METABOLIC EFFECT OF CONTRACEPTIVE AGENTS ON NIGERIAN WOMEN IN LAGOS METROPOLIS

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CERTIFICATION

This is to certify that the theses: **METABOLIC EFFECT OF CONTRACEPTIVE AGENTS ON NIGERIAN WOMEN IN LAGOS METROPOLIS** submitted to the School of Postgraduate Studies, University of Lagos for the award of the degree of

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DEDICATION

UNTO GOD; THE IMMORTAL, THE INVISCIBLE, THE ONLY WISE GOD BE ALL HONOUR, ADORATION, MAJESTY AND PRAISE, NOW AND FOR EVER MORE.

I am deeply indebted to my supervisors PROFESSOR O.A. MAGBAGBEOLA and PROFESSOR A.I. AKINWANDE whose help, stimulating suggestions and encouragements helped me in all the time of research for and writing of this thesis. My gratitude goes to all the other lecturers of the Department of Biochemistry of the College of Medicine of the University of Lagos, Idi Araba, Lagos., late PROF E. O. AKINRIMISI and DR C.J.OWUMI; PROF.R.O.OKOTORE who was my supervisor for my M.Phil, PROF G.O.GBENLE, PROF.V.I.OKOCHI, DR O.A.T. EBUEHI, DR S.O.ODESANMI and my friend, brother and co- supervisor DR A.A.OSUNTOKI. They had all individually and collectively impacted on my life God bless you all.

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and MOSOPEFOLUWA , they became biochemists by proxy, at least those long waiting was worth while, the end indeed justify the means. I am grateful to God for giving me Mosopefoluwa while on the course, what I researched on worked perfectly for me since my thesis was on Contraceptive agents. My prayer is that you will all grow to be greater than we your parents.

My activities; study and research years were years that brought me great satisfaction in my professional life in which I learned about "new" worlds, but also these years scared me since I did not want to disappoint the persons who believed in me. I sincerely hope I did not disappoint you all, thank you and God bless you indeed.

I will sing of the mercies of the Lord for ever Psalm lxxxix 1 William Jones 1726 - 1800 (of Nayland)

1. When all thy mercies, O my God,
   My rising soul surveys,
   Transported with the view, I'm lost
   In wonder, love and praise.

2. Unnumbered comforts to my soul
   Thy tender care bestowed,
   Before my infant heart conceived
   From whom these comforts flowed.

3. When worn with sickness, oft hast thou
   With health renewed my face
   And when in sins and sorrows sunk,
   Revived my soul with grace.

4. Ten thousand thousand precious gifts
   My daily thanks employ;
   Nor is the least a cheerful heart,
   That tastes those gifts with joy.

5. Through every period of my life
   Thy goodness I'll pursue;
   And after death, in distant worlds,
   The glorious theme renew.

6. Through all eternity to thee
   A joyful song I'll raise;
   For O eternity's too short
   To utter all thy praise! Joseph Addisson 1712

Tune Contemplation

ACKNOWLEDGEMENT

I acknowledge one and all, the Lord bless you and keep you, the Lord make his face to shine upon you and be gracious unto you, may He lift his countenance upon you and grant you His peace both now and ever more.

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ABSTRACT

Since the introduction of contraceptives in 1960, efforts have been directed to balance its risks and benefits. The first generation contraceptives were associated with several adverse effect, the most acute being stokes and thromboembolic events. In addition they were found to be associated with cardiovascular risk factors that promote myocardial infarctions in older populations who had used these contraceptives over time in an attempt to minimize these adverse occurrences, contraceptives with lower doses of oestrogen and less androgenic progestins were developed. Also several other methods of using these contraceptives were developed.

The objective of this research was to examine in Nigerian women within Lagos metropolis the effects of these contraceptive use especially the newer ones which hitherto had been banned both in Great Britain and USA but still being used here in Nigeria on lipid profile, glucose iron and copper and their carrier proteins and vitamin E and to also determine the rate of return to fertility after withdrawal of the contraceptives.

The study was conducted at the Family Planning Clinic, Lagos University Teaching Hospital, Idi-Araba, General Hospital, Lagos Family Planning Clinic Oshodi/Isolo Local Government Area, Isolo, Regina Mundi Catholic Church Clinic, Mushin, Lagos and The Planned Parenthood Federation of Nigeria, Palm groove, Lagos.

A total of 400 healthy women were recruited for the research, 50 Hormonal Implant users, 100 intrauterine devices users, 100 Injectable users 100 oral contraceptive users and 50 controls, age range of 15-49years were included in the study.

Subjects had glucose test, lipid profiles, iron and copper and their carrier proteins and vitamin E tests determined.

The tests were determined at the beginning of the research and thereafter every 6 months. Cholesterol, Low Density Lipoprotein-Cholesterol, triglyceride and glucose levels increased for Norplant, Injectable and oral contraceptives users significantly during the research when compared with the baseline and control. High Density Lipoprotein-Cholesterol level decreased significantly for the hormonal contraceptives. Iron and ferritin levels increased for the hormonal contraceptives while the values decreased for the intrauterine devices. Also copper and ceruloplasmin levels increased significantly for the Norplant, oral contraceptive and injectable users while there was a decrease with intrauterine users. The level of vitamin E increased significantly for hormonal contraceptive users and the rate of return of fertility for Norplant was higher than for Injectables.

In Nigeria women within Lagos metropolis contraceptive use is associated with an increased in markers for cardiovascular risk manifested by increased lipid profile and glucose and for those who still want to have more children the rate of return to fertility is delayed especially for injectable users.

1.0

CHAPTER ONE

INTRODUCTION

Ever since the dawn of history, women and men have wanted to be able to decide if and when to have a child. Contraceptives have been used in one form or another for thousands of years throughout human history and even pre-history. In fact, contraception has always been widely practiced, even in societies dominated by social, political, or religious codes that require people to "be fruitful and multiply" (Blundell, 1995; Wills, 2000; Planned Parenthood Federation of America, 2002).

The methods of contraception used before the 20th century were not always as safe or effective as those available today, but most importantly the past three decades have witnessed considerable improvements in reproductive health around the world; however, progress has been uneven in different regions, countries and even within countries.

Since the 1960s, the average number of babies born to women over their reproductive life time has been declining in both more developed and less developed countries. Fertility has fallen drastically since then among both group of countries and this has been mainly due to the improvements in the reproductive health of these women. (McDevitt, 1999).

In sub – Saharan Africa, oral contraceptive use account for about a quarter of all contraceptive use among both married and unmarried women aged 15 – 49years. Overall about 15% of unmarried women use family planning and slightly less than 4% use the pill. Among sexually active unmarried women about 43% use some contraceptive method and 10%

use the pill. In the developed world , 86% Canadian women aged 15 – 44 had used the oral contraceptive while 94% of Eastern German women aged 30 – 44 have taken the pill .(Lautmann and Starke, 1993 ;Boroditsky, *et al.*, 1996).

In 2000, the United Nations estimated global maternal mortality at 529,000 of which less than 1% occurred in the developed world. The risk of a woman dying as a result of pregnancy or childbirth during her lifetime is about 1 in 6 in the poorest parts of the world compared with about 1 in 30,000 in Northern Europe (Ronsmans, *et al.*, 2006).

The low maternal mortality achieved by the developed world is associated with better control of population growth. Also greater emphasis are on maternal and child health through effective contraception with resultant better reproductive health. The high mortality rate in the least developed and developing world including Nigeria arises from a high fertility rate linked to socio-economic and environmental factors such as poverty, level of education, nutritional status and inadequate level of infrastructure (World Health Report, 2005).

World Health Organization (WHO) defined health as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

In the context of this definition, reproductive health therefore, is not merely the absence of diseases or disorder of the reproductive processes, rather it is a condition in which the reproductive process is accomplished in a state of complete physical, mental and social well being. (Fathalla,1992). This implies that people have the ability to reproduce, that women can go through pregnancy and childbirth safely and that reproduction is carried to a successful outcome,

that is; the infants survive and grow up healthy; implicit in this are the right of men and women to be informed of and to have access to safe, effective, affordable and acceptable methods of fertility regulation of their choice, it implies further that people are also able to regulate their fertility without risks to their health.

Reproductive health is now recognized as a key issue for all the Millennium Development Goals (MDGs). (Glasier *et a.l*, 2006). Maternal and infant health have greatly improved in developed countries where infant mortality, adverse neonatal outcomes and maternal mortality has been substantially reduced. (Ronsmans and Graham, 2006 and Lawn *et al.,* 2005). This improvement has been accompanied by an increase in the use of contraceptives and a decrease in fertility rates in these countries. (Van Eijk *et al.,* 2008). However, many developing countries have not experienced such a progress.

The various elements of reproductive health are strongly inter-related and improvement of one can facilitate the improvement of others as indeed the deterioration of one leads to the deterioration of others. While all elements of reproductive health are individually important given the current socio-economic and environmental condition in the world; particularly in developing countries; fertility regulation is central to all other aspects of reproductive health (Fathalla, 1992). The reproductive health-care package covers the health needs of both women and men, but the burden of ill health associated with reproduction is divided unequally between the sexes with women bearing the brunt of it. (Fathalla,1992). According to World Health Organization, reproductive and sexual ill health account for 20% of the global burden of ill-health for women and 14% for men.

The most significant development in reproductive health over the past few decades has been the major expansion in contraceptive use world wide with potential benefits to individuals, families, societies and the world at large. (Fathalla,1990). Also, the world wide demand for family planning services is growing because of two trends: the burgeoning members of young people entering child bearing age and increasing adoption of contraceptive use (Kent, 2008)

The need to regulate fertility has been recognized by women and men living in the most varied social circumstances who have different needs and perspectives. Fertility regulation has diffused less rapidly through societies than the means to cure diseases and prevent death. (Potts and Campbell, 2002). Over the past few decades there has been an unprecedented steep decline in fertility in developing countries as a whole with a corresponding rise in contraceptive use. Among developing countries, contraceptive use is the highest in East Asia and lowest in Sub-Sahara Africa, (Shah, 2001).

In developing countries; the prevalence of contraceptive use, which is defined as the percentage of married women of reproductive age or their husband using any form of contraceptives rose from 9 percent between 1965 and 1970 to 50 percent between 1985 and 1990.(United Nations, 2001)

The total number of contraceptive users in developing countries is estimated to have risen from 31 million between 1960 and 1965 to 381 million between 1985 and 1990 (United Nations, 2001, The state of the World's children, 2002, Ross *et al*, 2002 and WHO, 2005).

In view of the world wide increases in the use of modern contraceptives, their safety have become a major important public health issue. The past two decades have witnessed a major global research effort on the safety of contraceptives particularly in developing countries (Bongaarts and Johansson, 2002). There are different types of contraceptive methods available to Nigerian women, namely:

1. Oral hormonal contraceptives
2. Injectable contraceptives
3. Hormonal implant contraceptives
4. Intrauterine devices
5. Barrier methods
6. Male and female sterilization

Like many drugs, contraceptives are not without various metabolic side effects. Several reports appearing in the literature have shown that contraceptives influence the metabolism of many nutrients as observed in biochemical studies of blood and urine. Contraceptives have been shown to induce changes in lipids, vitamins, carbohydrates and protein metabolism and consequently their requirements. (Wynn and Doar., 1966; Halsted, *et al.*, 1968; Spellacy, et al.,1975; Krauss, 1982; Lewis, 1983; Fortherlby, 1985;. Magbagbeola, 1988; Magbagbeola and Akinrimisi, 1996; Singh and Ratnan, 1997, Jordan, 2002, Kahn, *et al*, 2003, Frempong ,*et al.*, 2007; Damm, *et al.*, 2007 and Lopez, *et al.*, 2007) .

The changes are related to those associated with an increased risk of coronary heart disease, the risk of neoplasia, the emergence of type 2 diabetes among adolescent girls more than boys

(Fagot-Campagna, *et al.*, 2000) a rising prevalence of gestational diabetes (Ferrarci, *et al.*, 2001), and loss of bone mineral density (BMD) (ARHP, 2005).

There is a gradual wide spread acceptance of these contraceptive agents amongst Nigerian women because of the increasing awareness of the need for birth control. It has also been observed that a large percentage of Nigerian contraceptive users obtain them through commercial outlets and most of the time without prescription or any medical control. Also there is the introduction into the Nigerian society of newer methods of contraceptives like the Hormonal implants (Norplant). Meanwhile the manufacturer of Norplant stopped distributing the Norplant contraceptives in United State of America in July 2002. Norplant was also withdrawn from the United Kingdom in 1999 because of the several complaints of the side effects from the users. The question remains as to how enlightened our women are about the side effects and contraindications of these contraceptives and if the effects observed in the developed world are the same as in the developing countries such as Nigeria.

1.1 STATEMENT OF THE PROBLEM

Contraception has mainly remained the responsibility of women. The sexually active time during fertile period of life may last over 30 years.(WHO, 2005). A family would want to control the number of children they want to have during the period and therefore seek the convenient contraceptive methods when they do not want to get pregnant. The key features of the contraceptive methods are their convenience, efficacy, reversibility and the positive long term health effect.

The first hormonal contraceptive was approved for marketing in the 1960's. This contraceptive, known then and now as the "pill" was taken orally and consisted of an oestrogen and a progestin designed to be taken by women.

Female hormonal contraceptives administered by injection, transdermally, vaginally and released from a sub dermal implant are now available.

Before oral contraceptives were marketed, concern about their non-contraceptive health effects was expressed. A similar concern about the introduction of other forms of hormonal contraceptives exists.

All hormonal contraceptives designed for use by women involve exogenous administration of synthetic estrogen, progestin or both at doses that have been termed "unphysiologic" Administration of these exogenous estrogen and progestin has been shown to; alter secretion of hypothalamic, ovarian and other hormones and this theoretically affected multiple organ system and physiologic processes. Also, it is well known that there is a great difference in the nutritional value, composition and the availability of food available to Nigerian women as

compared to their counterpart in other developed countries and this can have several effects on the metabolism of the contraceptive. The need for studies on the metabolic effects of Contraceptive on women's health has arisen especially within Nigeria where there is a growing acceptance of contraceptives amongst women and couples who want fewer children than they once did .(Sedgh *et al.,* 2006). Considering the fact that women would be exposed to these exogenous hormones over many years, thus any metabolic effect of the contraceptives on the woman"s health condition might have enormous public health implications. It is therefore necessary to constantly researched into these metabolic effects. Presently there are no studies to determine the metabolic effects of these contraceptive agents in our environment. This study will therefore generate local data and provide a comprehensive and broader analysis of the metabolic effects of these contraceptive agents on Nigerian women

ABBREVIATIONS

HCG	-	Human Chorionic Gonadotropin
LH	-	Lutenizing Hormone
FSH	-	Follicle Stimulating Hormone
RH	-	Releasing Hormone
LHRH	-	Lutenizing Releasing Hormone
NADPH	-	Nicotinamide Adenine dinucleotide phosphate hydrogenase.
GnRH	-	Gonadotrophin Releasing Hormone

DEFINITION OF TERMS

Androgens: This is a term embracing any of the male sex hormones, substances that induce and maintain secondary sex characteristics in males. The principal androgens are testosterone and androsterone. They are found in the male testis and adrenal glands, in which they are produced, in the blood in which they circulate and in the urine in which they are excreted.

Contraceptives: Are birth control devices which are designed to prevent fertilization from taking place. For example condom, diaphragms, contraceptive sponges and cervical caps which provide physical barrier that keep sperm and egg from coming into contact, others are chemical methods, and they all prevent pregnancy.

Cyclic AMP: A cyclic form of adenosine monophosphate that activates enzymes in many hormone-induced biochemical reactions.

Corpus Luteum: A yellow mass of tissue that forms in a part of ovarian graafian follicles after ovulation in mammals and secretes the hormone progesterone. If no pregnancy is established: the corpus luteum degenerates: but it continues to secrete the hormone if pregnancy occurs.

Estrogen: These are hormones which are necessary for the development of the reproductive organs and of such secondary sexual characteristics; as the distribution of fat, widening of the pelvis, breast growth and pubic and axillary's hair.

Estradiol: An oesterogenic hormone present in the ovaries, produced synthetically as a component of oral contraceptive products and for treatment of oestrogen deficiency and breast cancer. Formula; $C_{18}H_{24}0_2$.

Oestriol: An oestrogen produced in the ovaries and secreted in the urine during pregnancy.

Oestrone: An oestrogenic hormone produced in the ovaries and synthesized for use in treating oestrogen deficiency and breast cancer. Formula: $C_{18}H_{24}0_2$.

Embryo: A human offspring in the early stages following conception up to the end of the eight week, after which it is classified as a foetus.

Female germ cell (Ovum): Is initially called an oogonium after final mitosis, the oogonia become oocytes which mature into an ovum or unfertilized egg.

Fallopian Uterine tube: One of two tubes in female mammals leading from the ovaries to the upper part of the uterus also known as oviduct .

Follicle – stimulating hormone (FSH): Are produced in both males and females and act upon the sex organs. It is secreted in greater quantity at puberty and it is responsible for the maturation of the reproductive organs and also stimulates the adolescent growth spurt. It is released at the beginning of the menstrual cycle and this stimulates growth of the follicle within each ovary.

Follicle: The germ cells which are formed early in life migrates to the developing gonads to become oogonia, this divide four times to give a cluster of 16 cells, one of these becomes the oocyte and the other 15 nurse cells. These 16 cells are surrounded by somatic, nongerm line cells called follicle cells.

Hypothalamus: The hypothalamus is that part of the brain from which the pituitary gland arises.

HCG: Human chorionic gonadotraphin (HCG) is a protein hormone elevated in urine only during pregnancy.

Hormones: these are chemical substances in animals and plant that regulates bodily processes such as growth, metabolism, reproduction and functioning of various organs. In animals hormones are secreted directly into the bloodstream by ductless endocrine glands.

Implants: Hormonal implant (Norplant): Six small capsules inserted by a health care professional under the skin of upper arm that deliver small amounts of hormone to prevent ovaries from releasing egg.

Implantation: The process by which or stage at which an embryo becomes embedded in the lining of the womb.

Male germ cell (sperm or spermatozoon): The male gonads, the testis are organs that contain germ cells which later develop into male gametes (spermatozoa).

Menstrual cycle: This is the cycle of ovulation and menstruation, the monthly process of ovulation and menstruation that occurs between puberty and menopause in women and females primates who are not pregnant.

Ovary: The ovaries are the female reproductive organs or gonads they are paired almond- shapes bodies situated on either side of the uterus.

Ovarian follicles: These produce the ova or eggs and also secrete a group of hormones called estrogens.

Pituitary gland: The primary gland or hypophysis consists of three lopes: the anterior lobe; the intermediate lobe, which in primates is present for only a short part of the life span; and the posterior lobe. It is situated at the base of the brain and has been called the "Master gland" the anterior and posterior lobes of the pituitary secrete different hormones.

Progesterone: A sex hormone produced in women, first by the corpus interior of the ovary to prepare the womb for the fertilized ovum and later by the placenta to maintain pregnancy. Formula: $C_{18}H_{24}0_2$.

Sex hormones: These are hormones in mammals that influence sexual differentiation and development.

Testosterone: This is the most important of the male hormones called androgens which is produced by the testes. Testosterone stimulates the development of secondary sex characteristics, influences the growth of the prostate secretory activity.

Tubal Ligation: Tying of fallopian tubes: a sterilization technique in which a woman's fallopian tubes are tied to prevent ova entering the uterus. so that the eggs produced cannot be fertilized by sperm after sexual intercourse.

Vasectomy: surgical cutting of spermduct; a surgical operation in which the vas deferens from testis is cut and tied to prevent transfer of sperm during ejaculation. It is the most common form of male sterilization.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BIRTH CONTROL

The history of birth control began with the discovery of the connection between coitus and pregnancy. Apart from abstinence the oldest forms of birth control included; coitus interruptus (withdrawal before ejaculation), pessaries, and the ingestion of herbs that were believed to be contraceptive or abortifacient (Lohiya *et al.*, 2002).

All non-seasonally breeding mammals have evolved mechanisms for the optimum spacing of pregnancies. The higher primates such as man and the chimpanzees are slow breeders and on the average reproduce every 3-5 years. Throughout most of human evolution, the total fertility rate was probably 5-6 (Potts and Bhiwandiwala, 1989). Studies confirm that the human reproductive system has been fine tuned by evolution to produce an optimum interval between two pregnancies. Both infant and maternal mortality and morbidity rise when traditional methods of breast-feeding are abandoned, thereby reducing pregnancy intervals.

In the world as a whole the percentage rate of growth in population has declined marginally in the last few decades, but so much demographic momentum has already been set up that the absolute annual increment in population continues to grow. (Potts and Bhiwandiwala, 1989).

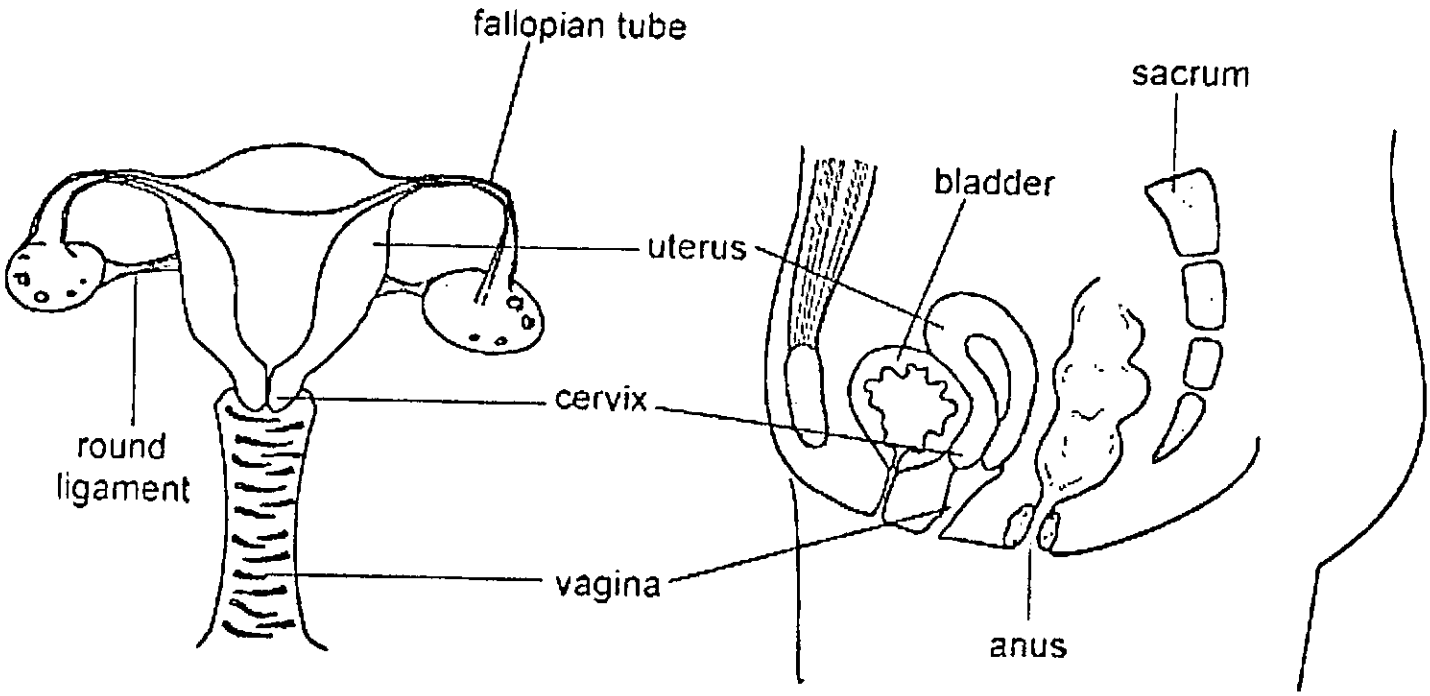
In developing countries fertility is high, and maternal mortality reaches appalling levels due to the lack of access to contraceptives, lower levels of female education and lower rates of female employment. Generally though fertility desires and levels are coming down. This requires contraceptive methods to prevent unwanted pregnancy. There is a close relationship between the level of fertility and contraceptive prevalence. (Shah,2002). The world fertility survey

conducted in the 1970s found a surprisingly strong desire for fertility control in nearly all societies. Almost without exception desired family size is less than achieved family size (Filshie and Guillebaud, 1989). Developing nations are putting increasing resources into family planning services and population programmes. Nevertheless the need for family planning around the world is great and growing and human fertility has been successfully controlled in a number of very different societies and against a variety of socioeconomic backgrounds, using the limited contraceptive methods available.(Chandhick *et al.*, 2003)

2.2 THE FEMALE REPRODUCTIVE SYSTEM

The means whereby mammals increase their numbers to ensure continuity of the species is characterized as sexual reproduction. This involves the union of the female germ cell (ovum) with the male germ cell (sperm or spermatozoon) during the process of copulation or sexual intercourse.

In the mature female mammal, the main reproductive organs are a pair of ovaries, one below each kidney. Each ovary is oval shaped and measures less than 6mm. It has a protective covering of connective tissues; blood capillaries and several thousands of germ cells which mature into eggs or ova during the life of the mammals. These ova are released during ovulation. The speed of ovulation is periodic and impacts directly on the length of a menstrual cycle . Close to each ovary is a fimbriated funnel-shaped structure, and the fallopian funnel which receives the eggs as they are released by the ovary. The funnel leads to a narrow spiraling tube called the Fallopian uterine tube or the oviduct and they have small hairs or cilia to help the egg cell travel. The uterine tube leads downwards to give rise to the pear shaped uterus which opens into the vagina. The lower narrower end of the uterus which leads to the vagina is called the cervix. (Tortora *et al*, 1984). See figure 1 and 2.



FEMALE REPRODUCTIVE SYSTEM

Figure 1: The female reproductive system

Source: Williams text book of endocrinology 7th ed, 1981

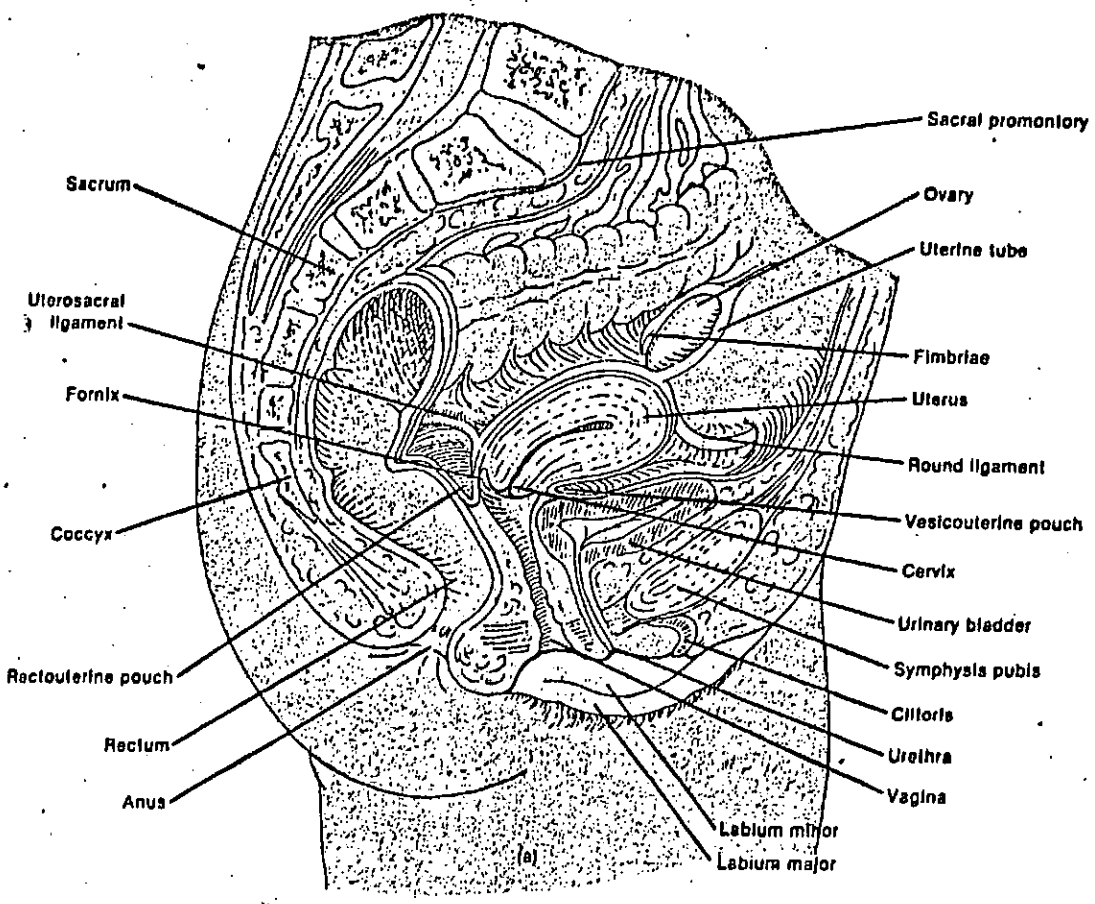


Figure 2; Female organs of reproduction and surrounding structures seen in sagittal section

Source; Yokochi and Rohen, 1979 Photographic Anatomy of the human body, 2nd ed Tokyo, New York.

2.3 THE NORMAL MENSTRUAL CIRCLE

Women during their fertile years are unique in having a more or less regular cycle of changes in their bodies. This cycle is caused by the ebb and flow in the blood stream of various hormones or chemical messengers which are released into it by certain glands. The whole process is controlled by the hypothalamus from the base of the brain (Guillebaud, 1987 , (b)) below which is the pituitary gland.

2.3.1 Egg release from the ovaries

The ovaries are about the same size as a peach-stone though much less hard. And like the testicles of a man they have two functions (a) the production and the release of special sex cells (eggs), and (b) of hormones into the bloodstream. There are several potential egg cells in the ovaries of a baby girl before birth, but by the age of puberty the number has greatly reduced. Normally only one egg is released from one or other ovary during each menstrual cycle which commonly lasts for twenty-eight days. Occasionally more than one egg is released leading, if pregnancy occurs, to multiple birth.

The egg is released from the largest one of several fluid filled egg cells called follicles and is picked up by the seaweed like fonds (or fimbrae) of the outer end of one of the uterine (Fallopian) tubes. It then starts its journey down the uterine tube partly by the whole tube contracting or because there are cilia within the tube which beat rhythmically in the direction of the uterus (Zucherman, 1962, and Guillebaud, 1987). This happens about the middle of a cycle, the time from egg release (ovulation) to the start of the next period is the only part of the cycle which is fixed in length and last between 12-16 days. The first day of menstrual bleeding is always called day 1 of the cycle .The cycle occurs because of a marvelously controlled

interaction of hormones, the most important ones, are two, produced by the pituitary gland – Follicle stimulating hormone (FSH) and Lutenizing hormone (LH) - and two from the ovaries:- Estrogen and Progesterone. Up to the time of egg release, the ovary produce only one female hormone called estrogen. It is made in cell within the wall of the follicle. These give their name to the first part of the menstrual cycle; the follicular phase. During the second part of the menstrual cycle, for almost fourteen days, the particular empty follicle from which the egg came that month now produces another hormone called progesterone as well as estrogen. It also turns yellow in colour and so is given the name corpus luteum and this part of the cycle is called the luteal phase (Zuchermann, 1962 and Guilleband, 1987) (Figure 3 and 4).

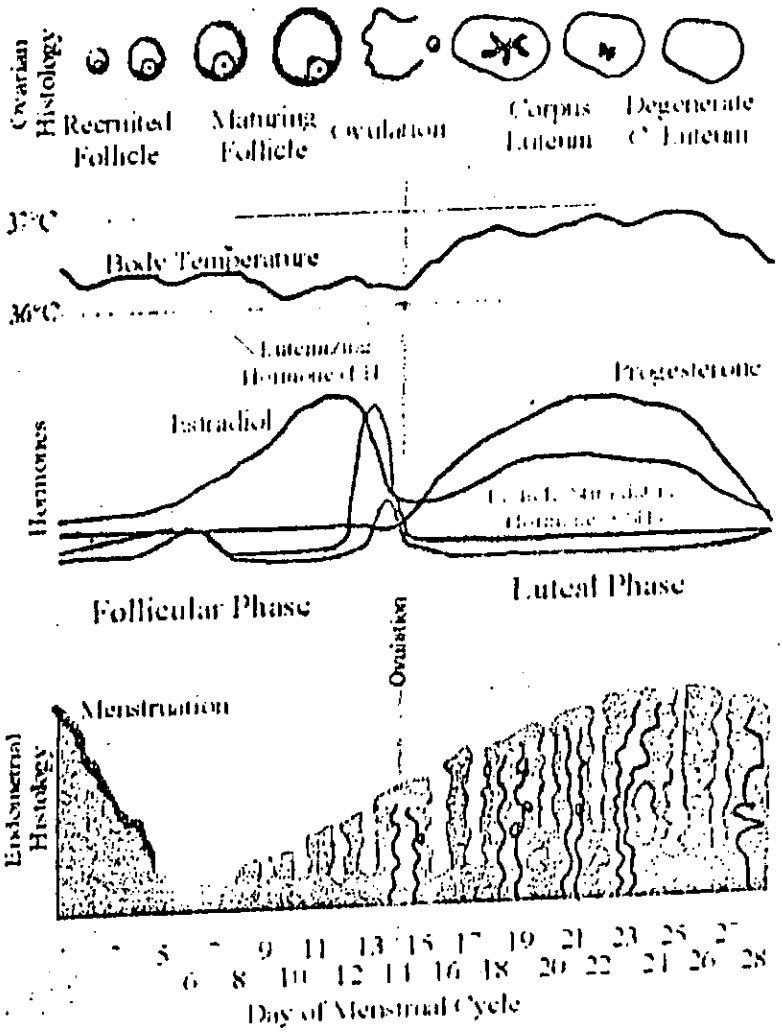


Figure 3: The Menstrual cycle

Source: Williams text book of Endocrinology 7th ed, 1981.

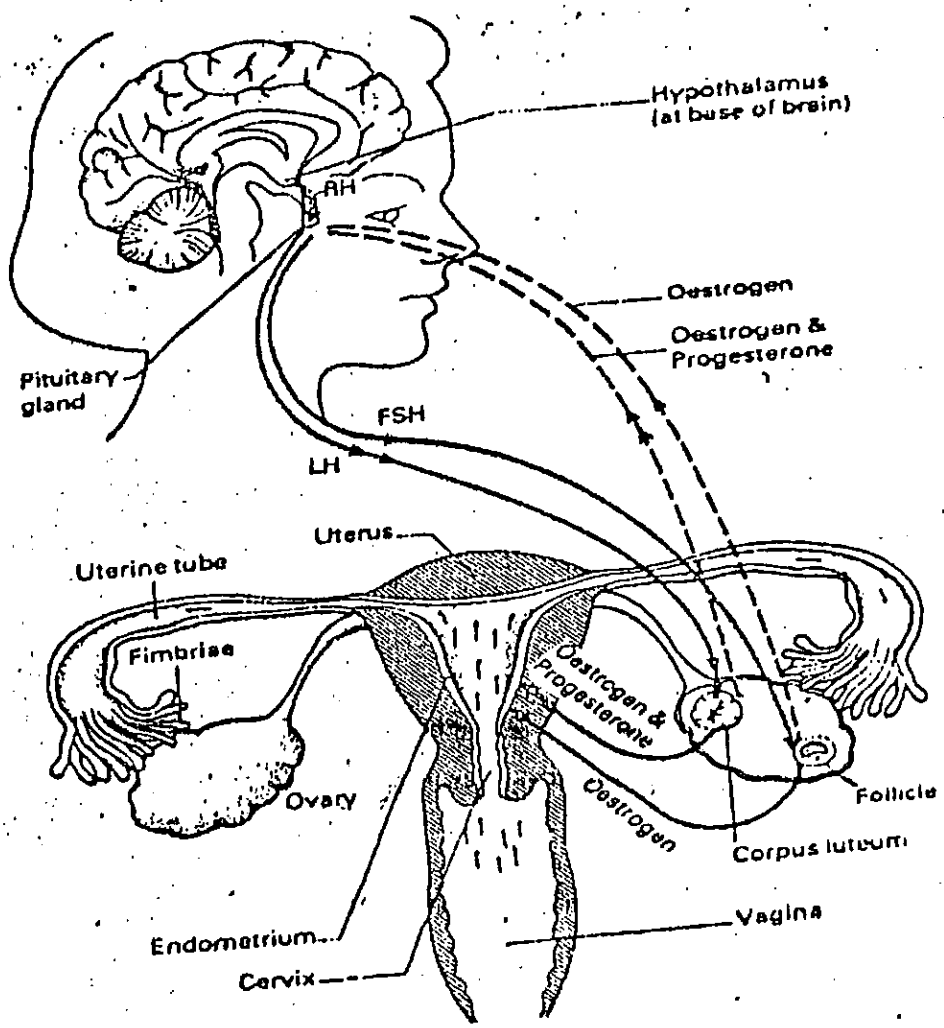


Fig: 4

The female reproductive system: control of the menstrual cycle

→ → the second half of the cycle (luteal phase)

→ events of the first half of the cycle (follicular phase)

... feedback effects.

RH releasing hormone

FSH follicle stimulating hormone

LH luteinizing hormone

Source: Williams Textbook of Endocrinology 7th ed ,1981.

2.3.2 **The uterus**

The estrogens and progesterone travel to the uterus during both phases of the cycle, to thicken its lining with extra glandular tissue and blood vessels so that it is ready just in case a pregnancy starts that month. If the woman has recently had unprotected sex, a sperm may reach the egg in the uterine tube and combine with it. This is called fertilization (Lloyd, 1964). The fertilized egg begins to divide on its journey along the uterine tube towards the uterus, and embeds itself in the prepared lining around the nineteenth day of the cycle. This is called implantation. If implantation fails, the ovary stops producing estrogen and progesterone. These rapid losses of the hormones which produce and maintain the lining of the uterus cause the lining to breakdown and leave the body through the cervix and vagina whereby a woman gets her period and the egg is flushed away. This causes the bleeding of the first day of the next period, this is Menstruation.

But if implantation continues, the embryo produces the special hormone called human chorionic gonadotrophin (hCG.). This sends messages to the corpus luteum to continue producing oestrogen and progesterone to ensure that the lining of the uterus is not shed so that it can continue to provide nourishment for the developing embryo (Lloyd and Weisz,1966).The developing embryo develops into a fetus and gestates until childbirth

2.4 THE OVARIAN HORMONES

Hormones are defined as informational molecules or chemical messengers which are secreted by certain organs of the body and released into the blood stream for transmission into various "target tissues" where they control diverse metabolic processes. The female reproductive system is regulated by gonadotropin, Follicular Stimulating Hormone (FSH) and Lutenizing Hormone (LH) with Releasing Factor or Hormone from the hypothalamus.

Hypothalamic control is exerted by Luteinizing Releasing Hormone (LHRH) or Gonadotrophin Releasing Hormone (GnRH) secreted into the portal hypophyseal vessels. LHRH is normally secreted in "episodic bursts" These bursts appear to be essential for normal secretion of the gonadotropins. (Ojeda *et al.*,2006). However Episodical administration of LHRH at a rate of one pulse per hour stimulates the secretion of LH or FSH. Another form of control is exerted by the Feedback effects of oestrogens and progesterone (steroidal hormones). Oestrogen inhibits FSH and LH secretion during the early part of the follicular phase of the menstrual cycle. The rise in circulating oestrogen 24 hours before ovulation initiates the burst of LH secretion that produces ovulation. FSH and LH secretion is again inhibited by the high circulation of oestrogen and progesterone levels during the luteal phase of the menstrual cycle. (figure 4).

Moderate, to constant level of circulating oestrogen level results in negative feedback effect of LH, while an elevated oestrogen level exerts a positive feedback effect-resulting in stimulation of LH secretion. When circulating levels of progesterone were high the positive feedback effects of oestrogen was inhibited (Ganong, 1983). The ovary is the target organ for both FSH and LH in the female. The granulosa cells of the ovary are the target cells for FSH. The ovarian FSH

receptors are localized on the granulosa cell membrane. The interaction of FSH with its ovarian receptor activates adenylate cyclase resulting in the elevation of Cyclic AMP levels within the cell with the attendant cascade of events. Such events results in the growth and development of multiple layers of granulosa cells accompanied by the formation of a follicle called antrum. Full development of the follicle, however also requires LH. Granulosa cell receptors for LH are increased by FSH stimulation (Ganong, 1983 and Filicori and Cognigni, 2002) demonstrating a synergistic role of FSH and LH in ovary.

2.5 SEX STEROIDAL HORMONES:

Steroids are heterocyclic lipid compounds found throughout the animal and plant kingdoms. The sex steroids are those secreted primarily by the gonads of mammals and they determine the phenotypic, physiologic and behavioural effects which distinguish male from female (Midgley, *et al.*, 1973).

The sex steroids can be divided into three major groups on the basis of structure and function. These groups are:-

1. The progestins (e.g. progesterone)
2. The androgens (e.g. testosterone)
3. The estrogens (e.g. 17β-Estradiol, Estrone and Estriol).

2.5.1 **Biosynthesis of Sex Steroids**

Steroids like other lipids are synthesized from the basic unit which is acetate. A series of reduced Nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) dependent condensation reaction that first yield a 6 carbon compound and then reduced to mevalonic acid that ultimately yield Squalene from activated two carbon fragments of acetyl coenzyme A, have been demonstrated. Subsequent oxidation, demethylations and double bond changes give rise to Hamosterol and finally cholesterol –a 27 carbon alcohol (Fig 5) .(Midgley, *et al*, 1973). This occurs in the liver and many other tissues beside the steroid forming glands.

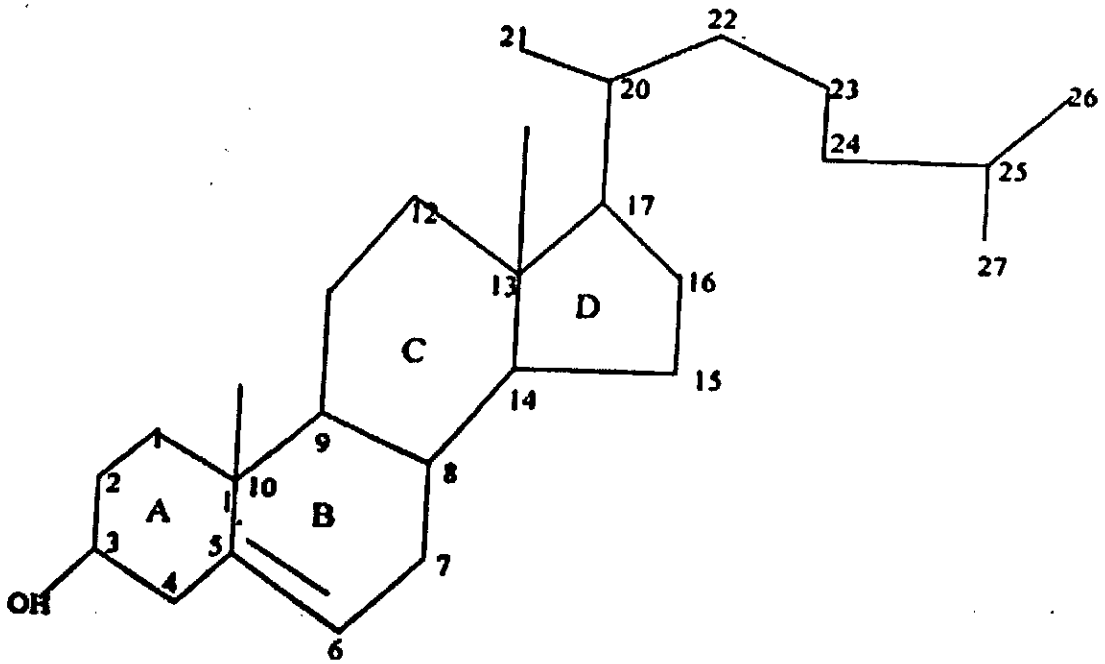
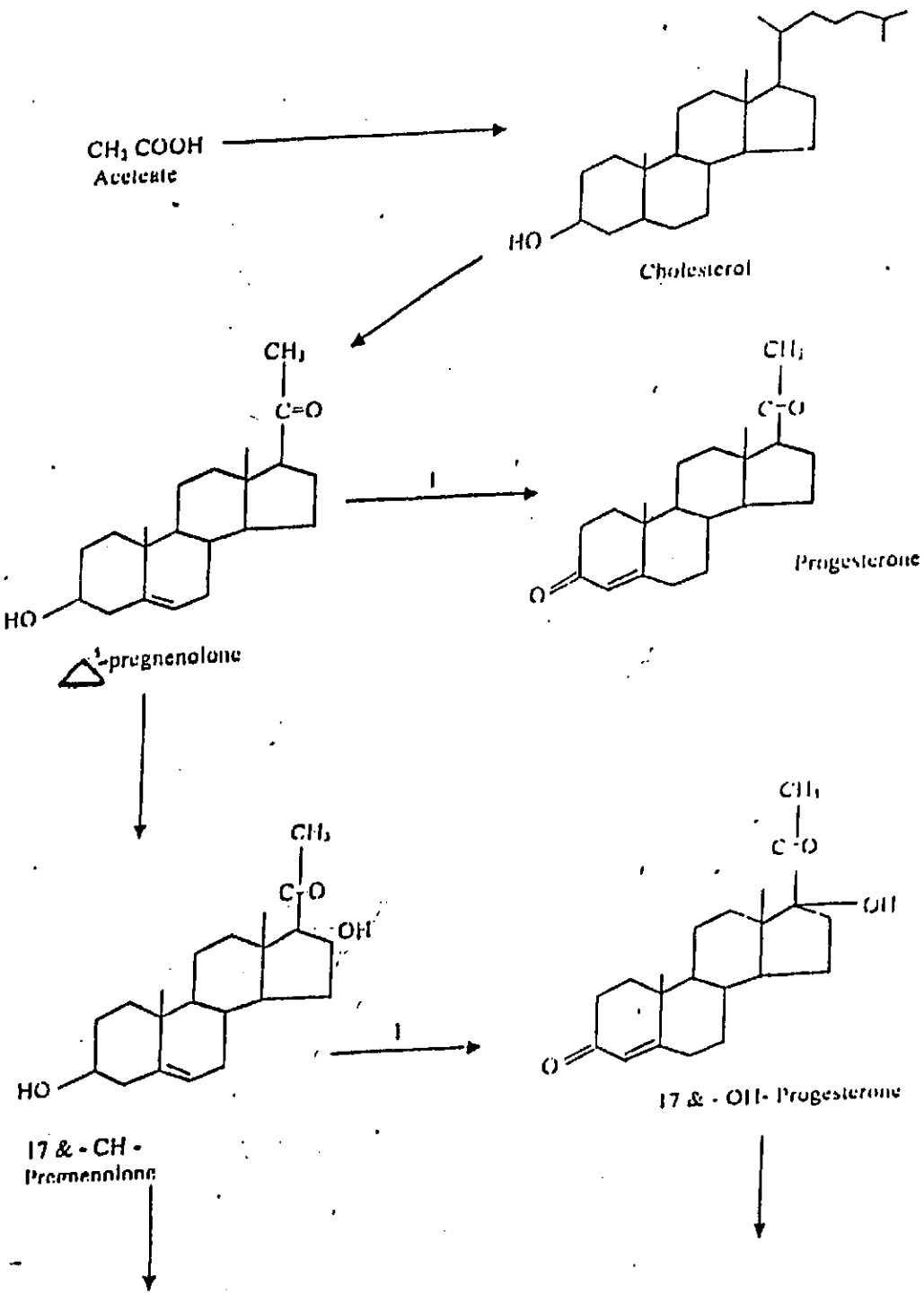


Fig 5: Cholesterol illustrating the Numbering sequence used to identify the carbon atoms and letter designations used for the 4-ring structure comprising cyclopentanophenantherene nucleus

There is a cleavage of the side chain of the cholesterol to yield a 5 – pregnenolone and isocaproic acid. Pregnenolone is a progestin precursor and it seems to be the common substrate for all of the hormonal steroids. Conversion of Pregnenolone requires 5,3, hydroxysteroid dehydrogenase which oxidizes the hydroxyl group at position 3 and an isomerase which changes the 5 double bond to 4 yielding progesterone . Thus progesterone can serve as a precursor for Androgens and oestrogens in both ovaries and the testes.(Figure 6)


CH3 COOH
Acetate
HO
Cholesterol
CH3
C=O
HO
Δ5-pregnenolone
I
Progesterone
OH
17 & - OH- Progesterone
17 & - CH -

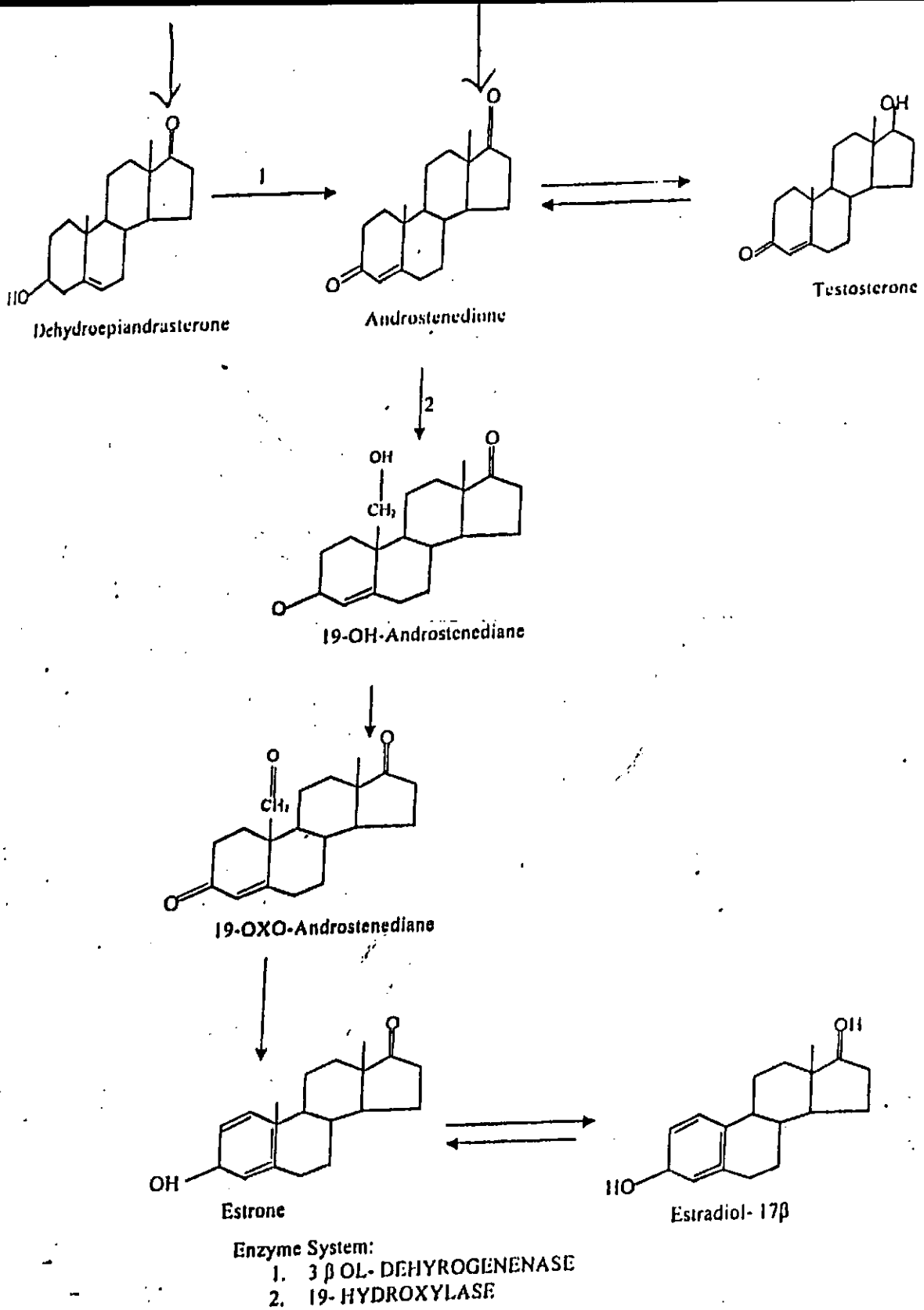


Figure 6: Biosynthesis of Oestrogen and Androgen

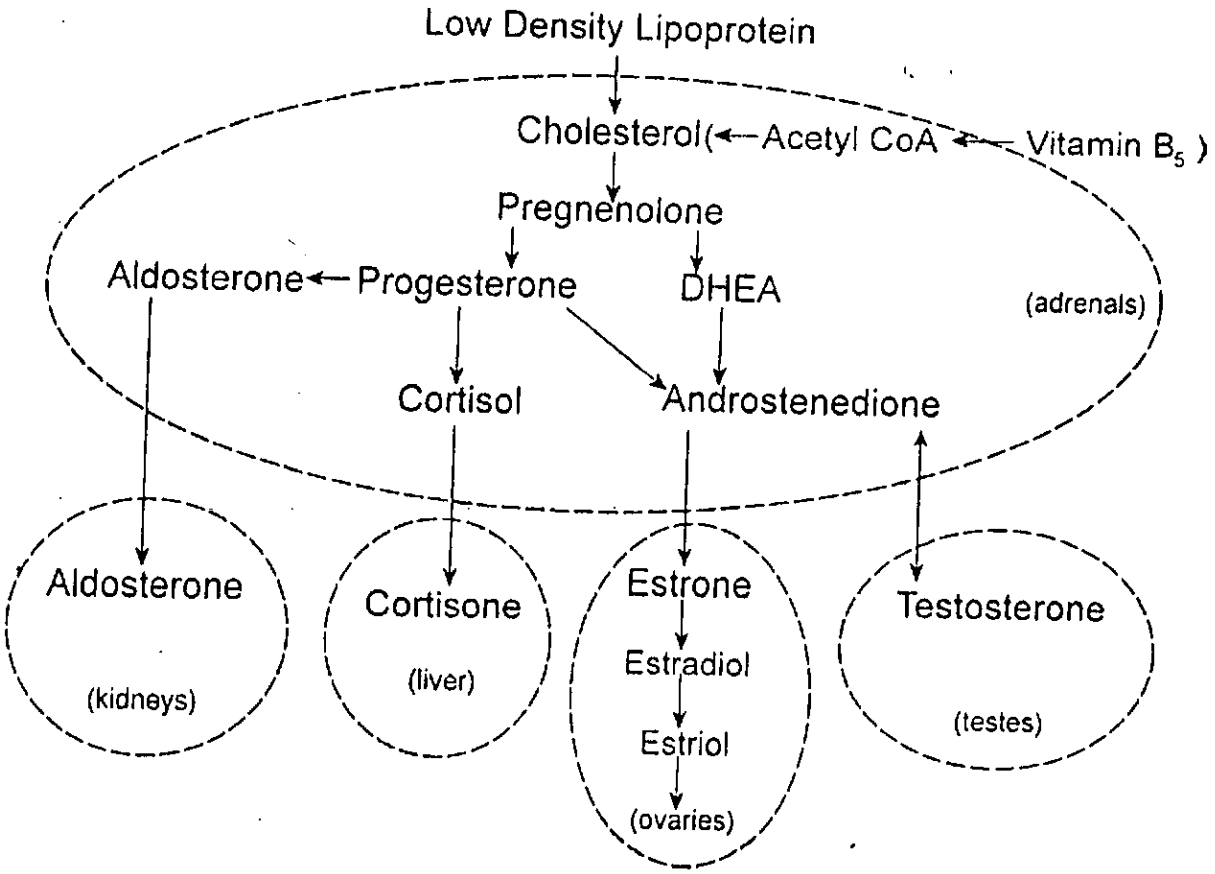


Fig 7: Major pathway of steroid hormone synthesis

Source: Book CGD & Marshall NJ. Essential Endocrinology 3rd ed, Blackwell Science 1996

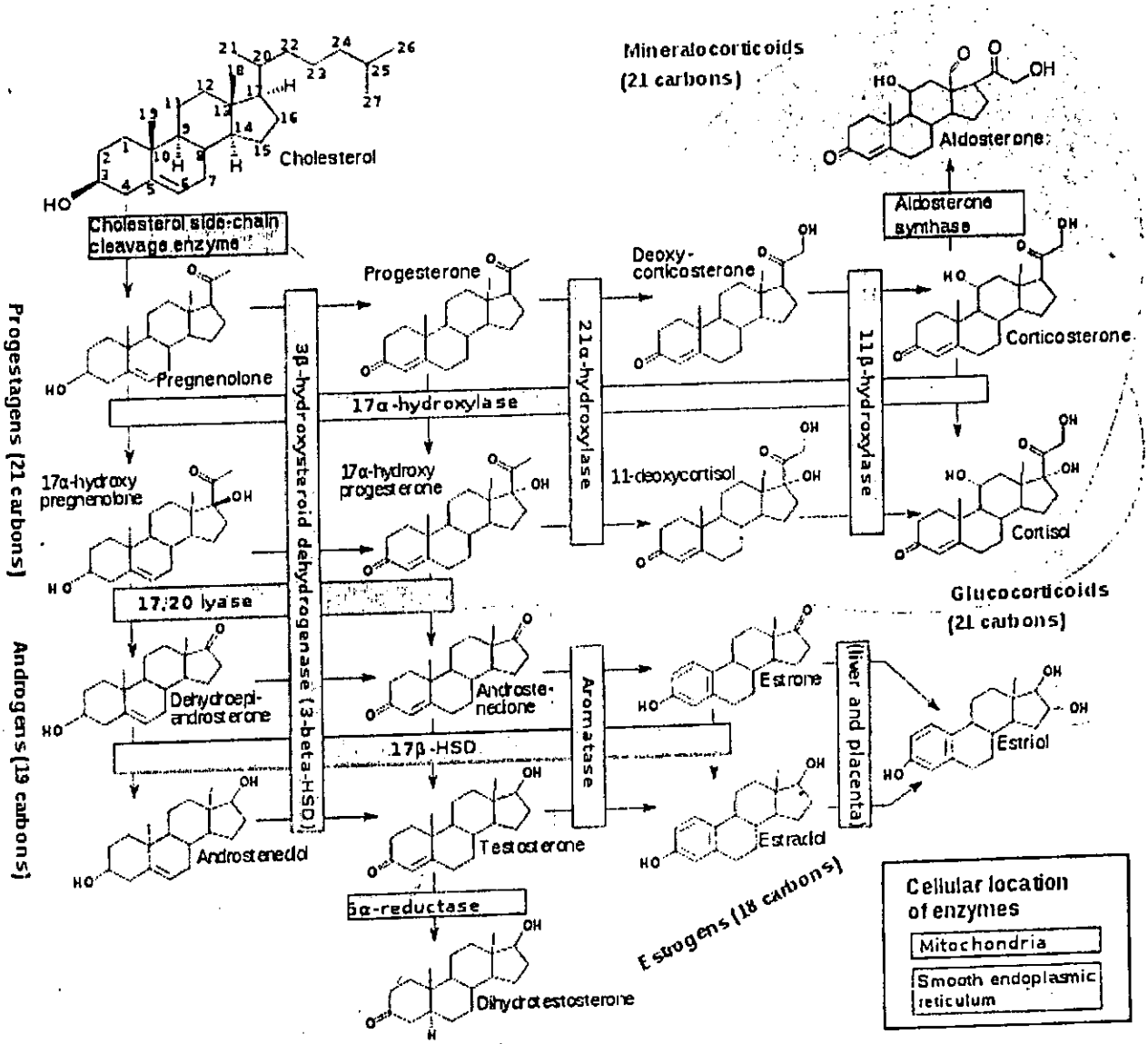


Fig 8: Steroidogenesis

2.6 Uses of Steroidal Hormones:

Progesterone has effects all over the body; for instance it is responsible for the slight rise in body temperature during the second half of the menstrual cycle. It also affects the lining of the uterus to prepare it for pregnancy. Progesterone means "Progestation", in favour of child bearing, it thickens the lining of the uterus and causes the glands in it to release a nutritious fluid (Kastner et al.., 1990, Jang and Yi, 2005).

Oestrogens are the fundamentally female hormones, which influence the whole body, producing rounded contours, breast development and many other features of feminity. Oestrogens also stimulate the uterus to grow new lining to replace the one that was shed at the previous menstrual period. The lining is made of many little glands set in several layers of cells that also contain arteries and veins. Oestrogen makes the glands grow and the layers of intertwining cells increase (Purdie, 2004).

2.7 CONTRACEPTIVES

World population almost doubled between 1950 and 1980 (Population Report, 1983). The implication of this increase and of the projected future growth of the population on the food supply, energy resources and political stability justify the present interest in fertility control. Indeed, an understanding of the methods of contraception and their applications, mode of action, effectiveness and side effects is of paramount importance.

The deliberate or planned effect to control fertility through spacing of pregnancies or determined intervals between birth is defined as contraception. The ultimate goal of contraception is to allow the satisfaction of a desire (sexual intercourse) without suffering the unwanted consequence (pregnancy) that is to prevent the spermatozoon from meeting the egg (ovum).

The discovery of oestrogen and progesterone and their potential contraceptive effects led to an enormous amount of research into fertility regulation in women.

A. Contraception may be classified into Modern and Traditional methods.

(i) Modern method of contraception includes:

* Male and female barrier contraceptives methods e.g. condoms, diaphragms and cap, spermicides.
* Intrauterine devices (IUDs)
* Hormonal contraceptives
* Implants
* Contraceptive surgery (tubal ligation and vasectomy)

(ii) While the traditional methods of contraception include

* Withdrawal

* Breast feeding

* Rhythm method

B. The modern method of contraception can further be sub-divided into temporary and permanent methods.

(i) The temporary methods of contraception include:

* Male and Female barrier contraceptive methods

* Hormonal Contraceptives

* Intrauterine contraceptive devices and

* Implants

(ii) The permanent methods of contraception include

* Contraceptive surgery which could be any of the following;

Tubal ligation for women, and

Vasectomy for men

Figure 7 below shows the various methods of fertility control and their primary targets (principal sites of action) in women. These methods include;

1. Hormonal contraceptive
2. Intrauterine device
3. Barrier methods
4. Natural family planning methods
5. Sterilization
6. Abortion (Carr, et al., 1981)

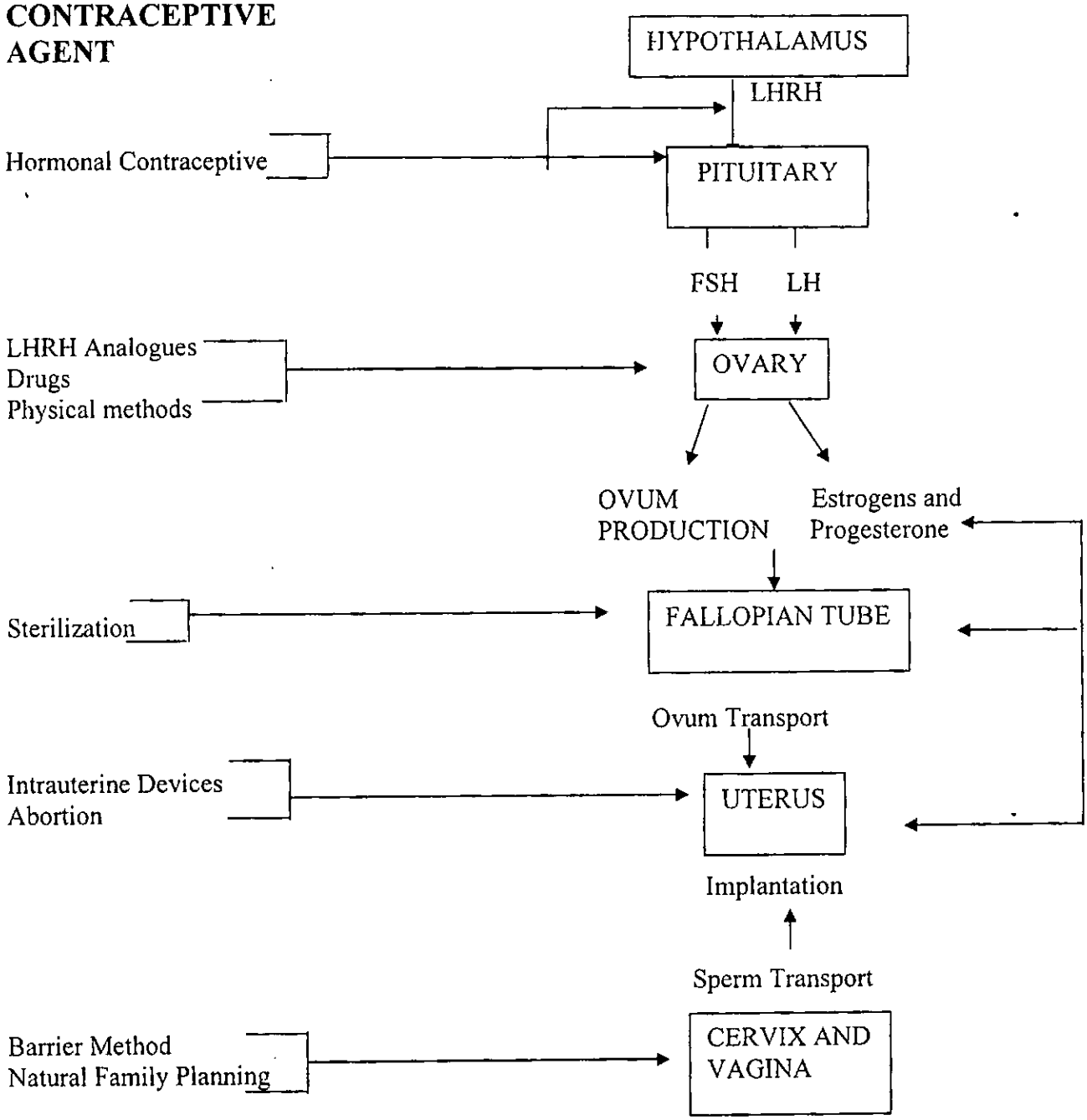


Fig 9: Principal sites of action of various contraceptives on the female reproductive tract.

2.8 DIFFERENT CONTRACEPTIVE METHODS

2.8.1: COMBINED ORAL CONTRACEPTIVE

The Combined oral contraceptive pill often referred to as the birth control pill or simply "the pill" is a combination of an estrogen (oestrogen) and a progestin (progestogen) taken orally to inhibit normal female fertility.

By the 1930s scientists had isolated and determined the structure of the steroid hormones and found that high doses of androgen, oestrogen or progesterone inhibited ovulation (Goldzieher and Rudel, 1974). In 1939, Russell Marker, a professor of organic chemistry at Pennyslvanian state University developed a method of synthesizing progesterone from plant steroid sapogenins and later from inedible Mexican yams.

Three chemical compounds with progestogenic activities were found in animals and they are ; Norethindrone, Norethynodrel and Norethandrolone

Norethynodrel (and Norethindrone) were subsequently discovered to be contaminated with a small percentage of the oestrogen ,Mestranol (an intermediate in their synthesis) further purifying lead to breakthrough bleeding after usage therefore 2.2% mestranol was incorporated into the first contraceptive that was tried on women in 1956(Junod and Mark,2002). The Norethynodrel and Mestranol combination was given the proprietary name ENOVID (Pincus *et al.*, 1958).

Enovid was first approved for contraceptive use in the United State in 1960, and was a very popular form of birth control. They are currently being used by more than 100 million women in the USA (Mosher *et al* ., 2004).These first set of contraceptives contain 2 to 5 times as much oestrogen and 5 to 10 times as much progestin as the oral contraceptives now in use.

Usage varies widely by country, age, education, and marital status. (UN population division, 2006).

Combined oral contraceptives should be taken at the same time each day. If one or more tablets are forgotten for more than 12 hours contraceptive protection will be reduced. Most brands of combined pills are packaged in one or two different packet sizes with days marked off for a 28 day cycle. For the 21- pill packet, a pill is consumed daily for 3 weeks followed by a week of no pills. For the 28 – pill packet 21 pills are taken followed by week of placebo or sugar pills during which there is withdrawal bleeding, the woman is still protected from pregnancy during this week.

Combined oral contraceptives were developed to prevent ovulation by suppressing the release of gonadotrophins, they inhibit follicular development and prevent ovulation as their primary mechanism of action (Loose and Stancel ,2006; and Glasier , 2006). Progestagen negative feedback decreases the pulse frequency of gonadotrophin – releasing hormone (GnRH) release by the hypothalamus which decreases the release of follicle – stimulating hormone (FSH) and greatly decreases the release of Luteinizing hormone (LH) by the anterior pituitary..

Decreased levels of FSH inhibit follicular development, preventing an increase in estradiol levels. Progestagen negative feedback and the lack of estrogen positive feedback on LH release prevent a midcycle LH surge. Inhibition of follicular development and the absence of LH surge prevent ovulation (Speroff and Darney, 2005; and Loose and Stancel, 2006).

Estrogen was originally included in oral contraceptive for better cycle control (to stabilize the endometrium and thereby reduce the incidence of breakthrough bleeding) but

was also found to inhibit follicular development and help prevent ovulation. Estrogen negative feedback on the anterior pituitary greatly decrease the release of FSH, which inhibit follicular development and helps prevent ovulation (Speroff and Darney, 2005; and Loose and Stancel, 2006).

A secondary mechanism of action of all progestagen – containing contraceptives is inhibition of sperm penetration through the cervix into the upper genital tract (uterus and fallopian tubes) by decreasing the amount of the increasing the viscosity of the cervical mucus (Rivera *et al.*, 1999).

2.8.2 HORMONAL IMPLANTS

Subdermal implants are an additional approach to meeting the worldwide need for more effective and acceptable birth control, they may be divided into two main groups; non – biodegradable and biodegradable. Subdermal implants are contraceptive systems that release low, stable amounts of synthetic progestin from silastic or other materials for a period of months to several years. Unlike other hormonal delivery system they do not cause unnecessary peaks in progestin level and do not use oestrogens (Darney , 1994). Contraceptive implants have been approved in more than 60 countries and are currently being used by millions of women around the world (Raymond, 2007).

The first progestin only contraceptive implant placed on the market was Norplant, a multiunit system and it was approved by Food and Drug Administration in 1990. This non – biodegradable system make use of a suitably inert carrier for the contraceptive steroid. It

consisted of a set of six small silicone capsules each filled with levonogestrel - a progestin, implanted in the upper arm .

The implant once in place, slowly and steadily release the progestagen into the blood stream. The implant provide birth control for up to 5 years and it is effective within 24 hours after insertion (Bongaarts and Johansson, 2002).

Norplant works by preventing ovulation by thickening the mucus of the cervix which prevent sperm from penetrating and by thinning of the uterus which makes implantation of an egg less likely (Croxatto, 2000). The mechanism of action also include cervical mucus blockade and prevention of sperm penetration; suppression of oestradiol – induced cyclic maturation of the endometrial lining, causing hypotrophic changes; and ovarian effects ranging from disordered luteal phase through complete anovulation to persistent follicles.

Norplant has been recently replaced by other products in the United State of America. The newer products are Implanon, Jadelle and Sino – Implant (I) and all of these are just as effective as Norplant. Implanon consist of one hormone releasing rod containing etonogestrel and it provide contraception for 3 years. Jadelle and Sino implant (I) are made up of 2 hormone releasing rods and provide contraception for 5 and 4 years respectively. Both contain levonorgestrel as their progestagen.

There are currently four progestogens used in implantable contraceptives; levonogestrel in Norplant and Jadelle, etonogestrel (3- keto-desogestrel) in Implanon;, nestorone in Elcometrine and normegestrol acetate in Uniplant and Surplant (Jordan , 2002)

2.8.3 INJECTABLE CONTRACEPTIVES

Steroids can be administered by almost any route including oral, subcutaneous, intramuscular, intravenous, vaginal, intrauterine, intracervical, rectal, and intranasal. Injectable contraceptives contains synthetic steroid hormones that are administered by deep intramuscular injection. Two types of injectable contraceptives are available; progestin – only injectable contraceptives and combined injectable contraceptives that contain both a progestin and an estrogen hormone. An estimated 16 million women throughout the world are currently on injectable steroids for contraception. The choice of injectable methods include products effective for 3 months, 2months or 1 month.(d'Arcangues and Snow, 1999)

The administration of steroids by deep intramuscular injection provides effective contraception which can last for some time and the progestogens that have been used -are ; medroxyprogesterone acetate (MPA), norethisterone enanthate (norethindrone enanthate, chlormadinone acetate and hydroxyprogesterone hexanoate (caproate). Majority of the compounds are esters of progestogens .The esterification of these compounds delays their absorbtion amd metabolism thereby making them suitable as long acting contraceptives given by intramuscular injection (Elder, 1984). Depot Medroxyprogesterone acetate (DMPA) (Depo Provera) was first used as a long – acting injectable contraceptive in 1963 and it was given every three months, while Norethisterone enanthate (NET – EN) is every 2 months. Both act on hypothalamus – pituitary- ovarian axis inhibiting ovulation, as well as on the endometrium and on the cervical mucus.

Following the first injection of DMPA, MPA levels are at maximum concentration within the first 3 weeks (5 – 22 days) post injection, at 15 – 26 nmol/l , decreasing to <1.0nmol/l in the majority of women by 90 – 190 days post injection (Garza – Flores *et al.*,1994). NET – EN levels falls more rapidly than MPA falling below detectable levels in most women within 46 – 100 days after injection.

Injection of DMPA inhibit ovulation for 14 weeks, suppressing both FSH and LH, cervical mucus is thickened , decreasing sperm penetration, the endometrium is atrophied with inactive glands and decreased tubal motility.(d'Arcangues and Snow, 1999).

2.8.4 INTRAUTERINE DEVICES

Intrauterine devices (IUD) are birth control device placed in the uterus to prevent pregnancy and they have been in use for centuries. The modern intrauterine devices are medicated, containing copper which has a spermicidal effect or a progestin to enhance the contraceptive efficacy of the device.(Treiman et *al.*,1995;and Thonneau and Almont, 2008). These medicated intrauterine devices are referred to as intrauterine contraceptives (IUC) while the progestin containing device is also referred to as intrauterine systems (IUS). The Intrauterine devices are the world's most widely used reversible birth control currently used by nearly 160 million women. The device has to be fitted inside the uterus and it remains in place the entire time pregnancy is not desired. Depending on the type a single IUD is approved for 5 – 10 years and trials have demonstrated the copper T380A to be effective for at least 12 years (WHO, 1997).

Most non - hormonal IUD's have a plastic T shaped frame that is wound around with pure electrolytic copper wire and/ or has copper collar / sleeves. The arms of the frame hold the IUD

in place near the top of the uterus. All copper containing IUD's have a number as part of their name. This is the surface area of copper (in square millimeters) the IUD provides. (Keller, 1996)

The copper IUCs are non hormonal contraceptive devices which are placed inside the uterus. They are composed of a polyethylene frame shaped like a "T" – the arms of the T hold the device in place. The exposed surface area is made of copper and is approved for 10 years of use. The contraceptive action of the intrauterine device is due to production of local sterile inflammatory reaction caused by the presence of foreign body in the uterus. This is said to change the reception of the endometrium for the indication of blastocyst, preventing implantation and also the device prompts the release of leukocytes and prostaglandins by the endometrium. These substances are hostile to sperm and eggs, the presence of copper increases this spermicidal effect. (Keller, 1996). Also copper ion impede sperm transport and sperm viability in the cervical mucus hence ovum is not fertilized.

The current medical consensus is that spermicidal and ovicidal mechanisms are the only way in which IUD's work (Grime, 2007). There are other suggestions that IUDs have a secondary effect of interfering with the development of pre – implanted embryo (Stanford and Mikolajczyk, 2002). The progestin - -IUCs not only exert its contraceptive effect through the foreign body reaction , but also prevents cervical mucus from thinning, thus preventing sperm transport from the cervix to the uterine cavity.

2.9 METABOLIC EFFECTS OF CONTRACEPTIVES

Contraceptives were shown to cause a number of changes in haemostatic function which at least on theoretical ground could be expected to increase the risk of thrombosis. In the early 1960's there were reports of various thromboembolism in women taking oral contraceptives (Jordan, 1961). These were followed later in the decade by data which confirmed the suspicion and suggested a link between the oestrogen content of the oral contraceptive and the incidence of venous thomboembolic disease (Inman *et al*, 1970).

Combined oral contraceptives contain oestrogen and progestin and are the most widely used type of hormonal contraception. While the oestrogen is always ethinyl estradiol, the type and dose of progestin varies. Generally ethinyl estradiol has no effect on glucose tolerance and insulin sensitivity but even the lowest ethinyl estradiol dosage may adversely influence hemostatic and rennin – angiotensin system to increase thrombosis risk and blood pressure .(Petersen, 2002).

Ethinyl estradiol also affect lipid metabolism; increasing triglyceride and HDL – C levels and decreasing LDL –C level. (Godsland *et al.*,1990 and Petersen, 2002).The metabolic effects are dose dependent with respect to the amount of oestrogen used in the combined oral contraceptive. The progestin most widely used nowadays are either the second generation levonorgestrel or norgestimate or third generation desogestrel or gestogen. The third generation progestin have less androgenic side effects.

Progestin decrease glucose tolerance and insulin sensitivity. Importantly progestins have no influence on blood pressure or clotting factors; they may however modify the effects of oestrogen by mechanism still to be elucidated. The progestin effects on lipids tends to antagonize the oestrogen effect that is; lowering of triglycerides, HDL – C and increasing LDL – C.(Petersen ,2002).

Other studies show that compared with non users low dose combined oral contraceptive users have an increased risk of venous and cerebral thrombotic episodes and myocardial infarction (Baillargeon *et al*, 2005). The ethinyl estradiol dose in the combined oral contraceptive formulation appears to be of greater importance for thrombotic risk than the generation of progestin (Lidegaard and Kreiner, 2002 and Lidegaard *et al*., 2003).

Oestrogen have the ability to reduce low density lipoprotein cholesterol and increase high density lipoprotein cholesterol thereby altering the LDL: HDL cholesterol ratio in a direction associated with a decreased atherogenic risk. Also combined pills were found to cause elevated triglyceride concentration (Filshie and Guilleband, 1989).

Interest in the effects of combined oral contraceptives on lipid metabolism stem from two sets of epidemiological data. Firstly, the recognition of an increased risk of ischaemic heart disease in women using these preparation and secondly the association between high blood lipid concentration and the risk of atherosclerotic heart disease (Lewis 1983).

Sex hormone binding globulin increases significantly with Combined oral contraceptives containing same amount of ethinyl estradiol and either levonorgestrel or desogestrel; after 2 months on each treatment (vanRooijen *et al.*, 2004)

Progestrogen – only contraceptives do not usually influence blood pressure or blood coaguability (Filshie and Guilleband ,1989).

Progestogen – only preparation containing norethisterone or norgestrel has been reported to be associated with lower HDL/LDL cholesterol concentration (Wynn and Nithyananthan, 1982)

Progestin agents can be administered intramuscularly or subcutaneously as an implant to deliver long acting and efficacious contraceptive protection. There are two subcutaneous implant system; Norplant with six levonogestrel rods lasting 5 years and Implanon with one etonorgestrel rod lasting 3 years. Intramuscular progestin compound include depo –provera (depot medroxyprogesterone acetate) DPMA. Which are given every 3 month or norethindrone which is given monthly. DPMA has in several studies shown to have more adverse effects on lipids and insulin resistance (Fahmy *et al.*, 1991 ;Kahn *et al.*, 2003) compared with minimal effects of Norplant(Singh *et al.*, 1992). Some other studies found that the use of DPMA increases the risk of diabetes and the risk was further increased with more than 1 year of use (Kim *et al.*, 2001 and Xiang *et al.*, 2006).

The intrauterine device is a reversible contraceptive method without significant metabolic disturbance. It has not been associated with any increased risk of pelvic inflammatory disease (Kimmerle *et al.*, 1995). There are two types either copper or levonogestrel containing IUD. The levonogestrel releasing IUDS have high contraceptive efficacy and low frequency of bleeding disturbances (French *et al.*,2001).

Furthermore, menstrual bleeding is reduced by progesting medicated atrophy of the uterine lining. The hormonal release is low and does not cause significant metabolic effects in normal women (Sturridge and Guillebaud, 1996). Randomised trial of women with type 1 diabetes did not show any influence on blood glucose, , daily insulin dose. (Rogovskaya *et al* ., 2005). More recent research suggests that the reduced oestrogen doses have been associated with a lessening of the incidence of thromboembolic disease. (Gray *et al.*, 2006).

It has been known for more than a century that pregnancy produces a diabetogenic stress .(Filshie and Guilleband, 1989), also early studies demonstrated that oral contraceptives like pregnancy caused mild impairment of glucose tolerance and an increase in circulating metabolites such as pyruvate despite enhanced insulin secretion and an apparently paradoxical fall in plasma glucose (Wynn and Doar, 1966).

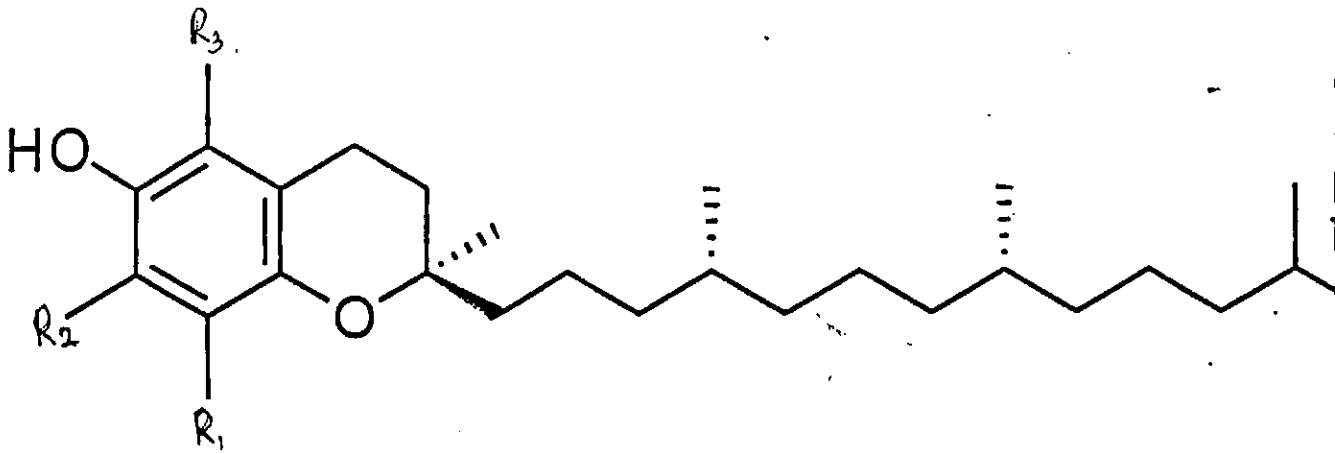
Subsequent studies in animals and men have, however demonstrated that oestrogen alone does not have any significant effect on glucose tolerance or insulin secretion but that the effects observed in pregnancy can be reproduced by progestogen treatment.

Other studies on low-oestrogen and progestogen preparations show that these produce very minor effects on insulin secretion and little or no change in glucose tolerance. (Spellacy *et al,*.1981).

2.10. VITAMIN E

During feeding experiments with rats Herbert McLean Evans concluded in 1992 that beside vitamin B and C, an unknown vitamin existed (Evans and Bishop, 1992). Although every other nutrient was present the rats were not fertile. This condition was changed by adding wheat germ to their feed. Several years later the substance that enhances the fertility was isolated from wheat germ and the formular was determined – C_{29} H_{50} O_2. The vitamin was given its name from the Greek word meaning "to bear young" (Evans *et al* ., 1936)

Vitamin E existed in eight different forms; four tocopherols and four tocotrienols. They are fat soluble vitamins with antioxidant properties. (Herrera and Barbas, 2001) All the eight forms feature chromanol ring, with a hydroxyl group that can donate an hydrogen atom to reduce free radicals and a hydrophobic side chain which allows for penetration into biological membranes . Both the tocopherols and tocotrienols occur in alpha, beta, gamma and delta forms, this is determined by the number of methyl groups on the chromanol ring . Each form has slightly different biological activity. (Burton and Ingold, 1981), which is the measure of potency or functional use in the body. (Traber and Packer, 1995).



α Tocopherol, R1 =R2 = R3 = CH_3

α. Tocotrienol, RI = R2 =R= R3 = CH_3

β Tocopherol, R1 = R3 = CH_3 = R2 = H

β Tocotrienol, R1 = R3 = CH_3 = R2 = H

γ Tocopherol, R1 = R2 = CH_3 = R3 = H

γ Tocotrienol, R1 = R2 = CH_3 = R3 =H

δ Tocopherol, R1 = R2 = R3 = H

δ Tocotrienol, R1 = R2 = R3 = H

Fig 10: VITAMIN E

Alpha tocopherol is the most active form of vitamin E that is preferentially absorbed and accumulated in humans (Rigotti, 2007), it is also a powerful biological antioxidant (Packer *et al.*, 2001), and has the highest bioavailability (Brigelius – Flohe and Traber, 1999). Tocotrienols with four d- isomers, also belong to the vitamin E family. The four tocotrienols have structures corresponding to the four tocopherols, except with an unsaturated bond in each of the three isoprene units that form the hydrocarbon tail. Tocopherols have a saturated phytyl tail.

Conventional medical studies use either a synthetic all- racemic ("d , l"-) alpha tocophenyl ester (acetate or succinate) or a semi – synthetic d- alpha tocophenyl ester. Proponents of mega vitamin, orthomolecular and naturally based therapies have advocated for the last two thirds of a century and have used the natural tocopherols , often mixed tocopherols with an additional 25% - 200 % w/w d- beta-, d- gamma-, and d- delta-tocopherol (Gaziano, 2004).

Researches advanced the belief that oxidative modification of LDL – C promotes blockage in coronary arteries that may lead to atherosclerosis and heart attack. Vitamin E may help prevent or delay coronary heart disease by limiting the oxidation of LDL- C. It may also help prevent the formation of blood clots which could lead to a heart attack (Vivekananthan *et al.*,2003).Vitamin E has also been shown to play a role in immune function, in DNA repair and other metabolic processes(Farrell and Roberts, 1994). Other forms of vitamin E have their own unique properties, for example gamma- tocopherol is a nucleophile that may react with electrophilic mutagens (Brigelius – Flohe, 1999). The tocotrienols have specialized roles in protecting neurons from damage (Sen *et al.*,2006), cancer prevention (Malafa, 2008), and cholesterol reduction by inhibiting the activity of the regulatory enzyme of cholesterol synthesis.

2.11 COPPER AND CERULOPLASMIN

Copper is an integral component and essential cofactor for many metalloenzymes, including cytochrome oxidase and suproxide dismutase, but it is toxic in its unbound form (Twomey *et al.*,2005) The important protein metallothionein binds copper as well as other heavy metals. A variety of pathological conditions have been attributed to loss of cuproenzyme activity; they include failure of pigmentation, and connective tissue cross – linking defects. Copper deficiency impairs iron absorption , and anemia accompanies severe copper deficiency.

Ceruloplasmin (ferroxidase EC 1.16.3.1) is a blue coloured plasma protein that binds up to 95% of circulating copper. The proposed physiological functions of ceruloplasmin include Cu transport, oxidation of organic amines, ferroxidase activity, regulation of cellular iron levels, glutathione peroxidase and ascorbate oxidase activities and an antioxidant activity. Furthermore, it has also been reported that ceruloplasmin may scavange reactive oxygen species such as superoxide singlet and hydroxyl radicals. However, it has also been shown to have prooxidant activities which involve a distinct active site from the antioxidant sites.(Healy and Tipton,2007). Ceruloplasmin, also oxidizes ferrous iron to its ferric state prior to its binding by plasma transferring.

Copper absorption in humans occurs primarily in the stomach and duodenum and its toxicity is characterized by nausea , vomiting , epigastric burning and diarrhea. Toxicity can occur subsequent to ingestion of copper contaminated solutions and the use of copper containing intrauterine devices

Oestrogens increase serum copper levels probably by increasing hepatic ceruloplasmin synthesis. Serum copper is normally higher in women than in men, and this difference increases further during pregnancy and in women taking oestrogenic oral contraceptives (Mason KE, 1979 and Tietz NW, 1983)

The vast majority of serum copper is transported bound to ceruloplasmin, the rest , bound to albumin, transcriprein, and copper – amino complexes.

2. 12. IRON AND FERRITIN

The total iron content of the body is about 3 – 3.5 g. Of this amount, about 2.5 g is in the hemoglobin, virtually all of which is contained within erythrocytes or their precursors in the bone marrow. Most of the remainder is storage iron . However numerous cellular enzymes and coenzymes require iron notably peroxidases and cytochromes that are also heme proteins, many of the enzymes of the krebs cycle, and monoamine oxidase which is involved in neurotransmission.

Free iron is toxic to cells as it acts as a catalyst in the formation of free radicals from reactive oxygen specie through the Fenton Reaction (Orino *et al.*, 2001). Hence organisms have evolved an elaborate set of protective mechanisms to bind iron in various tissue compartments. Within cells iron is stored complex to protein as ferritin or hemosiderin. Ferritin is a globular protein complex consisting of 24 protein subunits and is the main intracellular iron storage protein in both prokaryotes and eukaryotes, keeping it in a soluble and non toxic form. Ferritin which is not combined with iron is called apoferritin. Apoferritin binds to free ferrous iron and stores it in the ferric form. Each ferritin complex can store about 4500 ferric ions. Under steady state conditions the serum ferritin level correlates with

total body iron stores, thus the serum ferritin is the most convenient laboratory test to estimate iron stores (Cook *et al.*, 1974; Kaltwasser and Werner, 1989, and Willet, 1990).

Active ferrous iron is a highly effective promoter of lipid peroxidation in cell free medium and amplifies the prooxidant capacity of vascular cells (Fuhrman *et al.*, 1994). Also excess iron may further enhance the atherogenicity of LDL by stimulating the synthesis of lipoprotein with low antioxidative reserve in the liver and by promoting minimal surface modification in circulation. Such lipoproteins may be primed for further oxidation and remain unrecognized by the otherwise liver clearance system. Serum ferritine emerged as one of the strongest risk predictors of 5 year progression of carotid atherosclerosis. (Kiechl *et al.*, 1997).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 Subject Selection

Subjects were recruited from the following centers after approval has been given by the various ethical committees of the organizations

- The Planned Parenthood Federation of Nigeria, Palmgrove Lagos.
- Family Planning Clinic, Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos.
- Family Planning Clinic Oshodi-Isolo Local Government Area, Isolo Lagos.
- Regina Mundi Catholic Church Clinic, Mushin, Lagos.
- General Hospital, Lagos.

The subjects were categorized into

- Implant Contraceptive users
- Injectable Contraceptive users
- Oral Contraceptive users.
- Copper Contraceptive users.
- Non-Contraceptive users/Control group.

3.2 Sample Size Estimation

The prevalence of contraceptive use in developing countries is 50%(WHO,2005).

To determine minimum sample size (N) for this study

$N = Z^2Pq/d^2$ (Oyeka, 1992)

Where

N = Minimum sample size

Z = Confidence limit = 95% =1.96

P = Prevalence = 50% = 0.5

q = Complementary probability = 1 – P = 1-0.5 =0.5

d = Absolute sampling error that can be tolerated = 5% = 0.05

$$\underline{N = (1.96)^2 \times 0.5 \times 0.5}$$

$$(0.05)^2$$

$$\underline{0.96} \quad = \quad 384$$

$$0.0025$$

The sample size was rounded off to 400

3.3 Questionnaire Administration

Relevant data were collected from 400 women with the aid of a questionnaire (Appendix I) this was used to gather information about the volunteer's medical, contraceptive and reproductive history at the time of blood sampling. The volunteers were made up of heterogeneous groups. Blood samples were collected from 400 women which included;

50 women who were hormonal implant contraceptive users.

100 women who were copper T. (Intrauterine devices) users.

100 women who were injectable (Depo medroxyprogesterone acetate) users

100 women who were oral contraceptive users, and

50 women who were used as control; they were not on any contraceptive.

Women who were recruited for this research were just being introduced to these contraceptive agents. Considering the fact that the research seek to measure the metabolic effects of the contraceptives, new subjects were used. Also the control group were analysed once because of difficulties encountered in getting their co -operations and the results were compared with the baseline value.

3.4 **Specimen Collection**

20ml of venous whole blood were collected from the volunteer women by vein puncture. Blood sampling was standardized according to time of day by collecting almost at the same time of the day. This was done to eliminate effect of different timing. Blood was collected from the subjects between 9.00am and 10.00am.

The 20ml blood was divided into EDTA, fluoride oxalate and plain bottles.

Blood was collected from every volunteer at 6 monthly intervals till the end of the research, and this lasted for 12months for each group , that is three times ; 0 month, 6 month and 12 month respectively.

The blood was allowed to clot at room temperature for 1 hour before centrifugation with a Beckman table top centrifuge at 2000 rpm for 30 minutes at 2-8^{0}C

The serum/plasma which was drawn by aspiration using a disposable sterilized micropipette, was stored at 2-8^{0}C in the biofreezer until needed for analysis.

3.5 Method for the Quantization of Triglycerides.

Triglycerides Determination was by the Enzymatic method of (glycerol – 3- phosphate oxidase method) Tietz (1995).

Principle:-

The hydrolysis of triglycerides to form glycerol is achieved by lipase. The glycerol produced by hydrolysis was converted to glycerol-3-phosphate, this was followed by oxidation to release hydrogen peroxide. The hydrogen peroxide was then reacted upon by peroxidase to give a red quinonemine dye which was then measured spectrophotometrically at 505nm.

1. Tryglyceride + $3H_20$ $\xrightarrow{\text{Lipase}}$ Glycerol + 3 free fatty acid

2. Glycerol + ATP $\xrightarrow[\text{Kinase}]{\text{Glycerol}}$ Glycerol -3- Phosphate + ADP

3. Glycerol -3- Phosphate + 0_2 $\xrightarrow[\text{Oxidase}]{\text{Glycerol Phosphate}}$ diohydrox-acetone phosphate + H_20_2

4. H_20_2 + 4-chlorophenol +4- aminophenazone $\xrightarrow{\text{peroxidase}}$ HCl + $2H_20$ + 4-(P- -benzoquinomphenazone).

Procedure

Serum samples were analysed for triglycerides by setting up assay system in three tubes labeled, blank, sample and standard. 0.02ml of distilled water, 0.02ml of serum and 0.02ml of standard were pipetted into the tubes. Then 0.2ml of working reagent was added to all the tubes. The mixture was gently mixed and allowed to stand for 5 minutes at 37^0C absorbance was then measured at 505nm wavelength.

3.6 Method for the Determination of Cholesterol

Cholesterol assay was determined by the direct enzymatic procedure of Tietz (1995).

Principles:-

The cholesterol was determined after hydrolysis of cholesteryl esters at C-3 to form free cholesterol and the subsequent oxidation step to produce hydrogen per oxide and cholest – 4 – ene – 3 – one. The hydrogen peroxide reacted with phenol and 4-amino- phenazone in the presence of peroxidase to form an o-quinoneimine dye (Trinder reaction). The intensity of the colour formed is proportional to the cholesterol concentration and was measured photometrically at 500nm.

Procedure

Serum or plasma samples could be used but serum was analysed for cholesterol by setting up assay system in three tubes namely blank, standard and sample at room temperature. 0.1ml of distilled water, standard and serum were pipetted into the tubes respectively. Then reagent mixture (0.30mMol/L 4 – aminoantipyrine, 6mMol/L phenol, 0.5µg/ml peroxidase. 0.15µg/ml

cholesterol esterase 0.1μg/ml oxidase) was added to the tubes these were mixed gently and incubated for 5 minutes at room temperature the absorbance was taken at 500nm within 1hour.

Calculation;

$$\text{Cholesterol concentration} = \frac{\text{Absorbance of sample x Concentration of Standard}}{\text{Absorbance of standard}}$$

3.7 Determination of high-density lipoprotein cholesterol

High-density lipoprotein cholesterol was determined using, the precipitation method of Warnick *et al.*, (1979).

Principle:-

HDL is isolated from other major classes of lipoproteins by the formation of insoluble complexes of lipoproteins; polyanions and divalent cations. In the presence of Mn (II) and heparin these other major classes are selectively precipitated, leaving only HDL-C containing supernatant.

Procedure

1.0ml of serum and control are pipetted into tubes: 0.1ml of the working precipitation reagent (0.6ml of sodium heparin solution and 10ml $Mncl_2.4H_20$ 1.06ml/L) was added into each tube, this was mixed thoroughly and allowed to stand for 10 minutes centrifuged at 1500 x g for 30 minutes and the clear supernatant transferred to a clean tube for cholesterol concentration analysis as previously described.

3.8 Determination of Low-Density lipoprotein cholesterol

Low-Density lipoprotein cholesterol was carried out using Friedwald formular (1972).

Low-density lipoprotein can be fairly accurately estimated using the Friedwald formular. This is based on the assumption that very low density lipoprotein cholesterol is present in concentration equal to one-fifth of the triglyceride concentration

After the determination of total cholesterol, HDL-C and Triglyceride level of the blood samples. The Friedwald formular was used to calculate the LDL-C level of the samples.

LDL-C = Total cholesterol – HDL- C – triglyceride/5

3.9 Determination of Vitamin E:

Serum Free Vitamin E was determined by the method of Henry *et al.*,(1974)

Principles

After precipitation of serum protein the vitamin was extracted into hexane and quantitated by measuring relative fluorescence at specific activation and emission wavelength.

PROCEDURE

The following tubes containing 0.2ml distilled water as blank; 0.2ml working standard and 0.2ml unknown were set up. 1.0ml of distilled water was added to the blank and unknown while 1.2ml of distilled water was added to the tube and tubes were mixed for 30 seconds. 5.0ml of hexane was later added to the tubes which were thoroughly mixed then centrifuged at 1500 x g for 5 minutes. The hexane supernatant was then measured using spectrophotofluoremeter with the activation wavelength at 295mm and the emission wavelength at 330mm.

Calculation

$$\mu g \text{ free vitamin E/ml} = \frac{Fx - Fb \times 20}{Fs - Fb}$$

Fb = Fluorescence of blank

Fx = Fluorescence of unknown

Fs = Fluorescence of standard

3.10 Glucose assay determination:

Determination of glucose using the glucose oxidase methods as described in Tietz (1995)

Principle

The enzyme glucose oxidase catalyses the oxidation of glucose to gluconolactone and hydrogen peroxide. Addition of the enzyme perioxidase and a chromogenic oxygen acceptor results in formation of colour that can be measured at 570nm.

Reaction of the assay:-

$$\text{Glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-Glucono-}\delta\text{- lactose} + H_2O_2$$

$$\text{o-Diamisidine} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{oxidised} - \text{o} - \text{diamisidine} + H_2O$$

(Colourless) (Coloured)

Procedure

0.1ml of serum sample and 2.0ml of protein precipitant were added, mixed and centrifuged at 1500 x g for 5 minutes 1ml of supernatant was taken and 3.0ml of colour reagent was added, the same treatment was done for the blank used, where distilled water was used as the blank. All the

tubes were incubated at 37^0C for 10 minutes with occasional shaking to ensure adequate aeration. Absorbance was taken at 510mm.

3.11. Serum Ferritin assay:-

Serum ferritin was determined using an enzyme immunoassay procedure of Tietz (1995).

Principle

The binding of human serum ferritin to a solid phase antihuman ferritin and the simultaneous binding of the purified antihuman ferritin conjugated with alkaline phosphatase to immobilized solid phase. Phosphatase in a subtrate solution consisting of phenyphosphate disodium and 4-amino-antipyrine was added. Following the addition of potassium fericyanide a colour develops, the optical density at 510nm was measured and this is directly proportional to the ferritin concentration of the sample.

Procedure

200µl of conjugated antihuman ferritin was pipetted into all micro wells. This was incubated on a vibrator at room temperature beginning with micro well C1 and 10µl of prediluted ferritin calibrator solution was pipette into the well,10 µl serum sample in duplicate was pipetted into separate wells. Micro well A1 and B1 measure non-specific binding containing only the conjugated antihuman ferritin. The wells were washed with deionised water, 200µl of the substrate solution was added to each well; colour was developed with 100µl of 0.74% potassium ferricyanide, absorbance was read at 510nm.

3.12. Determination of serum Ceruloplasmin;

Ceruloplasmin concentration was determined using an Enzyme immunoassay procedure of Tietz (1995).

The Ceruloplasmin was assayed for by the Enzyme linked immunosorbent assay which was based on a solid phase Enzyme immunoassay which used affinity purified anti- ceruloplasmin antibodies for solid phase immobilization and horse- radish peroxidase conjugated anti ceruloplasmin antibodies for detection.

3.13. Determination of serum Copper concentration;

Atomic Absortion Spectrophotometry (AAS) was used to determine the copper concentration. Serum was diluted with an equal volume of deionized water, this was aspirated directly into AAS flame, copper concentration was calculated against copper standard in a glycerol matrix (10ml/dl) according to the method of Weinstock and Uhlemain (1981).

3.14. Determination of serum Iron concentration;

Iron was determined by the method in Tietz (1995).

Iron is released from transferrin by a decrease in pH of serum. Proteins are removed by precipitation and centrifugation. The Fe(III) of the supernatant is reduced to Fe(II) with thioglycolic acid and is complexes with a chromogen. The absorbance of the iron-chromogen complex is proportional to the iron concentration in the serum.

Procedure:-

2ml of serum was added to 2ml of protein precipitant solution (33.3ml) thioglycolic acid, 98g of trichlroacetic acid, and 400ml of water swirl to dissolve, slowly add 2ml of Hcl was added (192ml of concentrated HCl add water to volume) in a 15ml centrifuge tube, this was allowed to stand for 5 minutes and then centrifuged at 1500xg for 10minutes, 2ml of the clear supernatant was transferred into a clean tube, two other tubes were also labeled and these had 1ml of water solution and 1ml of standard and 1ml of solution respectively. To each of these 3 tubes was added 2ml chromogen solution (Bathophenanthroline) Absorbance was measured for the 3 tubes at 534nm.

Calculation:-

Serum iron = $\frac{(A_u - A_b) \times C_s}{(As - Ab)}$

Concentration

(µg/dl)

Where; A_a = Absorbance of serum

A_b = Absorbance of blank

A_s = Absorbance of standard

C_s = Concentration of standard

3.15 DETERMINATION OF RATE OF REVERSAL OF METABOLIC EFFECTS FOLLOWING CONTRACEPTIVE WITHDRAWAL.

Sixteen women who were Injectable and Norplant contraceptive users withdrew to conceive. All subjects were free from known medical disorders and were between the ages of 24 – 34 years. There were 8 women in each group and they were monitored for 12 months. The assays listed above were carried out on their blood samples after 6 and 12 months, also pregnancy was monitored by testing for human chorionic gonadotropin (HCG) in urine; a positive reaction indicates that pregnancy has taken place and fertility has returned.

3.16 Urine Human chorionic gonadotropin (HCG) was determined by the method of Tietz (1995) .

Human chorionic gonadotrophin is a glycoprotein composed of two nonidentical, noncovalently bound glycoproteins subunits. It plays an important role in maintaining the function of the corpus luteum during the first weeks of pregnancy until progesterone production occurs. Quantitative tests for hcG was primarily used for the confirmation of pregnancy.

3.17 Data Analysis.

The experimental assays were done at 6 monthly intervals and data are reported as mean standard deviation (SD). Significant difference were determined by Fisher's protected least significant difference t-test with two tail probabilities of less than 0.05 considered significant

CHAPTER FOUR

4.0 RESULTS

4.1 Age distribution of the control and subject groups:

From the analysis of the questionnaires, Table 1 shows the age distribution amongst the contraceptive agent users and the control. The age range for all the groups was between 19 years and 49years. For control the highest number of subjects was 13 and this was found among the age range, 29-33years followed by 12 for the age range 34 – 38 years, while the lowest number of subjects was 3 amongst 19 - 22 years. The use of implant was highest among the age range 34-38 years old where there were 21 subjects while the lowest was within the age range of 19-22 years where 2 subjects participated, meanwhile for this research no respondent was found within the age range 44 – 49 years. For intrauterine device or Copper T, the highest number of users was found within the age range of 29-33years where there were 39 subjects while the lowest was within the age range of 19-22 years old where there was only one subject . The highest number of injectable users was amongst the age range 34-38 years old and there were 40 subjects while the lowest was amongst the age range of 19-22 years. For oral contraceptive for this research the highest percentage of users were 33 subjects and they were within the age range 23-28 years and the lowest was between the age range 44-49 years where only one subject was found

Table 1: Age distribution from the questionnaire of control women and those on different contraceptive agents.

Contraceptive	19-22yrs	23-28yrs	29-33yrs	34-38yrs	39-43yrs	44-49yrs
Control (50)	3	10	13	12	8	4
Norplant (50)	2	5	7	21	16	-
Copper T(100)	1	13	39	36	6	5
Injectable (100)	1	6	28	40	21	4
Oral contraceptive(100)	15	33	31	17	3	1

4.2 Educational classes of control and contraceptive users

Table 2 showed the education profile of control women and those on different contraceptive agents from the analysis. Generally contraceptive use was highest amongst those with post secondary school education. For control group, 25 subjects (50%) had post secondary education followed by 10 (20%) subjects with secondary / Modern School Certificate while 7 respondents (13.3%) had no formal education. For Norplant contraceptives the highest number of users was found to be 28 (56%) amongst the post secondary school education class while the lowest number 10 (10%) was amongst those with no formal education. Intrauterine device or Copper T use was highest amongst those with post secondary school education, 48 and lowest 10 amongst those with no formal education. Also for injectable contraceptive users, the highest use prevalence for this study was amongst those with post secondary education and the number was 44 and lowest amongst those with primary school education where the number of respondent was found to be 12. For oral contraceptive users, the highest number of subjects was found to be 54 , amongst post secondary school education class and lowest 6 amongst those with primary school education.

Table 2: The education profile from the questionnaire of control women and women on different contraceptive agents.

Contraceptive	Primary	Secondary / Modern School	Post secondary	No formal education
Control (50)	8	10	25	7
Norplant (50)	10	7	28	5
Copper T(100)	18	24	48	10
Injectable (100)	12	29	44	15
Oral contraceptive (100)	6	32	54	8

4.3 Number of children "born" by the control and contraceptive users

From table 3, the number of children by these subjects was between 0 and 9 children. The percentage of women in the control group who had 3 children was 23.3%, those with 4 children was also 23.3% while 20.1% had no children. For Norplant users 28% of the women had 2 children while 26% had 3 children, whereas 2% of the women had 1, 7, and 8 children respectively. . Amongst the Copper T users the highest percentage of the women 32% had 4 children followed by 18% of the women with 2 and 3 children respectively while the lowest was 2% for women with no children. Also all the injectable users had children with the highest percentage 28% for 5 children and followed by 26% of the women with 4 children and the lowest percentage 1% with eight children.

The percentage of women in the oral contraceptive group was 28% for a child and 2 children respectively and followed by 18% who had no child while 2% of the women had 6 children.

Table 3: Number of children "born" by the control and contraceptive users.

Contraceptive	No of children (percentage)									
	None	1	2	3	4	5	6	7	8	9
Control	20.1	6.7	13.3	23.3	23.3	13.3	-	-	-	-
Norplant	-	2	28	26	24	16	-	2	2	-
Copper T	2	10	18	18	32	8	9	3	-	-
Injectable	-	2	9	25	26	28	5	2	1	2
Oral contraceptive	18	28	28	10	9	5	2	-	-	-

4.4 The mean weight of the control and contraceptive users at the beginning and end of the experiment

From table 4, the mean weight for Norplant contraceptive users the before the experiment was 67.9±1.7kg while there was a significant increase at the end of the experiment to 73.9±1.9kg. The mean weight for Copper T. users before and after the experiment was 71.7±1.27kg and 72.3±1.27kg respectively; there was an insignificant increase in the values of the mean weight. For injectable users before and after the experiment the mean weight values are 64.7±1.2kg and 70.5±1.3kg respectively and there was a significant increase in these values just like for Norplant contraceptive users. There was also a significant increase in the mean weight for oral contraceptive users before and after the experiment the weight increased from 58.9±1.4kg to 63.4±1.4kg in the course of the experiment also see Fig 6.

Table 4: The mean weight of the different groups of women at the beginning and end of the experiment.

Groups	Mean weight at the beginning of the experiment (kg)	Mean weight at the end of the experiment (kg)
Control[a]	75.0	N.D
Norplant[a]	67.9 ± 1.70	73.9 ± 1.90
Copper T[b]	71.7 ± 1.27	72.3 ± 1.27
Injectable[b]	64.7 ± 1.20	70.5 ± 1.30
Oral contraceptive[b]	58.9 ± 1.40	63.4 ± 1.40

N.D – not determined.

Mean ± standard deviation

n = a, b = 50 women

n = c, d and e = 100 women

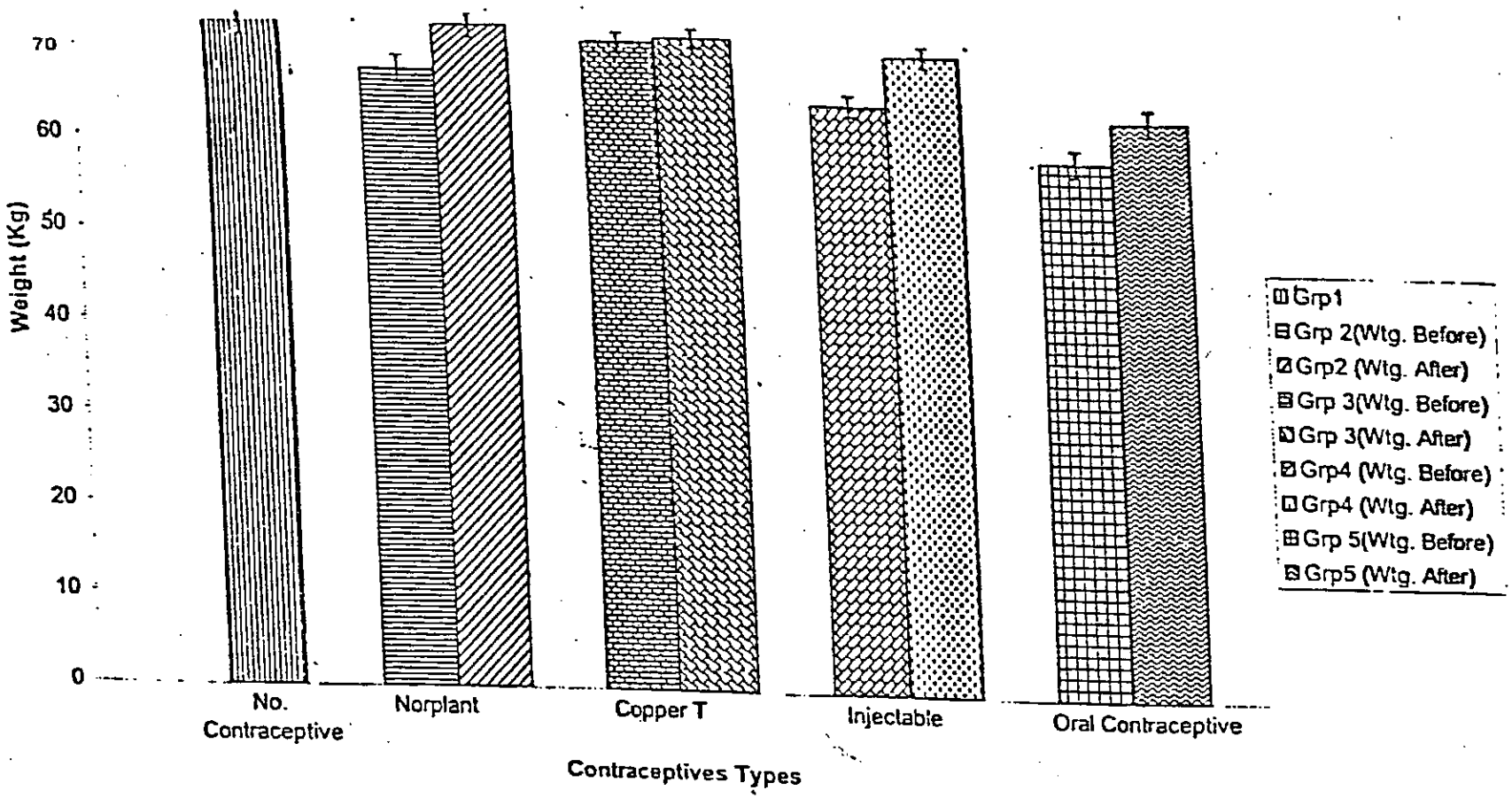


FIGURE 9:WEIGHT DIFFERENCE IN THE SUBJECTS BEFORE AND AFTER ADMINISTRATION OF DIFFERENT CONTRACEPTIVES

4.5 Serum Lipid and glucose concentration for the control and contraceptive users..

4.5.1 Serum Cholesterol concentration

Table 5 depicts the serum lipid profile of the control and contraceptive users for the duration of the experiment (that is 0, 6months, and 12months).For Norplant users the cholesterol concentration were found to be 187.46±2.4mg/dl, 200.4±2.1mg/dl and 211.8±2.6mg/dl respectively; for Copper T, the values were 183.1±2.9mg/dl, 186.4±2.1mg/dl and 189.9±2.6mg/dl; for injectables the values were 170.6±2.2mg/dl, 181.8+-1.0mg/dl and 193.4±1.8 mg/dl respectively and the values for oral contraceptive were 168.4±2.3mg/dl, 178.0±2.0mg/dl and 195.2±2.0mg/dl, respectively. There was significant increase in the serum cholesterol concentration level after 12 months of norplant and injectable usage when compared to baseline value. Whereas there was no significant difference between the baseline value and that at 12 months for Copper T and oral contraceptives usage, however the values were significantly higher than that of the control $p<(0.001)$.

4.5.2 Serum Low – density lipoprotein cholesterol concentration .

The Low density lipoprotein concentration for Norplant users was 114.0±2.0mg/dl, 135.2±2.1mg/dl and 146.74±2.4mg/dl at 0, 6months, and 12months respectively while for Copper T it was 126.3±3.2mg/dl, 124.4±2.0mg/dl and 123.5±4.4mg/d respectively; for injectables it was 93.4±2.3mg/dl, 124.5±1.8mg/dl and 135.75±1.8 mg/dl respectively and oral contraceptive was 120.7±2.2mg/dl, 119.1±2.1mg/dl and 126.4±1.75mg/dl respectively. There was a significant increase for norplant and injectable a usage while concentration decreased at 12 months for Copper T but the decrease increased

slightly at 12 months for oral contraceptive users when compared to the base line concentration.

4.5.3. Serum Triglycerid and High – density Lipoprotein concentration;.

The Triglyceride concentration for Norplant users was found to be 173.6±2.3mg/dl; 180.0±1.8mg/dl and 184.6±1.9mg/dl at 0, 6months and 12months respectively. That of Copper T was 85.0±3.4mg/dl, 94.5±3.0mg/dl and 107.7±3.7mg/dl respectively; for injectables the values were 170.5±2.0mg/dl, 160.0±3.1mg/dl and 178.25±1.2mg/dl respectively and lastly for oral contraceptives it was 72.2±3.3mg/dl, 167.2±2.2mg/dl and 178.6±2.3mg/d respectively. .All these values increased at 12 months when compared with the baseline There was a significant increase $p<(0.001)$ in Triglycerides values for Norplant users, injectable users and oral contraceptive users while there is a significant decrease $p<(0.05)$ in the values for Copper T. users when compared with the control. High density lipoprotein cholesterol concentration for Norplant users was found to be 38.7±1.7mg/dl, 29.2±0.95mg/dl and 28.2±0.91mg/dl at 0, 6months, and 12months respectively. The values for Copper T users were 39.8±1.4mg/dl; 43.1±1.2mg/dl and 44.9±3.0mg/dl respectively. For injectables the values were 43.1±1.4mg/dl; 25.3±0.7mg/dl and 22.0±0.7mg/dl respectively and for oral contraceptives it was 33.1±0.9mg/dl; 25.5±0.7mg/dl and 23.1±0.3mg/dl respectively.

These values were significantly lower $p>(0.005)$ for Norplant, oral contraceptives and injectables while for Copper T there was a significant increase when compared to the control. While there was decrease with norplant , injectable and oral contraceptive usage when compared with the baseline concentration.

4.5.4 Serum Glucose concentration

The serum glucose concentration in Norplant users was found to be 88.6±1.2mg/dl, 97.5±0.98mg/dl, and 104.18±1.0mg/dl at o, 6, 12months respectively. For Copper T the values were 85.6±1.36mg/dl, 90.6±0.98mg/dl and 94.4±1.1mg/dl respectively. For injectable the values were 91.3±0.8mg/dl, 98.9±1.5mg/dl and 104.6±1.0mg/dl respectively and finally for oral contraceptive users they were, 83.4±1mg/dl ,86.0+ 1.0mg/dl and 89.10+ 1.1 mg/dl respectively. After 6 months there was a significant decrease in the values of glucose amongst the oral contraceptive and Copper T users $p>(0.05)$ while there was an increase for Norplant and Injectable users after 6 months when compared with the control. There were significant increase when all the contraceptives were compared with the base line concentration. .

Table 5: Lipid profile concentration for control and contraceptive users

Parameter	Time interval (months)	Control[a]	Contraceptives			
			Norplant[b]	Copper T[c]	Injectables[d]	Oral contraceptives[e]
Cholesterol mg/dl	0	167.6±2.4	187.46±2.4	183.1±2.9	170.6±2.2	168.4±2.3
	6		200.4±2.1	186.4±2.1	181.8±1.0	178.±2.0
	12		211.8±2.6	189.9±2.6	193.4±1.8	185.2±2.0
LDL-C mg/dl	0	104.0±1.0	114.0±2.0	126.3±3.2	93.4±2.3	120.7±2.2
	6		135.2±2.1	124.4±2.0	124.5±1.8	119.1±2.1
	12		146.74±2.44	123.5±4.4	135.75±1.8	126.4±1.75
Triglyceride mg/dl	0	115.0±3.2	173.6±2.3	85.0±3.4	170.5±2.0	72.2±3.3
	6		180.0±1.8	94.5±3.0	160.0±3.1	167.2±2.2
	12		184.6±1.9	107.7±3.7	178.25±1.2	178.6±2.3
HDL-C mg/dl	0	40.6±3.2	38.7±1.7	39.8±1.4	43.1±1.4	33.3±0.9
	6		29.2±0.95	43.1±1.2	25.3±0.7	25.5±0.7
	12		28.2±0.91	44.9±3.0	22.0±0.7	23.1±0.3

Index; mean ± SEM

n = a, b = 50 women

n = c, d and e = 100 women

Table 6: Glucose concentration for control and contraceptive users

Parameter	Time interval (months)	Control[a]	Contraceptives			
			Norplant[b]	Copper T[c]	Injectables[d]	Oral contraceptives[e]
Glucose mg/dl	0	95.0±1.2	88.6±1.2	85.6±1.34	91.3±0.8	83.4±0.9
	6		97.5±0.98	90.6±0.98	98.9±1.5	86.0±1.0
	12		104.18±1.0	94.4±1.1	104.6±1.0	89.1±1.1

Index; mean ± SEM

n = a, b = 50 women

n = c, d and e = 100 women

4.6 Concentration of Serum Iron, Copper and their carrier proteins and vitamin E.

Table 7 and 8 showed the concentration of serum iron, copper, their carrier proteins and vitamin E analyzed at 0,6 month and 12 monthly intervals for the control and contraceptive users.

4.6.1 Serum Iron and Ferritin concentration ;

The serum iron concentration amongst the Norplant users was 84.8±3.5µg/dl, 129.6±3.5µg/dl and 134.9±3.7µg respectively and ferritin concentration was 60.9±1.5µg/dl, 118.0±1.9µg/dl and 120.0±2.6µg/dl respectively. For Copper T users, the values are 106.4±2.8µg/dl, 80.4±1.62µg/ml, 71.5± 1.64µg/ml respectively for serum iron concentration and 92.0±2.2µg/ml, 70.4±1.8µg/ml and 62.0±1.0µg/ml respectively for Ferritin concentration. For injectable users the values are 87.7±2.6µg/dl, 128.0±2.5µg/dl and 134.8±2.6µg/dl respectively for serum iron concentration; 62.0±1.8µg/dl, 108 ±2.4µg/dl and 116.0±2.1µg/ml respectively for Ferritin concentration. The values for oral contraceptive users are 81.9±2.5µg/dl, 155.6±2.5µg/dl and 176.8±1.1µg/dl respectively for serum iron concentration and 79.2±1.9µg/ml 120.6±1.8µg/ml and 142.4±2.2µg/ml respectively for ferritin concentration There is a significant increase $p<(0.001)$ in the values of serum iron and ferritin for Norplant, injectables and oral contraceptive users whereas at the end of 12 months compared to the base line value there was a significant decrease for serum iron and ferritin amongst the Copper T contraceptive users

4.6.2 Serum concentration of copper and ceruloplasmin

The values for serum copper concentration amongst the Norplant users are as follows: 96.0±1.8μg/dl, 107.0±2.2μg/dl and 110.2±2.2μg/dl at 0, 6, 12 months respectively. For Copper T, the values are 108.9±2.0μg/dl 96.2±1.3μg/dl respectively, while for Injectable users the values were 100.7±1.6μg/dl, 108.0±1.9μg/dl and 111.4±2.0μg/dl respectively. For oral contraceptive users, 96.0±1.4μg/dl, 126.3±1.6μg/dl and 138.9±1.9μg/dl respectively. These values were significantly higher $p< 0.001$ for iron amongst all the contraceptive users after 6 months of usage except for Copper T users where there was a significant decrease, $p<(0.05)$, also the trend was the same for ferritin. For Copper, the values were significantly lower amongst the contraceptive users after 6 months of usage except for oral contraceptive users where there was significant increase $p< 0.005$ after 6 months of contraceptive use.

For serum ceruloplasmin, the concentration was 35.0±1.3 mg/dl, 45.0±1.1mg/dl and 50.3±1.0mg/dl amongst the Norplant users at 0, 6, 12 months respectively .while for copper T users the values were 47.0±1.5mg/dl, 28.9±0.9mg/dl and25.0±4.4mg/dl; respectively. For Injectable users the values were 34.9±1.9mg/dl, 38.2±2.0mg/dl and 40.2±2.0mg/dl respectively, while the values were 33.9±1.3mg/dl, 44.3±1.2mg/dl and 50.6±1.3mg/dl respectively for oral contraceptive users.

The values for ceruloplasmin was significantly lower amongst the Copper T users after 6 months of usage. For Norplant users and oral contraceptive users at 6 months there was no significant difference compared to control ,whereas there was a significant increase for both when compared to control and base line at 12 months

4.6.3 Serum free Vitamin E concentration;

There was a significant increase $p<0.05$ in the value of vitamin E for Norplant users 0.70±1.1μg/ml, 0.79±0.9μg/ml and 0.81±1.6μg/ml at 0, 6 months and 12 months respectively; for Copper T there was no significant difference in the values at 0 and 6 months 0.64±1.0μg/ml and 0.63±0.9μg/ml respectively while the value decreased significantly 0.56±1.4μg/ml at 12 month. There was a significant decrease $p< 0.001$ in the values for injectable users; 0.55±1.2μg/ml, 0.56±1.1μg/ml and 0.40±1.0μg/ml and a significant increase $p < 0.05$ amongst the oral contraceptive users 0.54±1.5μg/ml 0.71±1.2μg/ml and 1.10±0.9μg/ml compared with control.

Table 7: Iron, Copper and their carrier proteins concentration for contraceptive users and control

Parameter	Time interval (months)	Control[a]	Contraceptives			
			Norplant[b]	Copper T[c]	Injectables[d]	Oral contraceptives[e]
Iron µg/dl	0	108.0±31.4	84.8±3.5	106.4±2.8	87.8±2.6	81.9±2.5
	6		129.6±3.5	80.4±1.62	128.0±2.5	155.6±2.5
	12		134.9±3.7	71.5±1.64	134.8±2.6	176.8±1.1
Copper µg/dl	0	122.0±4.0	96.0±1.8	108.9±2.0	100.7±1.6	96.0±1.4
	6		107.0±2.2	96.2±1.3	108.0±1.9	126.3±1.6
	12		110.2±2.2	93.5±1.2	111.4±2.0	138.9±1.9
Ceruloplasm mg/dl	0	45.0±1.6	35±1.3	47.0±1.5	34.9±1.9	33.9±1.3
	6		45.0±1.1	28.9±0.9	38.2±2.0	44.3±1.2
	12		50.3±1.0	25.0±4.4	40.2±2.0	50.6±1.3
Feritin mg/dl	0	21.9±16.1	60.9±1.5	92.0±2.2	62±1.8	79.2±1.9
	6		118.0±1.9	70.4±1.8	108±2.1	120.6±1.8
	12		120.0±2.6	62.0±1.0	116.0±2.4	142.4±2.2

Index; mean ± SEM

n = a, b = 50 women

n = c, d and e = 100 women

Table 8: Vitamin E concentration for contraceptive users and control

Parameter	Time interval (months)	Control[a]	Contraceptives			
			Norplant[b]	Copper T[c]	Injectables[d]	Oral contraceptives[e]
Vit E mg/dl	0	0.65.5±1.1	0.70±1.1	0.64±1.0	0.55.0±1.2	0.5.4±1.5
	6		0.79±0.9	0.6.3±0.9	0.5.6±1.1	0.7.1±1.2
	12		0.81±1.6	0.5.6±1.4	0.4.0±1.0	1.1.0±0.9

Index; mean ± SEM

n = a, b = 50 women

n = c, d and e = 100 women

Table 9: Cholesterol, HDL-C, LDL-C, Triglyceride, Glucose and Vitamin E concentration for Injectable and Norplant users after 6 months of withdrawal

Contraceptives	Cholesterol	HDL-C	Triglyceride	LDL-C	Glucose	Vitamin E
Injectable[a]	207.2±0.9mg/dl	37.0±0.6mg/dl	197.3±0.5mg/dl	197.2±1.3mg/dl	107.0±1.1mg/dl	0.50±1.4mg/dl
Norplant[a]	169.4±1.7mg/dl	27.1±1.1mg/dl	168.3±1.0mg/dl	165.6±1.2mg/dl	85.0±0.4mg/dl	0.70±1.7mg/dl

Mean ± SEM

n = a = 8

Total number of women = 16

Sixteen women who were on hormonal contraceptives voluntarily withdrew from using these hormonal contraceptives to get pregnant. Their lipid profile, glucose and Vitamin E analysis were considered after 6 months of contraceptive withdrawal

The analysis of the cholesterol, HDL-C, LDL-C, triglyceride ,Glucose and Vitamin E concentration at 6 months after the withdrawal of these hormonal contraceptives which were injectable and Norplant were compared with results got after 6 months of usage of these contraceptives. For Norplant contraceptives, cholesterol concentration, LDL-C and Glucose were significantly lower $p<0.005$ compared to 6 months after taking the contraceptive while, HDL-C and Vitamin E concentration did not change while Triglyceride concentration was significantly higher $p> 0.001$. For injectables users when the values were compared with the values at 6 months of contraceptive usage. Total cholesterol, Triglyceride LDL-C and glucose were significantly higher $p>0.001$ while HDL-C concentration was lower $p<0.005$ and Vitamin E concentration did not change.

Table 10 Analysis after contraceptive use withdrawal

Contraceptive	**Time interval(return of menstruation)**	**% menstruating**	**% pregnant**
Injectable	6 months	12.5	None
Norplant	6 months	37.5	10
Injectable	12 months	37.5	25
Norplant	12 months	75.0	62.5

Total no of women= 16

Analysis after drug withdrawal

Sixteen injectable and Norplant contraceptive users were analysed for the time it took for menstruation to return and pregnancy achieved. At six months only 12.5% of the women had started menstruating amongst the injectables while for Norplant it was 37.5%. This increased to 37.5% and 75.0% for injectable and Norplant respectively at 12 months. (Table 7)

At the same time none of injectable users got pregnant after 6 months of withdrawal but 25% got pregnant after 12 months while for Norplant users at the end of 6 months only 10% of the women got pregnant, This increased to 62.5% after 12 months.

SUMMARY OF RESULTS

- The age distribution amongst the control and contraceptive users was between 19-48 years.
- Highest percentage of the subjects in this study had post secondary education.
- The highest weight increase was found in the injectable users.
- Serum cholesterol, LDL-C, triglyceride and glucose levels increased with hormonal contraceptive usage.
- Serum HDL-C level decreased with the hormonal contraceptives usage.
- Iron, ferritin, copper and ceruloplasmin serum level increased in the hormonal contraceptives users but decreased in the non hormonal contraceptive users.
- Level of serum Vitamin E increased in the hormonal contraceptives users
- Fertility returned for both groups of contraceptive users but the rate of return was longer than the time of contraceptive usage which was 12 months.

CHAPTER FIVE

5.0 DISCUSSION

Research on current contraceptive practice worldwide shows that 61% of all women of reproductive age 15 – 44 who are married or in a consensual union are using contraceptives (Reproductive Health and HIV Research Unit, 2006), and that in developing countries longer – acting, highly effective methods are more popular. The proportion of women aged 15 – 44 currently using contraceptives increased from 56 % in 1982 to 64% in 1995 and then declined slightly to 61 % in 2001 (RHHRU, 2006). It was also found that 43 million women of reproductive age or 7 in 10 are sexually active and do not want to become pregnant but could become pregnant if they or their partners fail to use a contraceptive method (Mosher, 2004). Other studies have shown that most unplanned pregnancies occur among teenagers and that the reproductive age or the child years spans from 15 – 49 years (The State of The World Children, 2000).

This present study found that contraceptive choice vary markedly with age. The use of Norplant, Copper T and injectable contraceptive were highest between the age range of 23 to 43 years. This is probably due to the fact that these contraceptives offer longer years of contraception for this group, some of who might have finished having children .It was also found that there was a marked acceptance of oral contraceptives among the younger age range 19 – 22 years. This is in consonant with other research, Martin and Wu, (2000), and the reason could be because the contraceptive of choice among women younger than 30 years was the reversible oral contraceptive. In previous studies Mosher *et al.*, (2004) found that oral contraceptive

is the most widely used method by women who were never married and with at least a college degree In addition poor and low income women are more than twice likely as higher income women to use injectable contraceptives but this study observed that the highest percentage of contraceptive users had post secondary education. This may be due to the location of the study and also consistent with the well known negative relationship between higher education and fertility. This study also found that contraceptive use increased with educational attainment. The more educated women may know more about their contraceptive options and may better understand the health implication of the various methods.

An increase in the mean weight of the contraceptive users especially the hormonal contraceptives was observed in this study, these results are in consonance with previous studies that reported similar weight gain. Brache *et al.*,(2003) reported that among Norplant users there was an increase of 0.4 – 1.5 kg/year. Also Espey et al., (2000) reported that cross sectional and longitudinal studies of Injectables have generally found increased mean weight while Bahamondes, (2003) also reported that Depot Medroxyprogesterone Acetate (DMPA) users had significantly higher weight increase when compared to Intrauterine devices (IUD) users ($p<0.001$).

Studies have shown that the effect of hormonal contraceptives on metabolic variables are related to the type and dose of the contraceptive steroid (Dorflinger, 2002; Kiran *et al.*, 2003 and Taneepanichskul and Phupong, 2007). It was observed that HDL – C level decreased significantly during a 24 month Norplant use but that the value of LDL – C and total cholesterol rose after an initial decrease (Asher *et al.*, 1992). Also research comparing data on women using Norplant and DMPA

Injectable shows that total cholesterol and HDL – C was much lower than in the Injectable group while the values of Triglyceride and LDL – C were higher in the injectable than Norplant users (Anwar *et al.*, 1994).

Total cholesterol, LDL – C and Triglyceride level increase in Norplant, Copper T, Iniectable and oral contraceptive users the increase rose from the base line throughout the research. This increase in total cholesterol LDL – C and Triglyceride may be due to oestrogen, as previous study has demonstrated that exposure to oestrogen increase hepatic synthesis and secretion of triglyceride rich particles (Kruss and Burkman, 1992;Sharrett *et al.*, 2001 and Sarwar ., 2007)

Many studies have demonstrated that increased triglyceride and LDL – C levels are associated with an increased risk of cardiovascular disease. Therefore by these increase in cholesterol, LDL – C and Triglyceride levels, the contraceptives may be worsening cardiovascular risk (Sarwar *et al.*, 2007). HDL – C levels decreases for the hormonal contraceptives users and increased for Copper T users. Hepatic Lipase activity was promoted by the androgenic effect of progesterone present in these hormonal contraceptives this led to an increased clearance of HDL – C and lowering circulating HDL – C levels. Because the hormonal contraceptive use led to decreased HDL – C it would be anticipated that these contraceptives might increase cardiovascular risk amongst the users.

From this study glucose level also increased in all the contraceptive users. Consistent with this finding, Kahn *et al.*,(2003) found that of eight studies that performed sequential oral glucose tolerance test after 6 months of injectable or Norplant use, seven had significant glucose elevation. This glucose elevation could

be an indication of glucose intolerance and the long term effect could be a predictor for diabetes. This is in contrast to the study by Damm *et al.*, (2007), who found that in healthy population epidemiological studies on oral contraceptive users did not demonstrate an increased risk to develop diabetes.

It was also observed in this study that serum iron, ferritin, copper and cerruloplasmin concentration increased for all the hormonal contraceptives users while these values decreased for Copper T contraceptive users. This observation is in agreement with previous studies by Bo *et al.*, (2008). Who reported that copper has both pro and anti – oxidant effects and active ferrous iron is a highly effective promoter of lipid peroxidation in cell free medium and amplified the prooxidant capacity of vascular cells. Significant increase in plasma concentration were also noted in women taking oral contraceptives. It is probable that the increase is as a result of increased synthesis of ceruloplasmin in response to oestrogen (Buchwald, 2008). These increase in micronutrients and their carrier protein levels in hormonal contraceptive users are strong predictors of atherosclerosis. Vitamin E level increased in this study in Norplant and oral contraceptive users but decreased in Copper T and Injectable users. Vitamin E is a fat soluble antioxidant and research has led to a widely held view that it may help prevent or delay coronary heart disease by limiting the oxidative modification of LDL – C which may promote blockage of coronary arteries thereby leading to atherosclerosis and help in preventing the formation of blood clot which could lead to a heart attack (Buchwald, 2008).

In this study the effect of contraception on fertility in Norplant users and Injectable users differ significantly. The time to get pregnant in Norplant users was shorter

than in Injectable users. This is consistent with the report of Croxatto (2002); and RHRU (2006), found that all implants deliver low doses of progestogen that clear rapidly from the circulation after implant removal with consequent resumption of ovarian function. Hassan and Killick, (2004) reported that women have to wait to conceive, partly because injectable remain in the blood stream for several months after the last injectable would have been given, and also amenorrhea may persist for several months after women discontinue injectables.

Similarly, previous use of the injectable was associated with a significant reduction in subsequent pregnancy (Kaunitz, 1976,1998). The delay in return to fertility was more than a year for injectables in this study. This could partly be due to residual ovarian suppression. It has also been shown that contraceptive methods that act principally by ovarian suppression have transient residual negative effect on subsequent fertility particularly in women who already have potentially compromised ovarian function.

A retrospective review of clinical data on 363 Australian women suggested a mean interval from the end of effective dose of DMPA to conception of 10.8 months (Fraser and Dennerstein, 1994).

Individual and regional variation in time to return to fertility after DMPA use are noteworthy, variance on the mean time until return to fertility are wide and regional reports differ significantly (d'Arcangues and Snow, 1999). Regional differences may reflect underlying differences in the fecundability of different population groups, differences in client motivation to conceive or more fundamental differences in pharmacokinetics

5.1 CONCLUSION

This study for the first time has tried to compare the metabolic effects of the different contraceptive agents which are readily available within Nigeria and to Nigerian women. In this study contraceptive use was found to be associated with an increase in markers of cardiovascular risk, manifested by increased cholesterol, LDL – C and Triglyceride levels . As each of these factors may promote adverse cardiovascular outcomes, it is possible that these contraceptive agents are presenting risk to Nigerian women.

CONTRIBUTIONS TO KNOWLEDGE

1. Local data for the first time has been generated from this study on the possible metabolic effects of the different contraceptive agents available in Nigeria using samples from many centers
2. From this study it was found that hormonal contraceptive use was associated with an elevated cholesterol, LDL – C and Triglycerides levels. As each of these factors may promote adverse cardiovascular outcomes, it is possible that these contraceptive agents could present a risk to Nigerian women
3. Hormonal contraceptives increased the serum level of iron, copper and their carrier proteins in the users whereas the levels decreased in non hormonal contraceptive users (Copper T)
4. The norplant has the greatest effects on the lipid profile of the users followed by injectables and then oral contraceptive whereas the oral contraceptive has the greatest effects on the serum iron, copper and their carrier proteins and vitamin E, followed by the norplant and then the injectables and then IUD has the least effect.
5. The study also evaluated the impact of each of the commonly used hormonal contraceptive methods on subsequent fecundity as measured by the conception rates for users of DMPA and Norplant method after discontinuing contraception. DMPA has a transient residual negative effect on subsequent fertility.

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QUESTIONNAIRE

Mark X for the correct answer

1. Status: Married

   Single [ ]

   Separated [ ]

   Divorced [ ]

   Widowed [ ]

2. Age: Below 20 years [ ]

   20 – 29 years [ ]

   30 – 39 years [ ]

   40 – 49 years [ ]

   50 and above [ ]

3. Height: [ ] (Meters) Weight: [ ] (kg)

4. General health status: Very healthy [ ]

   Fairly healthy [ ]

   Healthy [ ]

   Unhealthy [ ]

5. Do you Smoke: Yes: [ ] No: [ ]

6. If yes: Heavily (Over 10 sticks per day) [ ]

Lightly (under 10 sticks per day) [ ]

7. Do you drink alcohol: Often [ ]

Occasionally [ ]

8. Do you suffer from any specific disease or illness?

(Please include any history)

9. Are you on any medication (Please state)

10. Are you on any vitamin therapy (e.g.)

Vitamin tablets [ ]

Blood tonic [ ]

Sliming tablets [ ]

Iron tablets [ ]

Others (Specify) .....

11. Do you use contraceptive? (OC) Yes: [ ] No: [ ]

12. Have you ever heard of any of these methods?

METHOD	YES	NO
Pill: women can take a pill every day		
IUD: women can have a loop or coil placed inside them by a doctor or a nurse		
Injection: women can have an injection by a doctor or nurse which stops them becoming pregnant for several months		
Diaphragm/Foam/Jelly: women can place sponge, suppository, diaphragm, jelly or cream inside them before intercourse		
Condom: men can use a rubber sheath during sexual intercourse		
Female Sterilization: women can have an operation to avoid having any more children		
Male Sterilization: men can have an operation to avoid having any more children		
Periodic abstinence: couples can avoid having sexual intercourse on certain days of the month when the women is more likely to become pregnant		
Withdrawal: men can be careful and pull out before climax		
Norplant		
Any other methods? Have you heard of any other ways of methods that women or men can use to avoid pregnancy? (specify)		

13. Have you ever used any of these method?

METHOD	YES	NO
Pill		
IUD		
Injection		
Diaphragm/Foam/Jelly		
Condom		
Female Sterilization		
Male Sterilization		
Periodic abstinence		
Withdrawal		
Norplant		

14. Where would you go to obtain each of the above listed methods if you wanted to use them? (Mark the methods preferred against the places you would go in the boxes provided.

METHOD	GOVT HOSPITAL	HEALTH CENTRE	FPC	PRIVATE DOCTOR	PRIVATE HOSPITAL	PHARMACY	CHEMIST SHOP	CHURCH	FRIENDS	NO WHERE
PILL										
IUD										
INJECTION										
DIAPRAGM/ FOAM/JELLY										
CONDOM										
FEMALE STERILIZATION										
MALE STERILIZATION										
PERIODIC ABSTINENCE										
WITHDRAWAL										
NORPLANT										

15. In your opinion what is the main problem if any, with each of these methods (using the underlisted problems as guide, fill in appropriate problems for each method)

METHOD	N.E	H.D	H.C	A/A	C.M	I.U	M.P	O.T	N.N
Pill									
IUD									
Injection									
Diaphragm/Foam/Jelly									
Condom									
Female Sterilization									
Male Sterilization									
Periodic abstinence									
Withdrawal									
Norplant									
Others									

Key: N.E. – Not Effective
H.D. – Husband disapproves
A/A – Access/ Availability
C.M. – Cost too much
M.P – Method permanent
O.T – Others
H.C. – Health concerns
I.U – Inconvenient to use
N.N. – None

16. Are you currently using any method to avoid getting pregnant?

Yes [ ] No [ ]

17. Do you intend to use a method to avoid getting pregnant in the future?

Yes [ ] No [ ]

18. Which method would you prefer to use?

19. Do you intend to use the preferred method in the next 12 months?

Yes [ ] No [ ]

20. Is it acceptable or not acceptable to you for family planning information to be provided on radio set or television?

Acceptable [ ] Not Acceptable [ ] Don't Know [ ]

21. Can you recall your diet in the past 24 hours?

Breakfast Lunch Dinner