptors (Bischot and Islami, 2003). Some examples of female hormones are: estrogen, progesterone, prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Granner, 2000; Luborsky et al., 2002).

2.6.1.1. FEMALE PROTEIN HORMONES -THE GONADOTROPINS

These hormones are produced by different tissues (pituitary and placenta) and their main functions are gonadic regulations. The gonadotropins include follicle stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropin (HCG) (Bischot and Islami, 2003).

2.6.1.2. FOLLICLE STIMULATING HORMONE (FSH)

FSH is a dimeric glycoprotein with an alpha and beta protein subunits (Horsman et al 2000). It is produced by the pituitary gland and has a molecular weight of about 30.000 daltons (Bischot and Islami, 2003). The alpha subunit is bound non-covalently to a beta

subunit and together confers biological specificity (Horsman et al., 2000). There are two carbohydrate chains N-linked to asparagine residues on each FSH subunit (Baenziger and Green, 1988) and these comprise 28% of the hormone mass. The alpha subunit (89 amino acids) is common to all gonadotropins, while the beta subunit (188 amino acids) is specific for FSH. The main function of FSH is to promote and sustain the ovarian follicular growth in women (Bischot and Islami, 2003). There is marked variability in the oligosaccharide compositions in the branching pattern and overall change of the carbohydrate structures, which give rise to a large array of glycoforms of FSH (Baenziger and Green, 1988).

FSH stimulates the synthesis of its own receptor on the granulosa and sertoli cells and the LH receptor on granulosa cells. The evidence regarding endocrine control and physiological importance of these glycoforms of FSH has been reviewed (Luborsky et al., 2002). The particular acidity of the FSH glycoforms in blood varies considerably at different stages of the menstrual cycle most markedly at mid-cycle when there is an increase in the less acidic, less complex species (Williams and Stance, 1996). FSH is responsible for "the choke of the dominant follicle". FSH synthesis and secretion by the hypophysis is controlled by different regulators, such as; gonadotropin releasing hormone (GNRH), ovarian estrogens, and inhibin (both of gonadic origin) (Bischot and Islami, 2003).

2.6.1.3. LUTEINIZING HORMONE (LH)

LH is a glycoprotein with a molecular weight of approximately 30,000 daltons and produced by the hypothalamus (Bischot and Islami, 2003). The release of LH is controlled by the concentration of gonadotropin releasing hormone (GNRH) (Granner, 2000) and ovarian estrogens and progesterone (Bischot and Islami, 2003). LH binds to specific plasma membrane receptors and stimulates the production of progesterone by the corpus luteum cells (Williams and Stance, 1996). At puberty, the pulsatile release of GNRH stimulates LH and this causes a dramatic increase of ovarian hormone production (Granner, 2000). The principal functions of LH are: promoting androgen synthesis in the thecal cells of the ovaries, inducing ovulation (by stimulating the cascade of proteolytic enzymes leading to the rupture of the basement membrane of the follicle) and maintaining the corpus luteum during the menstrual cycle (Bischot and Islami, 2003). Gonadotropin releasing hormone analogues such as triptorelin, leuprolide and nafarelin can be used in controlling ovarian

hyper-stimulation to prevent LH peak and to provide better follicular growth and maturation (Hill et al., 1992). Ovarian hyper-stimulation may cause excessive luteinization even with low LH levels (Speroff et al., 1994).

2.6.1.4. HUMAN CHORIONIC GONADOTROPIN (HCG)

HCG is a glycoprotein with a molecular weight of about 43,000 daltons, produced by the syncytrophoblast (Granner, 2000; Bischot and Islami, 2003). It is a heterodimer composed of two different sub-units α and β. The specific α sub-unit contains 145 amino acids and can be distinguished from the β sub-unit of LH only by 30 amino acids in the C terminal part of the molecule (Bischot and Islami, 2003). The function of HCG is to maintain the corpus luteum of pregnancy and its progresterone secretion (Bischot and Islami, 2003). It increases in the blood and urine shortly after implantation; hence its detection is the basis of many pregnancy tests (Granner, 2000).

2.6.1.5. PROLACTIN (PRL)

PRL is secreted by the anterior pituitary into the general circulation. It is a non-glycosylated protein which contains a simple polypeptide chain of 198 amino acids (Bischot and Islami, 2003). Like oxytocin (OT), it is a peptide hormone with one very clearly established physiological and classical hormonal function in the promotion of lactation. PRL has been reported as having more than 300 functions across vertebrates, a substantial amount of which relate to reproduction (Bole- Feysol et al., 1998).

In the humans, low sexual desire is a common symptom of hyperprolactinaemia. Although hyperprolactinaemia is also commonly associated with ovarian dysfunction in women, impaired sexual desire can occur in hyperprolactinemia without obvious gonadal steroid deficiency. Studies of sexual arousal by PRL in response to erotic stimuli not involving direct erectile stimulation or orgasm have been few; however Rosen et al. (1995) reported some increase in male PRL, in contrast, to other studies in which masturbation or sexual activity was found to result in orgasm, with increase in PRL following the orgasm (Exton et al., 1999). The post-orgasmic rise in PRL acts as a feed-back control of sexual drive contributing to the post-orgasmic refractory period. The increase in PRL following orgasm

dopamine (DA) activity, and not a hormonal mechanism of functional significance (UNICEF/WHO, 1999).

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. The release and synthesis of prolactin is under neuroendocrinal control, primarily through prolactin realeasing factor and prolactin inhibiting factor. Women normally have slightly higher basal prolactin levels than men; apparently, there is an estrogen-related rise at puberty and a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation (Bole- Feysol et al., 1998).

Prolactin also suppresses gonadal function. During pregnancy, prolactin levels increase progressively to between 10 and 20 times normal values, declining to non- pregnant levels by 3-4 weeks post-partum. Breast-feeding mothers maintain high levels of prolactin and it may take several months for serum concentrations to return to non-pregnant levels. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High prolactin levels are commonly associated with galactorrhea and amenorrhea. Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanism (Bole- Feysol et al., 1998).

Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of prolactin is episodic and demonstrates diurnal variation. Mildly elevated prolactin concentrations should be evaluated taking these considerations into account. Prolactin concentrations may also be increased by drugs such as chlorpromazine and reserpine and may be lowered by bromocyptine and L-dopa (Bole-Feysol et al., 1998).

2.6.1.6. OXYTOCIN (OT)

It is produced in a specific cell type in the hypothalamus and transported by axoplasmic flow to nerve endings in the posterior pituitary where, upon appropriate stimulation the hormones are released into the circulation (Granner, 2000). It is generally accepted that oxytocin plays a key role during lactation, facilitating the milk ejection reflex. It may also play a role facilitating uterine contraction during parturition (Granner, 2000). For such purposes, OT is produced in the magnocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus, which project axons into the posterior pituitary and thence into the peripheral circulation. OT has been proposed as a key factor in affiliative behaviour (Insel, 1992). Centrally administered OT has also been used as an index of sexual arousal (Argiolas, 1999). Dopamine agonists may enhance sexual response by increasing central oxytocinergic transmission (Argiolas, 1999). Studies with OT antagonists indicate that OT facilitates lordosis, an effect apparently dependent on progesterone priming (Insel, 1992). This may be related to the effects of vagino-cervical stimulation (VCS) (Komisaruk and Samsone, 2003).

The fundamental role of OT in sexual behaviour is questioned by the effects of OT gene ablation studies. Carmichael et al. (1990), found that plasma OT increased around the time of orgasm in women and remain raised for at least 5min after orgasm. It was postulated that OT has a facilitatory role on egg transport by increasing smooth muscle contractility in the reproductive tracts (Carmichael et al., 1990). In a study, Blaicher et al. (1999) found in women an increase in OT one minute after orgasm but the levels were close to base line by 5min post orgasm. It is possible that this OT rise will affect the experience of orgasm by influencing uterine and other reproductive tract smooth musculature. Caldwell (2002) has proposed that OT is a satiety hormone that acts by decoupling the S-protein and hence reducing sexual arousability.

2.6.2.0 FEMALE STEROID HORMONE: ESTROGEN.

Three steroid hormones estrone (E1), estradiol (E2) and estriol (E3) are known collectively by their functions as estrogens (Speroff et al., 1994; Bischot and Islami, 2003). Estradiol is the most physiologically active estrogen of ovarian origin in non-pregnant women (Kuiper

and Gustafission, 1997). Its potency is 12 times that of estrone and 80 times that of estriol. In pregnant women, estrogens are also produced in the placenta (Leon et al., 1994). Estrogens are excreted in the urine as glucuronides or sulphates (Bischot and Islami, 2003).

2.6.2.1. ESTRADIOL (E2)

Estradiol (E2) is a C18 steroid homone with a phenolic ring. This steroid hormone has a molecular weight of 272.4gm/mol. It is the most potent natural estrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes (Ratcliffe et al., 1983). Estradiol (E2) is secreted into the blood stream where 98% of it circulates bound to sex hormone binding globulin (SHBG). To a lesser extent it is bound to other serum proteins such as albumin. Only a tiny fraction circulates as free hormone or in the conjugated form. Estrogenic activity is affected via estradiol-receptor complexes, which trigger the appropriate response at the nuclear level in the target sites. These sites include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin.

In non-pregnant women with normal menstrual cycles, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. The rising estradiol concentration is understood to exert a positive feedback influence at the level of the pituitary where it influences the secretion of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are essential for follicular maturation and ovulation respectively. Following ovulation, estradiol levels fall rapidly until the luteal cells become active resulting in a secondary gentle rise and plateau of estradiol in the luteal phase (Dow and Gallasher, 1989). During pregnancy, maternal serum estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy (Granner, 2000).

Serum estradiol measurements are a valuable index in evaluating a variety of menstrual dysfunctions such as delayed puberty in girls and primary and secondary amenorrhea and menopause. Estradiol levels have been reported to be increased in patients with feminizing syndromes, gynaecomastia and testicular tumors (Bischot and Islami, 2003; Granner, 2000).

In cases of infertility, serum estradiol measurements are useful for monitoring induction of ovulation following treatment with, for example, clomiphene citrate, LH- releasing hormone (LH-RH), or exogenous gonadotropins. During ovarian hyperstimulation for in vitro fertilization (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (HCG) administration and oocyte collection. Estradiol (E2) enzyme immuno assay (EIA) is designed for the measurement of total estradiol in human serum or plasma (Granner, 2000).

In women of reproductive age, E2 is essentially produced by the enzymatic conversion of androgen (androstenedione and testosterone) (Granner, 2000). The androgens are produced by the thecal cells under the influence of LH and their conversion to E2 occurs in the grannulosa cells of the follicle, through the enzyme aromatase (Bischot and Islami, 2003; Granner, 2000). Aromatase activity in the post menopausal women, with low level of E2 is provided by peripheral tissues (liver, fat and muscular tissue), by the coversion ie aromatization of androgens secreted by the adrenal glands. Luborsky et al. (2002) have strongly advocated that the sexual effects of testosterone in women result from the consequent increase in free estradiol. Also estradiol and progesterone can in addition have much more rapid effect, directly on the cell membrane (Duty and Vincent, 1980). The principal function of E2 in women is the mitotic effect on the uterine mucosa and on the breast with the feedback (positive and negative) on pituitary gonadotropins. It also has a role in bone mineralization (Bischot and Islami, 2003).

2.6.2.2. ESTRONE (E1)

In women of reproductive age, E1 is mainly produced from the enzymatic conversion of androstenedione which is secreted under the influence of LH by the thecal cells (Bischot and Islami, 2003) and it is synthesized in numerous tissues (Granner. 2000). The aromatase activity depends on FSH. In menopausal women E1 and its sulphate represent the main circulating estrogens (Bischot and Islami, 2003). The biological function of E1 is related to the regulatory effect that the conversion of E1 into E2 has on the degree of estrogenisation.

2.6.2.3. ESTRIOL (E3)

In women of reproductive age, the very low concentrations of E3 are produced by hepatic hydroxylation of E1 and E2 (Bischot and Islami, 2003). Approximately 90% of estriol excretion can be accounted for by dehydroepiandrosterone sulphate (DHAS) production by the fetal adrenal gland during pregnancy (Leon et al., 1994). The E3 concentration strongly increases during pregnancy and a low E3 and a positive stress test indicate a foetus in jeopardy (Leon et al., 1994).

2.6.3. PROGESTERONE (P4)

In non-pregnant women of reproductive age P4 is essentially of ovarian origin and is produced and secreted by corpus luteum (Granner, 2000). The participation of the adrenal cortex is negligible (Bischot and Islami, 2003). P4 is largely produced by the corpus luteum until about 10 weeks of gestation (Csapo et al., 1973). The amount of progesterone produced in pregnancy depends upon placental maternal cooperation. In the middle of the menstrual cycle, it is the LH peak which induces biochemical and phenotypical changes of granulosa cells. This process called luteinisation makes the granulosa cells capable of producing progesterone (Bischot and Islami, 2003). The biological role of P4 is to transform the uterine mucosa already stimulated by E2, into a secretory mucosa, on the receptor on which to receive a fertilized ovum (Bischoft and Islami, 2003). Progesterone inhibits uterine contractions which is useful for successful implantation (Ratcliffe et al., 1983).

Progesterone is a C21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via activities of dehydrogenase and isomerase, to progesterone. The principal production sites are the adrenals, ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys (Ratcliffe et al., 1983).

Progesterone exhibits a wide variety of end organ effects. The primary role of progesterone is exhibited by the reproductive organs. In males, progesterone is a necessary intermediate

for the production of corticosteriods and androgens. In females, progesterone remains relatively constant throughout the follicular phase of the menstrual cycle. The concentration then increases rapidly following ovulation and remains elevated for 4-6 days and decreases to the initial level 24 hours before the onset of menstration. In pregnancy, placental progesterone production rises steadily to levels of 10 to 20 times those of the luteal phase peak (Bischot and Islami, 2003). Measurements of levels of progesterone are thus performed to determine ovulation as well as to characterize luteal phase defects. Monitoring of progesterone therapy and the early stage pregnancy evaluations comprise the remaining uses of progesterone assays. The progesterone EIA is designed for the measurement of total progesterone in human serum or plasma.

2.6.4. TESTOSTERONE (T)

In women of reproductive age, testosterone is produced by the thecal cells which surround the follicle. The androgen (T) serves as a substrate for the synthesis of E2, but it is also detected in circulation even in very low concentrations (Bischot and Islami, 2003). Clinical studies of women with low sexual interest or arousability have been demonstrated to have low testosterone levels. Bischot and Islami, (2003) found a marginally lower free androgen index (FAI) in women complaining of life long absence of sexual drive than in controls. Although Carney et al., (1978) found some significant level of testosterone, other comparable studies failed to replicate this effect (Dow and Gallasher, 1989). Women vary in the extent to which their sexuality is influenced by testosterone. Testosterone and sexuality was most apparent in women whose sexuality was unproblematic (Bancroft and Carwood, 1996).

Testosterone (17β- hydroxyandrost- 4- ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17; this steroid hormone has a molecular weight of 288.4gm/mol (Liewellyn- Jones, 1998). Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the leydig cells of the testes: in females 50% of circulating testosterone is derived from peripheral conversion of androstenedione, 25% from the ovary and 25% from the adrenal glands. Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the

hypogonadal states (Chen et al., 1991; Lambert et al., 1998). In women, high levels of testosterone are generally found in hirsutism and virilization, polycystic ovarian tumors, adrenal tumors and adrenal hyperplasia. A mean, high level of testosterone is associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer. Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, testicular feminization, orchidectomy and cryptorchidism, enzymatic defects and some autoimmune diseases. The testosterone EIA is designed for the measurement of total testosterone in human serum (Chen et al., 1991). Testosterone may play a role in the sexuality of many women; its effects can easily be obscured by the co-existence of other psychological or affective factors. The biological role of testosterone in women is to favour follicular atresia, a follicle which has a diminished capacity of aromatization or that cannot aromatize, causing all androgens to become atretic (Bischot and Islami, 2003). Like all androgens, testosterone crosses the placental barrier. Therapy with testosterone during any period of pregnancy is absolutely contraindicated because of its virilizing effects on the female foetus which vary from a simple clitoromegaly, to fusion of the outer lips and atrophy of muller's duct (Mecacci et al, 2000).

2.7.0 VITAMINS AS ANTI-OXIDANTS

Vitamins are organic nutrients that are required in small quantities for many different biochemical functions. They generally cannot be synthesized by the body and are therefore supplied by the diet. Humans require either milligram or microgram quantities of each vitamin per day, in order to maintain health. The vitamins are classified into two main groups: water – soluble vitamins and fat – soluble vitamins.

Water – soluble vitamins. include ascorbic acid (vitamin C) and vitamin B complex, which comprises of thiamin, riboflavin, niacin, pantothenic acid, vitamin B6, biotin, vitamin B12 and folic acid. Water – soluble vitamins are absorbed into the hepatic portal vein and any surplus of most of them is excreted in the urine. Thus, there is little storage of the free vitamin, which in most instances, needs to be continually supplied in the diet (Bruckner, 1997). **Fat – soluble vitamins** are vitamins A, D, E and K, which are present in

food lipids of both plant and animal origin. They are digested with fat and absorbed by the intestine and incorporated into chylomicrons (Brites et al., 1999).

Vitamins as anti-oxidants are both top line factors in immune function and play supportive roles that enable the immune system to work at its peak. The vitamins share characteristics with other anti-oxidants such as, minerals and other phytochemicals whose activities serve to protect the cells against the damaging effects of highly reactive molecules known as free radicals (Balakrishan and Anuradha, 1998). Free radicals have the ability to chemically react with and damage many structures in the body. Particularly susceptible to oxidative damage, are the cell membranes of virtually all cells and the very source of our genetic material – DNA. Free radical reactions and oxidative damage have been linked to many diseases such as cancer. Non-availability of vitamins, whether due to dietary or other reasons (e.g. defects in absorption), results in characteristic deficiency syndromes. Anti-oxidants are claimed to have the following: cellular protection, cancer prevention, promotes vision, heart disease prevention and enhanced immune function. A few studies have clearly documented the beneficial effects of dozens of anti-oxidant nutrients. Increased dietary intake of significant anti-oxidant nutrients such as, vitamins C and E, minerals such as, selenium and various phytonutrients such as, extracts from grape seed, pine bark and green tea, have all been linked to reduced rates of oxidative damage as well as reduced incidence of chronic diseases such as. heart disease and cancer (Bazzarre et al., 1992; Brites et al., 1999; Bruckner, 1997).

In the world of immune function, one of the known anti-oxidants is vitamin C. It is involved in antibody production and helps in the function and activity of white blood cells. It further helps synthesize adrenal hormones, which help the body to deal directly with stress, mineral balances and inflammation. Research has shown that natural killer cell (NK cell) activity was enhanced as much as ten-fold in 78% of patients given buffered, vitamin C, while T- and B- cell counts were restored to normal levels (Keen et al., 1994). Vitamin E is thought to protect the thymus gland and circulating white blood cells, in addition to protecting the immune system from oxidative stress. At the typically recommended levels, the majority of anti-oxidants appear to be quite safe. For example, vitamin C is one of the most powerful membrane bound antioxidants; it also has one of the best safety profiles.

Intravenous doses of 100 – 400 IV units have been linked to significant cardiovascular benefits with no side effects. Vitamin C, as a powerful anti-oxidant, can help to protect and restore the anti-oxidant activity of vitamin E and is considered safe up to doses of 500 – 1,000mg/day. Higher doses of vitamin C are not recommended because of concerns that such levels may cause an "imbalance" of the oxidative systems and actually promote oxidative damage instead of preventing it (Clarkson, 1995; Clarkson and Thompson, 2000).

2.7.1 VITAMIN E

For many years, vitamin E was described as the "vitamin in search of a disease". While its deficiency diseases are still virtually unknown, its metabolic role in the body has become better understood in recent years. Vitamin E is actually a family of related compounds known as tocopherols and tocotrienols. Although alpha-tocopherol is the most common form found in dietary supplements, vitamin E also exists with slightly different chemical structures as beta -, gamma – and delta –tocotrienols (Behl, 1999). Vitamin E was discovered in the early 1930's when rats fed a diet free of vegetable oils (the primary dietary source of vitamin E), resulted in reproductive problems. Although vitamin E doesn't have exactly the same reproductive effects in humans, it is commonly thought of as a "virility" vitamin for men (Behl, 1999).

Vitamin E can be obtained as a supplement in natural or synthetic form. In most cases, the natural and synthetic forms of vitamins and minerals are identical, but in the case of vitamin E; the natural form is clearly superior in terms of absorption and retention in the body. The synthetic "d– form" is the most common form found in dietary supplements, although many manufacturers are switching over to the more potent (and expensive) natural "d – form" (Benzie et al., 1998). Dietary sources of vitamin E include plant products, especially cold-pressed vegetable oils such as wheat germ oil and cotton seed oil, safflower oil which contains a good amount of vitamin E (about two-thirds of the RDA in ¼ caps). But there is very little vitamin E in either corn-oil or soybean oil; leafy green vegetables and whole-grain cereals. Although animal products contain little vitamin E, the best sources are liver, heart, kidney and eggs. Anti-oxidant activity is one of the roles of vitamin E: it boosts immune system function, reduces cancer risk, wound healing and reduced risk of cardiovascular disease (Kushi et al., 1996).

2.7.2.1 BIOLOGICAL ACTIVITY OF VITAMIN E:

Vitamin E is one of the most potent fat-soluble anti-oxidants in the body. As such, vitamin E protects cell membranes from the damage caused by free radicals. High doses of vitamin E have also been linked to a decreased ability of the blood to clot, which may be beneficial in those individuals at risk for heart disease by reducing the risk of heart attack. Most of the evidence for its anti-oxidant function have been obtained from in vitro experiments, but there are also data from studies of various tissues that showed that tocopherols have antioxidant potential in, in vivo. Many enzyme activities in plasma are altered during severe vitamin E deficiency because tissue necrosis causes release of cellular enzymes (Catignani, 1980).

2.7.2.2 PATHOLOGY OF VITAMIN E DEFICIENCY:

The most notable aspect of vitamin E deficiency in animals is the marked degree of species specificity. There is a good diversity of tissue and organ functions affected by low tocopherol level. Nelson has reviewed the histopathology of the reproductive organs in vitamin E deficient animals (Nelson, 1980). In various animals, vitamin E deficiencies can be associated with sterility, muscular dystrophy, central nervous system changes and megaloblastic anemia. In humans however, the symptoms are usually limited to increased fragility of red blood cell membrane (presumably due to peroxidation of the membrane components). In general, tissue necrosis is a major feature and is accompanied by accumulation of lipo-pigments thought to represent peroxidized lipid. A wide variety of epidemiological and prospective studies have shown health benefits associated with higher than average vitamin E consumption, although high dose of alpha-tocopherol supplements may displace body stores of the other naturally occurring vitamin E forms. In one study vitamin E appeared to offer protection only when taken up from the diet (mostly gamma) and not when taken in supplements (which are primarily alpha) (Kushi et al., 1996). While investigating the effect of diet on the reproductive cycle of rats, they found that rats could not reproduce when given a purified diet that contained all nutrients except vitamin E. However, when fresh green leaves or dried alfa was added to the diet, fertility was restored in the female but not in the male. The tissue affected in the rat was the placenta and testicle tissue.

The search for a clinical correlate of vitamin E deficiency in humans has been based largely on the understanding of the pathologic disturbances in animals on synthetic diets. In humans, rapid development of vitamin E deficiency apparently does not occur except in unusual clinical circumstance. Overall, it appears that a balanced intake of each of the naturally occurring forms of vitamin E may be the most prudent approach in terms of overall health benefits.

2.7.3. VITAMIN C (ASCORBIC ACID)

Vitamin C also known as ascorbic acid is a water soluble vitamin needed by the body for hundreds of vital metabolic reactions. The vitamin C deficiency disease, scurvy, is prevented by adequate intake of ascorbic (meaning "without scurvy") acid (Halliwell, 1996: 1999). As a dietary supplement, vitamin C is consumed by more people than any other vitamin, mineral or herbal product. Good food sources of vitamin C include all citrus fruits (oranges, grape fruit, and lemons) tomatoes, broccoli, pepper and cantaloupe. Vitamin C is a fairly "fragile" vitamin and can be easily destroyed by cooking or exposure of food to oxygen. Vitamin C functions as an anti-oxidant, boosts immunity, prevents cold, promotes wound healing, protects against effects of stress and prevents cancer. Due to the wide variety of reactions in which vitamin C plays a role, many claims of its function can be made for supplements. Perhaps the most well known function of vitamin C is as one of the key nutritional anti-oxidants – where it protects the body from free radical damage. As a water soluble vitamin, ascorbic acid, performs its anti-oxidant functions within the aqueous compartments of the blood and inside cells and can help restore the anti-oxidant potential of vitamin E (Halliwell, 1999). Support of immune cell function, is a key role performed by vitamin C and an effect which may help fight infections in their early stages.

2.7.3.1 ASCORBIC ACID AND PREGNANCY

Plasma ascorbic acid levels of women decrease during pregnancy, primarily because of hemodilution. However, the plasma ascorbic acid levels of the fetus and neonates are some 50% higher than those of the mother, indicating active placental transport and a relatively higher body pool of the vitamin. The increased maternal requirement for ascorbic acid based solely on the weight of a near – term fetus, would be about 3 to 4mg/day, although

ascorbic acid turnover in the growing fetus is believed to be greater than that for adults. The RDA of 1989 therefore includes additional intake of 10mg/day for pregnant women to compensate for ascorbic acid losses during pregnancy and to maintain an adequate body pool (Harlap et al., 1980; Halliwell, 1996).

As a water-soluble vitamin, ascorbic acid is extremely safe even at relatively high doses (because most of the excess is excreted in the urine). At high doses (over 1000mg/day), some people can experience gastro-intestinal side effects such as stomach cramps, nausea and diarrhea that may increase the risk of developing kidney stones (Halliwell, 1999). As a dietary supplement, vitamin C is the most popular single nutrient supplement. It is typically included in all multi-vitamin blends, but at widely varying levels from less than 30mg to over 1000mg. As a single nutrient supplement, typical doses range from 100-500mg/tablet. The body, however, can only absorb and retain about 20mg of vitamin C at one time – the rest is simply washed out in the urine. This means that the most effective approach to supplementing with vitamin C is to take it in divided doses throughout the day (Jacob et al., 1992; 1996). Although the RDA for vitamin C has recently been raised from 60mg to 75-90mg (higher for men), it is well established that almost everybody can benefit from higher levels. Even though vitamin C is well absorbed, the percent absorbed from supplements decreases with increasing dosages and optimal absorption is achieved by taking several small doses (about 200mg/dose) throughout the day (for a total daily intake of 200mg to 1000mg). Full blood and tissue saturation is achieved with daily intakes of 200mg to 500mg/day (in 2 to 3 divided doses) (Sharma and Mathur, 1995).

2.7.4. MICRO- NUTRIENTS AS ANTI-OXIDANTS: IRON IN WOMEN.

A mature woman has a total body iron content of between 3500 and 4500mg. Of this, 75% is held in the erythrocytes as hemoglobin, 20% in the body stores, mainly in the bone marrow and reticuloendothelial system as a ferritin complex, and the remaining 5% is held in muscles, mainly in the form of myoglobin (Liewellyn-Jones, 1984). The iron content is not static, and that portion in the erythrocytes particularly, is in constant flux. This is because the average life of an erythrocyte is 100 – 120 days. Each day erythrocytes die and disintegrate, and new erythrocytes are formed. The disintegrating erythrocytes release about 27mg of iron and approximately 1mg is lost to the body through cells shed from the

gastrointestinal tract, the skin, hair, nails and genito- urinary tract. Since the developing erythrocytes require about 27mg of iron daily, a deficit of 1 mg results. Iron is also lost each month by women in the menstrual flow (ie 30mg or 1mg daily) thus the non- pregnant woman needs to absorb 2mg of iron a day to maintain iron balance, and to keep her body stores full (Liewellyn-Jones, 1998). A non – anemic woman absorbs between 14 and 20% of the iron available in food, provided the diet is the 'mixed' diet usually eaten in the developed nations. When the diet is predominantly cereal in nature, very much less iron is absorbed. Thus, to obtain the 2mg required daily, a woman eating a diet containing 25% of animal food needs 12 mg of dietary iron, whilst a woman eating a cereal diet needs between 20 and 28mg of dietary iron each day (Liewellyn-Jones, 1984;1998).

2.7.4.1 IRON METABOLISM IN PREGNANCY

Pregnancy imposes additional demands for iron. Although, menstruation ceases in pregnancy and lactation, the increased red cell mass and additional muscle formation (including that of the uterus) requires about 425mg of iron spread out over the 40 weeks of pregnancy; relatively little iron being needed before this (Liewellyn-Jones, 1984; Finch, 1994). Pregnancy thus places an additional demand for iron on the mother of 250mg less the 750mg 'saved' by cessation of menstruation, a total of 500mg of iron being needed. The developing fetus in the second trimester takes 3 – 4 mg iron per day from the maternal stores and 6 mg/day in the last trimester. Although the red cell mass and muscle needs occur evenly throughout pregnancy, the fetal demand for iron is only marked after the 30th week, so that the daily amount of iron needed varies in the first 29 weeks, the total daily iron intake required is about 2.5mg and in the last 10 weeks, the quantity needed rises to 4.5mg (Table 5) (Liewellyn-Jones, 1984). About 20 to 30% of the iron presented to the intestinal mucosa is absorbed, the larger percentage occurring in the last quarter of pregnancy. For this reason, the diet of the pregnant woman needs to contain 15 to 20mg of elemental iron (Table 5). Once nursing, the baby again challenges the mother's iron stores. Frequent pregnancies without supplemental iron can result in iron deficiency anemia for both mother and child.

Table 5: Iron requirement in pregnancy.

	Over Period		Daily needs	
Week	Net maternal need (mg)	Fetoplacental needs (mg)	Total daily need (mg)	Daily intake required (mg)
1 – 9	90	40	2.3	10 – 12
10 – 19	112	65	2.5	10 –12
20 – 29	112	120	3.3	14 – 16
30 – 39	112	200	4.5	18 - 22

Note: The net daily need is calculated as follows: Assuming a daily utilization rate of 20 – 25% of dietary iron.

Replacement of iron lost from epithelial cell:	1mg/day
Increase in red cell mass and muscle development:	1.6mg/day
Less saving due to amenorrhea:	1mg/day
Net daily need:	1.6mg/day.

Adapted from: Finch (1994)

2.7.4.2. IRON DEFICIENCY AND IMMUNITY

The importance of iron deficiency on public health is based ultimately on the seriousness of its consequences on health. The most extensively investigated consequence of iron deficiency involves work function and immune function. The significance of the effect on work performance is generally accepted. However, data on the influence of iron deficiency on immune function are often perceived as being confusing and contradicting. From re-examination of relevant literature, it seems safe to conclude that abnormalities in cell – mediated immunity and ability of neutrophils to kill several types of bacteria are well established under experimental conditions in iron deficient patients. It remains uncertain whether these abnormalities result in an increased incidence and duration of infection, an area that still requires careful study (Sharma and Mathur, 1995).

The two major effects of iron deficiency, both with and without anaemia, relate to T – lymphocyte-dependent cellular immunity and intracellular microbial killing by polymorphonuclear leucocyte (Sharma and Mathur, 1995). The synthesis of T-lymphocytes in response to stimulus or mitogensis results in "blastic transformation" and the production of lymphokines that are important for immune regulation. Continuous supply of iron is required for the activity of the mammalian ribonucleotide reductase that reduces the sugar group of nucleotides to corresponding deoxy-derivatives, the precursor of DNA. If this enzyme is decreased, DNA synthesis will be impaired with resultant effects on all cell functions (Sharma and Mathur, 1995). There was preliminary recent evidence that iron deficiency alters the function and proportion of various T-cell subsets (Finch, 1994). Occasionally, the total lymphocyte count in the peripheral blood is also low. All these changes are reversed after iron therapy. Occasionally, an impaired immune response after iron administration may indicate the existence of an unsuspected functional iron deficiency (Sharma and Mathur, 1995). Collectively, the data on iron deficiencies indicate the following: reduced blastogenic responses of lymphocytes, reduced number of circulating T cells, reduced antibody titers, long – term impairment of humoral immunity in pre- and postnatal iron – deficient rats if not corrected by proper iron administration, impaired cell mediated immunity (CMI) and impaired microbial killing by neutrophils (Finch , 1994).

2.7.4.3 EFFECTS OF IRON DEFICIENCY ON PREGNANCY OUTCOME

Iron deficiency anemia, the most severe form of iron deficiency has important consequences for pregnant women and generally females of child-bearing age. Pregnant women with low iron stores may be at increased risk of iron deficiency anemia because pregnancy increases requirements for iron (Finch, 1994). Since iron deficiency occurs in stages, stage 1 and stage 2 may have little effects on pregnancy outcome. However, stage 3 (iron deficiency anemia) has adverse effects both on the mother and foetus. They are both at risk and except iron supplementation is given, the effect on the foetus may affect it for life.

2.7.4.4 CHANGES IN IMMUNE REACTIVITY DURING PREGNANCY

The fetus contains antigens derived from the father and is therefore an allograft, which is foreign to the mother. The reasons why the mother's immune system does not reject the fetus are not fully understood. One suggestion is that, as there is no expression of either classical class I or class II MHC antigens by human chorionic villous trophoblast throughout gestation, cell mediated rejection is not activated. The immunological changes in pregnancy seem to be relatively minor. They include a 30% increase in neutrophils, a decrease in helper T –cells, a slight reduction in IgG, an increase in IgD and a slight depression in cell –mediated immunity, see tables 1, 2a and b. These and other unknown factors that may limit, rather than abolish the immunological response, which may be necessary to prevent excessive growth of the placenta thereby, restraining fetal rejection process. Many diseases which tend to have an autoimmune basis tend to remit in pregnancy (Sharma and Mathur, 1995).

2.7.5.0 ZINC IN NON- PREGNANT WOMEN.

Zinc is a potent mediator by which the host presents resistance to infection, by modulating immune function. It is also known to be essential for all highly proliferating cells in the human body, especially the immune system. A variety of *in-vivo* and *in-vitro* effects of zinc on immune cells mainly depend on the zinc concentration. All kinds of immune cells show decreased function after zinc depletion (Apgar, 1992).

Furthermore, when peripheral blood mononuclear cells are incubated with zinc in-vitro, the release of cytokines such as interleukins (IL)-1 and -6, tumor necrosis factor-alpha, soluble IL-2R and interferon-gamma is induced. In a concentration of 100µmol/L, zinc suppresses natural killer cell and T-cell functions. Whereas monocytes are activated directly and at a concentration of 500µmol/L, zinc evokes a direct chemotactic action on neutrophil granulocytes (Apgar, 1992).

2.7.5.1 BIOCHEMICAL AND PHYSIOLOGICAL ROLES OF ZINC IN HUMANS.

Zinc is recognized to have a multitude of diverse functions and has been identified in numerous enzymes. It is a component of biomembranes, is thought to be necessary for RNA, DNA and ribosome stabilization, is involved in the binding of a number of

transcription factors, stabilizes some hormone receptor complexes, and may have a regulatory role in tubulin polymerization. Given its multitude of functions, it is not surprising that a deficit of this element can pose serious physiological challenges (Vallee, 1983). With regard to metalloenzymes, zinc plays a catalytic, structural or regulatory role. Examples are carbonic anhydrase, superoxide dismutase, and fructose bisphosphatase. Zinc has been reported to occur in over 200 biological systems and zinc enzymes are found in all the six enzyme classes of the International Union of Biochemistry (IUB) (Vallee, 1983; Hambidge et al., 1986).

A critical function of zinc is its role in the structure and function of biomembranes. Investigators have argued that a reduction in the concentration of zinc in biomembranes underlies some of the disorders associated with zinc deficiency, with a loss of zinc from the membrane resulting in an increased susceptibility to oxidative damage, structural strain alterations in specific receptor sites and transport systems. The influence of zinc on biomembrane structure and function may be due in part to, its ability to (1) stabilize thiol groups and phospholipids, (2) occupy sites that might otherwise contain transition metal with redox potential (such as iron) and (3) to be involved in the quenching of free radicals through its association with metallothionein (Wilson et al., 1999; Wellinghausen, 2001). Biomembrane accumulation of zinc can also result in alterations in membrane structure and function; for example, the release of histamine from mast cells is reduced when zinc masks receptor sites for histamine release agents (Hambidge et al., 1986). In addition, zinc has putative structural role in biomembranes and in many enzymes (Hambidge et al., 1986). Moreover several transcription factors have been reported to contain "zinc finger" regions of repeated cysteine- and histidine- containing domains that bind zinc in a tetrahedral configuration. It is currently thought that zinc- finger- zinc complex regions are needed for binding of these transcription factors to DNA; one suggested mechanism of transcription control might involve the binding and release of zinc from these regions in response to meal-associated changes in nuclear zinc content (Halliwell, 1999). Zinc may also have regulatory role in the polymerization of tubulin. Zinc has been shown to stabilize neurotubules in vitro, possibly through the formation of zinc mercaptide bridge between the tubulin dimer subunits. The rate of tubulin polymerization is decreased in brain extract of zinc-deficient rats and pigs; zinc deficiency induced reduction in the rate of tubulin polymerization has been postulated to underlie some of the deficiencies (Halliwell, 1999).

2.7.5.2 IMMUNOLOGY OF GESTATIONAL ZINC DEFICIENCY

The trace element zinc is an essential micronutrient for the proper functioning of the immune system. Zinc deficiency leads to impaired function of the unspecific and specific immune response and consequently to an increased susceptibility to bacterial, viral and fungal infections.

Immunological defects are not only seen in pronounced deficiency but even in marginal and moderate zinc deficiency. Lack of zinc is especially harmful for the development of the immune system, which stresses the importance of a balanced zinc level during pregnancy. However, gestational zinc deficiency due to an imbalance between intake and increased requirement is a common problem worldwide. Gestational zinc deficiency results in reduced thymic and spleen size and depressed active and passive immunity in the infants, depressed immunoglobulin levels, altered antibody repertoire, reduced proliferative response of lymphocytes and diminished neutrophil functions have been reported (Wellinghausen, 2001). Interestingly, immune defect caused by prenatal zinc deficiency, such as depressed antibody levels and lymphocyte proliferation may even persist in subsequent generations and are not reversible by postnatal zinc administration.

Since gestational zinc deficiency is a common problem throughout all cultures and socioeconomic levels, it might have immense consequences for the health status of the population (Wellinghausen, 2001).

2.7.5.3 ZINC INFLUENCE ON ANTI-THYRIOD ANTIBODIES

Anti-thyroglobulin antibodies are auto-antibodies directed to thyroid antigens. Thyroglobulin is a water-soluble glycoprotein thyroid hormone, which stimulates the development of the foetus. The anti-thyroglobulin being an auto-antibody is secreted by the specific immune response during zinc depletion due to one factor or the other. It was proposed that zinc deficiency and zinc depletion could cause an increase in anti-phospholipids auto-antibodies along with other auto-antibodies in the human system (Wellinghausan, 2001). Zinc deficiency was confirmed to depress antibody levels and lymphocyte proliferation (Wellinghausen. 2001). Also a high dosage of zinc evokes

negative effects on the immune cells and shows alterations that are similar to those observed during zinc deficiency (Halliwell, 1999).

2.7.5.4 MATERNAL PLASMA ZINC CONCENTRATION AND PREGNANCY OUTCOME

During the past few decades, many investigators have evaluated the relationship between maternal zinc nutrition and pregnancy outcome in humans (Swanson and King, 1987; Apgar, 1992). It is well known that zinc deficiency during pregnancy causes fetus growth retardation and malformations (Keen et al., 1994). Plasma zinc concentration declined as gestation progressed and also the requirement of zinc increases during pregnancy. However, a firm consensus has never been reached as to whether there is a positive association between maternal zinc nutrition and pregnancy outcome (Apgar, 1992).

The deficiency of zinc in pregnant women has been observed to impair the immune function during pregnancy, causing complications of pregnancy, such as pregnancy-induced hypertension, prematurity, prolonged labour and intra-partum hemorrhage (Keen and Hurley, 1989).

CHAPTER THREE

3.0 METHODOLOGY

3.01 MATERIALS:

Agglutination Assay – Anti-thyroid Auto-antibodies (ATA)

-Electric microscopic plate reader by OLYMPUS, U.S.A

-Magnifier – Viewer by OLYMPUS, U.S.A

-Pipette delivering 10-100 µl by IMPERIAL III U.S.A

-1.0ml, 5ml serological pipettes, graduated in 1/100ml by OLYMPUS U.S.A

- Autotray serial dilution tray or disposable clear plastic microtitraton ELISA trays with round bottomed (U-shaped) wells; (tray should be free from dust).

Triacylglycerol and Cholesterol assays

-Spectrophotometer by Militon Roy comp. Germany

-Cuvette by IMPERIAL III U.S.A

-Water bath with thermometer by Heraeus spatech, Germany

-Test tubes in racks by INTERNATIONAL EQUIPMENT CO. U.S.A

Natural killer cell- (Nka) assay

Incubator by IMPERIAL III U.S.A

- Fine wood sterile work

- Inverted microscope by OLYMPUS U.S.A

- Flow cytometer by FLOWJO.FACS CALBUR E3050 GERMANY

-Tissue culture flasks by IMPERIAL III U.S.A

- Hemocytometer neubauer for WBC counting by OLYMPUS U.S.A

- Centrifuge by INTERNATIONAL EQUIPMENT CO. U.S.A

- Disposable sterile 10 and 25ml pipettes by OLYMPUS U.S.A

- Disposable 15ml tubes by OLYMPUS U.S.A

- Disposable 12x75 B.D plastic tubes by OLYMPUS U.S.A

- Automated pipettes by (Eppendorf) Germany.

Progesterone, testosterone, estradiol ELISA assays

- Precision pipettes 25µl, 50µl, 100µl, and 200µl and 1.0ml by OLYMPUS U.S.A

- Disposable pipette tips by OLYMPUS U.S.A
- Vortex mixer - Gene2 Scientific Ind. U.S.A
- Absorbent paper by OLYMPUS U.S.A
- Microtiter plate reader by OLYMPUS U.S.A

ATG (Anti-thyroglobulin), TPO (thyroperoxidase) ELISA IgG auto-antibodies in human serum. (All from -Serodia Fujirebio Inc.)

-Micro pipettes 10 µl, 100 µl, by Eppendorf Germany.
-Disposable pipette tips ,,
-Buffer bottle (0.5L) ,,
-Sample test tubes (polystyrene) (12x75mm) ,,
-Microplate reader with 450nm wave-length absorber by OLYMPUS U.S.A

3.0.2 REAGENTS:

Antithyroid autoantibodies (ATA) agglutination assay

- Saline solution (0.85%) **(from -Serodia Fujirebio Inc.)** Germany.
- Sterile distilled water ,,
- (a) Serodia ATG- Thyroglobulin antibody test kit ,,
  (b) Serodia AMC- Thyroid microsomal antibody test kit ,,
- Serum diluent. ,,
- Sensitized particles and unsensitized reactive controls ,,

Biochemical assay: (Cholesterol, assay),

-4- Aminoantipyrine (0. 30 mol/L) **from Quimica ClinicaAplicada South.Africa.**
-Phenol (6m mol/L) ,,
-Peroxidase (0.5 U/ml) ,,
-Cholesterol esterase (0.15 U /ml) ,,
-Cholesterol oxidase (0.1 U/ml)
-PIRES Buffer (80 mol/L or pH 6.8 ,,
-Standard " (5.17 mol /L or 200mg/L) ,,

Biochemical assay: (Triglyceride assay),

-PIPES buffer pH 7.5 (40mM) **from Randox Laboratories U.S.A.**

-4 – Chlorophenol	(6mM)	,,
-4- Aminoantipyrine	(0.5mM)	,,
-ATP	(1mg)	,,
-$MgCl_2$	(5 mg)	,,
-Glycerol Kinase	(400 U/L)	,,
-Glycerol – 3- phospate oxidase	(1500 U/L)	,,
-Peroxidase	(500 U/L)	,,
-Lipases	(150 U/L)	,,

Biochemical assay: (Total protein assay),

Biuret reagent / Blank reagent:		**from Randox Laboratories U.S.A.**
Sodium hydroxide	(100 mmol/L)	,,
Na-K-tartarate	(16 mmol/L)	,,
Potasium iodide	(15 mmol/L)	,,
Cupric sulphate	(6 mmol/L)	,,
Standard; Protein	(60 g/l , 6.0 g/dl)	,,

Biochemical assay: (glucose oxidase assay),

Phosphate buffer	0.1 mol/L, pH 7.0	**from Randox Laboratories U.S.A**
Phenol	11mmol/L	,,
4-aminophenazone	0.77 mmol/L	,,
Glucose oxidase	1.5 U/L	,,
Peroxidase	1.5 U/L	,,
Standard; Glucose	5.55 mmol/L (100mg/dl).	,,

Biochemical assay: (Glutamate-oxaloacetate transaminase / Glutamate-pyruvate transaminase assay),

Buffer:		**from Randox Laboratories U.S.A.**
Phosphate buffer	100mmol/L pH 7.4	,,
L-alanine (GPT)	200 mmol/L	,,
L-aspartate (GOT)	100 mmol/L	,,
α-oxoglutarate	2.0 mmol/L	,,
2,4-dinitrophenylhydrazine	2.0 mmol/L	,,

Natural Killer cell Activity (Nka) assay

-K562 cell line (ATCC) **U.S.A.**

-RPMI – 1640 media (Sigma) **U.S.A**

-Hystopaque 1077 (Sigma) **U.S.A**

-3.3, Dioctadecyloxacarbocyanine perchlorate (Dio) (Sigma) **U.S.A**

-Propidium iodide (iv) (PI) (Sigma) **U.S.A**

-Iv1g (intravenous immunoglobulin (Bexter) **U.S.A**

-Fetal calf serum (FCS) heat inactivated (Sigma) **U.S.A**

-Complete medium (CM)- RPMI-1640 +10% Fetal calf serum (FCS) (Sigma) **U.S.A**
+ 100u/ml penicillin +100u/ml streptomycin (Cellgro), filtered, sterilized

-Dio Solution-in DMSO (Dimethyl sulfoxide), aliquot 50:1 in conical vials (3ml).
and kept refrigerated at 2-8° C until used

-PI solution- dissolved in CM working solution, 10mg/100ml.
kept refrigerated in the dark

-Phospate buffered saline (PBS)

(ATG (Anti-thyroglobulin) and TPO (Anti-thyroperoxidase) ELISA IgG auto-antibodies in human serum)

-IgG calibrators' nominal concentration (kit items from kronus, **U.S.A**.)

(a) ATG :- (0, 90,300,900 and 5000 iu/ml) blue colour

(b) TPO: (0, 30, 90,300 and 1000 iu/ml) red colour

- Microplate (a) Human thyroglobulin coated 96 wells.
(b) Human thyroperoxidase coated 96 wells.

- Enzyme conjugate;

(a) ATG IgG conjugate – Rabbit and Human IgG labeled Horseradish peroxidase Blue

(b) TPO IgG conjugate –Rabbit anti- human IgG labeled Horseradish peroxidase Red

- Control (a) ATG positive control (human serum with anti-thyroglobulin antibodies)
(b) TPO positive control (human serum with anti-thyroperoxidase antibodies)

- Sample diluents – Bovine serum albumin in Tris buffer (Blue or Red)

- Chromogenic substrate – 3^1, 5^1-tetramethylbenzene (TMB)

- STOP reagent – 1.0 N sulphuric acid

- WASH buffer - Tris buffer, concentrate.

Progesterone ELISA Assay, **kit from Diagnostic Automation Inc, U.S.A**

-Goat anti-rabbit IgG coated microtiter wells, 96 wells,

-Progesterone reference standard; 0, 0.5, 3.0, 10, 15 and 50ng/ml

-Rabbit anti-progesterone reagent (Pink colour) 7ml

-Progesterone- HRP conjugate concentrate (IIX), 1.3 ml

-Progesterone- HRP conjugates dilute, 13ml

-Progesterone control 1and 2

-TMB reagent (Chromogen)

-Stop solution (2N HCl) (11ml)

Testosterone ELISA Assay, **kit from Diagnostic Automation Inc. U.S.A**

-Goat anti rabbit IgG coated microtiter 96 wells,

-Testosterone reference standard 0, 0.1, 0.5, 2.0, 6.0, 18 ng/ml

-Rabbit anti- testosterone reagent (pink colour)

-Testosterone- HRP conjugate reagent (blue colour)

-Testosterone control 1 and 2

-TMB reagent (Chromogen)

-Stop solution (2N HCl)

Estradiol ELISA Assay, **kit from Diagnostic Automation Inc., U.S.A**

-Goat anti-rabbit IgG – coated microtiter wells, 96wells,

-Estradiol reference standards 0,10,30,20 and 1000 ng/ml

-Rabbit anti- Estradiol reagent (pink colour)

-Estradiol – HRP conjugate reagent (blue colour)

-Estradiol control 1 and 2

-TMB reagent (Chromogen)

-Stop solution (2N HCl).

PROLACTIN ASSAY, **kit from Diagnostic Automation Inc., U.S.A**

-Antibody – coated microtiter 96wells,

-Human prolactin reference standard 0, 7.5, 25, 60, 120, 240 ng/ml

-Enzyme conjugate reagent- HRP conjugate reagent

-TMB substrate (Chromogen) / -Stop solution (2N HCl)

3.1.1. STUDY AND CONTROL VOLUNTEERS - QUESTIONNAIRE

290 euthyroid women were used in this study. They were classified into two (2) groups namely the test group and the control groups. The test group consist of 34 primary infertile women, 46 secondary infertile women and 46 recurrent spontaneous aborters, from the Obstetric and Gynecology units, of LUTH –Lagos University Teaching Hospital ,Idi-Araba and the Ayinke house- (Lagos State University Teaching Hospital) Ikeja. The control groups included, 60 pregnant women undergoing antenatal care and 58 multiparous women, at the Family planning unit of the same hospitals and 46 non-pregnant nulligravida women volunteers. Special note was taken of the ages of the women and year of marriage for the possible age distribution in the year of marriage brackets.

Relevant data were collected from 630 women with the aid of a questionnaire (Appendices 1A and 1B) and their case notes were reviewed, to ascertain that the diagnoses of the women did not include the exclusion factors (ie known cause of reproductive failure). Thereafter, the 290 study subjects' individual blood samples were collected. The study was permitted and conducted according to the ethical standards of the Research and Ethics Committee of the LUTH –Lagos University Teaching Hospital, Idi-Araba and Lagos State University Teaching Hospital, Ikeja.

Exclusion factors: All women with diagnosed known causes of infertility (e.g. thyroid, adrenal disorders, severe nutritional disturbances, significant weight loss etc, see table 4) in all the test groups were excluded from the study. Only euthyroid women of no established causes of reproductive failure (i.e unexplained infertility) were included.

3.1.2 SPECIMEN COLLECTIONS:

Venous whole blood (10ml) was collected from each of the women volunteer, the week after the last day of menses in all the non-pregnant women and for pregnant women in the 1st or 2nd trimester by vein puncture, into both universal bottles and heparin bottles. All samples in the universal bottles were allowed to stand for at least 1/2 hour; others were allowed to stay on the table for 15minutes, before they were centrifuged at 2000 rpm for 30 minutes, in a Beckman table top centrifuge. The serum / plasma were decanted, drawn by aspiration using a disposable sterile micro- pipette, and stored at 2-8°C if necessary, until needed for analysis (Isselbacher and Tisdale, 1970).

3.2.0 **METHODS**

3.2.1. **AGGLUTINATION ASSAY FOR ANTI-THYROID (THYROGLOBULIN AND MICROSOMAL) ANTIBODIES (ATA),** (Pratt et al., 1993b, Tan, 1989).

3.2.1.1 PRINCIPLE:

The thyroglobulin and thyroid microsomal antibody determination was based on the agglutination of gelatin particles which have been sensitized with thyroid microsomal antigen, extracted and purified from human thyroid tissue.

Serum containing specific antibodies will react with thyroglobulin sensitized coloured gelatin particles to form a smooth mat of agglutinated particles in the micro titration tray.

Negative reactions are characterized by a compact button formed by the setting of the non agglutinated particles (Cayzer et al., 1978).

3.2.1.2 PROCEDURE:

Each serum being run along with the control were processed at the same time using 8-10 wells each of a micro titration ELISA tray.

The ELISA tray was well labelled with identification number of serum to the left of the first well in each row and noted on work sheets. Serum diluents (50µl) were pipetted into well numbers 1 and 2 while 75µl of serum diluents were placed in numbers 3-12. Then10 µl of each serum was placed into well number 1 of the appropriately marked row and mixed well. This was followed by the serial dilutions of each serum by removing 25µl from each well and adding it consecutively across each dilution row per serum.

Thereafter, 25 µl of unsensitised dilute particles was placed in well number 1 while 25µl of sensitized particles was placed in each of wells 2-12.

They were incubated for 3 hours at room temperature and the setting patterns read under a tray viewer (Tan, 1989; Pratt et al., 1993b).

3.3.0 BIOCHEMICAL ASSAY:

3.3.1 **TRIGLYCERIDE DETERMINATION** by Enzymatic Colourimetric Method Glycerol 3-Phosphate Oxidase Method of Trinder (1989) in Tietz (1995).

PRINCIPLE: Triglyceride is determined in a coupled reaction of enzymatic hydrolysis with oxidation reactions. The end point indicator quinoneimine is formed from the

reaction of hydrogen peroxide and 4-amino-antipyrine catalyzed by peroxidase', in the presence of the non reactive stabilizer, 4-Chlorophenol.

$$\text{Triglyceride} \xrightarrow{\text{Lipase}} \text{Glycerol + fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol -3- Phosphate +ADP}$$

$$\text{Glycerol -3- Phosphate + } O_2 \xrightarrow{\text{Glycerol 3-Phosphate oxidase}} \text{Dihydroxyacetone – Phosphate + } H_2O_2$$

$$2H_2O_2. + \text{4 Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + HCl + } H_2O$$

ASSAY PROCEDURE:

Serum samples were analyzed for triglycerides by setting up an assay system in three (3) test tubes labelled, blank, sample and standard (Std). Serum (0.02ml) and 0.02ml of standard were pipetted into the labelled tubes. Then 0.2ml of working reagent (containing 40mol/L PIPES buffer pH 7.5, 6mmol/L of chlorophenol, 0.5nmol/L 4-aminoantipyrine, 1mmol/L ATP, 5mmol/L Mg Cl_2, with 400U/L glycerol kinase, 1500U/L lipase) were added to all the tubes, except the blank that contain 0.22ml of the working reagent. The solution was gently mixed and allowed to stand for 5 minutes at 37^0C, before absorbance measurements were taken at 500nm wavelength in a 1cm light-path cuvette against the reagent blank.

Calculation:

$$\text{Triglycerides in Sample} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \text{ X } \text{Conc. of standard}$$

3.3.2 **CHOLESTEROL ASSAY by Enzymatic End Point method of Trinder, (1988)** in Tietz, (1995).

PRINCIPLE: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4 amino antipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol-ester} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholestene - 2-one} + H_2O_2$$

ASSAY PROCEDURE:

Serum samples were analyzed for cholesterol levels. Three different screw-capped cuvettes, of 1cm light path were placed in a rack at a temperature of 37 °C. The screw-cap cuvettes was labeled (1) blank containing 1.1ml of reagent mixture, (2) standard containing 0.1ml of standard (std) cholesterol solution and (3) containing 0.1ml of serum sample.

Then, a 1ml reagent mixture (containing 0.30mmol/L 4-aminoantipyrine, 6.0 mmol/L phenol, 0.5 U/ml peroxidase, 0.15 U/ml cholesterol esterase, 0.1 U/ml oxidase) was pipetted into cuvettes 2 and 3 and were mixed gently and incubated for 5 minutes at 37^0C. The absorbance of the assay mixture was taken at 500nm (546nm Hg) against reagent blank (cuvette 1) within 60 minutes.

Calculation:

$$\text{Cholesterol in Sample (mg/dl)} = \frac{\text{Absorbance of Sample} \times \text{Conc. of standard}}{\text{Absorbance of Standard}}$$

Standard reference is 200 mg/dl

3.3.3 DETERMINATION OF SERUM ALBUMIN

Dye binding method using bromocresol green (BCG) (Spenser and Price, 1971) in Tietz (1995).

PRINCIPLE: Many dyes are known to bind proteins. Bromocresol green at a pH below the isoelectric point of albumin reacts with albumin to cause a change in colour, which is proportional to the amount of albumin present.

PROCEDURE: Zero point five (0.5) milliliter of working dye solution were pipetted into a series of numbered cuvettes one for each of the five albumin working standards and one

for each unknown 0.5ml of 0.075 molar succinate buffer was pipetted into similar series of numbered cuvettes to serve as blank, 2.5ml of each standard as well as each unknown were added to appropriately numbered tubes in each series.They were mixed thoroughly and allowed to stand for 10min at room temperature. Using a spectrophotometer at 628nm, the absorbance of the first tube was used to adjust to zero absorbance. Absorbance (Ab) of the standards and the unknown were then measured.

CALCULATION:

Serum albumin (mg/dL)=

$$\frac{\text{Absorbance of Sample} \quad x \quad \text{concentration of standard}}{\text{Absorbance of standard.}}$$

3.3.4 DETERMINATION OF BLOOD GLUCOSE. Glucose oxidase method using 4-aminophenazone as oxygen acceptor (Trinder et al., 1986) in Tietz (1995).

PRINCIPLE: In the presence of glucose oxidase, glucose is oxidized to gluconic acid and hydrogen peroxide. The hydrogen peroxide in the presence of peroxidase oxidizes a suitable oxygen acceptor to give chromogenic oxidation products, the intensity of which is proportional to the amount of glucose initially present.

PROCEDURE: Protein precipitant (2.9ml) was added to 0.1ml of blood samples in labelled tubes (0.1ml of low standard, medium standard and high standard protein were treated similarly). These were mixed and centrifuged at 2000 rpm for 5 minutes, then 1ml of supernatant in each tube was collected and 3ml of colour reagent was added, (the blank used, was treated similarly with 1ml distilled water). All tubes were incubated at 37°C for 10 minutes with occasional brief shaking to ensure adequate aeration. Absorbances were read at 510nm wavelength .by setting the instrument to zero with the blank.

CALCULATION:

Serum glucose (mg/dl) =

$$\frac{\text{Absorbance of Sample} \quad x \quad \text{Concentration of standard}}{\text{Absorbance of Standard}}$$

3.3.5 DETERMINATION OF BLOOD ALKALINE PHOSPHATASE

Isselbacher et al. (1970) in Tietz (1995)

PRINCIPLE:

This is an enzyme found in mammalian (human) serum which hydrolyses synthetic phosphate esters at pH 9.

p-nitrophenyl phosphate + H_2O $\xrightarrow{\textbf{ALP}}$ Phosphate + p-nitrophenol

PROCEDURE.: Three screw cap cuvettes were labelled 1,2,3. Into cuvette (1) 3.5 ml reagent 1 mol/L diethanolamine buffer in 0.5mmol/L $MgCl_2$, pH 9 was pipetted The screw-cap cuvettes labeled (2) contained 0.5ml of standard (std). An aliquot of 0.5ml of heparinised plasma sample was pipetted into the sample cuvette (of 1cm light path) alongside 3.0ml of the reagent (1mol/L diethanolamine buffer; in 0.5mmol/L $MgCl_2$, pH 9). The substrate 0.1ml p-nitrophenyl phosphate was added to the reagent mixture which was then mixed and the initial absorbance read (at 405nm) the absorbance was later read after 1 min, 2 min and 3 min using a timer.

CALCULATION:

Plasma alkaline phosphatase (units/L)=

$$\frac{\text{Absorbance of Sample} \times \text{Concentration of standard}}{\text{Absorbance of Standard}}$$

3.3.6 DETERMINATION OF BLOOD TRANSAMINASES

3.3.6.1 **PRINCIPLE:** ALANINE TRANSAMINASE (GLUTAMIC PYRUVATE TRANSAMINASE- GPT) (E.C 2.6.1.2); Tietz (1995); Eissenthal and Danson (1992).

DL- Alanine + α – ketoglutarate $\xrightarrow{\text{ALT}}$ pyruvate + glutamate.

Glutamic pyruvate transaminase (GPT) catalyzes the above reaction. The pyruvate formed is then determined colorimetrically after reaction with 2.4 – dinitrophenyl hydrazine.

3.3.6.2 **PRINCIPLE** : ASPARTATE TRANSMINASE (GLUTAMATE OXALOACETATE TRANSAMINASE (GOT) (E.C.2.6.1.1.)

DL Aspartate + α-ketoglutarate $\xrightarrow{AST}$ oxaloacetate + glutamate

Glutamate oxaloacetate transaminase (GOT) catalyzes the above reaction. The oxaloacetate formed is then determined colorimetrically after reaction with 2,4-dinitrophenyl hydrazine.

Procedure:

The same procedure used for glutamate – pyruvate transaminase (GPT) in Appendix II is also applicable for glutamate-oxaloacetate transaminase (GOT). Two sets of test tubes were prepared and labeled R (for reagent blank) and U (for unknown). The following volumes were then pipetted into them.

	Reagent blank (R)	Unknown (U)
Sample	-	0.1ml
(Substrate) Reagent	0.5ml	0.5ml
Distilled water	0.1ml	-

The tubes were then mixed and incubated for thirty (30) minutes exactly at 37°C. The colour reagent (0.5ml) was also added to the reagent blank (R) and to the unknown. The tubes were again mixed and allowed to stand for exactly twenty (20) minutes at room temperature (25°C), then 0.1ml of 0.1N NaOH was added to the test tube containing the reagent blank and 0.5ml to that of the unknown. The test tubes were mixed and the absorbance of the unknown and standard reaction mixture in the kit was read against the reagent blank after five (5) minutes at 546nm.

CALCULATION:

Plasma GOT (units/L) =

$$\frac{\text{Absorbance of Sample} \times \text{Concentration of standard}}{\text{Absorbance of Standard}}$$

3.4. ANTI-THYROGLOBULIN (Tg Ab) OR ANTI-THYROID PEROXIDASE (TPO Ab), ELISA. (Pfahl et al., 1988, in Beever et al., 1989).

PRINCIPLE: Polystyrene wells are coated with purified human thyroglobulin Tg (or thyroid peroxidase TPO). Serum to be tested is incubated in the well. Anti-thyroglobulin (or TPO) antibodies, if present are bound to the thyroglobulin (antigen) coated solid phase. After washing, rabbit anti-human IgG conjugated with horse-radish peroxidase is added. At the end of a second incubation, unbound conjugate is removed by washing. When enzyme substrate is added, a blue colour develops if anti-thyroglobulin (or TPO) antibodies are present in the well. Sulfuric acid is added to stop the enzyme reaction and the absorbance of calibrators, controls or samples are measured using a plate reader with wavelength set at 450nm (Goodburn et al., 1981).

ASSAY PROCEDURE:

All reagents and materials were brought to room temperature for 30 minutes. The appropriate number of coated well strips was separated into a well labeled micro-titer grid. A 100µl of calibrators of 0 – 5000 iu/ml of Tg (or 0 to 1000 iu/ml of TPO) was pipetted into the appropriately labeled wells. Also 100µl of the positive control was placed in the so marked well, 100µl of each 1:100 sample dilution was added into the well marked out for each, with the first well serving as blank with 100µl of diluent only. The wells were incubated at room temperature for 30 minutes having been properly covered. The content after incubation was discarded by gentle inversion and washing using 300µl of buffer. This was repeated two times. The strips were inverted on a paper towel and blotted after each washing to dryness by tapping vigorously on the paper towel avoiding bubbles. 100µl of enzyme conjugate was added to the wells including blank wells. The wells were then covered and incubated at room temperature for 30 minutes, after which another series of washing and drying were carried out. A 100µl chromogenic substrate ($3,3^1$ $5,5^1$)-tetramethylbenzidine) (TMB) was added to all the wells and allowed to react for 30 minutes in each well at room temperature and a blue colour was developed. At 30minutes, expiration time, 100µl of stop solution was added to each well including the blank wells in a step wise manner. At the last 30 minutes of stoppage time, the well was tapped and the colour changed from blue to yellow. The micro-titer plate was read at 450nm using a micro-titer plate reader (Pfahl et al., 1989 in Beever et al., 1989).

The bench marks to determine the groups that tested positive for thyroglobulin auto-antibodies were with values ranging from 2.0 to > 200 units / ml) and thyroidperoxidase auto-antibodies were with values ranging from 2.0 to > 40 units / ml).

3.5.1 NATURAL KILLER CELL ACTIVITY (Nka) ASSAY:

PRINCIPLE: (Hatam et al., 1994 and Chang et al., 1993)

Flow cytometric method reliably distinguishes unique population of target (K562) and effector cells in patients' peripheral mononuclear blood cells (PMBC), allowing for an accurate assessment of NK functional activity. The target cells pre-stained with $3,3^1$-, dioctadecyloxacarbocyanine perchlorate (Dio) are mixed with the effector cells in a target cell / effector cell (T/E) ratio, of 1:50. Propidium iodide (PI) was used to mark the dead cells and the cells were analysed by fast analysis counter scan (FACS). Ivig (intravenous immunoglobulin) was used to measure NK cell response to suppression.

Natural killer cells (NK) are a subset of peripheral blood lymphocytes that mediate non-MHC (major histocompatibility complex)- restricted cytoxicity of foreign target cells.

Flow cytometric assay avoids the problems associated with the use of radioactivity.

The analysis of NK functional activity becomes of more practical use to certain chemical specialties and is useful in predicting patient outcome and treatment as well.

Percent lysis is calculated by the following equation:

% lysis = (quadrant events/quadrant z + quadrant z events) x 100

Where z = background control.

3.5.2. PROCEDURE:

Preparation of Target cells (K562)

K562 cells were grown in complete medium (CM) at 37°C in 5% CO_2 for 3 days prior to the assay, cells were sub- cultured every 24 hours, to ensure they are in the Log phase. The content of the culture flask was poured into 15ml plastic tube and washed in PBS. They were re-suspended in 1ml PBS at a concentration of $1x10^6$ /ml, then the cells were stained with10µl of 3mM Dio and incubated for 20 minutes at 37°C in 5% CO_2. Afterwards they were washed twice with PBS and resuspended in CM, counted and adjusted to $1x10^6$ /ml.

Effector Cell Preparation

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient technique, using Hystopaque – 1077 on the heparinized blood in ratio 1:1.5 with 5ml Hypaque in 15ml conical tube and 1:1 blood in PBS. These were centrifuged at 2000 rpm for 30 minutes and the interface ring was collected. The interface was washed twice in plain medium RPMI – 1640 and was suspended in CM. The cells were counted and adjusted to a concentration of $5x10^6$/ml.

STEP 1: Three (3) sets of tubes were used for patient samples. In the first set of tubes, 10µl of target cells and 500µl of PBMC effector cells were added ($5x10^6$ cells/ml) to create a T:E ratio of 1:50. 10µl of target cells were placed in a separate tube (for background control) and incubated for 2 hours at 37°C in 5% CO_2.

STEP 2; In the second set of tubes, was placed 1.5 mg/dl Ivig (10µl 5% IvIg) and incubated for 2 hours.

STEP 3; In the third set of tubes, was placed 6mg/dl Iv1g (diluted 1:2 from undiluted solution). They were centrifuged at 1000xg for 35 sec to pellet the B and T cells.

Then all tubes were incubated at 37°C in 5% CO_2 and were analysed on the cytometer. After 2 hours, 100µl of PI was added to all the tubes, 15 minutes before analysis, in which the control for the background contained only Dio-stained target (T) cells and PI (Chang. et al.. 1993; Hatam et al., 1994).

3.6.0 HORMONAL ASSAY:

3.6.1. PROLACTIN ELISA ASSAY (Utiola et al., 1981 in Tietz, 1995).

PRINCIPLE:

The quantitative prolactin analysis is based on a solid phase enzyme- linked immuno sorbent assay (ELISA). The analysis system utilizes one anti-prolactin antibody for solid phase (micro-titer wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horse-radish peroxidase- HRP) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubating for 60 minutes at room temperature the wells were washed with water to remove unbound labeled antibodies. A solution of TMB (3,3^1, 5,5^1- tetramethylbenzidine) is added and incubated for 20 minutes resulting in the development of a blue colour. The colour development is stopped with the addition of IN HCl and the colour is changed to yellow

and measured spectrophotometrically at 450nm. The concentration of prolactin is directly proportional to the colour intensity of the test sample.

3.6.2. PROLACTIN ELISA ASSAY PROCEDURE:

The number of the coated wells needed was arranged and labeled on a micro-titer plate, with appropriate data sheet for sample identification.

Fifty microliters (50µl) of standard, specimens, and control test samples were measured using micropipette into appropriate wells. Then 100µl of enzyme conjugate reagent was added into each well and thoroughly mixed for 10 seconds before it was incubated at room temperature for 60 minutes. The plates were washed 5 times using distilled water by repeated rinsing and flicking on an absorbent paper to remove all residual water droplets.

After this, 100µl of TMB solution was added to each well, gently mixed for 5 seconds before it was incubated for 20 minutes at room temperature.

The reaction was stopped by the addition of 100µl of stop solution (2N HCl) and mixed for 5 seconds. The mixture was read at 450nm on a micro-titer plate reader (Utiola et al., 1981 in Tietz, 1995).

3.6.3. PROGESTERONE, TESTOSTERONE AND ESTRADIOL ELISA ASSAY

PRINCIPLE: (Chen et al. (1991); Tietz (1995)).

The progesterone, testosterone or estradiol (E_2) enzyme immuno assay (EIA) is based on the principle of competitive binding between progesterone (testosterone or estradiol) in the test specimen and progesterone or testosterone, or E2 . HRP conjugate for a constant amount of rabbit anti-progesterone, testosterone or E_2 antibody.

Incubation: The goat anti rabbit IgG coated wells were incubated with 25µl progesterone, testosterone or E2, standards, controls, patient samples, 100µl progesterone, testosterone or E2, HRP conjugate reagent and 50µl rabbit anti-progesterone testosterone, E2, reagent at room temperature for 90minutes. The fixed amount of horse-radish peroxidase (HRP) labeled with the progesterone or testosterone, or E_2 plate, serve as the standard sample, or fixed quantity as control for a fixed number of binding sites for the specific progesterone, testosterone or E_2 as the specimen increases.

Unbound progesterone, testosterone or E_2 peroxidase conjugate was removed and the wells washed. Next a solution of TMB (3,3,5,5 tetramethylbenzidine) reagent was then added and incubated at room temperature for 20 minutes. This resulted in the development of a blue colour. The colour development was stopped with the addition of a stop solution (1N HCl), and the absorbance was measured spectrophotomerically at 450nm. The intensity of the colour formed was proportional to the amount of enzyme present and is inversely related to the amount of unlabelled progesterone or testosterone, or E_2 in the sample (Carreres, and Mooney, 1992).

3.6.4. PROGESTERONE ASSAY

PROCEDURE: Methods of Tietz (1995) improvement on Johansson and Jonasson (1971). The desired number of coated wells was secured in the holder tray, into which 25µl of standards, specimens and controls were put with appropriate markings and recordings. Then 100µl of working progesterone HRP conjugate reagent was added to each well. The first wells washing was done by flooding with distilled-deionized water and tapped on absorbent tissue paper in a repeated manner 5 times.

Thereafter, 50 µl of rabbit anti progesterone reagent was added to each well and thoroughly mixed for 30 seconds and incubated for 90 minutes at room temperature. The wells were washed again by flooding with distilled-deionized water and tapped on absorbent tissue paper in a repeated manner 5 times.

100µl of TMB reagent was later added into each well and was gently mixed for 10 seconds. The mixture was incubated for 20 minutes at room temperature. The reaction was terminated by adding 100µl of stop solution into each well, and gently mixed for 30 seconds before the plate was read at 450nm with a micro-titer plate reader within 15 minutes.

3.6.5. TESTOSTERONE ASSAY

PROCEDURE:

The desired numbers of coated wells were arranged and well labeled in the micro titer plates. Into each of the wells, was accurately measured 10µl of standard, patient samples and controls using a micropipette, after which 100µl of testosterone – HRP conjugate reagent was added to each well. This was followed by the addition of 50µl of rabbit anti-

testosterone reagent to each well. The mixture was thoroughly mixed for 30 seconds and then incubated for 90 minutes at 37° C.

The micro titer plates were rinsed and flicked on the absorbent 5 times repeatedly to wash the wells, using distilled – deionized water. Then 100µl of TMB reagent was added into each well and gently mixed for 5 seconds. These were incubated at room temperature for twenty minutes after which the reaction was stopped by the addition of 100µl of stop solution. After gently mixing for 30 seconds the plate was read at 450nm on a micro titer plate reader within 15 minutes (Chen et al., 1991).

3.6.6. ESTRADIOL ASSAY

PROCEDURE: Methods of Tietz (1995) improvement on Johansson and Jonasson (1971).

The desired number of coated wells was secured in the holder tray, into which 25µl of standards, specimens and controls were put with appropriate markings and recordings. Then 100µl of working estradiol HRP conjugate reagent was added to each well. The first wells washing was done by flooding with distilled-deionized water and tapped on absorbent tissue paper in a repeated manner 5 times.

Thereafter, 50 µl of rabbit anti-estradiol reagent was added to each well and thoroughly mixed for 30 seconds and incubated for 90 minutes at room temperature. The wells were washed again by flooding with distilled-deionized water and tapped on absorbent tissue paper in a repeated manner 5 times.

100µl of TMB reagent was later added into each well and was gently mixed for 10 seconds. The mixture was incubated for 20 minutes at room temperature. The reaction was terminated by adding 100µl of stop solution into each well, and gently mixed for 30 seconds before the plate was read at 450nm with a micro-titer plate reader within 15 minutes.

3.7.1 DETERMINATION OF ASCORBIC ACID (Halliwell, 1999)

PRINCIPLE

The most frequently used specimen is plasma collected with oxalate as anticoagulant. Serum is also used. EDTA or heparin can be used as, an anticoagulant for the collection of plasma samples.

In the dinitrophenylhydrazine method, the ascorbic acid content in whole blood remains stable for about 3 hours if the specimen is refrigerated. Vitamin C is stable for two weeks if the plasma specimen is promptly diluted with 6g/100ml of metaphosphoric acid and stored at – 20°C.

Tricholoracetic acid (10.0g/10ml) can also be used in place of metaphosphoric acid to prepare a protein – free filtrate of plasma or serum.

In the method commonly used for the determination of ascorbic acid; the acid reacts with 2,4 dinitrophenylhydrazine after oxidation of vitamin C to dehydroascorbic acid by either cupric sulfate or 2, 6-dichlorophenol-indophenol.

The dehydroascorbic acid in a strongly acid solution reacts with 2, 4-dinitrophenyl-hydrazine to form a dinitrophenylhydrazone. The hydrazone in the presence of concentrated sulfuric acid solution develops a red colour which can be measured spectrophotometrically. Thiourea is added to the dinitrophenyhydrazine reagent to prevent the oxidation, of the dinitrophenyhydrazine reagent by interfering substances.

3.7.1.2: PROCEDURE: Protein free filtrates of clear supernatant were obtained as follows; A 2.0ml freshly prepared metaphosphoric acid (6.0 gm/100ml) was added to each of the well labelled tubes containing 5.0ml of plasma samples. These were vortex mixed and centrifuged at 2500 rpm for 10 minutes.

Three sets of reaction tubes were set up as blank, standard, and the sample filtrate tubes. The blank tubes contained 1.2ml of metaphosphoric acid, the standard tubes contained 1.2ml of working solution of ascorbic acid, while 1.2ml of the sample filtrates were in the third sets of tubes.

0.4ml of dinitrophenylhydrazine in thio CU_2SO_4 was added to the three sets of tubes and were mixed and incubated at 37 °C for 3 hours . Afterwards the tubes were chilled in ice bath for 10 minutes and 2.0ml of cold H_2O_2 were slowly added and further vortex mixed at room temperature.

Absorbance's of samples and standard were read against the reagent blank to zero at 520nm.

$$\text{Calculation: vitamin C of test mg/dl} = \frac{\text{Absorbance of test- blank x dilution factor}}{\text{Absorbance of Standard- blank (ml)}}$$

3.7.2.1: DETERMINATION OF SERUM- FREE VITAMIN E

PRINCIPLE: Serum vitamin E content was determined by the method of Henry et al. (1974) in Utiola, et al. 1981). After precipitation of serum proteins the vitamin E is extracted into hexane and then quantified by measuring relative fluorescence at a specific activation and emission wavelength.

PROCEDURE: Three centrifuge tubes were well labeled as water blank, standard, and sample. Into the water blank tube nothing was placed, while 0.2ml of the working standard solution (μmol/dl) was placed in the standard tube and 0.2ml serum sample (unknown) was placed into the sample tube. Distilled water (1.2ml) was added to the blank, while 1.0ml distilled water was added to the standard tube and unknown tubes, all tubes were mixed for 30 seconds in a vortex mixer.

Also 1.8ml distilled ethanol was added to the blank, unknown tubes, and the standard tube with mixing for 30 seconds in a vortex mixer, and then 0.5ml of hexane was added to all tubes. These were shaken for 5 minutes by hand (or alternatively in a vortex mixer). The tubes were centrifuged for 5 minutes. Hexane layer was transferred to a quarz cuvette. The spectrofluorometer was set, with the activation wavelength at 295nm and the emission wavelength was determined as F value.

$$\text{Calculation: vitamin E of test } \mu g/dl = \frac{Fx - Fblk}{Fstd - F\,blk} \times 20$$

(key; Fx =sample , Fblk= reagent blank, Fstd= Vitamin E)

3.7.3.1: DETERMINATION OF PLASMA IRON AND ZINC BY ATOMIC ABSORPTION SPECTROMETRY (AAS)

PRINCIPLE

Atomic absorption spectrometry (AAS) is a technique used in the determination of at least 70 elements in amounts as low as 10-14ng with reasonable selectivity. Little manipulation by AAS is used in the measurement of the radiant energy absorbed by free atoms in the gaseous atoms species which consist of well-defined narrow lines at wavelength characteristic of the element involved. In practice, the samples (solution or solid) are vapourized and on further heating, the vapour dissociates into free atoms. The vapour is then allowed to absorb radiant energy of a characteristic wavelength. Lamps,

electrically heated and inductively coupled plasmas with lasers are commonly used to convert the sample into atom vapour (Hassan et al., 1984 in Biney et al., 1994).

The basic components of atomic absorption spectrophotometer are:

1. Radiation source (e.g. hollow – cathode or electrode-leads discharge amp) to emit the spectral line of the element of interest.
2. Atomization system (e.g. flame or furnace) to provide sufficient energy for analytic dissolution and vapourization as free atoms (2 main types, flame and electrothermal).
3. Monochromator for spectral dispersion and isolation of the spectral line to be measured.

Detector and data – logging device to measure and display result (Hassan et al., 1984 in Biney et al., 1994).

3.7.3.2: **PROCEDURE**

The Buck Scientific' 210VGP atomic absorption spectrophotometer located at Rotas Soilab Limited, Ibadan, was used. The 210VGP is a powerful, compact and cost effective solution to all atomic analyses. This spectrometer comes with instructions and these were strictly adhered to. Flame was used to convert the sample into atomic vapour and the wavelength was set to 248.5nm (5ppm). The values obtained were then calculated to give the value in milligram per litre (mg/L) and then to the corresponding microgram per deciliter (μg/dL).

3.8.0 STATISTICAL ANALYSIS:

Analysis of variance (ANOVA) and Pearson correlation with the two way test (two tailed Fisher's exact test) was utilized for the statistical analysis between groups. The null hypothesis was used within groups.

An important set condition was that the level combination of factors is not observed, when the number of subject is less than 2 in the sub groups of positive and negative, thus the corresponding population marginal mean is not estimable.

Ref. WWW.SPSS.COM 11.0, for Windows.

CHAPTER FOUR

4.0 RESULT ANALYSIS

4.1 Bio-data of the women studied:

The women who volunteered to take part in this study were grouped into their reproductive capabilities based on the medical bio-data and information collected from the administered questionnaires (appendix ii). The results are as shown in Tables 6 and 7. The analysis of the medical bio-data was such that, in the control group of 164 women, 46 (28 %) were nulligravida, 58 (35 %) were multiparous and 60 (37 %) were pregnant women. In the test group, out of 126 women, 34 (27 %) were primary infertile, 46 (36.5%) were secondary infertile and 46 (36.5%) were recurrent spontaneous aborter (RSA) group.

The age bracket of 20 – 28 years was designated as early marriage age category, of this nulligravida group had the highest number of women with the mean age value of 26.55 $\pm$ 7. 64 years. The middle age bracket of 29 – 37 years was designated as late marriage age category; of this, the pregnant women had the highest number of women with the mean age value of 31.15 $\pm$ 5.25 years compared with the multiparous women with the mean age of 36.55 $\pm$ 7.65 years, within the control group. On the other hand, majority of the women in the test subject group were in the late marriage age category, with the primary infertile women mean age of 32.65 $\pm$ 5.66 years, the secondary infertile women with mean age of 32.05 $\pm$ 5.60 years and the RSA group mean age of 31.42 $\pm$ 4.25 years. From the results in Tables 6 and 7, the mean age range of the selected euthyroid women used in this study showed that, for those who claimed that they married early, the inability to reproduce had changed their dream or hope to stop child bearing early in life.

Table 6: Biodata of the control and test groups

Euthyroid women	Nulligravida N= 46	Multiparous Non pregnant N= 58	Pregnant N= 60	Recurrent spontaneous Aborter N= 46	Secondary Infertile N= 46	Primary infertile N= 34
Age (years) **X±SE**	26.55 ± 7.64	36.55 ± 7.65	31.15±5.25	31.42 ± 4.25	32.05 ±5.60	32.65 ±5.66
Nos children (mean)	-	3.0±0.05	2.0± 0.06	0.25±0.09	1.0 ± 0.07	-
Pregnancy stage (trimester)	-	-	1st & 2nd	-	-	-
Years of infertility	-	-		4.27 ± 2.26	5.76 ± 0.63	5.76 ± 0.63

The values represent mean ± standard error **(X±SE)**
N = Number of patients.

Table7: Age distribution of women used in the study:

GROUPS Age Bracket (years)	Early marriage 20 – 28	Late marriage 29 – 37	Others >37 – 55
Control group (Total 164)			
46 Nulligravida	46	-	-
60 Pregnant	15	44	1
58 Multiparous	3	51	4
Test group (Total 126)			
34 Primary infertile	2	30	2
46 Secondary infertile	9	33	4
46 Recurrent spontaneous aborter	9	36	1

Note: The figures in the table represent number of women in the control and test groups.

4.2 Biochemical Parameters:

Table 8 depicts the mean serum concentration of cholesterol obtained in the control and test groups.The control group showed that the nulligravida had 125.61 ± 46.78 mg/dl value, representing the marginal mean of cholesterol in women with anti-Tg and anti-TPO (which comprises of the mean serum cholesterol concentration in the nulligravida that are anti-TPO positive with 125.94 ± 68.07 mg/dl value and those that are anti-TPO negative with 125.28 ± 64.18 mg/dl value, in which the positive was not significantly higher than the negative (P>0.05)). In the multiparous women the mean serum concentration of cholesterol was 169.87 ± 82.32 mg/dl representing the marginal mean of cholesterol in women with anti-Tg and anti-TPO (which comprises of the mean serum cholesterol concentration in the multiparous women that were anti-TPO positive with 166.74 ± 48.91 mg/dl and those that were anti-TPO negative with 173.00 ± 57.27 mg/dl, in which the positive was not significantly higher than the negative (P>0.05)). In the pregnant group, the mean serum concentration of cholesterol was found to be 1237.30 ± 72.47 mg/dl, representing the marginal mean of cholesterol in women with anti-Tg and anti-TPO (which comprises of the mean serum cholesterol concentration in the pregnant women that were anti-TPO positive with 1267.60 ± 49.71 mg/dl value and those that were anti-TPO negative with 1207.00 ± 36.15 mg/dl, in which the positive was significantly higher than the negative (P<0.05)).

The mean serum concentration of cholesterol obtained in the test group showed that, the cholesterol concentration was 185.08 ± 99.23 mg/dl, in the primary infertile women, representing the marginal mean of cholesterol in women with anti-TPO and anti-Tg (which comprises of the mean serum cholesterol concentration in the primary infertile women, that were anti-Tg positive with 198.16 ± 48.14 mg/dl and those that were anti-Tg negative with 172.00 ± 92.54 mg/dl, in which the positive was significantly higher than the negative (P<0.05)). In the secondary infertile

women the mean serum concentration of cholesterol was 176.50 ± 38.19 mg/dl, representing the marginal mean of cholesterol in women with anti-TPO and anti-Tg (which comprises of the mean serum cholesterol concentration in the secondary infertile women that were anti-Tg positive with 170.00 ± 47.40 mg/dl and those that were anti-Tg negative with 183.00 ± 72.29 mg/dl, in which the positive was significantly lower than the negative ($P<0.05$)). In the RSA group the mean serum concentration of cholesterol was 1766.50 ± 46.70 mg/dl, representing the marginal mean of cholesterol in women with anti-TPO and anti-Tg (which comprises of the mean serum cholesterol concentration in the RSA women that are anti-Tg positive and positive anti-TPO). The pregnant control women serum cholesterol concentration, were significantly higher than the values obtained in the test groups, except in the RSA women where a significantly higher value was obtained at $P< 0.05$.

The mean serum concentrations of triglyceride are presented in Table 9. Among the women in the control group, the pregnant group showed the highest mean triglyceride concentration of 617.87 ± 37.19 mg/dl representing the marginal mean of triglyceride in women with anti-Tg and anti-TPO (which comprises of the mean serum triglyceride concentrations in the pregnant women that were anti-TPO positive with 588.73 ± 25.51 mg/dl which was significantly lower than those that were negative to anti-TPO with 647.00 ± 69.86 mg/dl at $P< 0.05$). The mean serum concentration of triglycerides obtained for the nulligravida women was 150.32 ± 24.00 mg/dl, representing the marginal mean of triglyceride in women with anti-Tg and anti-TPO (which comprise of the mean serum triglyceride concentrations in the nulligravida women that are anti-TPO positive with 146.75 ± 34.93 mg/dl which was not significantly different from those who were negative to anti-TPO with 153.89 ± 32.93 mg/dl ($P> 0.05$)). In the multiparous women the mean serum concentration of triglyceride was 160.48 ± 42.24 mg/dl representing the

marginal mean of triglyceride in women with anti-Tg and anti-TPO (which comprise of the mean serum triglyceride concentrations in the multiparous women that were anti-TPO positive with 163.97 ± 25.10 mg/dl which was not significantly different from those that were negative to anti-TPO with 157.00 ± 80.67 mg/dl (P> 0.05)).

Among the test group, the women in the RSA group had mean serum triglyceride concentration of 271.35 ± 23.96 mg/dl representing the marginal mean of triglycerides in women with only positive anti-Tg and anti-TPO. That is the RSA women of the test group mean serum triglyceride were higher than that in the women in the primary infertile group. The primary infertile women, had mean serum triglyceride concentration of 193.14 ± 50.92 mg/ml representing the marginal mean of triglyceride in women with anti- TPO and anti- Tg (which comprises of the mean serum triglyceride concentration in the primary infertile group that are anti-Tg positive with 197.28 ± 24.70 mg/dl which was not significantly different from that of the negative anti-Tg with 189.00 ± 98.80 mg/dl (P > 0.05)). The mean serum concentration of triglyceride in the women of the secondary infertile group was 204.17 ± 70.91 mg/dl representing the marginal mean of triglyceride in women with anti- TPO and anti- Tg (which comprises of the mean serum triglyceride concentration in the secondary infertile group that are anti-Tg positive with 199.33 ± 24.32 mg/dl which was significantly lower than those that are anti-Tg negative with 209.00 ± 39.73 mg/dl, (P> 0.05)). The statistical comparison showed that the value obtained for the pregnant group was significantly higher than those of the infertile group especially the women in the RSA group at P< 0.05.

The mean serum concentrations of the fasting blood glucose obtained are presented in Table 10. The mean serum glucose concentration of the women in the nulligravida group was 66.31 ± 5.91

mg/dl representing the marginal mean of serum glucose in women with anti-Tg and anti-TPO, (which comprise of the mean serum glucose concentration in the nulligravida women that were anti-TPO positive with 67.63 ± 8.60 mg/dl and those that were anti-TPO negative with 65.00 ± 8.10mg/dl, in which the value for those that were positive was significantly higher than those that were negative at (P<0.05)). In the multiparous group, the mean serum glucose concentration was 51.98 ± 10.39 mg/dl, as the marginal mean of glucose in women with anti-Tg and anti-TPO (which comprise of the mean serum glucose concentration in the multiparous women that were anti-TPO positive with 41.97 ± 6.18 mg/dl and those that were anti-TPO negative with 62.00 ± 1.98 mg/dl, here the anti-TPO positive women's value was significantly lower than that for the anti-TPO negative (P<0.05)). In the pregnant group the mean serum glucose concentration was 47.32 ± 9.15 mg/dl as the marginal mean of glucose in women with anti-Tg and anti-TPO (which comprises of the mean serum glucose concentration in the pregnant group that are anti-TPO positive with 45.13 ± 6.28 mg/dl and those that were anti-TPO negative with 49.50 ± 1.72 mg/dl, here the anti-TPO positive was significantly higher than the anti-TPO negative (P<0.05)).

The mean serum glucose concentration in the women in the primary infertile group was 46.02 ± 1.25 mg/dl, as the marginal mean of glucose in women with anti-TPO and anti-Tg (which comprise of the mean serum glucose concentration in the primary infertile women that were anti-Tg positive with 66.03 ± 6.08 mg/dl and those that were anti-Tg negative with 26.00 ± 2.43 mg/dl. Here, the anti-Tg positive value was significantly higher than the anti-Tg negative (P<0.05)). In the secondary infertile the mean serum glucose concentration was 77.85 ± 1.74 mg/dl, as the marginal mean of glucose in women with anti-TPO and anti-Tg (which comprise of the mean serum glucose concentration in the secondary infertile women that were anti-Tg positive with 76.70 ± 5.99 mg/dl and those that were anti-Tg negative with 79.00 ± 3.44 mg/dl. In this regard the anti-Tg positive value was significantly lower than the anti-Tg negative

(P<0.05)). In the RSA, the mean serum glucose concentration was 93.18 ± 5.90 mg/dl representing the marginal mean of glucose in women with anti-TPO and anti-Tg. The values of the serum glucose concentration in all the women in the control groups were significantly lower than that for the women in the secondary infertile group and the RSA group, at P< 0.05.

The mean serum concentrations of albumin in the women are as shown in Table 11.This showed that the test groups except women in RSA group had higher values of albumin concentrations than the control groups. In the primary infertile women the mean serum albumin concentration was 3.99 ± 0. 23 mg/dl representing the marginal mean of serum albumin concentrations in women with anti-TPO and anti-Tg (which comprise of the mean serum albumin concentration in the primary infertile women that were anti-Tg positive with 4.05 ± 0.11 mg/dl and those that were anti-Tg negative with 3.95 ± 0.45 mg/dl, here the anti-Tg positive women value was not significantly different from the anti-Tg negative value (P>0.05)). The mean serum albumin concentration in the secondary infertile women was 4.48 ± 0.32 mg/dl representing the marginal mean of albumin concentration in the women with anti-TPO and anti-Tg (which comprise of the mean serum albumin concentration in the primary infertile women that were anti-Tg positive with 4.16 ± 0.11 mg/dl and those that were anti-Tg negative with 4.80 ± 0:63 mg/dl. here the anti-Tg positive value was not significantly different from the anti-Tg negative value (P>0.05)). The mean serum albumin concentration in the RSA group was 2.83 ± 0.11g/dl representing the marginal mean of albumin in women with anti-TPO and anti-Tg which are both positive .

In the control group, the mean serum albumin concentration in the women of the nulligravida group was 3.97 ± 0.11 mg/dl, representing the marginal mean of albumin in women with anti-Tg and anti-TPO (which comprise of the mean serum albumin concentration in the nulligravida

women that were anti-TPO positive with 3.90 ± 0.16 mg/dl and those that were anti-TPO negative with 4.04 ± 0.15 mg/dl, here the anti-TPO positive value was not significantly different from that for the anti-TPO negative). In the multiparous women the mean serum concentration of albumin was 3.58 ± 0.19 mg/dl representing the marginal mean of albumin in women with anti-Tg and anti-TPO (which comprise of the mean serum albumin concentration in the multiparous women that were anti-TPO positive with 3.44 ± 0.11 mg/dl and those that were anti-TPO negative with 3.73 ± 0.37 mg/dl, here the anti-TPO positive value was not significantly different from that for the anti-TPO negative). In the pregnant women the mean serum albumin concentration was 3.84 ± 0.17 mg/dl representing the marginal mean of albumin in women with anti-Tg and anti-TPO (which comprise of the mean serum albumin concentration in the pregnant women that were anti-TPO positive with 3.82 ± 0.12 mg/dl and those that were anti-TPO negative with 3.85 ± 0.32 mg/dl, here the anti-TPO positive value was not significantly different from the anti-TPO negative ($P>0.05$)). Statistical comparison showed that, the values for control groups were significantly lower than that for the infertile group, but higher than that for the RSA, at $P< 0.05$.

The Pearson (2 – tailed) correlation analysis showed that between the anti-TPO value and the serum glucose, serum cholesterol and serum albumin concentration there was no significance. Whereas there was significant correlation at 0.01 levels with serum triglyceride concentration. The correlation between anti-Tg value and the serum glucose and triglyceride concentration showed significant correlation at 0.01 levels, see Table 27 in appendix iii .

Table 8: COMPARATIVE ANALYSES OF CHOLESTEROL CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	**Cholesterol (mg/dl)**	**Cholesterol (mg/dl)**		**Cholesterol (mg/dl)**	
SUBJECTS	Statistical marginal mean	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	125.61 ± 46.78[c]	125.28 ± 64.18 n=18[a]	125.94 ± 68.07 n=16[a,b]	125.61 ± 46.78[c] n=18[b]	n.s.v n=16[a,b]
Multiparous Women n=34	169.87 ±82.32[c]	173.00 ±57.27 n=3[a]	166.74 ± 48.91 n=31[a, b]	169.87 ±82.32[c] n=3[b]	n.s.v n=31[a,b]
Pregnant Women n=34	1237.30 ± 72.47[c]	1207.00 ± 36.15 n=4[a]	1267.60 ± 49.71 n=30[a,b]	1237.30 ± 72.47[c] n=4[b]	n.s.v n=30[a,b]
Primary Infertile Women n =34	185.08 ± 99.23[c]	n.s.v n=2[a,b]	185.08 ± 99.23[c] n=32[b]	172.00 ± 92.54 n=2[a,b]	198.16 ± 48.14 n=32[a]
Secondary Infertile Women n =34	176.50 ± 38.19[c]	n.s.v n=1[a,b]	176.50 ± 38.19[c] n=33[b]	183.00 ± 72.29 n=1[a,b]	170.00 ± 47.40 n=33[a]
Recurrent Spont. **Aborter n=34**	1766.50 ± 46.70[c]	-	1766.50 ± 46.70[c] n=34[b]	-	1766.50 ± 46.70 n=34[a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant [a]P<0.05 = comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 = test vs. control group. C = marginal mean value n.s.v = no statistical value of any significance.

Table 9 : COMPARATIVE ANALYSES OF TRIGLYCERIDES CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROI WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	**Triglycerides (mg/dl)**	**Triglycerides (mg/dl)**		**Triglycerides (mg/dl)**	
SUBJECTS	Statistical marginal mean	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	150.32 ± 24.00 [c]	153.89 ± 32.93 n=18 [a]	146.75 ± 34.93 n=16 [a,b]	150.32 ± 24.00 [c] n=18 [b]	n.s.v n=16 [a]
Multiparous Women n=34	160.48 ± 42.24 [c]	157.00 ± 80.67 n=3 [a]	163.97 ± 25.10 n=31 [a,b]	160.48 ± 42.24 [c] n=3 [b]	n.s.v n=31 [a]
Pregnant Women n=34	617.87 ± 37.19 [c]	647.00 ± 69.86 n=4 [a]	588.73 ± 25.51 n=30 [a,b]	617.87 ± 37.19 [c] n=4 [b]	n.s.v n=30 [a]
Primary Infertile Women n = 34	193.14 ± 50.92 [c]	n.s.v n=2 [a,b]	193.14 ± 50.92 [c] n=32 [b]	189.00 ± 98.80 n=2 [a,b]	197.28 ± 24.70 n=32 [a]
Secondary Infertile Women n =34	204.17 ± 70.91 [c]	n.s.v n=1 [a,b]	204.17 ± 70.91 [c] n=33 [b]	209.00 ± 39.73 n=1 [a,b]	199.33 ± 24.32 n=33 [a]
Recurrent Spont. **Aborter n=34**	271.35 ± 23.96 [c]	-	271.35 ± 23.96 [c] n=34 [b]	-	271.35 ± 23.96 n34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c= marginal mean value

Table 10: COMPARATIVE ANALYSES OF FASTING GLUCOSE CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	**Fasting glucose (mg/dl)**	**Fasting glucose (mg/dl)**		**Fasting glucose (mg/dl)**	
SUBJECTS	Statistical marginal mean	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	66.31 ± 5.91 [c]	65.00 ± 8.10 n=18 [a]	67.63 ± 8.60 n=16 [a,b]	n.s.v n=18 [a]	66.31 ± 5.91 [c] n =16 [b]
Multiparous Women n=34	51.98 ± 10.39 [c]	62.00 ± 1.98 n= 3 [a]	41.97 ± 6.18 n=31 [a,b]	n.s.v n= 3 [a]	51.98 ± 10.39 [c] n =31 [b]
Pregnant Women n=34	47.32 ± 9.15 [c]	49.50 ± 1.72 n=4 [a]	45.13 ± 6.28 n=30 [a,b]	n.s.v n=4 [a]	47.32 ± 9.15 [c] n=30 [b]
Primary Infertile Women n =34	46.02 ± 1.25 [c]	n.s.v n=2 [a,b]	46.02 ± 1.25 [c] n=32 [b]	26.00 ± 2.43 n=2 [a]	66.03 ± 6.08 n=32 [a,b]
Secondary Infertile Women n =34	77.85 ± 1.74 [c]	n.s.v n-1 [a,b]	77.85 ± 1.74 [c] n=33 [b]	79.00 ± 3.44 n=1 [a]	76.70 ± 5.99 n=33 [a,b]
Recurrent Spont. **Aborter n=34**	93.18 ± 5.90 [c]	-	93.18 ± 5.90 [c] n=34 [b]	-	93.18 ± 5.90 n=34 [a,b]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance. [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c= marginal mean value

Table 11: COMPARATIVE ANALYSES OF ALBUMIN CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	**Albumin (mg/dl)**	**Albumin (mg/dl)**		**Albumin (mg/dl)**	
SUBJECTS	Statistical marginal mean	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	3.97 ± 0.11^{c}	4.04 ± 0.15 n=18 [a]	3.90 ± 0.16 n=16 [a,b]	3.97 ± 0.11^{c} n=34 [b]	-
Multiparous Women n=34	3.58 ± 0.19^{c}	3.73 ± 0.37 n=3 [a]	3.44 ± 0.11 n=31 [a,b]	3.58 ± 0.19^{c} n=34 [b]	-
Pregnant Women n=34	3.84 ± 0.17^{c}	3.85 ± 0.32 n=4 [a]	3.82 ± 0.12 n=30 [a,b]	3.84 ± 0.17^{c} n=34 [b]	-
Primary Infertile Women n = 34	3.99 ± 0.23^{c}	n.s.v n=2 [a,b]	3.99 ± 0.23^{c} n=32 [b]	3.95 ± 0.45 n=2 [a,b]	4.05 ± 0.11 n=32 [a]
Secondary Infertile Women n =34	4.48 ± 0.32^{c}	n.s.v n=1 [a,b]	4.48 ± 0.32^{c} n=33 [b]	4.80 ± 0.63 n=1 [a,b]	4.16 ± 0.11 n=33 [a]
Recurrent Spont. **Aborter n=34**	2.83 ± 0.11^{c}	-	2.83 ± 0.11^{c} n=34 [b]	-	2.83 ± 0.11 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance. [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c= marginal mean value.

4.3 The metabolic and physiological enzyme:

The mean serum acid phosphatase activities in Table 12, showed that the value in the control nulligravida women was 59.04 ± 4.45 unit/L, being the marginal mean of acid phosphatase activity in women with anti-Tg and anti- TPO (this included those women that were anti-TPO positive with 58.29 ± 7.36 unit /L and those that were anti-TPO negative with 59.80 ± 5.03 unit /L, where the anti TPO positive value was not significantly different from that of the anti-TPO negative (P>0.05)). In the multiparous group the mean serum acid phosphatase activity was 60.20 ± 7.22 unit/L, being the marginal mean of acid phosphatase activity in women with anti-Tg and anti- TPO (this included those women that are anti-TPO positive with the value which was 57.40 ± 4.35 unit /L and anti-TPO negative with 63.00 ± 13.76 unit /L, where the anti TPO positive value was significantly lower than that for the anti-TPO negative (P<0.05)). In the pregnant group, the mean serum acid phosphatase activity was 58.71 ± 6.05 unit/L, being the marginal mean of acid phosphatase activity for women with anti-Tg and anti- TPO (this included those women that are anti-TPO positive with 54.42 ± 4.47 unit /L and anti-TPO negative with 63.00 ± 11.24 unit /L, where the value for the anti TPO positive women was significantly lower than those that were anti-TPO negative (P<0.05)).

The mean serum acid phosphatase activity in the test group, namely the primary infertile group was 68.90 ± 7.22 unit/L, being the marginal mean of acid phosphatase activity in women with anti- TPO and anti- Tg (this included those women that are anti-Tg positive with 80.80 ± 4.35 unit /L and anti-Tg negative with 57.00 ± 13.76 unit /L, while the value for the women that were anti- Tg positive was significantly higher than for those that were anti-Tg negative (P<0.05)). In the secondary infertile group, the mean serum acid phosphatase activity was 93.68 ± 4.15 unit/L, being the marginal mean of acid phosphatase activity of women that were anti-TPO and anti- Tg positive). In the women in the RSA group the mean serum acid phosphatase activity was 54.55 ±

4.15 unit/L, being the marginal mean of acid phosphatase activity for the women positive to anti-TPO and anti- Tg). The statistical analysis showed that the women in the primary infertile and spontaneous aborter groups had values that were significantly lower than those that were multiparous and pregnant at $P< 0.05$.

The mean serum alkaline phosphatase activities are as shown in Table 13. In the control nulligravida group the activity was 18.61 $\pm$ 2.41 unit/L, being the marginal mean of alkaline phosphatase activity in the women with anti-Tg and anti- TPO (this included those women that were anti-TPO positive with 24.86 $\pm$ 3.97 unit /L and anti-TPO negative with 12.35 $\pm$ 2.71 unit /L, where the value for the anti TPO positive women was significantly higher than that for the anti-TPO negative ($P<0.05$)). In the multiparous group the mean serum alkaline phosphatase activity was 122.51 $\pm$ 3.90 unit/L, being the marginal mean of alkaline phosphatase activity for women with anti-Tg and anti- TPO (this included those women that were anti-TPO positive with 113.37 $\pm$ 0.44 unit /L and the anti-TPO negative with 131.65 $\pm$ 7.43 unit /L, where the value in women who were anti TPO positive was significantly lower than those that were anti-TPO negative ($P<0.05$)). In the pregnant women group, the mean serum alkaline phosphatase activity was 207.19 $\pm$ 3.26 unit/L, being the marginal mean of alkaline phosphatase activity in women having anti-Tg and anti- TPO (this included those women that were anti-TPO positive with 134.44 $\pm$ 24.11 unit /L and anti-TPO negative with 279.93 $\pm$ 6.07 unit /L, where the anti TPO positive value was significantly lower than that for the anti-TPO negative ($P<0.05$)).

In the test groups, the mean serum alkaline phosphatase activity in the primary infertile women group was 61.17 $\pm$ 3.90 unit/L, being the marginal mean of alkaline phosphatase activity for women with anti-TPO and anti- Tg (this included those women that were anti-Tg positive with 71.35 $\pm$ 2.35 unit /L and those that were anti-Tg negative with 51.00 $\pm$ 7.43 unit /L, where the

anti TPO positive value was significantly high (P<0.05)). In the secondary infertile group the mean serum alkaline phosphatase activity was 127.17 ± 2.24 unit/L being the marginal mean of alkaline phosphatase activity in women who were anti-TPO and anti- Tg positive). In the women of the RSA group the mean serum alkaline phosphatase activity was 103.89 ± 2.24 unit/L being the marginal mean of alkaline phosphatase activity for anti-TPO and anti- Tg positive women. At $P < 0.05$, the values for the secondary and primary infertile women were significantly higher than the value for the women in the control groups.

The mean serum glutamate oxaloacetate transaminase activities are as shown in Table 14. In the control, the women in the nulligravida group had mean serum glutamate oxaloacetate transaminase activity of 12.89 ± 2.76 unit/L, being the marginal mean of glutamate oxaloacetate transaminase activity for anti-Tg and anti-TPO (this included those women that are anti-TPO positive with 15.57 ± 4.55 unit /L and anti-TPO negative with 10.20 ± 3.11 unit /L, where the value for the women who were anti-TPO positive was significantly higher than those that were anti-TPO negative (P<0.05)). In the multiparous group, the mean serum glutamate oxaloacetate transaminase activity in the women was 8.00 ± 4.47 unit/L being the marginal mean of glutamate oxaloacetate transaminase activity for women with anti-Tg and anti- TPO (this included those women that were anti-TPO positive with 15.00 ± 2.76 unit /L and anti-TPO negative with 10.00 ± 8.52 unit /L, where those that were anti TPO positive had significantly higher value than the anti-TPO negative (P<0.05)). In the pregnant group, the mean serum glutamate oxaloacetate transaminase activity was 9.90 ± 3.74 unit/L, being the marginal mean of glutamate oxaloacetate transaminase activity for women having anti-Tg and anti-TPO (this included those women that are anti-TPO positive with 12.79 ± 2.76 unit /L and those that were anti-TPO negative with 7.00 ± 6.96 unit /L, where the anti TPO positive women's value was significantly higher than that for the anti-TPO negative (P<0.05)).

The obtained glutamate oxaloacetate transaminase activity in the test group were as follows; in the primary infertile women the mean serum glutamate oxaloacetate transaminase activity was 17.35 ± 4.46 unit/L, being the marginal mean of glutamate oxaloacetate transaminase activity in women having anti-TPO and anti- Tg (this included those women that are anti-Tg positive with 12.05 ± 2.69 unit /L and anti-Tg negative with 23.00 ± 8.52 unit /L, where the anti Tg positive value was significantly lower than the anti-Tg negative value ($P<0.05$)). In the secondary infertile group the mean serum glutamate oxaloacetate transaminase activity in the women was 6.00 ± 2.57 unit/L, being the marginal mean of glutamate oxaloacetate transaminase activity for the women that were anti-TPO and anti- Tg positive). In the RSA group, the mean serum glutamate oxaloacetate transaminase activity was 13.41 ± 2.57 unit/L being the marginal mean of glutamate oxaloacetate transaminase activity for the women who were anti-TPO and anti- Tg positive). When this was compared, there was no statistical significance between the test groups and control groups, ($P> 0.05$).

The mean serum glutamate pyruvate transaminase activity obtained was as presented in Table 15. In one of the control groups, that is, the nulligravida group the mean serum glutamate pyruvate transaminase activity was 11.87 ± 0.45 unit/L, being the marginal mean of glutamate pyruvate transaminase activity in women having anti-Tg and anti- TPO (this included those women that were anti-TPO positive with 12.00 ± 0.75 unit /L and anti-TPO negative with 11.73 ± 0.51 unit /L, where, the value in the anti TPO positive women was not significantly higher than that in the anti-TPO negative, ($P>0.05$)). In the multiparous group, the mean serum glutamate pyruvate transaminase activity was 11.20 ± 0.73 unit /L, being the marginal mean of glutamate pyruvate transaminase activity for the women having anti-Tg and anti- TPO (this included those women that are anti-TPO positive with 11.40 ± 1.44 unit /L and anti-TPO negative with 11.00 ±

1.40 unit /L, where the value in the women who were anti -TPO positive was not significantly different from those that were anti-TPO negative, (P>0.05)). In the pregnant group the mean serum glutamate pyruvate transaminase activity was 12.40 ± 0.61 unit/L, being the marginal mean of glutamate pyruvate transaminase activity for women having anti-Tg and anti- TPO (this included those women that are anti-TPO positive with 12.79 ± 0.45 unit /L and anti-TPO negative with 12.00 ± 1.14 unit /L, where the activity in the anti TPO positive women was not significantly higher than those that were anti-TPO negative, (P>0.05)).

In the primary infertile group, the mean serum glutamate pyruvate transaminase activity level was 15.63 ± 0.73 unit/L, being the marginal mean of glutamate pyruvate transaminase activity for the women having anti-TPO and anti- Tg (this included those women that were anti-Tg positive with 14.25 ± 0.44 unit /L and anti-Tg negative with 17.00 ± 1.40 unit /L, where the value in the women who were anti Tg positive was significantly lower than the anti-Tg negative at P<0.05). In the secondary infertile group the mean serum glutamate pyruvate transaminase activity was 12.95 ± 0.42 unit/L, being the marginal mean of glutamate pyruvate transaminase activity in the women positive to both anti-TPO and anti-Tg). In the RSA group, the mean serum glutamate pyruvate transaminase activity was 14.50 ± 0.42 unit/L, being the marginal mean of glutamate pyruvate transaminase activity in women positive to both anti-TPO and anti-Tg). Overall, at P < 0.05, the control groups' value were significantly lower than that of the test groups.

The Pearson (2 – tailed) correlation performed between the anti-TPO values showed significant correlation at 0.01 with only serum glutamate pyruvate transaminase activity. Whereas there was significant correlation at 0.01 between anti-Tg value with both serum glutamate pyruvate transaminase and acid phosphatase activity as shown in Table 28 (appendix iii).

Table 12: COMPARATIVE ANALYSES OF THE SERUM ACID PHOSPHATASE ACTIVITIES IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Acid Phosphatase Units/L	Acid Phosphatase Units/L		Acid Phosphatase Units/L	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=22	59.04 ± 4.45 [c]	59.80 ± 5.03 n=15 [a]	58.29 ± 7.36 n=7 [a,b]	59.04 ± 4.45 [c] n=15 [b]	n.s.v n=7
Multiparous Women n=22	60.20 ± 7.22 [c]	63.00 ± 13.76 n=2 [a]	57.40 ± 4.35 n=20 [a,b]	60.20 ± 7.22 [c] n=2 [b]	n.s.v n=20
Pregnant Women n=22	58.71 ± 6.05 [c]	63.00 ± 11.24 n=3 [a]	54.42 ±4.47 n=19 [a,b]	58.71 ± 6.05 [c] n=3 [b]	n.s.v n=19
Primary Infertile Women n = 22	68.90 ± 7.22 [c]	n.s.v n=2	68.90 ± 7.22 [c] n=20 [b]	57.00 ± 13.76 n=2 [a,b]	80.80 ± 4.35 n=20 [a,b]
Secondary Infertile Women n =22	93.68 ± 4.15 [c]	-	93.68 ± 4.15 [c] n=22 [b]	-	93.68 ± 4.15 n=22 [b]
Recurrent Spont. Aborter n=22	54.55 ± 4.15 [c]	-	54.55 ± 4.15 [c] n=22 [b]	-	54.55 ± 4.15 n=22 [b]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ – significant, n.s.v = no statistical value of any significance [a]$P<0.05$ comparing positive (+) and negative (-) Anti-TPO /Tg [b]$P<0.05$ test vs. control group. c= marginal mean value.

Table 13: COMPARATIVE ANALYSES OF THE SERUM ALKALINE PHOSPHATASE ACTIVITIES IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Alkaline Phosphatase Units/L	Alkaline Phosphatase Units/L		Alkaline Phosphatase Units/L	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=22	18.61 ± 2.41 [c]	12.35 ± 2.71 n=15 [a]	24.86 ± 3.97 n=7 [a,b]	18.61 ± 2.41 [c] n=15 [b]	n.s.v n=7
Multiparous Women n=22	122.51 ± 3.90 [c]	131.65 ± 7.43 n=2 [a]	113.37 ± 0.44 n=20 [a,b]	122.51 ± 3.90 [c] n=2 [b]	n.s.v n=20
Pregnant Women n=22	207.19 ± 3.26 [c]	279.93 ± 6.07 n=3 [a]	134.44 ± 24.11 n=19 [a,b]	207.19 ± 3.26 [c] n=3 [b]	n.s.v n=19
Primary Infertile Women n =22	61.17 ± 3.90 [c]	n.s.v n=2	61.17 ± 3.90 [c] n=20 [b]	51.00 ± 7.43 n=2 [a,b]	71.35 ± 2.35 n=20 [b]
Secondary Infertile Women n =22	127.17 ± 2.24 [c]	-	127.17 ± 2.24 [c] n=22 [b]	-	127.17 ± 2.24 n=22 [b]
Recurrent Spont. Aborter n=22	103.89 ± 2.24 [c]	-	103.89 ± 2.24 [c] n=22 [b]	-	103.89 ± 2.24 n=22 [b]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ – significant, n.s.v = no statistical value of any significance, [a]$P<0.05$ comparing positive (+) and negative (-) Anti-TPO /Tg [b]$P<0.05$ test vs. control group. c= marginal mean value.

Table 14 : COMPARATIVE ANALYSES OF THE SERUM GLUTAMATE OXALOACETATE TRANSAMINASE ACTIVITIES IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY

PARAMETER	Glutamate Oxaloacetate Transaminase (GOT) Units/L	Glutamate Oxaloacetate Transaminase (GOT) Units/L		Glutamate Oxaloacetate Transaminase (GOT) Units/L	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=22	12.89 ± 2.76^{c}	10.20 ± 3.11 n=15 [a,b]	15.57 ± 4.55 n=7 [a,b]	12.89 ± 2.76^{c} n=15 [b]	n.s.v n=7
Multiparous Women n=22	8.00 ± 4.47^{c}	10.00 ± 8.52 n=2 [a,b]	15.00 ± 2.76 n=20 [a,b]	8.00 ± 4.47^{c} n=2 [b]	n.s.v n=20
Pregnant Women n=22	9.90 ± 3.74^{c}	7.00 ± 6.96 n=3 [a,b]	12.79 ± 2.76 n=19 [a,b]	9.90 ± 3.74^{c} n=3 [b]	n.s.v n=19
Primary Infertile Women n =22	17.35 ± 4.46^{c}	n.s.v n=2	17.35 ± 4.46^{c} n=20 [b]	23.00 ± 8.52 n=2 [a,b]	12.05 ± 2.69 n=20 [a,b]
Secondary Infertile Women n =22	6.00 ± 2.57^{c}	-	6.00 ± 2.57^{c} n=22 [b]	-	6.00 ± 2.57 n=22 [b]
Recurrent Spont. Aborter n=22	13.41 ± 2.57^{c}	-	13.41 ± 2.57^{c} n=22 [b]	-	13.41 ± 2.57 **n=22** [b]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ – significant, n.s.v = no statistical value of any significance, [a]$P<0.05$ comparing positive (+) and negative (-) Anti-TPO /Tg [b]$P<0.05$ test vs. control group. c= marginal mean value.

Table 15 : COMPARATIVE ANALYSES OF THE SERUM GLUTAMATE PYRUVATE TRANSAMINASE ACTIVITIES IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Glutamate Pyruvate Transaminase (GPT) Units/L	Glutamate Pyruvate Transaminase (GPT) Units/L		Glutamate Pyruvate Transaminase (GPT) Units/L	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=22	11.87 ± 0.45^{c}	11.73 ± 0.51 $n=15^{a,b}$	12.00 ± 0.75 $n=7^{a,b}$	11.87 ± 0.45^{c} $n=15^{b}$	n.s.v n=7
Multiparous Women n=22	11.20 ± 0.73^{c}	11.00 ± 1.40 $n=2^{a,b}$	11.40 ± 1.44 $n=20^{a,b}$	11.20 ± 0.73^{c} $n=2^{b}$	n.s.v n=20
Pregnant Women n=22	12.40 ± 0.61^{c}	12.00 ± 1.14 $n=3^{a,b}$	12.79 ± 0.45 $n=19^{a,b}$	12.40 ± 0.61^{c} $n=3^{b}$	n.s.v n=19
Primary Infertile Women n = 22	15.63 ± 0.73^{c}	n.s.v n=2	15.63 ± 0.73^{c} $n=20^{b}$	17.00 ± 1.40 $n=2^{a,b}$	14.25 ± 0.44 $n=20^{a,b}$
Secondary Infertile Women n =22	12.96 ± 0.42^{c}	-	12.96 ± 0.42^{c} $n=22^{b}$	-	12.96 ± 0.42 $n=22^{b}$
Recurrent Spont. Aborter n=22	14.50 ± 0.42^{c}	-	14.50 ± 0.42^{c} $n=22^{b}$	-	14.50 ± 0.42 $n=22^{b}$

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ -- significant, n.s.v = no statistical value of any significance, $^{a}P<0.05$ comparing positive (+) and negative (-) Anti-TPO /Tg $^{b}P<0.05$ test vs. control group. c= marginal mean value

4.4.1 The agglutination evaluation:

Table 16 depicts the agglutination evaluation results, which showed the presence of anti-thyroglobulin (Tg Ab) and anti-thyroperoxidase (TPO Ab) antibodies in the serum of control and test groups. The results established the presence of auto-antibodies to thyroglobulin (Tg Ab) with the highest number in the women in the primary infertile group of 86% and 75% in the RSA group. The anti-thyroperoxidase was found in 80% of women in the infertile group and 79% in the RSA group ie among the women experiencing reproductive failure.

Table 16: AUTO-ANTIBODY IMMUNOPHENOTYPIC AGGLUTINATION VALUES OF Tg Ab AND TPO Ab OF CONTROL AND TEST GROUPS

AGGLUTINATION (Euthyroid Women) Tg Ab and TPOAb	CONTROL GROUP (Nulligravida) Tg Ab N=26 (46) TPOAb N=23 (46)	TEST GROUP Primary infertile N=28 (34)	TEST GROUP RSA N=33 (46)
Tg Ab ++	2 (8%)	24 (86%)	25 (75%)
Tg Ab +	5 (19%)	4 (14%)	7 (22%)
Tg Ab -	19 (73%)	0	1 (3%)
ND	20	6	13
TPO Ab ++	1 (4%)	22 (80%)	26 (79%)
TPO Ab +	3 (13%)	4 (15%)	7 (21%)
TPO Ab-	19 (83%)	2 (5%)	0
ND	23	6	13

Note: The figures in the table represent numbers of women in the control and test groups.

KEY:
++ve - Strongly positive
+ve - slightly positive
-ve - negative
ND - not determined.

4.4.2 The serum anti-thyroglobulin and thyroperoxidase auto-antibody mean titer values:

The obtained mean titer values of the autoimmune species of anti-thyroperoxidase (anti-TPO), considered to be positive were those values that were above the benchmark of the normal level of < 40 U/ml. The results on Table 17 showed that within the control group, the multiparous women had higher serum anti-TPO positive mean titer value of 76.01 $\pm$ 0.97 Unit/ml compared to others, having anti-TPO negative within the normal range. Comparing also the serum anti-TPO positive mean titer of 88.88 $\pm$ 1.35 Unit /ml of the women in the nulligravida group and 62.50 $\pm$ 0.90 Unit /ml in the pregnant group, within the control groups, compared with that of the negative anti-TPO of the other control group there was no significant difference at $P < 0.05$. Whereas, comparing the mean titer of 1503.50 $\pm$ 1.16 Unit /ml in the primary infertile women and 747.41 $\pm$ 0.99 Unit /ml in the recurrent spontaneous aborter and 613.39 $\pm$ 0.99 Unit /ml in the secondary infertile women against control groups, they were significantly higher at $P < 0.05$. The anti-thyroperoxidase anti-TPO negatve mean titer values obtained in the control group showed that, the women in the nulligravida group had 22.48 $\pm$ 1.47 Unit /ml, the multiparous group had 33.60 $\pm$ 1.31 Unit /ml and the pregnant group had 33.50 $\pm$ 3.37 Unit /ml . They were all within the normal range. The mean serum titer value of the anti- throglobulin (anti- Tg) in the women studied are as shown in Table 17. The bench marks for the determination of the anti-Tg level that was above normal level, of < 200 Unit /ml. The anti- Tg values were all negative, in the women of the control group with 42.48 $\pm$ 3.16 Unit /ml in the nulligravida group, 32.02 $\pm$ 2.82 Unit /ml in the multiparous group and 31.90 $\pm$ 2.77 Unit /ml in the pregnant group. The titer value in the test group positive to anti-Tg was 539.59 $\pm$ 3.79 Unit /ml in the primary infertile, 809.65 $\pm$ 3.23 Unit /ml in the secondary infertile and 490.00 $\pm$ 3.20 Unit /ml in the spontaneous aborter group. These were significantly higher ($P<0.05$) than the negative anti-Tg in the control group, as well as within the test group with negative anti-Tg of 144.00 $\pm$ 15.17 Unit /ml in the primary infertile, 138.00 $\pm$ 15.17 Unit /ml in secondary infertile and 122.00 $\pm$ 21.45 Unit /ml in the spontaneous aborter group.

TABLE 17 : COMPARATIVE ANALYSES OF SERUM THYROGLOBULIN AND THYROPEROXIDASE AUTO ANTIBODY ELISA VALUES OF THE WOMEN STUDIED.

	Overall marginal mean /Number of Women with		**Mean TgAb (unit/ml) x + S.E**		**Mean TpoAb (unit/ml) x ± S.E.**		**Age range (years) / Duration of years of infertility**	
PARAMETERS N=290	Anti-Tg	Anti-TPO	Anti-Tg(-) N=169	Anti-Tg(+) N=121	Anti-TPO(-) N=35	Anti-TPO(+) N=255	Age range	Duration
CONROL GROUP								
Nulligravida Women	42.48 ± 3.16 n=46	55.68 ± 1.00 n=46	42.48 ± 3.16 n=46	nill	22.48 ± 1.47 n=21	88.88±1.35 n=25	20-50	-
Multiparous Women	32.02 ± 2.82 n=58	54.81 ± 1.17 n=58	32.02 ± 2.82 n=58	nill	33.60 ± 1.31 n=10	76.01 ± 0.97 n=48	20-28	-
Pregnant Women	31.90 ± 2.77 n=60	48.00 ± 1.74 n=60	31.90 ± 2.77 n=60	nill	33.50 ±3.37 n=4	62.50 ± 0.90 n=56	20-46	-
TEST GROUP								
Primary Infertile Women	341.80 ± 7.82 n=34	1503.50 ± 1.16 n=34	144.00 ± 15.17 n=.2	539.59 ± 3.79 n=32	nill	1503.50±1.16 n=34	20-48	2-17
Secondary Infertile Women	473.82 ± 7.75 n=46	613.39 ± 0.99 n=46	138.00 ± 15.17 n=2	809.65± 3.23 n=44	nill	613.39 ± 0.99 n=46	21-48	0.5-10
Recurrent Spont. Aborter	306.00 ± 10.84 n=46	747.41 ± 0.99 n=46	122.00± 21.45 n=1	490.00.± 3.20 n=45	nill	747.41 ± 0.99 n=46	20-39	1-8

Data are expressed as mean ± SEand analyzed by ANOVA (Two-way test)

P<0.05 – significant [a]P<0.05 comparing Anti-Tg/ Anti-TPO positive vs negative, [b]P<0.05 infertile women vscontrol group.

4.5 Flow cytometric CD counts;

Table 18 depicts the result obtained for the natural killer cells (NK) determined by flow cytometric technique. The NK cell value of CD 56 + 16 obtained in the women of the multiparous group with anti-TPO positive was 7.24 ± 0.57 %, this was significantly lower than the value obtained in the women of the primary infertile group with anti-TPO positive of 13.31± 1.11 % and anti-Tg positive of 14.92 ± 0.70 % at $P < 0.05$. The titer value of CD 56 + 16 obtained in the women of the RSA group with anti-TPO positive was 14.57 ± 0.65 % which when compared at $P < 0.05$, there was no significant difference between this value of the test group and the value of CD 56 + 16 obtained in the women of the nulligravida group with anti-TPO positive which was 14.55 ± 0.86%.

The CD56 count obtained in the nulligravida women positive to anti-TPO in the control group was 6.21 ± 0.51 % and in the multiparous women was 6.67 ± 0.34%, which was significantly higher when compared with the CD56 count obtained in the primary infertile women positive to anti-TPO of 2.78 ± 0.66 % and the count obtained in the RSA women women positive to anti-TPO and anti-Tg of 2.60 ± 0.46 % marginal mean ($P<0.05$).

Conversely the CD19 count determined in the nulligravida women positive to anti-TPO was 11.43 ± 0.90 % which was significantly lower when compared with the CD19 count in the multiparous women positive to anti-TPO as against the the CD19 count in the women positive to anti-TPO with 22.85 ± 1.17 % and positive to anti-Tg with 20.85 ± 0.74 % in the primary infertile women as well as the CD19 count in the women positive to anti-TPO with 19.29 ± 0.81% in the RSA women,at $P < 0.05$.

The CD3 count obtained in the nulligravida women that were negative to anti–TPO was 70.96 ± 0.87 % and anti-Tg was 72.72 ± 0.88 %. Also the CD3 count obtained in the multiparous women that were negative to anti-Tg was 70.68 ± 1.14 % and anti-TPO was 67.81 ± 1.41 %. These were

significantly lower ($P < 0.05$), when compared to the CD3 count obtained in the primary infertile women with 82.16 ± 1.12% in the anti-Tg positive and 85.58 ± 1.76 % in the anti-TPO. The CD3 count of 80.67 ± 1.22 % was obtained in the recurrent spontaneous aborter women positive to anti-Tg, while 83.10 ± 1.22 % was obtained in the same group positive to the anti-TPO.

The CD8 count obtained in the nulligravida women positive to anti–TPO was 27.03 ± 1.11 % and CD8 count obtained in the multiparous women positive to anti–TPO was22.72 ± 0.74 % which were significantly higher than the CD8 count in the primary infertile women with anti-TPO positive of 13.85 ± 1.43 %, in the presence of anti-Tg positive of 13.36 ±0.90 % and the CD8 count obtained in the recurrent spontaneous aborter women with marginal mean count of 13.84 ± 0.99 % at $P < 0.05$.

The CD4 count obtained in the nulligravida women of the control group positive to the anti-TPO was 43.58 ± 1.36 %, while the CD4 count obtained in the multiparous group positive to the anti-TPO was 45.16 ± 0.91 % compared with the 53.85 ± 1.76 % CD4 count obtained in the anti-TPO positive women of the primary infertile group, in the presence of CD4 count 55.11 ± 1.11 % of anti-Tg positive, as well as the 54.71 ± 1.22 % marginal mean CD4 count obtained in the RSA women, which were significantly higher ($P < 0.05$).

The Pearson (2 – tailed) correlation showed that there was significant correlation at 0.01 between each of the anti – TPO and anti –Tg value with all the species of T –lymphocyte CD counts determined, as obtained in Table 29 (appendix v). There was however no significant correlation at 0.01 between serum concentration of testosterone and estradiol with all the species CD counts determined whereas, there was significant correlation at 0.01 with CD 56 and CD 3 and at 0.05 between the CD 56 + 16 and progesterone concentration. There was significant correlation at 0.01 between all species of CD – lymphocyte count except CD 3 and serum estradiol concentration.

TABLE 18 : COMPARATIVE ANALYSES OF THE CLUSTERS OF DIFFERENTIATION COUNT ON T- CELL OF CONTROL AND TEST GROUP OF WOMEN WITH AND WITHOUT ANTI-THYROGLOBULIN ANTIBODY.

	Nulligravida (Control) N=30		**Multiparous (Control) N=30**		**Primary infertile (Subject) N=20**		**Recurrent spontaneous aborter (Subject) N=15**	
PARAMETERS (CD T -cells)	Auto-antibodies negative (-) n =18	Auto-antibodies positive (+) n =12	Auto-antibodies negative (-) n = 3	Auto-antibodies positive (+) n = 27	Auto-antibodies negative (-) n = 0	Auto-antibodies positive (+) n = 20	Auto-antibodies negative (-) n = 0	Auto-antibodies positive (+) n = 15
CD3 (%) Anti-TPO	70.96 ±0.87	n.s.v	67.81 ± 1.41	n.s.v	78.57 ± 1.74	85.58 ± 1.76	n.s.v	83.10 ± 1.22
Anti-Tg	72.72 ± 0.88	n.s.v	70.68 ± 1.44	n.s.v	82.00 ± 3.35	82.16 ± 1.12	n.s.v	80.67 ± 1.22
CD19(%) Anti-TPO	10.28 ±0.74	11.43 ± 0.90	11.60 ± 1.81	11.90 ± 0.60	n.s.v	22.98 ± 1.17	n.s.v	19.29 ± 0.81
Anti-Tg	10.86 ± 0.58	n.s.v	11.75 ± 0.95	n.s.v	25.10 ± 2.21	20.85 ± 0.74	n.s.v	19.29 ± 0.81
CD56 +CD16(%) Anti-TPO	14.62±0.70	14.55 ±0.86	5.50± 1.72	7.24 ± 0.57	n.s.v	13.31± 1.11	n.s.v	14.57± 0.65
Anti-Tg	14.59± 0.56	n.s.v	6.37± 0.91	n.s.v	11.70 ±2.11	14.92 ±0.70	n.s.v	14.57 ±0.65
CD56(%) Anti-TPO	6.29 ±0.42	6.21 ±0.51	5.97 ± 1.03	6.67 ± 0.34	n.s.v	2.78 ± 0.66	n.s.v	2.60 ± 0.46
Anti-Tg	6.25 ± 0.33	n.s.v	6.32 ± 0.54	n.s.v	2.85 ± 1.26	2.70 ±0.42	n.s.v	2.60 ± 0.46
CD4(%) Anti-TPO	42.03 ± 1.11	43.58 ± 1.36	46.47 ± 2.73	45.16 ± 0.91	n.s.v	53.85 ± 1.76	n.s.v	54.71 ± 1.21
Anti-Tg	42.81 ±0.88	n.s.v	45.81 ± 1.44	n.s.v	52.60 ±3.34	55.11 ± 1.11	n.s.v	.54.71 ± 1.22
CD8(%) Anti-TPO	24.04 ± 0.90	27.03 ± 1.11	24.67 ± 2.21	22.72 ± 0.74	n.s.v	13.85 ± 1.43	n.s.v	13.84 ± 0.99
Anti-Tg	25.53 ± 0.71	n.s.v	23.69± 1.17	n.s.v	14.35 ± 2.71	13.36 ± 0.90	n.s.v	13.84 ± 0.99

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ – significant, n.s.v-no statistical value.

4.6 Hormonal assay;

The serum hormonal concentration profile of the women in the control and test groups is presented in Table19. The mean serum progesterone concentration of the women in the primary infertile group was 2.93± 1.76 ng/ml, representing the marginal mean serum progesterone concentration of the women with anti-TPO and anti-Tg (made up the value of 3.02 ± 0.86 ng/ml for the anti-Tg positive and 2.85 ± 0.34 ng/ml for the anti-Tg negative with no significant difference between these values, (P> 0.05)). In the secondary infertile women the mean serum progesterone concentration was 0.29 ± 0.24 ng/ml, representing the marginal mean serum progesterone concentration of the women with anti-TPO and anti-Tg (made up of 0.26 ± 0.08 ng/ml for the anti-Tg positive and 0.33 ± 0.05 ng/ml for anti-Tg negative with no significant difference between the values, (P>0.05)). The above values within the test groups when compared were significantly lower than that of the RSA women at P< 0.05, with the mean serum progesterone concentration of 16.88 ± 0.83 ng/ml representing the marginal positive mean of anti-TPO and anti-Tg.

The mean serum concentration of progesterone in the nulligravida women was 6.66 ±0.83 ng/ml, representing the marginal mean serum concentration of progesterone in the women with anti-Tg and anti-TPO (made up of 7.13 ± 1.21 ng/ml for the anti-TPO positive and 6.19 ± 1.14 ng/ml for the anti-TPO negative with no significant difference between the values, (P> 0.05)). This was significantly lower than the mean serum concentration of progesterone of the pregnant women that was 40.25 ± 1.28 ng/ml, representing the marginal mean serum concentration of progesterone in the women with anti-Tg and anti-TPO (made up of 40.75 ± 0.88 ng/ml for the anti-TPO positive and 39.75 ± 2.41 ng/ml for the anti-TPO negative, here the positive anti-TPO value was significantly higher than the negative value (P< 0.05)). Moreover the values were significantly higher when compared (P < 0.05) against the mean serum concentration of

progesterone of the multiparous women with titer value of 0.47 ± 0.15 ng/ml, representing the marginal mean serum concentration of progesterone in the women with anti-Tg and anti-TPO (made up of 0.53 ± 0.09 ng/ml for the anti-TPO positive and 0.41 ± 0.28 ng/ml for the anti-TPO negative with no significant difference between the values, (P>0.05)).

The mean serum concentration of estradiol presented in Table 20 for the women in the control group showed that, the nulligravida women mean serum concentration of estradiol was 19.08 ± 2.44 pg/ml, representing the marginal mean serum estradiol concentration in the women with anti-Tg and anti-TPO (made up of 19.01 ± 3.56 pg/ml for anti-TPO positive and 19.14 ± 3.35 pg/ml for anti-TPO negative with no significant difference between these values, (P> 0.05)). The mean serum concentration of estradiol of the multiparous women was 6.71 ± 0.43 pg/ml, representing the marginal mean serum estradiol concentration in the women with anti-Tg and anti-TPO (made up of 5.48 ± 2.55 pg/ml for anti-TPO positive and 7.93 ± 0.82 pg/ml for anti-TPO negative with no significant difference between these values (P > 0.05)). When the two groups above were compared, both were significantly lower (P < 0.05) than the mean serum concentration of estradiol in the pregnant women with 197.12 ± 3.78 pg/ml, representing the marginal mean serum estradiol concentration in the women with anti-Tg and anti-TPO (made up of 201.48 ± 2.60 pg/ml for anti-TPO positive and 192.75 ± 7.11 pg/ml for anti-TPO negative with no significant difference between these values, (P>0.05)). The mean serum estradiol concentration for the primary infertile women was 0.22 ± 0.05 pg/ml, representing the marginal mean serum estradiol concentration in the women with anti-Tg and anti-TPO (made up of 0.30 ± 0.25 pg/ml for anti-Tg positive and 0.14 ± 0.10 pg/ml for anti-Tg negative with no significant difference between these values, (P> 0.05)). The mean serum estradiol concentration in the secondary infertile women was 0.41 ±0.07 pg/ml representing the marginal mean serum estradiol concentration in the women with anti-Tg and anti-TPO (made up of 0.40 ± 0.25 pg/ml for anti-

Tg positive and 0.41 ± 0.14 pg/ml anti-Tg negative with no significant difference between these values (P> 0.05)). The mean serum estradiol concentration in the RSA women was 11.94± 2.44 pg/ml representing the marginal mean serum estradiol concentration in the women with positive anti-Tg and anti-TPO.

The concentration of the test group for the RSA women was significantly lower at $P < 0.05$, when compared with that of the nulligravida women and the pregnant womem in the control group. There was no significant difference between the estradiol concentrations in the women with negative anti-TPO within the test group except for the RSA women mean values, also there was no significant difference between the non- pregnant nulligravida women and multiparous women except with the pregnant women within the control group.

The mean serum concentration of testosterone obtained is as presented in Table 21. The mean serum concentration of testosterone in the primary infertile women was 315.96 ± 8.48 ng/ml, representing the marginal mean serum concentration of testosterone for women with anti-TPO and anti-Tg (made up of 304.43 ± 4.11 ng/ml for women who were anti-Tg positive and 327.50 ± 16.46 ng/ml for those who were anti-Tg negative with no significant difference between the values, (P>0.05)). This marginal mean value was significantly higher than the mean serum concentration of testosterone for the secondary infertile women with 1.90 ± 0.12 ng/ml, representing the marginal mean serum concentration of testosterone in the women with anti-TPO and anti-Tg (made up of 1.49 ± 0.04 ng/ml for anti-Tg positive and 2.30 ± 0.23 ng/ml for anti-Tg negative with no significant difference between the values, (P> 0.05)). The mean serum concentration of the testosterone of the RSA women was 14.20 ± 3.99 ng/ml, representing the marginal mean serum concentration of testosterone in the women that were anti-TPO and anti-Tg positive.

With the nulligravida women in the control group, the mean serum testosterone concentration was 1.43 ± 0.40 ng/ml, representing the marginal mean serum testosterone concentration in the women with anti-Tg and anti-TPO (madeup of 1.44 ± 0.58 ng/ml for anti-TPO positive and 1.41 ± 0.55 ng/ml anti-TPO negative with no significant difference (P> 0.05)). In the multiparous women the mean serum testosterone concentration was 4.10 ± 0.70 ng/ml, representing the marginal mean for women with anti-Tg and anti-TPO (made up of 3.13 ± 0.42 ng/ml for anti-TPO positive and 5.07 + 1.34 ng/ml for anti-TPO negative with no significant difference between these values, (P> 0.05)). In the pregnant women the mean serum testosterone concentration was 0.50 ± 0.06 ng/ml, representing the marginal mean for women with anti-Tg and anti-TPO (madeup of 0.53 ± 0.04ng/ml for anti-TPO positive and 0.47+ 0.11 ng/ml for anti-TPO negative with no significant difference between the values (P> 0.05)).

The value obtained for the primary infertile women was significantly higher than the values in the women of the control groups (P < 0.05).

The mean serum prolactin concentration obtained is as presented in Table 22. This showed that in the primary infertile women the mean serum prolactin concentration was 196.95 ± 23.16 ng/ml, representing the marginal mean serum prolactin concentration in the women with anti-TPO and anti-Tg (made up of 183.90 ± 11.23 ng/ml for anti-Tg positive and 210.00 ± 4.49 ng/ml for anti-Tg negative, here the value for anti-Tg positive was significantly lower than that for the negative (P< 0.05)). In the secondary infertile women, the mean serum prolactin concentration was 186.06 ± 32.25 ng/ml, representing the marginal mean serum prolactin concentration in the women with anti-TPO and anti-Tg (made up of 161.13 ± 11.06 ng/ml for anti-Tg positive and 211.00 ± 6.35 ng/ml for anti-Tg negative, here the value in the anti-Tg was significantly lower in the positive than in the negative (P< 0.05)). In the RSA women the mean serum prolactin

concentration was 279.80 ± 10.90 ng/ml, representing the marginal mean serum prolactin concentration in the women who were both anti-TPO and anti-Tg positive.

The serum prolactin concentration in the test group were all significantly higher ($P<0.05$). compared with the mean serum prolactin concentration in the multiparous women with 32.83 ± 19.21 ng/ml representing the marginal mean serum prolactin concentration in the women with anti-Tg and anti-TPO (made up of 35.32 ± 1.14 ng/ml for anti-TPO positive and 30.33 ± 3.37 ng/ml for anti-TPO negative with no significant difference ($P>0.05$)). In the nulligravida women the mean serum prolactin concentration was 13.72 ± 1.11 ng/ml, representing the marginal mean serum prolactin concentration in the women with anti-Tg and anti-TPO (made up of 12.98 ± 1.59 ng/ml for anti-TPO positive and 14.45 ± 1.49 ng/ml for anti-TPO negative with no significant difference ($P> 0.05$)). The pregnant women's mean serum prolactin concentration was 15.81 ± 1.69 ng/ml, representing the marginal mean serum prolactin concentration in the women with anti-Tg and anti-TPO (made up of 17.49 ± 1.17 ng/ml for anti-TPO positive and 14.13 ± 3.77 ng/ml for anti-TPO negative with no significant difference ($P> 0.05$)).

The Pearson (2 – tailed) correlation between each of anti -TPO and the anti -Tg with all the hormone concentrations determined showed significant correlation at 0.01 level. There was also significant correlation at 0.01 level, between serum concentration of testosterone and estradiol with all the species of the CD counts determined. Also, there was significant correlation at 0.01 between CD 56 and CD 3, between the CD 56 + 16 and serum progesterone concentration. There was significant correlation at 0.01 between all species of CD – lymphocyte count except CD 3 and serum estradiol concentration, see Table 30 appendix iii.

Table 19: COMPARATIVE ANALYSES OF SERUM PROGESTERONE- FEMALE HORMONAL CONCENTRATION PROFILE IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Progesterone (ng/ml)	Progesterone (ng/ml)		Progesterone (ng/ml)	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	6.66 ±0.83 [c]	6.19 ±1.14 n=18 [a]	7.13 ± 1.21 n=16 [a,b]	6.66 ±0.83 [c] n=18 [b]	n.s.v n=16 [a,b]
Multiparous Women n=34	0.47 ± 0.15 [c]	0.41 ± 0.28 n=3 [a]	0.53 ± 0.09 n=31 [a, b]	0.47 ± 0.15 [c] n=3 [b]	n.s.v n=31 [a,b]
Pregnant Women n=34	40.25 ± 1.28 [c]	39.75 ± 2.41 n=4 [a]	40.75 ± 0.88 n=30 [a,b]	40.25 ± 1.28 [c] n=4 [b]	n.s.v n=30 [a,b]
Primary Infertile Women n = 34	2.93 ±1.76 [c]	n.s.v n=2	2.93 ±1.76 [c] n=32 [b]	2.85 ± 0.34 n=2 [a,b]	3.02 ±0.86 n=32 [a]
Secondary Infertile Women n =34	0.29 ± 0.24 [c]	n.s.v n=1	0.29 ± 0.24 [c] n=33 [b]	0.33 ± 0.05 n=1 [a,b]	0.26 ± 0.08 n=33 [a]
Recurrent Spont. Aborter n=34	16.88 ± 0.83 [c]	-	16.88 ± 0.83 [c] n =34 [b]	-	16.88 ± 0.83 n =34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance, [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c=marginal mean value.

Table 20: : COMPARATIVE ANALYSES OF SERUM ESTRADIOL FEMALE HORMONAL CONCENTRATION PROFILE IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Estradiol (pg/ml)	Estradiol (pg/ml)		Estradiol (pg/ml)	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	19.08 ± 2.44 [c]	19.14 ± 3.35 n=18 [a]	19.01 ± 3.56 n=16 [a,b]	19.08 ± 2.44 [c] n=18 [b]	n.s.v n=16
Multiparous Women n=34	6.71 ± 0.43 [c]	7.93 ± 0.82 n=3 [a]	5.48 ± 2.55 n=31 [a,b]	6.71 ± 0.43 [c] n=3 [b]	n.s.v n=31
Pregnant Women n=34	197.12 ± 3.78 [c]	192.75 ± 7.11 n=4 [a]	201.48 ± 2.60 n=30 [a,b]	197.12 ± 3.78 [c] n=4 [b]	n.s.v n=30
Primary Infertile Women n = 34	0.22 ± 0.05 [c]	n.s.v n=2 [a,b]	0.22 ± 0.05 [c] n=32 [b]	0.14 ± 0.10 n=2 [a,b]	0.30 ± 0.25 n=32 [a]
Secondary Infertile Women n =34	0.41 ± 0.07 [c]	n.s.v n=1 [a,b]	0.41 ± 0.07 [c] n=33 [b]	0.41 ± 0.14 n=1 [a,b]	0.40 ± 0.25 n=33 [a]
Recurrent Spont. Aborter n=34	11.94 ± 2.44 [c]	-	11.94 ± 2.44 [c] n=34 [b]	-	11.94 ± 2.44 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance, [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c=marginal mean value.

Table 21: COMPARATIVE ANALYSES OF SERUM TESTOSTERONE (MALE FACTOR) HORMONAL CONCENTRATION PROFILE IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Testosterone (ng /ml)	Testosterone (ng /ml)		Testosterone (ng /ml)	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	1. 43 ± 0.40 [c]	1.41 ± 0.55 n=18 [a]	1.44 ± 0.58 n=16 [a,b]	1. 43 ± 0.40 [c] n=18 [b]	n.s.v n=16 [a,b]
Multiparous Women n=34	4.10 ± 0. 07 [c]	5.07 ± 1.34 n=3 [a]	3.13 ± 0.42 n=31 [a,b]	4.10 ± 0. 07 [c] n=3 [a]	n.s.v n=31 [a,b]
Pregnant Women n=34	0.50± 0.06 [c]	0.47 ± 0.11 n=4 [a]	0.53 ± 0.04 n=30 [a,b]	0.50± 0.06 [c] n=4 [a]	n.s.v n=30 [a,b]
Primary Infertile Women n =34	315.96 ± 8.48 [c]	n.s.v n=2	315.96 ± 8.48 [c] n=32 [b]	327.50 + 16.46 n=2 [a,b]	304.43 ± 4.11 n=32 [b]
Secondary Infertile Women n =34	1.90 ± 0.12 [c]	n.s.v n=1	1.90 ± 0.12 [c] n=33 [b]	2.30 + 0.23 n=1 [a,b]	1.49 ± 0.04 n=33 [b]
Recurrent Spont. Aborter n=34	14.20 ± 3.99 [c]	-	14.20 ± 3.99 [c] n=34 [b]	-	14.20 ± 3.99 n=34 [b]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance, [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c=marginal mean value.

Table 22: : COMPARATIVE ANALYSES OF SERUM PROLACTIN HORMONAL CONCENTRATION PROFILE IN THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Prolactin (ng /ml)	Prolactin (ng /ml)		Prolactin (ng /ml)	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	13.72 ± 1.11 [c]	14.45 ± 1.49 n=18 [a]	12.98 ± 1.59 n=16 [a,b]	13.72 ± 1.11 [c] n=18 [b]	n.s.v n=16
Multiparous Women n=34	32.83 ± 19.21 [c]	30.33 ± 3.37 n=3 [a]	35.32 ± 1.14 n=31 [a,b]	32.83 ± 19.21 [c] n=3 [b]	n.s.v n=31
Pregnant Women n=34	15.81 ± 1.69 [c]	14.13 ± 3.77 n=4 [a]	17.49 ± 1.17 n=30 [a,b]	15.81 ± 1.69 [c] n=4 [b]	n.s.v n=30
Primary Infertile Women n = 34	196.95 ± 23.16 [c]	n.s.v n=2 [a,b]	196.95 ± 23.16 [c] n=32 [b]	210.00 ± 4.49 n=2 [a,b]	183.90 ± 11.23 n=32 [a]
Secondary Infertile Women n =34	186.06 ± 32.25 [c]	n.s.v n=1 [a,b]	186.06 ± 32.25 [c] n=33 [b]	211.00 ± 6.35 n=1 [a,b]	161.13 ± 11.06 n=33 [a]
Recurrent Spont. Aborter n=34	279.80 ± 10.90 [c]	-	279.80 ± 10.90 [c] n=34 [b]	-	279.80 ± 10.90 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance, [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c=marginal mean value.

4.7 Anti-Oxidant levels:

The mean serum concentration determined for vitamin C was presented in Table 23. The mean serum vitamin C concentration in the nulligravida women was 2.83 ± 0.21 mg/dl, this represented the marginal mean serum vitamin C concentration in the women having anti-Tg and anti-TPO (which comprises of the value for the women who were positive to anti-TPO with 2.39 ± 0.31 mg/dl and those negative to anti-TPO with 3.32± 0.29 mg/dl, and the positive value was significantly lower than the negative (P<0.05)). In the multiparous women the mean serum vitamin C concentration was 2.32 ± 0.38 mg/dl, this represented the marginal mean serum vitamin C concentration in the women with anti-Tg and anti-TPO (which comprises of the value for the women who were positive to anti-TPO with 2.99 ± 0.22 mg/dl and negative anti-TPO of 1.67 ± 0.72 mg/dl, and the positive value was significantly higher than the negative (P<0.05)). In the pregnant women the mean serum vitamin C concentration was 2.24 ± 0.33 mg/dl, this represented the marginal mean serum vitamin C concentration in the women with anti-Tg and anti-TPO (which comprises of the value in the women who were positive to anti-TPO with 2.51 ± 0.23 mg/dl and negative anti-TPO with 1.98± 0.62 mg/dl, and the positive was significantly higher than the negative (P<0.05)).

On the other hand, in the test group, the mean serum vitamin C concentration in the primary infertile women was 1.72 ± 0.45 mg/dl, this represented the marginal mean serum vitamin C concentration in the women with anti-TPO and anti-Tg (comprises of the value in the women who were positive to anti-Tg with 1.95 ± 0.22 mg/dl and negative anti-Tg with 1.50 ± 0.88 mg/dl and the positive was significantly higher than the negative (P<0.05)). The mean serum concentration of vitamin C for the secondary infertile women was 2.11 ± 0.63 mg/dl, this represented the marginal mean serum vitamin C concentration in the women with anti-TPO and

anti-Tg (which comprises of value in the women who were positive to anti-Tg with 1.91 ± 0.22 mg/dl and negative anti-Tg of 2.30 ± 1.25 mg/dl and the positive was significantly lower than the negative (P<0.05)). The mean serum concentration of vitamin C for the RSA women was 0.75 ± 0.21 mg/dl this represent the marginal mean serum vitamin C concentration in the women with anti-TPO and anti-Tg that were both positive . At P < 0.05, the marginal mean serum concentration of the vitamin C in women in the control groups were significantly higher than the test groups. Also at P < 0.05, the negative anti – TPO of the control group was significantly higher than in the test groups.

The serum vitamin E antioxidant concentrations obtained were presented in Table 24, the mean serum vitamin E concentration in the nulligravida women was 12.38 ± 0.50 μg/ml, this was the marginal mean serum vitamin E concentration in the women having anti-Tg and anti-TPO (which comprises of the value for those women who were positive to anti-TPO with 11.81 ± 0.73 μg/ml and those that were negative to anti-TPO with 12.94 ± 0.69 μg/ml, and those that were positive had value that was significantly lower than the negative (P<0.05)). The mean serum concentration of vitamin E for the multiparous women was 8.95 ± 0.89 μg/ml, this value was the marginal mean serum vitamin E concentration in the women having anti-Tg and anti-TPO (which comprises of the value for those positive to anti-TPO with 9.23 ± 0.54 μg/ml and those negative to anti-TPO with 8.67 ± 1.69 μg/ml and the value for those positive was significantly higher than for those that were negative (P<0.05)). The mean serum concentration of vitamin E for the pregnant women was 7.05 ± 0.78 μg/ml, this value was the marginal mean serum vitamin E concentration in the women having anti-Tg and anti-TPO (which comprises of the value for those who were positive to anti-TPO with 7.10 ± 0.54 μg/ml and those negative to anti-TPO with 7.00 ± 1.47 μg/ml, which was not significantly different). The marginal mean value for the nulligravida and multiparous women were significantly higher (P<0.05) than the pregnant group within the control group positive to anti-TPO.

In the test groups, the mean serum concentration for vitamin E of the primary infertile women was 7.38 ± 1.07 μg/ml, this value was the marginal mean serum concentration for vitamin E in the women having anti-TPO and anti-Tg (which comprises of the mean serum vitamin E concentration in those women positive to anti-Tg with 8.75 ± 0.52 μg/ml and those negative to anti-Tg with 6.00 ± 2.07 μg/ml, the positive was significantly higher than the negative ($P<0.05$)). The mean serum concentration of vitamin E for secondary infertile women was 10.49 ± 1.49 μg/ml, this value was the marginal mean serum vitamin E concentration in the women having anti-TPO and anti-Tg (which comprises of the mean serum vitamin E concentration in those women positive to anti-Tg with 8.97 ± 0.51 μg/ml and those negative to anti-Tg of 12.00 ± 2.93 μg/ml where the positive was significantly lower than the negative ($P<0.05$)). The mean serum vitamin E concentration in the RSA women was 8.82 ± 0.50 μg/ml, this value was the marginal mean serum vitamin E concentration in those women having both anti-Tg and anti-TPO positive. Statistical analysis showed that values in the test group were significantly lower than in the non- pregnant control women, but higher than the pregnant women, at $P< 0.05$.

Table 25 depicts the mean serum concentration of zinc (Zn) in the women studied. The mean serum zinc (Zn) concentration in the nulligravida women was 0.61 ± 0.03 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-Tg and anti-TPO (which comprises the value of positive anti-TPO of 0.69 ± 0.03 μg/dl and negative anti-TPO of 0.53 ± 0.03 μg/dl, and the positive was not significantly different from the negative, ($P>0.05$)). The mean serum zinc (Zn) concentration in the multiparous women was 0.37 ± 0.04 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-Tg and anti-TPO (which comprises the value of positive anti-TPO of 0.39 ± 0.03 μg/dl and negative anti-TPO of 0.35 ± 0.08 μg/dl, and the positive was not significantly different from the negative ($P>0.05$)). The mean serum zinc (Zn) concentration in the pregnant women was 0.47 ± 0.04 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with

anti-Tg and anti-TPO (which comprises the value of positive anti-TPO of 0.48 ± 0.03 μg/dl and negative anti-TPO of 0.46 ± 0.07 μg/dl, and the positive was not significantly different from the negative (P>0.05)).

The mean serum zinc (Zn) concentration for the test group, in the primary infertile group was 0.55 ± 0.05 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-TPO and anti-Tg (which comprises the value of positive anti-Tg of 0.53 ± 0.02 μg/dl and negative anti-Tg of 0.56 ± 0.10 μg/dl and the positive was not significantly lower than the negative (P>0.05)). The mean serum zinc (Zn) concentration in the secondary infertile women was 0.50 ± 0.07 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-TPO and anti-Tg (which comprises the value of positive anti-Tg of 0.51 ± 0.02 μg/dl and negative anti-Tg of 0.49 ± 0.14 μg/dl and the positive was not significantly higher than the negative (P>0.05)). The mean serum zinc (Zn) concentration in the RSA women was 0.40 ± 0.02 μg/ml, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-TPO and anti-Tg that were both positive. At P< 0.05, the marginal mean serum zinc concentration in the primary and the secondary infertile women were significantly higher than the value in the pregnant and the multiparous women of the control group but lower than the value in the nulligravida women.

The mean serum concentration of iron (Fe) obtained were presented in Table 26. In the control group, the mean serum iron (Fe) concentration in the nulligravida women was 0.86 ± 0.04 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-Tg and anti-TPO (which comprises the value in the women positive to anti-TPO with 0.89 ± 0.05 μg/dl and negative anti-TPO of 0.82 ± 0.05 μg/dl, and the positive was not significantly

different from the negative (P>0.05)). The mean serum iron (Fe) concentration in the multiparous women was 0.54 ± 0.06 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-Tg and anti-TPO (which comprises the value in the women positive to anti-TPO with 0.63 ± 0.04 μg/dl and negative anti-TPO of 0.46 ± 0.12 μg/dl, and the positive was significantly higher than the negative (P<0.05)). The mean serum iron (Fe) concentration in the pregnant women was 0.99 ± 0.05 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-Tg and anti-TPO (which comprises the value in the women positive to anti-TPO with 0.95 ± 0.04 μg/dl and negative anti-TPO of 1.03 ± 0.10 μg/dl, where the positive was significantly lower than the negative (P<0.05)).

The obtained result within the test groups, of the mean serum iron (Fe) concentration in the primary infertile women was 0.92 ± 0.07 μg/ml, this represented the marginal mean serum iron (Fe) concentration in the women with anti-TPO and anti-Tg (comprising the value in the women positive to anti-Tg of 0.90 ± 0.04 μg/dl and negative anti-Tg of 0.93 ± 0.14 μg/dl and the positive was not significantly lower than the negative (P>0.05)). The mean serum iron (Fe) concentration in the secondary infertile women was 0.67 ± 0.10 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-TPO and anti-Tg (comprising the value in the women positive to anti-Tg of 0.85 ± 0.04 μg/dl and negative anti-Tg of 0.49 ± 0.20 μg/dl and the positive was significantly higher than the negative (P<0.05)). The mean serum iron (Fe) concentration in the RSA women was 0.92 ± 0.04 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-TPO and anti-Tg that are both positive. At P< 0.05, the women in the test groups were significantly higher than the non- pregnant multiparous women in the control group.

0.04 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-Tg and anti-TPO (which comprises the value of positive anti-TPO of 0.48 ± 0.03 μg/dl and negative anti-TPO of 0.46 ± 0.07 μg/dl, and the positive was not significantly different from the negative ($P<0.05$)).

The mean serum zinc (Zn) concentration for the test group, in the primary infertile group was 0.55 ± 0.05 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-TPO and anti-Tg (which comprises the value of positive anti-Tg of 0.53 ± 0.02 μg/dl and negative anti-Tg of 0.56 ± 0.10 μg/dl and the positive was not significantly lower than the negative ($P<0.05$)). The mean serum zinc (Zn) concentration in the secondary infertile women was 0.50 ± 0.07 μg/dl, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-TPO and anti-Tg (which comprises the value of positive anti-Tg of 0.51 ± 0.02 μg/dl and negative anti-Tg of 0.49 ± 0.14 μg/dl and the positive was not significantly higher than the negative ($P<0.05$)). The mean serum zinc (Zn) concentration in the RSA women was 0.40 ± 0.02 μg/ml, this represented the marginal mean serum zinc (Zn) concentration in the women with anti-TPO and anti-Tg that were both positive. At $P< 0.05$, the marginal mean serum zinc concentration in the primary and the secondary infertile women were significantly higher than the value in the pregnant and the multiparous women of the control group but lower than the value in the nulligravida women.

The mean serum concentration of iron (Fe) obtained were presented in Table 26. In the control group, the mean serum iron (Fe) concentration in the nulligravida women was 0.86 ± 0.04 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-Tg and anti-TPO (which comprises the value in the women positive to anti-TPO with 0.89 ±

0.05 μg/dl and negative anti-TPO of 0.82 ± 0.05 μg/dl, and the positive was not significantly different from the negative (P<0.05)). The mean serum iron (Fe) concentration in the multiparous women was 0.54 ± 0.06 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-Tg and anti-TPO (which comprises the value in the women positive to anti-TPO with 0.63 ± 0.04 μg/dl and negative anti-TPO of 0.46 ± 0.12 μg/dl, and the positive was significantly higher than the negative at P<0.05). The mean serum iron (Fe) concentration in the pregnant women was 0.99 ± 0.05 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-Tg and anti-TPO (which comprises the value in the women positive to anti-TPO with 0.95 ± 0.04 μg/dl and negative anti-TPO of 1.03 ± 0.10 μg/dl, where the positive was significantly lower than the negative at P<0.05).

The obtained result within the test groups, of the mean serum iron (Fe) concentration in the primary infertile women was 0.92 ± 0.07 μg/ml, this represented the marginal mean serum iron (Fe) concentration in the women with anti-TPO and anti-Tg (comprising the value in the women positive to anti-Tg of 0.90 ± 0.04 μg/dl and negative anti-Tg of 0.93 ± 0.14 μg/dl and the positive was not significantly lower than the negative (P<0.05)). The mean serum iron (Fe) concentration in the secondary infertile women was 0.67 ± 0.10 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-TPO and anti-Tg (comprising the value in the women positive to anti-Tg of 0.85 ± 0.04 μg/dl and negative anti-Tg of 0.49 ± 0.20 μg/dl and the positive was significantly higher than the negative (P<0.05)). The mean serum iron (Fe) concentration in the RSA women was 0.92 ± 0.04 μg/dl, this represented the marginal mean serum iron (Fe) concentration in the women with anti-TPO and anti-Tg that are both positive. At P< 0.05, the women in the test groups were significantly higher than the non- pregnant multiparous women in the control group.

The Pearson (2 tailed) correlation showed that there was significant correlation at 0.05 between anti – TPO value and serum vitamin C concentration only. Whereas there was significant correlation at 0.01 level between anti- Tg value and serum vitamin C concentration.

Table 23: COMPARATIVE ANALYSES OF ANTI-OXIDANT (VITAMIN-C) CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Vitamin C (mg/dl)	Vitamin C (mg/dl)		Vitamin C (mg/dl)	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	2.83 ± 0.21^{c}	3.32 ± 0.29 n=18 [a]	2.39 ± 0.31 n=16 [a,b]	2.83 ± 0.21^{c} n=18 [b]	n.s.v n=16 [a,b]
Multiparous Women n=34	2.32 ± 0.38^{c}	1.67 ± 0.72 n=3 [a]	2.99 ± 0.22 n=31 [a,b]	2.32 ± 0.38^{c} n=3 [b]	n.s.v n=31 [a,b]
Pregnant Women n=34	2.24 ± 0.33^{c}	1.98 ± 0.62 n=4 [a]	2.51 ± 0.23 n=30 [a,b]	2.24 ± 0.33^{c} n=4 [b]	n.s.v n=30 [a,b]
Primary Infertile Women n = 34	1.72 ± 0.45^{c}	n.s.v n=2	1.72 ± 0.45^{c} n=32 [b]	1.50 ± 0.88 n=2 [a,b]	1.95 ± 0.22 n=32 [a]
Secondary Infertile Women n =34	2.11 ± 0.63^{c}	n.s.v n=1	2.11 ± 0.63^{c} n=33 [b]	2.30 ± 1.25 n=1 [a,b]	1.91 ± 0.22 n=33 [a]
Recurrent Spont. Aborter n=34	0.75 ± 0.21^{c}	-	0.75 ± 0.21^{c} n=34 [b]	-	0.75 ± 0.21 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ – significant, n.s.v = no statistical value of any significance, [a]$P<0.05$ comparing positive (+) and negative (-) Anti-TPO /Tg [b]$P<0.05$ test vs. control group. c=marginal mean value.

Table 24: COMPARATIVE ANALYSES OF SOME ANTI-OXIDANT (VITAMIN -E) CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	Vitamin E (μ g/dl)	Vitamin E (μ g/dl)		Vitamin E (μ g/dl)	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	12.38 ± 0.50^{c}	12.94 ± 0.69 n=18 [a]	11.81 ± 0.73 n=16 [a,b]	12.38 ± 0.50^{c} n=18 [b]	n.s.v n=16
Multiparous Women n=34	8.95 ± 0.89^{c}	8.67 ± 1.69 n=3 [a]	9.23 ± 0.54 n=31 [a,]	8.95 ± 0.89^{c} n=3 [b]	n.s.v n=31
Pregnant Women n=34	7.05 ± 0.78^{c}	7.00 ± 1.47 **n= 4** [a]	7.10 ± 0.54 n=30 [a,b]	7.05 ± 0.78^{c} n= 4 [b]	n.s.v n=30
Primary Infertile Women n = 34	7.38 ± 1.07^{c}	n.s.v n=2 [a,b]	7.38 ± 1.07^{c} n=32 [b]	6.00 ± 2.07 n=2 [a,b]	8.75 ± 0.52 n=32 [a]
Secondary Infertile Women n =34	10.49 ± 1.49^{c}	n.s.v n=1 [a,b]	10.49 ± 1.49^{c} n=33 [b]	12.00 ± 2.93 n=1 [a,b]	8.97 ± 0.51 n=33 [a]
Recurrent Spont. Aborter n=34	8.82 ± 0.50^{c}	-	8.82 ± 0.50^{c} n=34 [b]	-	8.82 ± 0.50 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), $P<0.05$ – significant, n.s.v = no statistical value of any significance, [a]$P<0.05$ comparing positive (+) and negative (-) Anti-TPO /Tg [b]$P<0.05$ test vs. control group. c=marginal mean value.

Table 25: COMPARATIVE ANALYSES OF ANTI-OXIDANT ZINC (METAL) CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	**Zinc (zn) (μ g/dl)**	**Zinc (zn) (μ g/dl)**		**Zinc (zn) (μ g/dl)**	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	0.61 ± 0.02^{c}	0.53 ± 0.03 n=18 [a]	0.69 ± 0.03 n=16 [a,b]	0.61 ± 0.02^{c} n=18 [b]	n.s.v n=16 [a,b]
Multiparous Women n=34	0.37 ± 0.04^{c}	0.35 ± 0.08 n=3 [a]	0.39 ± 0.03 n=31 [a,b]	0.37 ± 0.04^{c} n=3 [b]	n.s.v n=31 [a,b]
Pregnant Women n=34	0.47 ± 0.04^{c}	0.46 ± 0.07 n=4 [a]	0.48 ± 0.03 n=30 [a,b]	0.47 ± 0.04^{c} n=4 [b]	n.s.v n=30 [a,b]
Primary Infertile Women n = 34	0.55 ± 0.05^{c}	n.s.v n=2	0.55 ± 0.05^{c} n=32 [b]	0.56 ± 0.10 n=2 [a,b]	0.53 ± 0.02 n=32 [a]
Secondary Infertile Women n =34	0.50 ± 0.07^{c}	n.s.v n=1	0.50 ± 0.07^{c} n=31 [b]	0.49 ± 0.14 n=1 [a,b]	0.51 ± 0.02 n=31 [a]
Recurrent Spont. Aborter n=34	0.40 ± 0.02^{c}	-	0.40 ± 0.02^{c} n=34 [b]	-	0.40 ± 0.02 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 -- significant, n.s.v = no statistical value of any significance. [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c=marginal mean value.

Table 26: COMPARATIVE ANALYSES OF ANTI-OXIDANT IRON (METAL) CONCENTRATION IN THE SERUM OF THE STUDIED EUTHYROID WOMEN WITH AND WITHOUT ANTI-MICROSOMAL AND ANTI-THYROGLOBULIN ANTIBODY.

PARAMETER	**IRON(Fe) (μ g/dl)**	**IRON(Fe) (μ g/dl)**		**IRON(Fe) (μ g/dl)**	
SUBJECTS	Marginal mean value	Anti-TPO(-)	Anti-TPO(+)	Anti-Tg(-)	Anti-Tg(+)
Nulligravida Women n=34	0.86 ± 0.04	0.82 ± 0.05 n=18 [a]	0.89 ± 0.05 n=16 [a,b]	0.86 ± 0.04 n=18 [b]	n.s.v n=16
Multiparous Women n=34	0.54 ± 0.06	0.46 ± 0.12 n=3 [a]	0.63 ± 0.04 n=31 [a,b]	0.54 ± 0.06 n=3 [b]	n.s.v n=31
Pregnant Women n=34	0.99 ± 0.05	1.03 ± 0.10 n=4 [a]	0.95 ± 0.04 n=30 [a,b]	0.99 ± 0.05 n=4 [b]	n.s.v n=30
Primary Infertile Women n = 34	0.92 ± 0.07	n.s.v n=2 [a,b]	0.92 ± 0.07 n=32 [b]	0.93 ± 0.14 n=2 [a,b]	0.90 ± 0.04 n=32 [a]
Secondary Infertile Women n =34	0.67 ± 0.10	n.s.v n=1 [a,b]	0.67 ± 0.10 n=31 [b]	0.49 ± 0.20 n=1 [a,b]	0.85 ± 0.04 n=33 [a]
Recurrent Spont. Aborter n=34	0.92 ± 0.04	-	0.92 ± 0.04 n=34 [b]	-	0.92 ± 0.04 n=34 [a]

Data are expressed as mean ± SE and analyzed by ANOVA (Two-way test), P<0.05 – significant, n.s.v = no statistical value of any significance, [a]P<0.05 comparing positive (+) and negative (-) Anti-TPO /Tg [b]P<0.05 test vs. control group. c=marginal mean value.

CHAPTER FIVE

5.0 DISCUSSION OF RESULTS

The etiology of reproductive failure has severally been implicated in the disturbance of thyroid homeostasis and attempt had been made to control it with the targeted treatment of the disease condition identified during investigations (Di-Siomone et al., 1999).

The importance of thyroid hormones in metabolic regulation and control of reproduction can not be over emphasized. A normal thyroid functioning system ie euthyroid tissue can still be subjected to stress due to derangement of immune system when auto-antibodies are raised against self (auto) antigens. In the euthyroid, the normal thyroid functioning and hormonal regulation still goes on, but the effective responses are truncated at another level. especially with immune interference (El-Roeiy and Gleicher,1988). It has been reported that Caucasian women with autoimmune disease have an increased frequency of reproductive failure (Coulam, 1992).

This study was designed, executed and thus provided the local views on the African women's immune reproductive assessment from the point of view of the results obtained from some volunteer Nigerian women residing in Lagos with recurrent spontaneous abortions (RSA) and infertility of immune etiology. The volunteer women in this study were grouped into the age brackets classified into early and late marriage ages, they were recruited from among the women attending the various clinics visited, that were within the reproductive age brackets (Tables 6 and 7). The reproductive age of an average woman in general, is put between 16 – 45 years, which represents the reproductive events between puberty and menopause. The age distribution in Table 7 showed that the majority of the pregnant and multiparous women in the control groups were within the same age brackets compared with the majority of women in the test groups of the primary infertile, secondary infertile and spontaneous aborter groups.

The biochemical indices determined in the women studied included cholesterol which is the building block of the steroid hormones. This was evaluated along side the obtained immune species parameter as determined, which may be involved in the lymphoid homeostasis, which also showed a significant correlation. The triglyceride concentrations obtained showed a significant correlation with the anti-Tg and anti-TPO titer values

obtained in this study. The cholesterol was higher in the pregnant women but was highest in the recurrent spontaneous aborter which is one of the indices suggestive of the patho-physiologic, mimicking state of pregnancy. The protein levels, (albumin index) determined, in the primary and secondary infertile women were higher than in the control women, which showed that the body system of the women was responding to increased protein synthesis which is one of the characteristics of immunological stress responses (Chao, 1996).

Acid phosphatase (ACP; E.C. 3.1.3.2) is of interest chiefly in the diagnosis of prostrate malignancies. The enzyme hydrolyses orthophosphate esters at an acidic pH of 4.0. Small increases in the total ACP are found in conditions such as acute myelogenous leukemia, thrombocythemia etc., that could promote oxidative stress which may affect immunological balance.The ACP activity in this study showed a significant correlation with the anti-Tg titer in the euthyroid women studied.

Alkaline phosphatase (ALP; E.C. 3.1.3.1) has an optimal activity at a pH of approximately 10. It is found in a number of tissues but is used in clinical diagnosis chiefly in connection with bone and liver damages. ALP is elevated in liver disease with cholestasis. The placental ALP will be found only during pregnancy which explained why it was highest in the pregnant group and significantly high in the secondary infertile group. However, the ALP activity did not show any significant correlation with the auto-antibody evaluated in this study.

Serum glutamate oxaloacetate transaminase (SGOT, EC.2.6 .1.1) and serum glutamate pyruate transaminase (SGPT, EC.2.6.1.2), are slightly lower in women than in men and elevations in transaminase activity occur in myocardial infarction, infectious hepatitis, cirrhosis and biliary obstruction (Tietz, 1995). The result obtained in the analysis of the liver function enzymes in this study showed that, the control groups had significantly lower values than the infertile groups. This support the suspicion that metabolic imbalance may be additional indices, for the elevated auto- anti-thyroid effects on hormonal imbalance causing infertility (Tietz, 1995).

Thyroid auto-antibodies have been predicted to be independent markers for pregnancies at risk for loss. The women who have significant anti-thyroid antibody levels miscarry at approximately twice the rate of women who have no anti-thyroid antibodies, therefore history of two or more pregnancy losses or unexplained infertility should be investigated for the presence of anti-thyroid antibodies (Hill and Chol, 2000).

This study agrees with previous report (Coulam, 1992), which had shown that, high titers of auto-thyroperoxidase in euthyroid patients (i.e. without clinical evidence of thyroid dysfunction), is a positive phenomenon, in association with recurrent pregnancy loss. In this study the anti-thyroglobulin (anti-TgAb) and anti-thyroperoxidase (anti-TPOAb) auto-antibody mean concentrations showed that the anti-TgAb and anti-TPOAb were signifcantly higher in test groups compared to the control groups, ($p < 0.05$). In addition, the anti-TgAb levels of the three test group were recorded to be significantly higher than the control groups. Moreover the secondary infertile women group, within the test group, had significantly higher anti-TgAb concentrations, compared to others ($p<0.05$) as shown in Table 17. The anti-TgAb mean concentrations in the secondary infertile, the spontaneous aborter as well as in the primary infertile women in this study that were significantly higher compared with the control groups thus provides the primary marker for the reproductive failure. In Table 17 the positive anti-Tg-Ab and anti-TPO-Ab mean concentrations in the control groups, made up of the pregnant group, multiparous group and nulligravida group were within the lower range of the bench mark (that is, those that tested positive for thyroglobulin auto-antibodies were within the bench mark range of 2.0 to > 200 units / ml) and thyroidperoxidase auto-antibodies were within range of the bench mark of 2.0 to > 40 units / ml), suggesting that low levels of antiTg-Ab and anti-TPO-Ab may not hinder reproductivity.

Flow cytometric techniques used in this study are reliable, reproducible and sensitive for evaluation of the levels of the subsets of the T-lymphocytes (Rosen, et al., 1995). The CD4 and CD8 monoclonal antibodies determined are recognized important subsets of cells of the monoclonal antibodies Nk T-cells. Moreover the CD4 cells recognize antigen in association with the class II MHC (HLA – D) molecules and CD8 cells recognize antigens in association with class I MHC (HLA – A, HLA - B and HLA – C) molecules, and antigen – specific T – cell responses are MHC restricted (Rosen et al., 1995). Abnormalities in the number of CD4 and CD8 cells can be associated with abnormalities in cognate as well as regulatory functions of T–cells and may lead to immune incompetence or auto-immunity (Rosen et al., 1995). The monoclonal antibodies against CD16 and CD56 even though they are not lineage specific, may be useful for the detection and enumeration of natural killer (NK) cells. The T-cell responses to the immunoglobulin IgG-Tg-Ab and TPO-Ab were also

expressed in the obtained results of the CD counts in table 18, for the Nka cells and T-suppressor cells. The NKa Cells expressing CD 4, 19 and 56+16 increased in the primary infertile and spontaneous aborter compared with the control groups. The T- suppressor cells, expressing CD 56 and 8 increased in control than test groups. In humans there is evidence that CD 8^+ T cells may recognize trophoblast in normal successful pregnancy or after T cell activation as occurs with immunization, (Rosen, et al., 1995). Activated CD 8^+ cells express progesterone receptors and in response to the hormone, further secrets a factor that inhibits activation of NK cells (Coulam et al., 1995).

This study has not only provided data that support the above views, but also showed the influence on hormone homeostasis, as responded to by the classes of CD – T cells that were evaluated. Thus the traditional hormone profile assessment in the clinics could be better interpreted with the CD – T cells evaluation in the etiology of infertility. The high anti-thyroid levels in women with recurrent miscarriage correspond to deficient activation of their CD 8^+ suppressor cell, in early stage of pregnancy which may account for loss of pregnancy. CD 56 + CD 16 cell represent conventional NK cells that are particularly effective in lysing troploblastic target cells when activated. From this study a significant elevation of CD 56 + CD 16 Nk cells in peripheral blood was associated with spontaneous aborter and primary infertility groups.

The physiological parameters and hormonal profiles of the various categories of euthyroid women were expected to further corroborate the lymphoid homeostasis being determined by the levels of anti-Tg-Ab and TPO-Ab. This is true where the immune function is better in female than in male vertebrae species and this dimorphism has been attributed to the presence of immunosuppressive androgens in males. Females exhibited the expected increase in antibody production, independent of hormone treatment condition (Coulam et al., 2003a). The progesterone concentrations of the early follicular phase of all the women in the study group except the pregnant women in their first trimester showed that the hormonal level was in synergism to the lymphoid homeostasis. The mean concentration of progesterone in pregnant women was significantly higher compared to that in the spontaneous aborter. In pregnancy the placenta continues to produce more progesterone, such that at the point of abortion, the level would be higher than in the non- pregnant state.

Testosterone and estradiol are said to enhance cell-mediated immunity in-vitro suggesting that sex steroid hormone may be enhancing immune function through direct actions on immune cells (Chao, 1996). This results in decreased lymphocyte proliferation to the T-cell mitogen, compared with control group with the level of testosterone and estradiol. Estradiol has immuno-enhancing effects on antibody production in-vitro, whereas testosterone has immunosuppressive effects on B and T -cell differentiation. Therefore, enhanced immune function in females has been hypothesised to be attributed at least in part to lack of the immunosuppressive effects of androgens (Chao, 1996). The mean serum estradiol concentration in the pregnant women studied and that in the spontaneous aborter were also significantly higher than the other test groups. Whereas the mean values of testosterone and prolactin in the test groups were significantly higher when compared to the control groups. This study to some extent agrees with other results that indicate that women with unexplained infertility secrete less progesterone in the early follicular phase than fertile women. Ratcliffe et al., (1983) found lower plasma progesterone concentrations in patients whose infertility was explained by male factors or tubal occlusion.

The influence of metabolites in reproduction are usually pronounced at the level of coenzymes or a metal co-factor that also may serve as immune enhancers because of their antioxidant properties. Vitamin E had been demonstrated to have some biological activities that are attributable to its major role in the prevention of lipid peroxidation in biological membranes and is an important intra-membrane antioxidant (Burton et al., 1983). A review of an in-vitro experiment has demonstrated antioxidant synergism between alpha-tocopherol regeneration and ascorbate in a protein renaturing system following free radical mop up, which revealed that ascorbate regenerate vitamin E by a non-enzymatic mechanism (Burton et al., 1983). This suggest that significant interaction occurs between water and lipid-soluble vitamins at the membrane cytosol interface and that ascorbic acid may as well have functioned in-vivo in the repair of membrane bound oxidized vitamin E (Chan, 1994). Deficiency of Vitamin E markedly impaired both humoral and cellular immunity thus emphasizing the importance of this micronutrient in the diet. A higher intake was needed to maintain and improve the decreased cellular immunity function (Karanth et al., 2003).

Oxidative stress represents an imbalance between the generation of free radicals and reactive oxygen species and protection by antioxidant molecules like vitamins E and C that provide additional significant protection. In this study, vitamin E and zinc levels in the test and control groups showed some association. A trial study in groups of women had indicated that zinc supplementation improved pregnancy outcome. Zinc nutriture of pregnant women has been assessed by measuring maternal zinc concentration in plasma or serum, leukocytes and amniotic fluid as well as by other indices including dietary zinc intake (Apgar, 1992). Zinc plays a very important and critical role in various functions of the human body including protein synthesis and nucleic acid metabolism (Valee and Flachuk, 1993). Deficiencies of zinc and iron often co-exist and supplements containing both could be of value in vulnerable population groups. Iron deficiency remains the most common micronutrient deficiency disorder worldwide. It is usually always an acquired condition resulting from a diet that contains insufficient bioavailable iron. The expression of hepcidin, a small cysteine rich cationic peptide, is induced independently by the accumulation of storage iron and by inflammation. Hepcidin ensures that body tissue receive the right amount of iron to meet their functional needs (Finch, 1994).

In the present study, the serum zinc concentration in the women that were positive to anti-Tg was more than the iron concentration. In the same way vitamin E concentration was more than the vitamin C concentration. Hence it may be concluded that serum zinc concentration may be of more importance than iron while vitamin E concentration will be more appreciable than vitamin C concentration determination in infertility study.

CHAPTER SIX

6.0 SUMMARY OF FINDINGS / CONCLUSIONS

These results have established that high concentrations of auto-anti-thyroglobulin is a significant index in the etiology of primary and secondary infertility in women, as well as recurrent spontaneous aborter women

The study also showed that low progesterone and estradiol as well as high testosterone and prolactin levels in association with anti-Tg and anti-TPO are markers of infertility in women. The findings generated in this study, support the fact that progesterone is necessary for effective pregnancy, and that elevated testosterone levels in the presence of anti-TPO are indeed risk factors for infertility in women as observed in the primary infertile women with anti thyroid antibodies.

In this study, autoimmunity has been directly associated with the etiology of the identified reproductive conditions including unexplained infertility and recurrent pregnancy loss. The autoimmune thyroid disorder is expressed specifically as anti-thyroglobulin and antithyroid peroxidase (microsomal) antibodies.

This study has been able to demonstrate the presence of immunological factors such as anti-Tg and anti-TPO and by extension, responses in both hormonal and biochemical indices as detectable makers in reproductive failure as compared with the controls. The determination of auto-antibodies supported with other parameters would serve as a valuable alternative to determining how the immune system affects reproductive outcome.

Finally the study has been able to show that the essential feature of a successful pregnancy outcome is very much dependent on the status of the immunological defense system.

6.1 CONTRIBUTIONS TO KNOWLEDGE

This work has established the presence of auto-antibodies (i.e. anti-thyroglobulin and anti-thyroperoxidase antibodies) in some euthyroid Nigerian women.

The high cholesterol concentration in the pregnant women the highest being found in the recurrent spontaneous aborter was indicative of infertility being one of the indices of the patho-physiology mimicking state of pregnancy.

The protein level, (albumin index), in the primary and secondary infertile women were higher than in the control women, suggesting that the women's body system responded to increased protein synthesis which characterizes immunological stress responses.

The high levels of the auto-antibodies (anti-thyroperoxidase and anti-thyroglobulin) in the test groups, primary infertile, secondary infertile as well as recurrent spontaneous aborter women, compared to the low levels of auto-antibodies in the control group women were strong factors against women carrying pregnancy to term .

The CD T-lymphocyte counts of killer cells, CD56+16; CD4 and CD19 were higher in primary infertile and recurrent spontaneous aborter than in the control women. Whereas the CD T-lymphocyte counts of the suppressor cells CD8 and CD56 were higher in the control women than in the primary infertile and recurrent spontaneous aborters. This reflected the lymphoid homeostasis to the anti- thyroid auto- antibody levels.

These results have established that the essential features of a successful pregnancy outcome were very much dependent on the auto antibody levels and the immunological correlates of the types of T-cells in the defense mechanism of the women studied.

The serum progesterone and estradiol levels in this study were significantly higher in the pregnant women than in the recurrent spontaneous aborters and lower in the primary and secondary infertile women than in the control women. This result for the first time has established hormone response as another marker to the patho-physiological effects of auto anti-thyroid antibody levels in successful pregnancy outcome.

In conclusion, for the first time, this study has generated locally-documented data on the immuno-reproductive assessment and biochemical correlates of the possible etiology of reproductive failure in euthyroid Nigerian women.

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6.3 APPENDIX I

QUESTIONAIRE

Study of the prevalence of thyroid autoantibody in Nigerian women with reproductive failures:- Questionnaire on clinical classification of patients

	Female (Wife)	Male (Spouse)
1. NAME	_____	_____
2. TRIBE/RACE	_____	_____
3. AGE/ D.O.B	☐☐ Years ☐☐☐☐ ☐☐ ☐☐☐☐	
4. OCCUPATION	_____	_____
5. LEVEL OF HIGHEST EDUCATION ATTAINED	☐ None ☐ Pry. ☐ Sec ☐ Tert.	☐ None ☐ Pry. ☐ Sec.
6. Have You Been Married To Another Person (s) Before:	Yes ☐ No ☐	Yes ☐ No ☐

If *"yes" answer Question 7:*

7. (i) Duration (ii) Reason for separation / divorce

From Month/Year to Month /Year

(i)

(ii)

8. Marital Status now: Sing ☐ Marr. ☐ Div. ☐ Sep. ☐

9. For how long have you been married to the present man? ☐☐ Year ☐☐ Month

10. Have you ever been pregnant before? Yes ☐ No ☐

if the answer is "Yes" answer Q 11. If the answer is "No" proceed to Q. 12.

11. Kindly tell us about the previous pregnancies (including abortions, miscarriages, still-births e.t.c).

	Year you were pregnant	Duration of pregnancy	Place of delivery	Outcome (Enter 1,2,3 or 4 as indicated below)	Complications ** (Enter 1,2,3,4 or 5 as indicated below)
I					
Ii					
Iii					
Iv					
V					
Vi					
Vii					
Viii					

- For Outcome: Enter 1 = Abortion, 1= Still-birth, 3=Normal delivery of live baby or 4 = others (specify)

** For Complications: Enter 1=None, 2=instrument was used to assist delivery or evacuation, 3=Heavy bleeding following delivery, 4=Infection after delivery or 5=others (specify).

- Abortion = Product of conception was delivered before 28 completed weeks of gestation.

+ Still-Baby was born after 28 completed weeks of gestation but with no signs of the life:

If the answer to Q. 10 is 'No" i.e. You have never been pregnant before), answer Question 12.

12.1) Since when have you wanted to become pregnant? M ☐☐ Y ☐☐

i) Do you and your spouse live together? Yes ☐ No ☐

ii) How frequently do you have coitus (intercourse)? Per W☐☐ Per M☐☐

13. Have you had any of the following tests or procedures performed on you?

	Yes No	If 'Yes" Date	Indication	Result (If

		done dd mm yy	(Why it was done)	Known)
Scan/Ultrasonography				
Hormonal assay				
Dilatation and Curetage (D & C)				
Hysterosalphingogram (HSG)				
Seminal Fluid Analysis (SFA)				
Others (Specify				

14. Have you ever had 'in- vitro fertilization' (IVF) done on you?

Yes ☐ No ☐

If 'yes'

Date of procedure	Where (Optional)	Outcome (Result)
i)		
ii)		

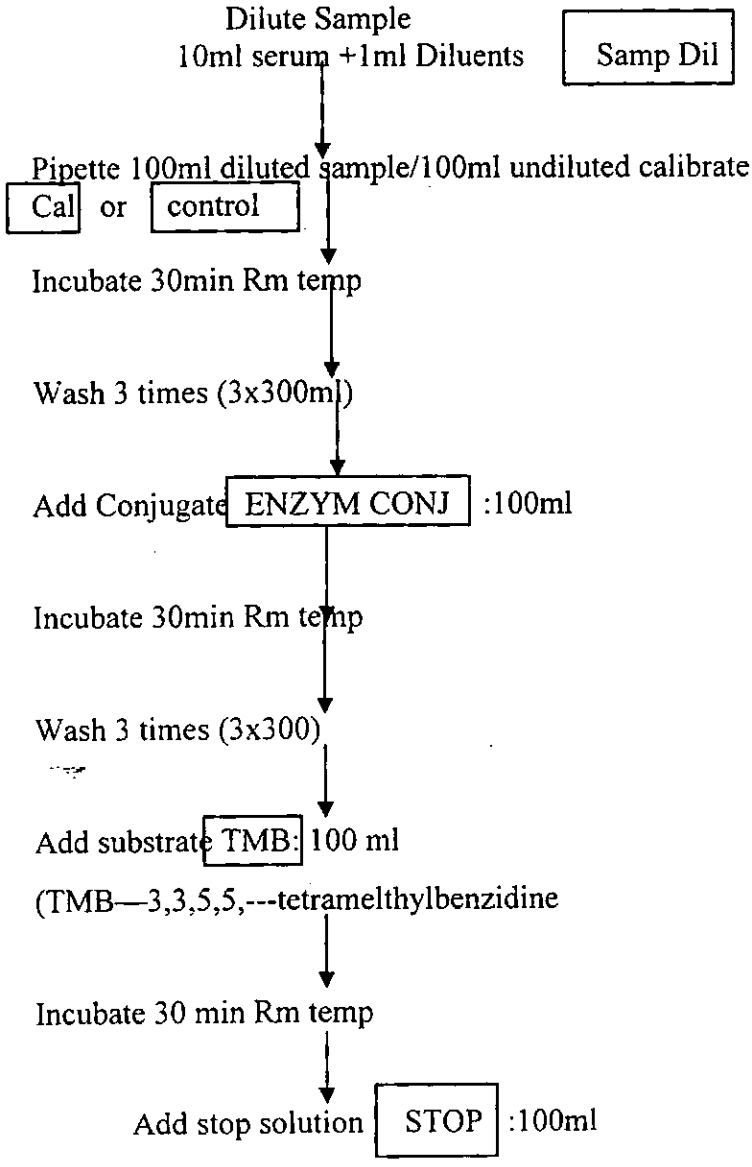
15. Have you ever been told that you have, or have had any of the following condition(s)

	Yes No	If 'yes' Data first diagnosed	Where	Treatment/Medication
Hypertension				
Diabetes Mellitus				
Asthma				
Drug Allergy				
SLE				
Thyroid disease				
Tubal Obstruction				
Fibroid				
Polycystic Ovaries				
Endometriosis				
Premature Ovarian Failure				

APPENDIX II

FLOW CHATS

1. THYROGLOBULIN (TG) / THYROPEROXIDASE (TPO) AUTO ANTIBODY ELISA ASSAY



2. THYROGLOBULIN (TG) / THYROPEROXIDASE (TPO) AUTO ANTIBODY

Agglutination Procedure

Well no	1	2	3	4	5	6 - - - - - - - - - - - 12
Sample Diluents (ML)	50	50	75	75	75	75 75
Semn Di Control (ML)	10	25	25	25	25	25 25
Serum Diluents	1.6	1:18	1:72	1:288	1:1152	1:4608
Unfersitized Particles (NL)		25				
Sensitized Particles (NL)			25	25	25	25 25
Final Dilution		1:27	1:100 $(10)^2$	1:400 $(20)^2$	1:1600 $(40)^2$	1:6400 $(80)^2$ (5120)

View under magnifier

Key
PI – Pro pidium Iodide
DMSO – dimeltyl suloride
D10 – 3, 3, Dioctadecyl oxacarbocyanine Rerchlorate

Nka CELLS CYTOFLOWMETER Assay

Preparation of Target Cells

Grown on stationary complete culture medium (CM)
At 37 °C in 5% CO_2 – for 3 days

Growth flask

- Poured into 15ml plastic tub
- Washed in PBS
- Resuspended in 1ml PBS

Stained

- With 3mM DIO (10 $_M$L)
- Incubated for 20 min at 37°C in 5% $C0_2$
- Washed twice in PBS
- Resuspended in 1 ml Cm

Effectors cell preparation
Peripheral Blood Mononuclear Cells

- Isolated by density gradient tech
  (Hysotopaque 1077
- 1: 1.55 – 5ml Hypaque in 15ml conical tube
  (= 1:1 blood in PBS (3.33ml)

Centrifuged

- at 2000 rpn for 30mins
- collected at the interface ring
- interface washed twice in RPMI
- suspended in CM

T. E. ration 1:50

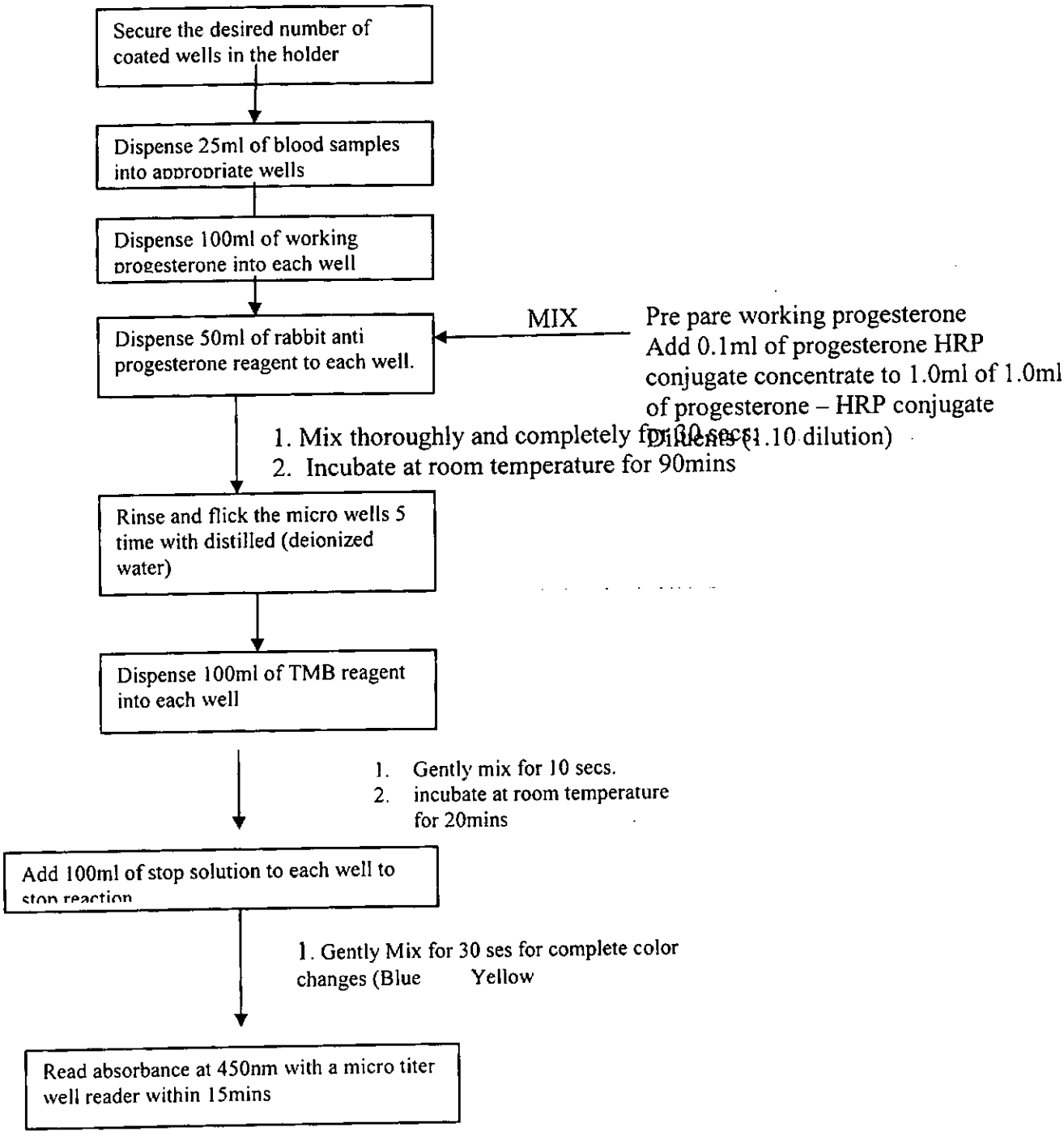
(1) Tubes
10 ml of target cells placed in separation tub
(For background control)
Incubated for 2hrs at 37 0c in 5% C02
(2) Tubes
12.5 mg/d/ Ivig (10 Ml - 5% 1vig)
Incubated for 2hrs
(3) tubes 6mg/d/ Ivig (diluted 1:2)
Centrifuged at 1000g for 35 sec
(Pallets the B and T cells)

Incubate

- Incubate all tubes at 370c in 5% C02
- analysis on Cytometer after 2 hrs
  100 Ml of PT were added to all tubes 15 minute 5c to re analyze

HORMONES ELISA ASSAY

(Progesterone, Estradiol Testosterone etc.)



VIT C (Ascobic Acid) DETERMINATION(Halliwell et al 1996)

Ascobic Acid--------- Dehydroascorbic---------- dinitrophenylic Hydrozone derivatives of dehydroscobic acid

0.5ml heparinized plasma(triplicate)

--2.0ml freshly prepared metaphosphoric acid
--(6.0g/100ml)--
-- vortex mixed
-- centrifuge 10mn at 2500rpm

Protein free Filtrate (clear supernatant)

--1.2ml working std of Ascobic acid
-- 1.2 ml metaphosphoric acid as black

X--- filtrate /std/ Blk—tubes

-- + 0.4 of DTCS reagent (Dinitrophenyl hydrazine in $ThiCU_2SO_4$)
--+mixed incubated at 37^0C for 3hrs

Mixture

- chill in ice bath 10mins
- slowly added 2.0 cold 12M H_2O_2
- Vortex mixed at room temp.

Absorbance against reagent blank at 520nm

REAGENTS:

1. Metaphosphoric acid solution, 6.0g/100ml, was dissolved in 30.0g of metaphosphoric acid ($HP0_3$) in distilled water and brought to a final volume of 500ml prepared immediately before use.
2. Sulfuric acid, 4.5 molar was added slowly of 250ml of concentrated sulfuric acid to 500ml of cold water in a litre flask and brought to a final volume of 1 litre with distilled water, and the flask was placed in ice bath.
3. 650ml of concentrated sulfuric acid (12 molar) was added to 300ml of cold water in a liter flask. Cool, and brought to a final volume of 1 litre with distilled water and refrigerated.
4. 2,4 Dinitrophenyhydrazine reagent, 2.0g/100ml in 4.5 molar sulfuric acid, prepared by dissolving 10g of 2,4- dinitrophenyhydradine in 4.5 molar sulfuric acid and diluted to a final volume of 500ml. it was allowed to stand in the fridge overnight then filtered.
5. Thiourea solution, 5.0g/100ml, was prepared by dissolving 5g of thiourea in glass-distilled water and diluted to a final volume of 100ml. This reagent was stable for one month at $4^{O}C$.
6. Copper sulfate solution, 0.6g/100ml, was prepared by dissolving 0.6g of anhydrous copper sulfate in glass-distilled water and diluted to a final volume of 100ml.
7. Dinitrophenyhydrazine – thiourea – copper sulfate (DTCS) reagent. Combine 5ml of the thiourea solution, 5ml of the copper sulphate solution, 100ml of the 2, 4-dinitrphenyhydrazine reagent. Store in a bottle at $4^{O}C$ for a maximum of one week.
8. Standard. All ascorbic standard were prepared fresh daily.
    a. Ascorbic acid stock standard, 50.0mg/100ml. Dissolve 50mg of ascorbic acid in metaphosphoric acid (6.0g/100ml) and bring to a final volume of 100ml with metaphosphoric, acid.
    b. Intermediate ascorbic acid standard 5.0mg/100ml prepared was by pipetting 100ml of stock standard into a 100ml volumetric flask and diluted to a volume of 100ml with metaphosphoric acid (6.0g/100ml).
    c. Working standards were obtained using a series of 25ml volumetric flask and pipetting the following amounts of intermediate standard. 0.5, 2.0, 4.0, 6.0, 10.0, 15.0, and 20.0ml, then brought to a final volume of 25ml with metaphosphoric acid (6.0g/100ml).

VITAMIN E DETERMINATION

0.2ml x – Serum sample/std/Blk

Into centrifuge tubes (15ml)
+ 1ml of H20 (dist) in sample/Blk or 1.2ml in std

Mixed for 30 second.

Vortex mixed for 30 second
+ 2ml of dist ethanol to Blk/samples or 1.8ml to std

Mixed for 30 seconds

Vortex mixed for 30 seconds
+ 5 ml of Hexane to all tubes

Shaken for 5minutes

Hand shaken or vortex mixed
+ Centrifuged for 5mins at 1500g

Transfer

Hexane layer transferred to a quart cuveh
Spectro photofluorometer {t 295mm activation}
F – values {at 330nm emission }

$$\text{VIT E Mg/Ml} = \frac{Fx - Fblk}{Fstd - F\,blk} \times 20$$

TRIACYLGLYCEROL (TAG) DETERMINATION

Enzymatic colorimetric Test (Glycerol – Phosphate oxidase) Method.
(Trinder 1987)

Triglycerides $\xrightarrow{\text{Lipases}}$ Glycerol + Falty Acids

Glycerol + ATP $\xrightarrow{\text{Gk}}$ Glycerol 3 – phosphate + ADP

Glycerol 3 phosphate + 0_2 $\xrightarrow{\text{GPO}}$ Dihydroxy acetone – phosphate + $H_2{}^0{}_2$

$2H_20_2$ + 4 Amino antipyrine $\xrightarrow{\text{POD}}$ Quinoneimine + Hd + $4H_20$

X – Serum/std/Blk
↓ + 0.02ml of x
↓ + 2.0ml of reagent

Mixture 5mm at 37^0c (10mm at 25^0c)
↓ Incubate
↓
Results – AB at 500nm against Blk within 1hr

Ref value:

TAG	< 200 mg/dl	- No
HDL Shol	< 35 mg/dl	- Yes
TAG	> 200 mg/dl	- Yes

CHOLESTEROL DETERMINATION

Cholesterol Enzymatic Endpoint Method
(Trinder (1987)

Cholesterol – ester + $H2^0$ $\xrightarrow[\text{Esterase}]{\text{Cholesterol}}$ cholesterol + Fatty acids

Cholesterol + 02 $\xrightarrow[\text{Oxidese}]{\text{cholesterol}}$ cholestene – zone + H_20_20

$2H_20_2$ + phenol + 4 Aminoantipyrine $\xrightarrow{\text{Peroxidese}}$ quinoneimine + H_20

X – serum/std/Blk
↓ + 10 ml of x
↓ + 1000 ml of Reagent mix

Mixture
↓ Incubate for 10mm 25^0c (or 5mm 37^0c)
↓ Results - Ab at 500nm agent Blk within 60 minutes

Ref levels in tuman < 5. 17 m Mol/L (200 mg/dl) normal level
5.17 - 6.18 m Mol/L (200 – 239mg/dl) – Bordelunt
> 6.20 m Mol/L (240 mg/dl) - High blood level

ALBUMIN DETERMINATION

(Bodensky et al)

X 2.5 of sample / std
-- 5.0 ml of sample/ std

--- 5.0 ml of working dye(Bromo cresol Green

Mixture
- stand for 10min room temp

Absorbance at 628nm against Blank

Albumin g/100ml =Albumin x conc of std

Ab std

GLUCOSE OXIDASE METHOD

Glucose + O2---------- Glucoronic acid +H2O2

2h2o+4 Aminophenazine+phenol ---------- Quionnamine +H2o
GLUCOSE
oXIDASE (red /violet colour)

PHOSPHATASE DETERMINATION

Phosphatasee (Acid &Alkaline) (issel bacher et al 1970)

ALP -------E.C 3;1, 3;1 AT p49.8

ine p--- nitrophenyl phosphate + H2O---------- PHOSPHATE + P---nitrophenyl

0.5 mr x ----(in curette 1cm light path)
+3.0 ml of the reagent
+ mixed
Absorbance at 405nm

TRANSAMINASES (GOT/ GPT) DETERMINATION

(Eisenthal and Danson 1992)

1) GOT----------E.C. 2.6.1.1 Aspartate transaminase

Aspartate + ketoglutarate---------- Oxaloacetate +Glutamate

Oxaloacetate + 2.4 dinitrophenyl hydrazine----------

2) GPT-----E.C 26.1.2 Alanine transaminate

Alanine + ketoglutarate ---------- pyruvate+ Glutamate

Pyruvate + 2.4 dinitrophenyl hydrazine

X ---0.1 ml sample / Blk----H2O

+ 0.5ml substrate (REAGENT) in unknown (0.1m in Blk)

Mixture

---incubated for 30 c at 37 c

--plus 0.5ml of colour reagent to unknown (1.0 ml in Blk)

Mixture stand for 20 min at room temp.

APPENDIX III

STATISTIC DATA

TABLE 27: THE PEARSON CORRELATION OF SOME SERUM BIOCHEMICAL PARAMETERS WITH THE AUTO-ANTIBODY IN THE WOMEN STUDIED

Correlations

		Study group	Serum glucose concentration (mg/dl)	Serum cholesterol concentration (mg/dl)	Serum triglyceride concentration (mg/dl)	Serum albumiin concentration (g/dl)	Anti thyroglobulin auto antiboby (U/ml)	Anti thyroperoxidase auto antibody (U/ml)
Study group	Pearson Correlation	1	.325	.495	.075	-.213	.654	.350
	Sig. (2-tailed)	.	.000	.000	.289	.002	.000	.000
	Sum of Squares and Cross-products	829.186	4281.000	121549.500	5340.000	-57.050	118127.052	139429.724
	Covariance	2.869	21.089	598.766	26.305	-.281	408.744	482.456
	N	290	204	204	204	204	290	290
Serum glucose concentration (mg/dl)	Pearson Correlation	.325	1	.105	-.148	-.032	.213	.041
	Sig. (2-tailed)	.000	.	.136	.034	.651	.002	.562
	Sum of Squares and Cross-products	4281.000	292282.686	569769.363	-234982.314	-189.333	608835.931	295097.270
	Covariance	21.089	1439.816	2806.746	-1157.548	-.933	2999.192	1453.681
	N	204	204	204	204	204	204	204
Serum cholesterol concentration (mg/dl)	Pearson Correlation	.495	.105	1	.445	-.404	-.034	-.029
	Sig. (2-tailed)	.000	.136	.	.000	.000	.630	.683
	Sum of Squares and Cross-products	121549.500	569769.363	101203380.877	13139798.363	-44766.916	-1803943.936	-3866452.679
	Covariance	598.766	2806.746	498538.822	64728.071	-220.527	-8886.423	-19046.565
	N	204	204	204	204	204	204	204
Serum triglyceride concentration (mg/dl)	Pearson Correlation	.075	-.148	.445	1	-.008	-.190	-.144
	Sig. (2-tailed)	.289	.034	.000	.	.911	.007	.040
	Sum of Squares and Cross-products	5340.000	-234982.314	13139798.363	8597398.686	-253.773	-2943252.069	-5637920.730
	Covariance	26.305	-1157.548	64728.071	42351.718	-1.250	-14498.779	-27773.009
	N	204	204	204	204	204	204	204
Serum albumiin concentration (g/dl)	Pearson Correlation	-.213	-.032	-.404	-.008	1	.074	.032
	Sig. (2-tailed)	.002	.651	.000	.911	.	.292	.654
	Sum of Squares and	-57.050	-189.333	-44766.916	-253.773	121.069	4314.969	4637.228

	Cross-products							
	Covariance	-.281	-.933	-220.527	-1.250	.596	21.256	22.843
	N	204	204	204	204	204	204	204
Anti thyroglobulin auto antiboby (U/ml)	Pearson Correlation	.654	.213	-.034	-.190	.074	1	.384
	Sig. (2-tailed)	.000	.002	.630	.007	.292	.	.000
	Sum of Squares and Cross-products	118127.052	608835.931	-1803943.936	-2943252.069	4314.969	39368387.556	33337451.159
	Covariance	408.744	2999.192	-8886.423	-14498.779	21.256	136222.794	115354.502
	N	290	204	204	204	204	290	290
Anti thyroperoxidase auto antibody (U/ml)	Pearson Correlation	.350	.041	-.029	-.144	.032	.384	1
	Sig. (2-tailed)	.000	.562	.683	.040	.654	.000	.
	Sum of Squares and Cross-products	139429.724	295097.270	-3866452.679	-5637920.730	4637.228	33337451.159	191652374.608
	Covariance	482.456	1453.681	-19046.565	-27773.009	22.843	115354.502	663157.006
	N	290	204	204	204	204	290	290

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

TABLE 28: THE PEARSON CORRELATION OF SOME SERUM ENZYMES ACTIVITY WITH THE AUTO-ANTIBODY IN THE WOMEN STUDIED

Correlations

		Study group	Anti thyroglobulin auto antiboby (U/ml)	Anti thyroperoxidase auto antibody (U/ml)	Serum glutamate oxaloacetate transaminase activity (U/L)	Serum glutamate pyruvate transaminase activity (U/L)	Serum alkaline phosphatase activity (U/L)	Serum acid phosphatase activity (U/L)
Study group	Pearson Correlation	1	.654	.350	-.059	.429	.169	.217
	Sig. (2-tailed)	.	.000	.000	.502	.000	.052	.012
	Sum of Squares and Cross-products	829.186	118127.052	139429.724	-161.000	220.000	4283.575	1171.500
	Covariance	2.869	408.744	482.456	-1.229	1.679	32.699	8.943
	N	290	290	290	132	132	132	132
Anti thyroglobulin auto antiboby (U/ml)	Pearson Correlation	.654	1	.384	-.115	.285	.140	.330
	Sig. (2-tailed)	.000	.	.000	.188	.001	.110	.000
	Sum of Squares and Cross-products	118127.052	39368387.556	33337451.159	-63714.273	29474.545	715434.957	359264.955
	Covariance	408.744	136222.794	115354.502	-486.368	224.997	5461.336	2742.481
	N	290	290	290	132	132	132	132
Anti thyroperoxidase auto antibody (U/ml)	Pearson Correlation	.350	.384	1	.072	.290	-.057	.159
	Sig. (2-tailed)	.000	.000	.	.413	.001	.515	.068
	Sum of Squares and Cross-products	139429.724	33337451.159	191652374.608	100721.568	76137.197	-742522.848	441178.511
	Covariance	482.456	115354.502	663157.006	768.867	581.200	-5668.113	3367.775
	N	290	290	290	132	132	132	132
Serum glutamate oxaloacetate transaminase activity (U/L)	Pearson Correlation	-.059	-.115	.072	1	-.048	-.014	-.188
	Sig. (2-tailed)	.502	.188	.413	.	.585	.875	.031
	Sum of Squares and Cross-products	-161.000	-63714.273	100721.568	19414.636	-174.273	-2491.916	-7181.227
	Covariance	-1.229	-486.368	768.867	148.203	-1.330	-19.022	-54.819
	N	132	132	132	132	132	132	132
Serum glutamate pyruvate transaminase activity (U/L)	Pearson Correlation	.429	.285	.290	-.048	1	.076	.084

	Sig. (2-tailed)	.000	.001	.001	.585	.	.387	.339
	Sum of Squares and Cross-products	220.000	29474.545	76137.197	-174.273	681.879	2558.648	601.455
	Covariance	1.679	224.997	581.200	-1.330	5.205	19.532	4.591
	N	132	132	132	132	132	132	132
Serum alkaline phosphatase activity (U/L)	Pearson Correlation	.169	.140	-.057	-.014	.076	1	.110
	Sig. (2-tailed)	.052	.110	.515	.875	.387	.	.208
	Sum of Squares and Cross-products	4283.575	715434.957	-742522.848	-2491.916	2558.648	1664427.617	39134.481
	Covariance	32.699	5461.336	-5668.113	-19.022	19.532	12705.554	298.736
	N	132	132	132	132	132	132	132
Serum acid phosphatase activity (U/L)	Pearson Correlation	.217	.330	.159	-.188	.084	.110	1
	Sig. (2-tailed)	.012	.000	.068	.031	.339	.208	.
	Sum of Squares and Cross-products	1171.500	359264.955	441178.511	-7181.227	601.455	39134.481	75509.295
	Covariance	8.943	2742.481	3367.775	-54.819	4.591	298.736	576.407
	N	132	132	132	132	132	132	132

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

TABLE29: THE PEARSON CORRELATION OF SOME SERUM T-CELL CD-COUNT WITH THE AUTO-ANTIBODY IN THE WOMEN STUDIED

Correlations

		Study group	Anti thyroglobulin auto antiboby (U/ml)	Anti thyroperoxidase auto antibody (U/ml)	Lyphocyte count (cd56+16) (%)	Lyphocyte count (cd19) (%)	Lyphocyte count (cd56) (%)	Lyphocyte count (cd3) (%)	Lyphocyte count (cd8) (%)
Study group	Pearson Correlation	1	.654	.350	.215	.722	-.656	.591	-.749
	Sig. (2-tailed)	.	.000	.000	.036	.000	.000	.000	.000
	Sum of Squares and Cross-products	829.186	118127.052	139429.724	165.116	659.832	-276.147	709.826	-803.947
	Covariance	2.869	408.744	482.456	1.757	7.019	-2.938	7.551	-8.553
	N	290	290	290	95	95	95	95	95
Anti thyroglobulin auto antiboby (U/ml)	Pearson Correlation	.654	1	.384	.306	.669	-.603	.610	-.653
	Sig. (2-tailed)	.000	.	.000	.003	.000	.000	.000	.000
	Sum of Squares and Cross-products	118127.052	39368387.556	33337451.159	29246.496	76151.612	-31624.747	91206.186	-87363.707
	Covariance	408.744	136222.794	115354.502	311.133	810.124	-336.433	970.279	-929.401
	N	290	290	290	95	95	95	95	95
Anti thyroperoxidase auto antibody (U/ml)	Pearson Correlation	.350	.384	1	.265	.430	-.366	.427	-.424
	Sig. (2-tailed)	.000	.000	.	.009	.000	.000	.000	.000
	Sum of Squares and Cross-products	139429.724	33337451.159	191652374.608	116268.158	224640.966	-88139.174	293290.463	-260389.124
	Covariance	482.456	115354.502	663157.006	1236.895	2389.798	-937.651	3120.111	-2770.097
	N	290	290	290	95	95	95	95	95
Lyphocyte count (cd56+16) (%)	Pearson Correlation	.215	.306	.265	1	.241	-.200	.436	-.239
	Sig. (2-tailed)	.036	.003	.009	.	.018	.052	.000	.020
	Sum of Squares and Cross-products	165.116	29246.496	116268.158	1968.665	566.212	-216.141	1344.101	-659.347
	Covariance	1.757	311.133	1236.895	20.943	6.024	-2.299	14.299	-7.014
	N	95	95	95	95	95	95	95	95
Lyphocyte count (cd19)	Pearson Correlation	.722	.669	.430	.241	1	-.589	.569	-.640

(%)	Sig. (2-tailed)	.000	.000	.000	.018	.	.000	.000	.000
	Sum of Squares and Cross-products	659.832	76151.612	224640.966	566.212	2796.875	-759.321	2092.715	-2102.857
	Covariance	7.019	810.124	2389.798	6.024	29.754	-8.078	22.263	-22.371
	N	95	95	95	95	95	95	95	95
Lyphocyte count (cd56) (%)	Pearson Correlation	-.656	-.603	-.366	-.200	-.589	1	-.490	.573
	Sig. (2-tailed)	.000	.000	.000	.052	.000	.	.000	.000
	Sum of Squares and Cross-products	-276.147	-31624.747	-88139.174	-216.141	-759.321	594.112	-830.644	867.862
	Covariance	-2.938	-336.433	-937.651	-2.299	-8.078	6.320	-8.837	9.233
	N	95	95	95	95	95	95	95	95
Lyphocyte count (cd3) (%)	Pearson Correlation	.591	.610	.427	.436	.569	-.490	1	-.499
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.	.000
	Sum of Squares and Cross-products	709.826	91206.186	293290.463	1344.101	2092.715	-830.644	4834.750	-2156.956
	Covariance	7.551	970.279	3120.111	14.299	22.263	-8.837	51.434	-22.946
	N	95	95	95	95	95	95	95	95
Lyphocyte count (cd8) (%)	Pearson Correlation	-.749	-.653	-.424	-.239	-.640	.573	-.499	1
	Sig. (2-tailed)	.000	.000	.000	.020	.000	.000	.000	.
	Sum of Squares and Cross-products	-803.947	-87363.707	-260389.124	-659.347	-2102.857	867.862	-2156.956	3863.964
	Covariance	-8.553	-929.401	-2770.097	-7.014	-22.371	9.233	-22.946	41.106
	N	95	95	95	95	95	95	95	95
Lyphocyte count (cd4) (%)	Pearson Correlation	.710	.655	.324	.241	.648	-.556	.546	-.632
	Sig. (2-tailed)	.000	.000	.001	.018	.000	.000	.000	.000
	Sum of Squares and Cross-products	840.347	96594.747	219339.374	733.901	2348.741	-928.702	2601.784	-2689.702
	Covariance	8.940	1027.604	2333.398	7.807	24.987	-9.880	27.679	-28.614
	N	95	95	95	95	95	95	95	95

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

TABLE 30: THE PEARSON CORRELATION OF SOME SERUM HORMONE CONCENTRATION WITH THE AUTO-ANTIBODY IN THE WOMEN STUDIED

Correlations		Study group	Anti thyroglobulin auto antiboby (U/ml)	Anti thyroperoxidase auto antibody (U/ml)	Serum progesterone concentration (ng/ml)	Serum estradiol concentration (pg/ml)	Serum testosterone concentration (ng/ml)	Serum prolactin concentration (ng/ml)
Study group	Pearson Correlation	1	.654	.350	.031	-.167	.158	.778
	Sig. (2-tailed)	.	.000	.000	.655	.017	.024	.000
	Sum of Squares and Cross-products	829.186	118127.052	139429.724	167.972	-4279.306	6351.250	31988.850
	Covariance	2.869	408.744	482.456	.827	-21.080	31.287	157.581
	N	290	290	290	204	204	204	204
Anti thyroglobulin auto antiboby (U/ml)	Pearson Correlation	.654	1	.384	-.324	-.368	.256	.493
	Sig. (2-tailed)	.000	.	.000	.000	.000	.000	.000
	Sum of Squares and Cross-products	118127.052	39368387.556	33337451.159	-375158.451	2044384.070	-2225821.851	4393646.801
	Covariance	408.744	136222.794	115354.502	-1848.071	-10070.857	10964.640	21643.580
	N	290	290	290	204	204	204	204
Anti thyroperoxidase auto antibody (U/ml)	Pearson Correlation	.350	.384	1	-.203	-.243	.477	.366
	Sig. (2-tailed)	.000	.000	.	.004	.000	.000	.000
	Sum of Squares and Cross-products	139429.724	33337451.159	191652374.608	-593896.381	3409145.228	-10455615.171	8252056.323
	Covariance	482.456	115354.502	663157.006	-2925.598	-16793.819	51505.493	40650.524
	N	290	290	290	204	204	204	204
Serum progesterone concentration (ng/ml)	Pearson Correlation	.031	-.324	-.203	1	.864	-.305	-.182
	Sig. (2-tailed)	.655	.000	.004	.	.000	.000	.009
	Sum of Squares and Cross-products	167.972	-375158.451	-593896.381	47923.764	198598.619	-109516.516	-67101.709
	Covariance	.827	-1848.071	-2925.598	236.078	978.318	-539.490	-330.550
	N	204	204	204	204	204	204	204
Serum estradiol concentration (pg/ml)	Pearson Correlation	-.167	-.368	-.243	.864	1	-.245	-.388
	Sig. (2-tailed)	.017	.000	.000	.000	.	.000	.000
	Sum of Squares and Cross-	-4279.306	-2044384.070	-3409145.228	198598.619	1103220.418	-422748.899	-686880.762

	products							
	Covariance	-21.080	-10070.857	-16793.819	978.318	5434.583	-2082.507	-3383.649
	N	204	204	204	204	204	204	204
Serum testosterone concentration (ng/ml)	Pearson Correlation	.158	.256	.477	-.305	-.245	1	.291
	Sig. (2-tailed)	.024	.000	.000	.000	.000	.	.000
	Sum of Squares and Cross-products	6351.250	2225821.851	10455615.171	-109516.516	-422748.899	2698766.374	805175.539
	Covariance	31.287	10964.640	51505.493	-539.490	-2082.507	13294.416	3966.382
	N	204	204	204	204	204	204	204
Serum prolactin concentration (ng/ml)	Pearson Correlation	.778	.493	.366	-.182	-.388	.291	1
	Sig. (2-tailed)	.000	.000	.000	.009	.000	.000	.
	Sum of Squares and Cross-products	31988.850	4393646.801	8252056.323	-67101.709	-686880.762	805175.539	2844637.946
	Covariance	157.581	21643.580	40650.524	-330.550	-3383.649	3966.382	14012.995
	N	204	204	204	204	204	204	204

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

TABLE 31: THE PEARSON CORRELATION OF SOME SERUM ANTI-OXIDANT CONCENTRATION WITH THE AUTO-ANTIBODY IN THE WOMEN STUDIED

Correlations

		Study group	Anti thyroglobulin auto antiboby (U/ml)	Anti thyroperoxidase auto antibody (U/ml)	Serum vitamin E concentration (mg/dl)	Serum vitamin C concentration (mg/dl)	Serum iron concentration (ug/dl)	Serum zinc concentration (ug/dl)
Study group	Pearson Correlation	1	.654	.350	-.249	-.477	.203	-.183
	Sig. (2-tailed)	.	.000	.000	.000	.000	.004	.009
	Sum of Squares and Cross-products	829.186	118127.052	139429.724	-285.500	-239.100	16.189	-10.000
	Covariance	2.869	408.744	482.456	-1.406	-1.178	.080	-.049
	N	290	290	290	204	204	204	204
Anti thyroglobulin auto antiboby (U/ml)	Pearson Correlation	.654	1	.384	-.104	-.248	.083	.032
	Sig. (2-tailed)	.000	.	.000	.138	.000	.239	.651
	Sum of Squares and Cross-products	118127.052	39368387.556	33337451.159	-25956.397	-26918.844	1431.509	377.218
	Covariance	408.744	136222.794	115354.502	-127.864	-132.605	7.052	1.858
	N	290	290	290	204	204	204	204
Anti thyroperoxidase auto antibody (U/ml)	Pearson Correlation	.350	.384	1	-.090	-.165	.099	.135
	Sig. (2-tailed)	.000	.000	.	.198	.018	.159	.054
	Sum of Squares and Cross-products	139429.724	33337451.159	191652374.608	-56846.654	-45307.634	4319.627	4037.231
	Covariance	482.456	115354.502	663157.006	-280.033	-223.190	21.279	19.888
	N	290	290	290	204	204	204	204
Serum vitamin E concentration (mg/dl)	Pearson Correlation	-.249	-.104	-.090	1	.110	-.125	.206
	Sig. (2-tailed)	.000	.138	.198	.	.119	.075	.003
	Sum of Squares and Cross-products	-285.500	-25956.397	-56846.654	2213.544	105.938	-19.234	21.738
	Covariance	-1.406	-127.864	-280.033	10.904	.522	-.095	.107
	N	204	204	204	204	204	204	204
Serum vitamin C concentration (mg/dl)	Pearson Correlation	-.477	-.248	-.165	.110	1	-.090	.095
	Sig. (2-tailed)	.000	.000	.018	.119	.	.198	.178
	Sum of Squares and Cross-products	-239.100	-26918.844	-45307.634	105.938	422.766	-6.081	4.360
	Covariance	-1.178	-132.605	-223.190	.522	2.083	-.030	.021

	N	204	204	204	204	204	204	204
Serum iron concentration (ug/dl)	Pearson Correlation	.203	.083	.099	-.125	-.090	1	.110
	Sig. (2-tailed)	.004	.239	.159	.075	.198	.	.117
	Sum of Squares and Cross-products	16.189	1431.509	4319.627	-19.234	-6.081	10.693	.806
	Covariance	.080	7.052	21.279	-.095	-.030	.053	.004
	N	204	204	204	204	204	204	204
Serum zinc concentration (ug/dl)	Pearson Correlation	-.183	.032	.135	.206	.095	.110	1
	Sig. (2-tailed)	.009	.651	.054	.003	.178	.117	.
	Sum of Squares and Cross-products	-10.000	377.218	4037.231	21.738	4.360	.806	5.025
	Covariance	-.049	1.858	19.888	.107	.021	.004	.025
	N	204	204	204	204	204	204	204

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

OTHER STATISTIC DATA

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	46
	2	Multigravida	58
	3	Pregnant	60
	4	Primary Infertile	34
	5	Secondary Infertile	46
	6	Spontaneous Aborters	46
tpoab status	-ve		35
	+ve		255

Descriptive Statistics
Dependent Variable: Anti thyroperoxidase auto antibody (U/ml)

Estimates
Dependent Variable: Anti thyroperoxidase auto antibody (U/ml)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	55.678	.997	-140.614	251.970
Multigravida	54.805	1.171	-175.709	285.319
Pregnant	48.000	1.744	-295.206	391.206
Primary Infertile	1503.500	1.156	1276.046	1730.954
Secondary Infertile	613.391	.993	417.843	808.940
Spontaneous Aborters	747.413	.993	551.864	942.962

a Based on modified population marginal mean.

2. Study group * tpoab status
Dependent Variable: Anti thyroperoxidase auto antibody (U/ml)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	22.476	1.470	-266.941	311.893
	+ve	88.880	1.348	-176.375	354.135
Multigravida	-ve	33.600	2.131	-385.805	453.005
	+ve	76.010	.973	-115.421	267.442
Pregnant	-ve	33.500	3.369	-629.638	696.638
	+ve	62.500	.900	-114.731	239.731
Primary Infertile	-ve	.	.	.	.
	+ve	1503.500	1.156	1276.046	1730.954
Secondary Infertile	-ve	.	.	.	.
	+ve	613.391	.993	417.843	808.940
Spontaneous Aborters	-ve	.	.	.	.
	+ve	747.413	.993	551.864	942.962

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

Multiple Comparisons
Dependent Variable: Anti thyroperoxidase auto antibody (U/ml)
Based on observed means.
* The mean difference is significant at the .05 level.

Anti thyroperoxidase auto antibody (U/ml)

	N	Subset		
Study group		1	2	3

Tukey HSD	Nulligravida	46	58.5652		
	Pregnant	60	60.5667		
	Multigravida	58	68.6983		
	Secondary Infertile	46		613.3913	
	Spontaneous Aborters	46		747.4130	
	Primary Infertile	34			1503.5000
	Sig.		1.000	.930	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 453964.883.
a Uses Harmonic Mean Sample Size = 46.679.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	46
	2	Multigravida	58
	3	Pregnant	60
	4	Primary Infertile	34
	5	Secondary Infertile	46
	6	Spontaneous Aborters	46
tgab status	-ve		169
	+ve		121

Descriptive Statistics
Dependent Variable: Anti thyroglobulin auto antiboby (U/ml)

Estimates
Dependent Variable: Anti thyroglobulin auto antiboby (U/ml)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	42.478	31.624	-19.772	104.728
Multigravida	32.017	28.163	-23.420	87.455
Pregnant	31.900	27.690	-22.606	86.406
Primary Infertile	341.797	78.165	187.933	495.661
Secondary Infertile	473.824	77.536	321.199	626.449
Spontaneous Aborters	306.000	108.427	92.568	519.432

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Anti thyroglobulin auto antiboby (U/ml)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Anti thyroglobulin auto antiboby (U/ml)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound

Nulligravida	-ve	42.478	31.624	-19.772	104.728
Multigravida	+ve	.	.	.	.
	-ve	32.017	28.163	-23.420	87.455
Pregnant	+ve	.	.	.	.
	-ve	31.900	27.690	-22.606	86.406
Primary Infertile	+ve	.	.	.	.
	-ve	144.000	151.663	-154.540	442.540
Secondary Infertile	+ve	539.594	37.916	464.959	614.229
	-ve	138.000	151.663	-160.540	436.540
Spontaneous Aborters	+ve	809.648	32.335	745.999	873.297
	-ve	122.000	214.484	-300.199	544.199
	+ve	490.000	31.973	427.062	552.938

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

Multiple Comparisons
Dependent Variable: Anti thyroglobulin auto antiboby (U/ml)
Based on observed means.
* The mean difference is significant at the .05 level.

Anti thyroglobulin auto antiboby (U/ml)

	Study group	N	Subset 1	2	3
Tukey HSD	Pregnant	60	31.9000		
	Multigravida	58	32.0172		
	Nulligravida	46	42.4783		
	Spontaneous Aborters	46		482.0000	
	Primary Infertile	34		516.3235	
	Secondary Infertile	46			780.4457
	Sig.		1.000	.972	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 46003.373.
a Uses Harmonic Mean Sample Size = 46.679.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum glucose concentration (mg/dl)

Estimates
Dependent Variable: Serum glucose concentration (mg/dl)

Study group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nulligravida	66.313	5.906	54.663	77.962
Multigravida	51.984	10.394	31.484	72.484
Pregnant	47.317	9.150	29.270	65.364
Primary Infertile	46.016	12.529	21.304	70.727
Secondary Infertile	77.848	17.448	43.434	112.263
Spontaneous Aborters	93.176	5.896	81.547	104.806

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum glucose concentration (mg/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum glucose concentration (mg/dl)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	66.313	5.906	54.663	77.962
Multigravida	+ve	.	.	.	.
	-ve	51.984	10.394	31.484	72.484
Pregnant	+ve	.	.	.	.
	-ve	47.317	9.150	29.270	65.364
Primary Infertile	+ve	.	.	.	.
	-ve	26.000	24.310	-21.948	73.948
Secondary Infertile	+ve	66.031	6.078	54.044	78.018
	-ve	79.000	34.380	11.191	146.809
Spontaneous Aborters	+ve	76.697	5.985	64.893	88.501
	-ve	.	.	.	.
	+ve	93.176	5.896	81.547	104.806

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum glucose concentration (mg/dl)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	65.000	8.103	49.017	80.983
Multigravida	+ve	67.625	8.595	50.673	84.577
	-ve	62.000	19.849	22.851	101.149
Pregnant	+ve	41.968	6.175	29.789	54.147
	-ve	49.500	17.190	15.596	83.404
	+ve	45.133	6.277	32.753	57.513
Primary Infertile	-ve	.	.	.	.
	+ve	46.016	12.529	21.304	70.727
Secondary Infertile	-ve	.	.	.	.
	+ve	77.848	17.448	43.434	112.263
Spontaneous Aborters	-ve	.	.	.	.
	+ve	93.176	5.896	81.547	104.806

a Based on modified population marginal mean.

b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum glucose concentration (mg/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum glucose concentration (mg/dl)

	Study group	N	Subset 1	2	3
Tukey HSD	Multigravida	34	43.7353		
	Pregnant	34	45.6471		
	Primary Infertile	34	63.6765	63.6765	
	Nulligravida	34	66.2353	66.2353	
	Secondary Infertile	34		76.7647	76.7647
	Spontaneous Aborters	34			93.1765
	Sig.		.080	.620	.364

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 1181.980.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum cholesterol concentration (mg/dl)
Estimates
Dependent Variable: Serum cholesterol concentration (mg/dl)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	125.608	46.779	33.345	217.870
Multigravida	169.871	82.319	7.510	332.232
Pregnant	1237.300	72.469	1094.367	1380.233
Primary Infertile	185.078	99.232	-10.641	380.797
Secondary Infertile	176.500	138.193	-96.062	449.062
Spontaneous Aborters	1766.500	46.698	1674.397	1858.603

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum cholesterol concentration (mg/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum cholesterol concentration (mg/dl)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	125.608	46.779	33.345	217.870
	+ve	.	.	.	.
Multigravida	-ve	169.871	82.319	7.510	332.232
	+ve	.	.	.	.
Pregnant	-ve	1237.300	72.469	1094.367	1380.233
	+ve	.	.	.	.
Primary Infertile	-ve	172.000	192.539	-207.750	551.750
	+ve	198.156	48.135	103.219	293.094
Secondary Infertile	-ve	183.000	272.291	-354.048	720.048
	+ve	170.000	47.400	76.512	263.488
Spontaneous Aborters	-ve	.	.	.	.
	+ve	1766.500	46.698	1674.397	1858.603

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum cholesterol concentration (mg/dl)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	125.278	64.180	-1.306	251.861
	+ve	125.937	68.073	-8.325	260.200
Multigravida	-ve	173.000	157.207	-137.065	483.065
	+ve	166.742	48.905	70.285	263.199
Pregnant	-ve	1207.000	136.145	938.476	1475.524
	+ve	1267.600	49.713	1169.549	1365.651
Primary Infertile	-ve	.	.	.	.
	+ve	185.078	99.232	-10.641	380.797
Secondary Infertile	-ve	.	.	.	.
	+ve	176.500	138.193	-96.062	449.062
Spontaneous Aborters	-ve	.	.	.	.
	+ve	1766.500	46.698	1674.397	1858.603

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum cholesterol concentration (mg/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum cholesterol concentration (mg/dl)

	Study group	N	Subset 1	2	3
Tukey HSD	Nulligravida	34	125.5882		
	Multigravida	34	167.2941		
	Secondary Infertile	34	170.3824		
	Primary Infertile	34	196.6176		
	Pregnant	34		1260.4706	
	Spontaneous Aborters	34			1766.5000
	Sig.		.891	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 74142.354.

a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum triglyceride concentration (mg/dl)
Estimates
Dependent Variable: Serum triglyceride concentration (mg/dl)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	150.319	24.004	102.975	197.664
Multigravida	160.484	42.242	77.168	243.800
Pregnant	617.867	37.188	544.520	691.213
Primary Infertile	193.141	50.921	92.707	293.574
Secondary Infertile	204.167	70.914	64.301	344.033
Spontaneous Aborters	271.353	23.963	224.090	318.616

a Based on modified population marginal mean.
Pairwise Comparisons
Dependent Variable: Serum triglyceride concentration (mg/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J)

2. Study group * tgab status
Dependent Variable: Serum triglyceride concentration (mg/dl)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound
Nulligravida	-ve	150.319	24.004	102.975	197.664
	+ve	.	.	.	.
Multigravida	-ve	160.484	42.242	77.168	243.800
	+ve	.	.	.	.
Pregnant	-ve	617.867	37.188	544.520	691.213
	+ve	.	.	.	.
Primary Infertile	-ve	189.000	98.802	-5.870	383.870
	+ve	197.281	24.700	148.564	245.999
Secondary Infertile	-ve	209.000	139.727	-66.587	484.587
	+ve	199.333	24.323	151.360	247.307
Spontaneous Aborters	-ve	.	.	.	.

	+ve	271.353	23.963	224.090	318.616

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.
Multiple Comparisons
Dependent Variable: Serum triglyceride concentration (mg/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum triglyceride concentration (mg/dl)

		N	Subset		
	Study group		1	2	3
Tukey HSD	Nulligravida	34	150.5294		
	Multigravida	34	163.3529		
	Primary Infertile	34	196.7941	196.7941	
	Secondary Infertile	34	199.6176	199.6176	
	Spontaneous Aborters	34		271.3529	
	Pregnant	34			595.5882
	Sig.		.697	.242	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 19523.550.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum albumiin concentration (g/dl)

Estimates
Dependent Variable: Serum albumiin concentration (g/dl)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	3.969	.109	3.755	4.184
Multigravida	3.584	.192	3.206	3.962
Pregnant	3.837	.169	3.504	4.169
Primary Infertile	3.998	.231	3.543	4.454
Secondary Infertile	4.482	.322	3.847	5.116
Spontaneous Aborters	2.827	.109	2.613	3.041

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum albumiin concentration (g/dl)

Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum albumiin concentration (g/dl)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	3.969	.109	3.755	4.184
	+ve	.	.	.	.
Multigravida	-ve	3.584	.192	3.206	3.962
	+ve	.	.	.	.
Pregnant	-ve	3.837	.169	3.504	4.169
	+ve	.	.	.	.
Primary Infertile	-ve	3.950	.448	3.066	4.834
	+ve	4.047	.112	3.826	4.268
Secondary Infertile	-ve	4.800	.634	3.550	6.050
	+ve	4.164	.110	3.946	4.381
Spontaneous Aborters	-ve	.	.	.	.
	+ve	2.827	.109	2.613	3.041

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum albumiin concentration (g/dl)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	4.039	.149	3.744	4.334
	+ve	3.900	.158	3.587	4.213
Multigravida	-ve	3.733	.366	3.012	4.455
	+ve	3.435	.114	3.211	3.660
Pregnant	-ve	3.850	.317	3.225	4.475
	+ve	3.823	.116	3.595	4.052
Primary Infertile	-ve	.	.	.	.
	+ve	3.998	.231	3.543	4.454
Secondary Infertile	-ve	.	.	.	.
	+ve	4.482	.322	3.847	5.116
Spontaneous Aborters	-ve	.	.	.	.
	+ve	2.827	.109	2.613	3.041

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum albumiin concentration (g/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum albumiin concentration (g/dl)

	Study group	N	Subset 1	2	3
Tukey HSD	Spontaneous Aborters	34	2.8271		
	Multigravida	34		3.4618	
	Pregnant	34		3.8265	3.8265
	Nulligravida	34			3.9735

Primary Infertile	34			4.0412
Secondary Infertile	34			4.1824
Sig.		1.000	.171	.193

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = .402.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	22
	2	Multigravida	22
	3	Pregnant	22
	4	Primary Infertile	22
	5	Secondary Infertile	22
	6	Spontaneous Aborters	22
tgab status	-ve		68
	+ve		64
tpoab status	-ve		20
	+ve		112

Descriptive Statistics
Dependent Variable: Serum glutamate oxaloacetate transaminase activity (U/L)

Estimates
Dependent Variable: Serum glutamate oxaloacetate transaminase activity (U/L)

	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Study group				
Nulligravida	12.886	2.757	7.428	18.343
Multigravida	8.000	4.467	-.843	16.843
Pregnant	9.895	3.742	2.487	17.302
Primary Infertile	17.525	4.467	8.682	26.368
Secondary Infertile	6.000	2.568	.916	11.084
Spontaneous Aborters	13.409	2.568	8.325	18.493

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum glutamate oxaloacetate transaminase activity (U/L)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum glutamate oxaloacetate transaminase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Study group	tgab status				
Nulligravida	-ve	12.886	2.757	7.428	18.343
	+ve	.	.	.	.
Multigravida	-ve	8.000	4.467	-.843	16.843
	+ve	.	.	.	.
Pregnant	-ve	9.895	3.742	2.487	17.302
	+ve	.	.	.	.

Primary Infertile	-ve	23.000	8.518	6.138	39.862
	+ve	12.050	2.694	6.718	17.382
Secondary Infertile	-ve	.	.	.	.
	+ve	6.000	2.568	.916	11.084
Spontaneous Aborters	-ve	.	.	.	.
	+ve	13.409	2.568	8.325	18.493

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum glutamate oxaloacetate transaminase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	10.200	3.110	4.043	16.357
	+ve	15.571	4.553	6.558	24.585
Multigravida	-ve	1.000	8.518	-15.862	17.862
	+ve	15.000	2.694	9.668	20.332
Pregnant	-ve	7.000	6.955	-6.768	20.768
	+ve	12.789	2.764	7.319	18.260
Primary Infertile	-ve	.	.	.	.
	+ve	17.525	4.467	8.682	26.368
Secondary Infertile	-ve	.	.	.	.
	+ve	6.000	2.568	.916	11.084
Spontaneous Aborters	-ve	.	.	.	.
	+ve	13.409	2.568	8.325	18.493

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
Multiple Comparisons
Dependent Variable: Serum glutamate oxaloacetate transaminase activity (U/L)
Based on observed means.
* The mean difference is significant at the .05 level

Serum glutamate oxaloacetate transaminase activity (U/L)

		N	Subset 1
	Study group		
Tukey HSD	Secondary Infertile	22	6.0000
	Nulligravida	22	11.9091
	Pregnant	22	12.0000
	Primary Infertile	22	13.0455
	Spontaneous Aborters	22	13.4091
	Multigravida	22	13.7273
	Sig.		.280

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 145.111.
a Uses Harmonic Mean Sample Size = 22.000.
b Alpha = .05.
Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	22
	2	Multigravida	22
	3	Pregnant	22
	4	Primary Infertile	22
	5	Secondary Infertile	22

	6	Spontaneous Aborters	22
tgab status	-ve		68
	+ve		64
tpoab status	-ve		20
	+ve		112

Descriptive Statistics
Dependent Variable: Serum glutamate pyruvate transaminase activity (U/L)

Estimates
Dependent Variable: Serum glutamate pyruvate transaminase activity (U/L)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	11.867	.452	10.973	12.761
Multigravida	11.200	.732	9.751	12.649
Pregnant	12.395	.613	11.181	13.608
Primary Infertile	15.625	.732	14.176	17.074
Secondary Infertile	12.955	.421	12.122	13.787
Spontaneous Aborters	14.500	.421	13.667	15.333

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum glutamate pyruvate transaminase activity (U/L)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum glutamate pyruvate transaminase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound
Nulligravida	-ve	11.867	.452	10.973	12.761
	+ve	.	.	.	.
Multigravida	-ve	11.200	.732	9.751	12.649
	+ve	.	.	.	.
Pregnant	-ve	12.395	.613	11.181	13.608
	+ve	.	.	.	.
Primary Infertile	-ve	17.000	1.395	14.238	19.762
	+ve	14.250	.441	13.376	15.124
Secondary Infertile	-ve	.	.	.	.
	+ve	12.955	.421	12.122	13.787
Spontaneous Aborters	-ve	.	.	.	.
	+ve	14.500	.421	13.667	15.333

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum glutamate pyruvate transaminase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	11.733	.510	10.725	12.742

Multigravida	+ve	12.000	.746	10.523	13.477
	-ve	11.000	1.395	8.238	13.762
Pregnant	+ve	11.400	.441	10.526	12.274
	-ve	12.000	1.139	9.745	14.255
Primary Infertile	+ve	12.789	.453	11.893	13.686
	-ve	.	.	.	.
Secondary Infertile	+ve	15.625	.732	14.176	17.074
	-ve	.	.	.	.
Spontaneous Aborters	+ve	12.955	.421	12.122	13.787
	-ve	.	.	.	.
	+ve	14.500	.421	13.667	15.333

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum glutamate pyruvate transaminase activity (U/L)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum glutamate pyruvate transaminase activity (U/L)

	Study group	N	Subset 1	2
Tukey HSD	Multigravida	22	11.3636	
	Nulligravida	22	11.8182	
	Pregnant	22	12.6818	
	Secondary Infertile	22	12.9545	12.9545
	Primary Infertile	22		14.5000
	Spontaneous Aborters	22		14.5000
	Sig.		.088	.106

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 3.894.
a Uses Harmonic Mean Sample Size = 22.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	22
	2	Multigravida	22
	3	Pregnant	22
	4	Primary Infertile	22
	5	Secondary Infertile	22
	6	Spontaneous Aborters	22
tgab status	-ve		68
	+ve		64
tpoab status	-ve		20
	+ve		112

Descriptive Statistics
Dependent Variable: Serum acid phosphatase activity (U/L)

Estimates
Dependent Variable: Serum acid phosphatase activity (U/L)

	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Study group				
Nulligravida	59.043	4.454	50.226	67.860
Multigravida	60.200	7.216	45.914	74.486
Pregnant	58.711	6.045	46.743	70.678
Primary Infertile	68.900	7.216	54.614	83.186

Secondary Infertile	93.682	4.149	85.468	101.896
Spontaneous Aborters	54.545	4.149	46.332	62.759

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum acid phosphatase activity (U/L)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum acid phosphatase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound
Nulligravida	-ve	59.043	4.454	50.226	67.860
	+ve	.	.	.	.
Multigravida	-ve	60.200	7.216	45.914	74.486
	+ve	.	.	.	.
Pregnant	-ve	58.711	6.045	46.743	70.678
	+ve	.	.	.	.
Primary Infertile	-ve	57.000	13.761	29.758	84.242
	+ve	80.800	4.352	72.185	89.415
Secondary Infertile	-ve	.	.	.	.
	+ve	93.682	4.149	85.468	101.896
Spontaneous Aborters	-ve	.	.	.	.
	+ve	54.545	4.149	46.332	62.759

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum acid phosphatase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	59.800	5.025	49.853	69.747
	+ve	58.286	7.356	43.724	72.847
Multigravida	-ve	63.000	13.761	35.758	90.242
	+ve	57.400	4.352	48.785	66.015
Pregnant	-ve	63.000	11.236	40.757	85.243
	+ve	54.421	4.465	45.583	63.259
Primary Infertile	-ve	.	.	.	.
	+ve	68.900	7.216	54.614	83.186
Secondary Infertile	-ve	.	.	.	.
	+ve	93.682	4.149	85.468	101.896
Spontaneous Aborters	-ve	.	.	.	.
	+ve	54.545	4.149	46.332	62.759

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum acid phosphatase activity (U/L)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum acid phosphatase activity (U/L)

	Study group	N	Subset 1	2
Tukey HSD	Spontaneous Aborters	22	54.5455	
	Pregnant	22	55.5909	
	Multigravida	22	57.9091	
	Nulligravida		59.3182	
		22		
	Primary Infertile	22		78.6364
	Secondary Infertile	22		93.6818
	Sig.		.965	.114

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 378.743.
a Uses Harmonic Mean Sample Size = 22.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	22
	2	Multigravida	22
	3	Pregnant	22
	4	Primary Infertile	22
	5	Secondary Infertile	22
	6	Spontaneous Aborters	22
tgab status	-ve		68
	+ve		64
tpoab status	-ve		20
	+ve		112

Descriptive Statistics
Dependent Variable: Serum alkaline phosphatase activity (U/L)
Estimates
Dependent Variable: Serum alkaline phosphatase activity (U/L)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	18.605	24.052	-29.007	66.218
Multigravida	122.510	38.968	45.369	199.651
Pregnant	207.186	32.644	142.564	271.808
Primary Infertile	61.174	38.968	-15.968	138.315
Secondary Infertile	127.166	22.405	82.813	171.519
Spontaneous Aborters	103.891	22.405	59.538	148.244

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum alkaline phosphatase activity (U/L)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).
2. Study group * tgab status

Dependent Variable: Serum alkaline phosphatase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound

Nulligravida	-ve	18.605	24.052	-29.007	66.218
	+ve	.	.	.	.
Multigravida	-ve	122.510	38.968	45.369	199.651
	+ve	.	.	.	.
Pregnant	-ve	207.186	32.644	142.564	271.808
	+ve	.	.	.	.
Primary Infertile	-ve	51.000	74.309	-96.103	198.103
	+ve	71.347	23.499	24.829	117.866
Secondary Infertile	-ve	.	.	.	.
	+ve	127.166	22.405	82.813	171.519
Spontaneous Aborters	-ve	.	.	.	.
	+ve	103.891	22.405	59.538	148.244

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum alkaline phosphatase activity (U/L)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	12.353	27.134	-41.361	66.068
	+ve	24.857	39.720	-53.773	103.487
Multigravida	-ve	131.650	74.309	-15.453	278.753
	+ve	113.370	23.499	66.852	159.888
Pregnant	-ve	279.933	60.673	159.824	400.042
	+ve	134.439	24.109	86.713	182.166
Primary Infertile	-ve	.	.	.	.
	+ve	61.174	38.968	-15.968	138.315
Secondary Infertile	-ve	.	.	.	.
	+ve	127.166	22.405	82.813	171.519
Spontaneous Aborters	-ve	.	.	.	.
	+ve	103.891	22.405	59.538	148.244

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum alkaline phosphatase activity (U/L)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum alkaline phosphatase activity (U/L)

		N	Subset 1	2
	Study group			
Tukey HSD	Nulligravida	22	16.3318	
	Primary Infertile	22	69.4977	69.4977
	Spontaneous Aborters	22	103.8909	103.8909
	Multigravida	22		115.0318
	Secondary Infertile	22		127.1659
	Pregnant	22		154.2795
	Sig.		.071	.088

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 11043.769.
a Uses Harmonic Mean Sample Size = 22.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum progesterone concentration (ng/ml)

Estimates
Dependent Variable: Serum progesterone concentration (ng/ml)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	6.657	.829	5.023	8.291
Multigravida	.470	1.458	-2.406	3.346
Pregnant	40.250	1.284	37.718	42.782
Primary Infertile	2.934E-02	1.758	-3.437	3.496
Secondary Infertile	.293	2.448	-4.534	5.121
Spontaneous Aborters	16.882	.827	15.251	18.514

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum progesterone concentration (ng/ml)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum progesterone concentration (ng/ml)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound
Nulligravida	-ve	6.657	.829	5.023	8.291
	+ve	.	.	.	.
Multigravida	-ve	.470	1.458	-2.406	3.346
	+ve	.	.	.	.
Pregnant	-ve	40.250	1.284	37.718	42.782
	+ve	.	.	.	.
Primary Infertile	-ve	2.850E-02	3.410	-6.698	6.755
	+ve	3.019E-02	.853	-1.651	1.712
Secondary Infertile	-ve	.330	4.823	-9.182	9.842
	+ve	.256	.840	-1.400	1.912
Spontaneous Aborters	-ve	.	.	.	.
	+ve	16.882	.827	15.251	18.514

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum progesterone concentration (ng/ml)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	6.189	1.137	3.947	8.431
	+ve	7.125	1.206	4.747	9.503
Multigravida	-ve	.410	2.784	-5.082	5.902
	+ve	.530	.866	-1.179	2.238
Pregnant	-ve	39.750	2.411	34.994	44.506
	+ve	40.750	.881	39.013	42.487
Primary Infertile	-ve	.	.	.	.
	+ve	2.934E-02	1.758	-3.437	3.496
Secondary Infertile	-ve	.	.	.	.
	+ve	.293	2.448	-4.534	5.121
Spontaneous Aborters	-ve	.	.	.	.
	+ve	16.882	.827	15.251	18.514

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum progesterone concentration (ng/ml)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum progesterone concentration (ng/ml)

	Study group	N	Subset 1	2	3	4
Tukey HSD	Primary Infertile	34	.03009			
	Secondary Infertile	34	.25853			
	Multigravida	34	.51912			
	Nulligravida	34		6.62941		
	Spontaneous Aborters	34			16.88235	
	Pregnant	34				40.63235
	Sig.		.998	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 23.260.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum estradiol concentration (pg/ml)

Estimates
Dependent Variable: Serum estradiol concentration (pg/ml)

Study group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nulligravida	19.073	2.443	14.255	23.891
Multigravida	6.708	4.299	-1.771	15.186
Pregnant	197.117	3.784	189.653	204.581
Primary Infertile	.220	5.182	-10.000	10.441
Secondary Infertile	.406	7.216	-13.827	14.639
Spontaneous Aborters	11.941	2.439	7.132	16.751

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum estradiol concentration (pg/ml)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum estradiol concentration (pg/ml)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	19.073	2.443	14.255	23.891
	+ve	.	.	.	.
Multigravida	-ve	6.708	4.299	-1.771	15.186
	+ve	.	.	.	.
Pregnant	-ve	197.117	3.784	189.653	204.581
	+ve	.	.	.	.
Primary Infertile	-ve	.138	10.054	-19.692	19.969
	+ve	.302	2.514	-4.656	5.259
Secondary Infertile	-ve	.408	14.219	-27.637	28.453
	+ve	.404	2.475	-4.478	5.286
Spontaneous Aborters	-ve	.	.	.	.
	+ve	11.941	2.439	7.132	16.751

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum estradiol concentration (pg/ml)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	19.139	3.351	12.529	25.749
	+ve	19.006	3.555	11.995	26.017
Multigravida	-ve	7.933	8.209	-8.258	24.125
	+ve	5.482	2.554	.445	10.519
Pregnant	-ve	192.750	7.110	178.728	206.772
	+ve	201.483	2.596	196.363	206.604
Primary Infertile	-ve	.	.	.	.
	+ve	.220	5.182	-10.000	10.441
Secondary Infertile	-ve	.	.	.	.
	+ve	.406	7.216	-13.827	14.639
Spontaneous Aborters	-ve	.	.	.	.
	+ve	11.941	2.439	7.132	16.751

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum estradiol concentration (pg/ml)
Based on observed means.

- The mean difference is significant at the .05 level.

Serum estradiol concentration (pg/ml)

	Study group	N	Subset 1	2	3	4
Tukey HSD	Primary Infertile	34	.29218			
	Secondary Infertile	34	.40397			
	Multigravida	34	5.69853	5.69853		
	Spontaneous Aborters	34		11.94118	11.94118	
	Nulligravida	34			19.07647	
	Pregnant	34				200.45588
	Sig.		.621	.462	.308	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 202.182.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

	Value Label	N
Study group	1 Nulligravida	34
	2 Multigravida	34
	3 Pregnant	34
	4 Primary Infertile	34
	5 Secondary Infertile	34
	6 Spontaneous Aborters	34
tgab status	-ve	105
	+ve	99
tpoab status	-ve	25
	+ve	179

Descriptive Statistics
Dependent Variable: Serum testosterone concentration (ng/ml)

Estimates
Dependent Variable: Serum testosterone concentration (ng/ml)

Study group	Mean	Std. Error	95% Confidence Interval Lower Bound	Upper Bound
Nulligravida	1.425	3.998	-6.461	9.311
Multigravida	4.099E-02	7.036	-13.837	13.919
Pregnant	.496	6.194	-11.721	12.713
Primary Infertile	315.964	8.482	299.235	332.693
Secondary Infertile	1.897	11.812	-21.400	25.194
Spontaneous Aborters	14.200	3.991	6.328	22.072

a Based on modified population marginal mean.
Pairwise Comparisons
Dependent Variable: Serum testosterone concentration (ng/ml)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).
2. Study group * tgab status
Dependent Variable: Serum testosterone concentration (ng/ml)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	1.425	3.998	-6.461	9.311
	+ve	.	.	.	.
Multigravida	-ve	4.099E-02	7.036	-13.837	13.919
	+ve	.	.	.	.
Pregnant	-ve	.496	6.194	-11.721	12.713
	+ve	.	.	.	.
Primary Infertile	-ve	327.500	16.457	295.041	359.959
	+ve	304.428	4.114	296.313	312.543
Secondary Infertile	-ve	2.300	23.274	-43.604	48.204
	+ve	1.494	4.051	-6.497	9.485
Spontaneous Aborters	-ve	.	.	.	.
	+ve	14.200	3.991	6.328	22.072

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum testosterone concentration (ng/ml)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	1.406	5.486	-9.414	12.225
	+ve	1.444	5.818	-10.032	12.920
Multigravida	-ve	5.067E-02	13.437	-26.452	26.553
	+ve	3.132E-02	4.180	-8.213	8.276
Pregnant	-ve	.467	11.637	-22.484	23.419
	+ve	.525	4.249	-7.855	8.906
Primary Infertile	-ve	.	.	.	.
	+ve	315.964	8.482	299.235	332.693
Secondary Infertile	-ve	.	.	.	.
	+ve	1.897	11.812	-21.400	25.194
Spontaneous Aborters	-ve	.	.	.	.
	+ve	14.200	3.991	6.328	22.072

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum testosterone concentration (ng/ml)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum testosterone concentration (ng/ml)

	Study group	N	Subset 1	2
Tukey HSD	Multigravida	34	.03303	
	Pregnant	34	.51853	
	Nulligravida	34	1.42353	
	Secondary Infertile	34	1.51765	
	Spontaneous Aborters	34	14.20000	
	Primary Infertile	34		305.78529

Sig.		.126	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 541.671.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum prolactin concentration (ng/ml)

Estimates
Dependent Variable: Serum prolactin concentration (ng/ml)

	Mean	Std. Error	95% Confidence Interval	
Study group			Lower Bound	Upper Bound
Nulligravida	13.716	10.915	-7.813	35.244
Multigravida	32.828	19.208	-5.057	70.713
Pregnant	15.809	16.910	-17.543	49.161
Primary Infertile	196.952	23.155	151.283	242.621
Secondary Infertile	186.064	32.246	122.464	249.663
Spontaneous Aborters	279.803	10.896	258.312	301.294

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum prolactin concentration (ng/ml)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum prolactin concentration (ng/ml)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound
Nulligravida	-ve	13.716	10.915	-7.813	35.244
	+ve	.	.	.	.
Multigravida	-ve	32.828	19.208	-5.057	70.713
	+ve	.	.	.	.
Pregnant	-ve	15.809	16.910	-17.543	49.161
	+ve	.	.	.	.
Primary Infertile	-ve	210.000	44.927	121.389	298.611
	+ve	183.903	11.232	161.750	206.056

Secondary Infertile	-ve	211.000	63.536	85.685	336.315
	+ve	161.127	11.060	139.313	182.942
Spontaneous Aborters	-ve	.	.	.	.
	+ve	279.803	10.896	258.312	301.294

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum prolactin concentration (ng/ml)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	14.450	14.976	-15.087	43.987
	+ve	12.981	15.884	-18.347	44.310
Multigravida	-ve	30.333	36.683	-42.017	102.684
	+ve	35.323	11.411	12.815	57.830
Pregnant	-ve	14.125	31.768	-48.532	76.782
	+ve	17.493	11.600	-5.386	40.373
Primary Infertile	-ve	.	.	.	.
	+ve	196.952	23.155	151.283	242.621
Secondary Infertile	-ve	.	.	.	.
	+ve	186.064	32.246	122.464	249.663
Spontaneous Aborters	-ve	.	.	.	.
	+ve	279.803	10.896	258.312	301.294

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
Multiple Comparisons
Dependent Variable: Serum prolactin concentration (ng/ml)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum prolactin concentration (ng/ml)

		N	Subset		
	Study group		1	2	3
Tukey HSD	Nulligravida	34	13.7588		
	Pregnant	34	17.0971		
	Multigravida	34	34.8824		
	Secondary Infertile	34		162.5941	
	Primary Infertile	34		185.4382	
	Spontaneous Aborters	34			279.8029
	Sig.		.744	.676	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 4036.869.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34

	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum vitamin E concentration (mg/dl)

Estimates
Dependent Variable: Serum vitamin E concentration (mg/dl)

Study group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nulligravida	12.378	.504	11.385	13.372
Multigravida	8.946	.886	7.199	10.694
Pregnant	7.050	.780	5.511	8.589
Primary Infertile	7.375	1.068	5.268	9.482
Secondary Infertile	10.485	1.488	7.551	13.419
Spontaneous Aborters	8.824	.503	7.832	9.815

a Based on modified population marginal mean

Pairwise Comparisons
Dependent Variable: Serum vitamin E concentration (mg/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum vitamin E concentration (mg/dl)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	12.378	.504	11.385	13.372
	+ve	.	.	.	.
Multigravida	-ve	8.946	.886	7.199	10.694
	+ve	.	.	.	.
Pregnant	-ve	7.050	.780	5.511	8.589
	+ve	.	.	.	.
Primary Infertile	-ve	6.000	2.073	1.912	10.088
	+ve	8.750	.518	7.728	9.772
Secondary Infertile	-ve	12.000	2.931	6.219	17.781
	+ve	8.970	.510	7.963	9.976
Spontaneous Aborters	-ve	.	.	.	.
	+ve	8.824	.503	7.832	9.815

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum vitamin E concentration (mg/dl)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	12.944	.691	11.582	14.307
	+ve	11.813	.733	10.367	13.258

Multigravida	-ve	8.667	1.692	5.329	12.004
	+ve	9.226	.526	8.188	10.264
Pregnant	-ve	7.000	1.466	4.109	9.891
	+ve	7.100	.535	6.045	8.155
Primary Infertile	-ve	.	.	.	.
	+ve	7.375	1.068	5.268	9.482
Secondary Infertile	-ve	.	.	.	.
	+ve	10.485	1.488	7.551	13.419
Spontaneous Aborters	-ve	.	.	.	.
	+ve	8.824	.503	7.832	9.815

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
D
ependent Variable: Serum vitamin E concentration (mg/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum vitamin E concentration (mg/dl)

	Study group	N	Subset 1	2	3
Tukey HSD	Pregnant	34	7.0882		
	Primary Infertile	34	8.5882	8.5882	
	Spontaneous Aborters	34	8.8235	8.8235	
	Secondary Infertile	34	9.0588	9.0588	
	Multigravida	34		9.1765	
	Nulligravida	34			12.4118
	Sig.		.066	.962	1.000

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 8.591.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum vitamin C concentration (mg/dl)

Estimates
Dependent Variable: Serum vitamin C concentration (mg/dl)

Study group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nulligravida	2.852	.214	2.430	3.274
Multigravida	2.332	.377	1.589	3.074
Pregnant	2.243	.332	1.589	2.896
Primary Infertile	1.723	.454	.828	2.619
Secondary Infertile	2.105	.632	.858	3.351
Spontaneous Aborters	.747	.214	.326	1.168

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum vitamin C concentration (mg/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum vitamin C concentration (mg/dl)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	2.852	.214	2.430	3.274
Multigravida	+ve	.	.	.	.
	-ve	2.332	.377	1.589	3.074
Pregnant	+ve	.	.	.	.
	-ve	2.243	.332	1.589	2.896
Primary Infertile	+ve	.	.	.	.
	-ve	1.500	.881	-.237	3.237
Secondary Infertile	+ve	1.947	.220	1.513	2.381
	-ve	2.300	1.246	-.157	4.757
Spontaneous Aborters	+ve	1.909	.217	1.481	2.337
	-ve	.	.	.	.
	+ve	.747	.214	.326	1.168

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum vitamin C concentration (mg/dl)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	3.317	.294	2.738	3.896
Multigravida	+ve	2.387	.311	1.773	3.002
	-ve	1.667	.719	.248	3.085
Pregnant	+ve	2.997	.224	2.556	3.438
	-ve	1.975	.623	.747	3.203
Primary Infertile	+ve	2.510	.227	2.061	2.959
	-ve	.	.	.	.
Secondary Infertile	+ve	1.723	.454	.828	2.619
	-ve	.	.	.	.

Spontaneous Aborters	+ve	2.105	.632	.858	3.351
	-ve	.	.	.	.
	+ve	.747	.214	.326	1.168

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum vitamin C concentration (mg/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum vitamin C concentration (mg/dl)

Study group	N	Subset 1	2	3
Tukey HSD Spontaneous Aborters	34	.7471		
Primary Infertile	34		1.9206	
Secondary Infertile	34		1.9206	
Pregnant	34		2.4471	2.4471
Nulligravida	34			2.8794
Multigravida	34			2.8794
Sig.		1.000	.505	.708

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 1.552.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum iron concentration (ug/dl)

Estimates
Dependent Variable: Serum iron concentration (ug/dl)

Study group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nulligravida	.858	.035	.790	.926
Multigravida	.540	.061	.420	.660
Pregnant	.988	.054	.882	1.093
Primary Infertile	.917	.073	.772	1.061
Secondary Infertile	.668	.102	.466	.869

Spontaneous Aborters	.920	.035	.852	.988

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum iron concentration (ug/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum iron concentration (ug/dl)

		Mean	Std. Error	95% Confidence Interval	
Study group	tgab status			Lower Bound	Upper Bound
Nulligravida	-ve	.858	.035	.790	.926
	+ve	.	.	.	.
Multigravida	-ve	.540	.061	.420	.660
	+ve	.	.	.	.
Pregnant	-ve	.988	.054	.882	1.093
	+ve	.	.	.	.
Primary Infertile	-ve	.931	.142	.650	1.211
	+ve	.903	.036	.833	.973
Secondary Infertile	-ve	.487	.201	9.008E-02	.884
	+ve	.849	.035	.779	.918
Spontaneous Aborters	-ve	.	.	.	.
	+ve	.920	.035	.852	.988

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum iron concentration (ug/dl)

		Mean	Std. Error	95% Confidence Interval	
Study group	tpoab status			Lower Bound	Upper Bound
Nulligravida	-ve	.823	.047	.729	.916
	+ve	.893	.050	.794	.992
Multigravida	-ve	.455	.116	.226	.684
	+ve	.625	.036	.554	.697
Pregnant	-ve	1.029	.101	.831	1.228
	+ve	.946	.037	.873	1.018
Primary Infertile	-ve	.	.	.	.
	+ve	.917	.073	.772	1.061
Secondary Infertile	-ve	.	.	.	.
	+ve	.668	.102	.466	.869
Spontaneous Aborters	-ve	.	.	.	.
	+ve	.920	.035	.852	.988

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.

Multiple Comparisons
Dependent Variable: Serum iron concentration (ug/dl)
Based on observed means.

* The mean difference is significant at the .05 level.

Serum iron concentration (ug/dl)

	Study group	N	Subset 1	2
Tukey HSD	Multigravida	34	.61041	
	Secondary Infertile	34		.83794
	Nulligravida	34		.85576
	Primary Infertile	34		.90435
	Spontaneous Aborters	34		.91997
	Pregnant	34		.95565
	Sig.		1.000	.157

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 4.050E-02.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.

Between-Subjects Factors

		Value Label	N
Study group	1	Nulligravida	34
	2	Multigravida	34
	3	Pregnant	34
	4	Primary Infertile	34
	5	Secondary Infertile	34
	6	Spontaneous Aborters	34
tgab status	-ve		105
	+ve		99
tpoab status	-ve		25
	+ve		179

Descriptive Statistics
Dependent Variable: Serum zinc concentration (ug/dl)

Estimates
Dependent Variable: Serum zinc concentration (ug/dl)

Study group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Nulligravida	.609	.024	.562	.655
Multigravida	.369	.042	.287	.451
Pregnant	.468	.037	.396	.540
Primary Infertile	.547	.050	.449	.646
Secondary Infertile	.503	.070	.366	.641
Spontaneous Aborters	.398	.024	.352	.445

a Based on modified population marginal mean.

Pairwise Comparisons
Dependent Variable: Serum zinc concentration (ug/dl)
Based on estimated marginal means
* The mean difference is significant at the .05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

b An estimate of the modified population marginal mean (I).
c An estimate of the modified population marginal mean (J).

2. Study group * tgab status
Dependent Variable: Serum zinc concentration (ug/dl)

Study group	tgab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	.609	.024	.562	.655
	+ve	.	.	.	.
Multigravida	-ve	.369	.042	.287	.451
	+ve	.	.	.	.
Pregnant	-ve	.468	.037	.396	.540
	+ve	.	.	.	.
Primary Infertile	-ve	.564	.097	.372	.755
	+ve	.531	.024	.483	.579
Secondary Infertile	-ve	.492	.137	.221	.763
	+ve	.514	.024	.467	.562
Spontaneous Aborters	-ve	.	.	.	.
	+ve	.398	.024	.352	.445

a This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
b Based on modified population marginal mean.

3. Study group * tpoab status
Dependent Variable: Serum zinc concentration (ug/dl)

Study group	tpoab status	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Nulligravida	-ve	.533	.032	.469	.596
	+ve	.685	.034	.617	.753
Multigravida	-ve	.348	.079	.192	.504
	+ve	.390	.025	.341	.439
Pregnant	-ve	.462	.069	.327	.597
	+ve	.475	.025	.425	.524
Primary Infertile	-ve	.	.	.	.
	+ve	.547	.050	.449	.646
Secondary Infertile	-ve	.	.	.	.
	+ve	.503	.070	.366	.641
Spontaneous Aborters	-ve	.	.	.	.
	+ve	.398	.024	.352	.445

a Based on modified population marginal mean.
b This level combination of factors is not observed, thus the corresponding population marginal mean is not estimable.
Multiple Comparisons
Dependent Variable: Serum zinc concentration (ug/dl)
Based on observed means.
* The mean difference is significant at the .05 level.

Serum zinc concentration (ug/dl)

	Study group	N	Subset 1	2	3
Tukey HSD	Multigravida	34	.38638		

Spontaneous Aborters	34	.39826		
Pregnant	34	.47309	.47309	
Secondary Infertile	34		.51382	.51382
Primary Infertile	34		.53309	.53309
Nulligravida	34			.60438
Sig.		.101	.467	.076

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 1.885E-02.
a Uses Harmonic Mean Sample Size = 34.000.
b Alpha = .05.