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Project paper on risk of Birth Defects associated with *in utero* exposure to Antiretroviral Drugs

The project paper is submitted to Addis Ababa University, College of Health Sciences, School of Medicine, Department of Anatomy in partial fulfillment for degree of Master of Science in Anatomy

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Lists of Abbreviations

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
Apgar	American pediatric gross assessment record
APR	Antiretroviral Pregnancy Registry
ART	Antiretroviral Therapy
ARV	Antiretroviral
AST	Aspartate Aminotransferase
AZT	Zidovudine
BDMP	Birth Defects Monitoring Program
BSID-II	Bayley Scales of Infant Development-II
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CDC	Centers for Disease Control and Prevention
d4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
DNA	Deoxyribonucleic acid
EFV	Efavirenz
FDA	Food and Drug Administration
GD	Gestational Day
GLUT4	Glucose transporter isoform 4
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
LBW	Low Birth Weight
LPV/r	Lopinavir/ritonavir
MACDP	Metropolitan Atlanta Congenital Defects Program
MBDR	Michigan birth defect registry
MD	Mitochondrial Dysfunction
MDI	Mental Development Index
mtDNA	Mitochondrial DNA
NFV	Nelfinavir
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NVP	Nevirapine
PACTG	Pediatric AIDS Clinical Trials Group
PDI	Psychomotor Development Index
PIs	Protease Inhibitors
PTD	Preterm Delivery
PMTCT	Prevention of Mother to Child Transmission
RTI	Reverse Transcriptase Inhibitors
UNAIDS	Joint United Nations Program on HIV/AIDS
US	United States
WHO	World Health Organization

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Summary

Birth defects are a global problem. This impact is particularly severe in middle and low income countries where more than 94 percent of the births with serious birth defects and 95 percent of the deaths of these children occur.

The aim of this project is to review and present outcomes of antiretroviral drugs exposed pregnancy.

Antiretroviral compounds differ from most other new pharmaceutical agents in that they have become widely prescribed in pregnancy in the absence of proof of safety. In this paper antiretroviral agents used in pregnant women infected with human immunodeficiency virus and their effects on the infants are reviewed**.** This review gives an overview about *in vivo* and *in vitro* developmental toxicity and teratogenicity of the anti-AIDS drugs (antiretrovirals), in experimental animals and humans.

Animal embryos exposed *in vivo* to antiretrovirals exhibited significantly increased pregnancy losses, drugs incorporation into the DNA of fetal organs, external abnormalities, skeletal defects, developmental toxicity, carcinogenicity, reduced weight, anemia, deaths and significant mitochondrial damage. The *in vitro* antiretrovirals exposure of animal cells or organs resulted in cytotoxicity, growth retardation, chromosomal aberrations, mutations, sister chromatid exchange and other genotoxic effects. In earlier human studies, management of AIDS positive pregnant women with antiretrovirals revealed exposure of their infants to such drugs with evidence of adverse events. However, recent publications present conflicting data about the associations between antiretrovirals and adverse pregnancy outcomes.

Because of the increasingly frequent use of highly active antiretroviral therapy during pregnancy, ongoing efforts are needed to monitor any long-term effects of *in utero* exposure to the multiple antiretroviral agents used.

Key words: Birth defects, ARV Drugs, HIV, Pregnancy.

1. Introduction

1.1. Overview of Birth Defects

1.1.1. Definition: Birth defects are structural and functional abnormalities present at birth, whether the abnormality is detected at the time of delivery or at a later time. For example, spina bifida is a structural birth defect clinically obvious at birth and hemophilia is a functional birth defect that may present clinically only in infancy or childhood (Global report on birth defect, 2006; Kim et al., 2012). A few birth defects, like Huntington disease, manifest only in adulthood (Christianson et al., 1981). Some birth defects are minor while others are life-threatening which have the potential to cause fetal and infant mortality and lifelong disability (Birth defect prevalence and mortality in Michigan, 2011). The term congenital disorder is considered to have the same definition; the two terms are used interchangeably (WHO, 2010).

1.1.2. Cause: It was only in the 20th century that the causes of particular birth defects began to be recognized. The causes of birth defects are many and complex; even now, approximately 50-70 percent of birth defects cannot be ascribed to a specific cause (Nelson and Holmes, 1989; Turnpenny and Ellard, 2005; Kim et al., 2012). However, known causes can be divided broadly into preconception and postconception factors (Global report on birth defect, 2006).

Preconception factors causing birth defects

Most birth defects originate before conception and are due to abnormalities of the genetic material-chromosomes and genes. Partially genetic birth defects are due to a combination of genes that puts the fetus at risk in the presence of specific environmental factors. Genetic abnormalities can be inherited, in which case they are found in families, or they can occur as an isolated event in a particular pregnancy. They include chromosomal abnormalities, single gene defects and conditions known as multi-factorial disorders, which are caused by the interactions of genes and the environment (Global report on birth defect, 2006; WHO, 2010).

Chromosomal abnormalities are due to changes in the number or the structure of chromosomes from the normal state that result in a gain or loss of genetic material. Such abnormalities account for approximately 6 percent of birth defects in industrialized countries (Turnpenny and Ellard, 2005). Single gene defects are caused by alterations in gene structure, called mutations, which result in abnormal cell functioning. More than 7,000 single gene defects have been described (Baltimore and Bethesda, 2000). All single gene defects combined account for an estimated 7.5 percent of all birth defects in industrialized countries (Turnpenny and Ellard, 2005; Global report on birth defect, 2006).

The concept of multi-factorial inheritance which is birth defects due to complex of genetic and environmental interactions is now broadly accepted as suggested by Passarge (1995). This category accounts for an estimated 20-30 percent of all birth defects, a number of which are lethal (Turnpenny and Ellard, 2005). Examples of multi-factorial birth defects are numerous, are usually malformations of a single organ system or limb and include congenital heart disease, neural tube defects, cleft lip and/or cleft palate, clubfoot and developmental dysplasia of the hip (Berry et al., 1999). Multi-factorial inheritance is also the cause of the many common diseases with a genetic predisposition that present later in life, are usually systemic and do not involve malformations. Included among these disorders are hypertension, diabetes, stroke, mental disorders and cancer (Global report on birth defect, 2006).

Postconception factors causing birth defects

Causes of birth defects originating after conception are primarily non-genetic. In these disorders, the genetic material inherited by the fetus is normal and the birth defect is caused by an intra-uterine environmental factors. These include teratogens that interefere with normal growth and development of the embryo or fetus, mechanical forces that deform the fetus and vascular accidents that disrupt the normal growth of organs. This category accounts for an estimated 5-10 percent of all birth defects (Nelson and Holmes, 1989; Turnpenny and Ellard, 2005). According to Seashore and Wappner (1996) and WHO (2010) teratogens are broadly categorized into five groups: (1) physical agents such as high dose of radiation, (2) certain environmental pollutants/chemicals like methyl mercury; (3) maternal illness or disturbances of the mother's metabolism such as maternal insulin-dependent diabetes mellitus or maternal iodine and folic acid deficiency; (4) maternal infectious diseases, including rubella, syphilis and toxoplasmosis which particularly are significant cause of birth defect in low and middle income countries; and (5) exposure to drugs, both medicinal and recreational drugs including alcohol and tobacco are other factors that cause birth defects.

Unknown Causes

As noted above, a specific cause cannot be designated in approximately 50-70 percent of all children born with birth defects. Some of these birth defects may be due to new autosomal dominant mutations, submicroscopic chromosome deletions or uniparental disomy (Turnpenny and Ellard, 2005). Causes for birth defects continue to be identified, so the percentage of birth defects of unknown cause can be expected to decrease in the future.

1.1.3. Prevalence: Since the importance of birth defects was recognized in 1960s, studies have been done to determine its prevalence mainly in developed countries. However, as determined by CDC surveillance systems, the Birth Defects Monitoring Program (BDMP), the Metropolitan Atlanta Congenital Defects Program (MACDP) (Erickson et al., 1974) and Michigan birth defect registry (MBDR) (Birth defect prevalence and mortality in Michigan, 2011), most of the studies were limited to a single institute or region and not nationwide. Therefore, the prevalence of birth defects varies considerably with respect to type of defect, time, place, and other demographic, genetic and environmental factors (Erickson et al., 1974; Kim, 1998; Chung et al., 2004; Birth defect prevalence and mortality in Michigan, 2011). The reported frequency of birth defects has been 2%-3% of all births and 25% of severe birth defects are multiple defects and the prevalence of birth defects is much higher in abortion or stillbirth (Chung and Myrianthopoulos, 1987; Cordero, 1994; Kim et al., 2012).

Global report on birth defect (2006) showed every year an estimated 7.9 million children-6 percent of total births worldwide are born with a serious birth defect of genetic or partially genetic origin. For example according to the report five common serious birth defects of genetic or partially genetic origin in 2001 were: (1) congenital heart defects (1,040,835 births); (2) neural tube defects (323,904 births); (3) the hemoglobin disorders, thalassemia and sickle cell disease (307,897 births); (4) down syndrome (trisomy 21) (217,293 births); and (5) glucose-6-phosphate dehydrogenase (G6PD) deficiency (177,032 births). Combined these five conditions account for about 25 percent of all of birth defects of genetic or partially genetic origin. An undetermined number, but undoubtedly additional hundreds of thousands more are born with serious birth defects of postconception origin, including maternal exposure to environmental agents (teratogens) such as alcohol, rubella, syphilis and iodine deficiency that can harm a developing fetus (Christianson and Modell, 2004).

According to Christianson and Modell (2004) birth defects of genetic or partially genetic origin as well as postconception birth defects are more common in low and middle income countries, where the potential for exposure to teratogenic agents is greater and fewer preventive measures are in place. But they are less likely to have systems to detect disorders arising from such exposure and quantification of such problems is limited than in high income countries (Global report on birth defect, 2006). Thus, they tend to have few, if any; regulations governing the use of some of these substances and their health services are not directed towards identifying and controlling exposure (Penchaszadeh, 2002; Christianson and Modell, 2004). Christianson and Modell's (2004) study

showed, the prevalence of birth defects is, therefore, about 20 percent higher in middle and low income countries than in high income countries.

Data from birth defect prevalence and mortality in Michigan (2011) showed the overall prevalence rate of birth defects reported by one year of life has increased slightly over the past 14 years. There were about 650 reported defects per 10,000 live births in 1992 and 830 reported defects per 10,000 live births in 2006 which means approximately 8% of the 127,537 Michigan newborns in 2006 were diagnosed with a birth defect by one year of age. This increase may in part be due to improved reporting and diagnostic techniques, or to changes in population demographics. Population changes may include a shift in the distribution of births by maternal age or race, or a change in the rate of preterm infants. In 2006 the majority of reported birth defects fell into three diagnostic categories: the heart and circulatory system (23%), the musculoskeletal system (20%), and the genitourinary system (17%). Other birth defects fell into the respiratory system (9%), the integument (8%), the digestive system (7%), and the central nervous system (CNS) (5%) categories. All other diagnostic categories had less than 5% of all reported birth defects. As stated; categories are not mutually exclusive, meaning that an infant could be counted more than once if diagnosed with birth defects in multiple categories. This means that the numbers, and therefore rates, of specific types of birth defects may not reflect the rates of Michigan children with birth defects because some children have multiple defects and are therefore counted more than once by the MBDR.

After the survey on birth defects was done in 2,348 medical institutions around the nation of Korea by Kim et al. (2012) there were 883,184 live births from 2005-2006 and 25,335 cases of birth defects were notified which is , equivalent to a prevalence of 286.9 per 10,000 live births. In addition, the total number of stillbirths in 2005-2006 was 5,079, and out of those, 640 had birth defects. Therefore, the total number of birth defects among total live births and stillbirths was 25,975 in 2005-2006. According to the survey anomalies of the circulatory system were the most common defects, accounting for 43.4% of birth defects with a prevalence of 124.5 per 10,000 live births. It was followed successively by the musculoskeletal system anomalies, the digestive system anomalies, and the urinary system anomalies. The five major birth defects based on the ranking of prevalence were atrial septal defect, ventricular septal defect, hydronephrosis, patent ductus arteriosus and cleft lip/palate. Birth defects in live births were associated with a high proportion of low birth weight, prematurity, multiple births and advanced maternal age (Kim et al., 2012).

A study that lasted 16 years period in Victoria, 1983 to 1998 by Riley & Halliday (1999) showed 32,445 babies were born with a birth defect at or after 20 weeks gestation. Another 2,402 were

identified at terminations of pregnancy before 20 weeks gestation. This gave an overall prevalence of 344/10,000 or 3.4%. From 1995–1998 the overall prevalence was 388/10,000 or 3.9% showing increment on the rate of occurrence of birth defect (Riley & Halliday, 1999).

Accordingly, the four most common defects for the period 1995–1998 are hypospadias, ventricular septal defect, congenital dislocation of the hip and obstructive defects of the renal pelvis (Riley & Halliday, 1999). Congenital dislocation of the hip is a very common birth defect, reported in 28.6/10,000 births or 1/350 births during 1983–1998, with female babies having a four-fold increased risk for this condition over male babies. The number of cases reported with obstructive defects of the renal pelvis continues to increase annually (Riley & Halliday, 1999). This is suggested to be due to increased reporting secondary to ultrasound detection in pregnancy. There has been an improved survival rate for babies with renal agenesis/dysgenesis with 55% between 1995–1998 surviving beyond 28 days compared with only 33% in 1983–1994 (Riley & Halliday, 1999).

The birth defect study in Victoria showed, advanced maternal age to be significantly associated with hydrocephalus, exomphalos, down syndrome and other chromosome abnormalities, while teenage mothers had a significantly increased risk of having babies with spina bifida, hydrocephalus and gastroschisis (Riley & Halliday, 1999). Similarly the study has shown, there are some significant gender differences in birth defect prevalence with males at higher risk compared with females. Birth defects significantly associated with males include cardiac (transposition of great vessels, tetralogy of fallot, coarctation of aorta) or renal (renal agenesis/dysgenesis, obstructive defects of renal pelvis) anomalies. Males are also at increased risk of cleft lip and palate and down syndrome, while females have an increased risk for anencephaly, cleft palate and congenital dislocation of the hip (Riley & Halliday, 1999).

The prevalence of having a baby affected by a birth defect increases with increasing age. Women aged 35 years or more, 11.7% of all mothers for the period 1983–1998, comprise 15% of all mothers who have a pregnancy affected by a birth defect for this same period. They have a relative risk of having a baby with a birth defect of 1.34 (1.3–1.38) compared with younger women which is statistically significant. If the chromosomal abnormalities involving down sydrome, patau syndrome and edward syndrome are excluded, the relative risk for women aged 35 years or more having a baby with a birth defect decreases to 1.13 (1.10–1.17) (Riley & Halliday, 1999).

Women from the Middle East are significantly more likely to have a baby with a birth defect than women from those in Victoria. This is not related to advanced maternal age and may reflect genetic factors (Cordero et al., 1994).

Women from rural regions have a statistically significant decreased likelihood of having a baby with a birth defect when compared to metropolitan regions (relative risk (RR), 0.9; 96% confidence interval (CI), 0.88–0.92; $p<0.0001$). This is a small decrease and may relate to ascertainment issues as much as a biological risk, such as the fact that they are generally younger than metropolitan mothers (Riley & Halliday, 1999).

The prevalence of birth defects in pregnancies of women identified as koori in the perinatal morbidity statistics system is significantly less than for those identified as non-koori (RR, 0.77;95% CI, 0.66–0.91; p, 0.001) (Riley & Halliday, 1999). Babies from multiple births are more likely to have a birth defect than babies from singleton births (RR, 1.55; 98% CI, 1.48–1.64; $p<0.0001$) (Riley & Halliday, 1999).

The overall prevalence rate of birth defects in Victoria, Australia for 2003 was 4.0%. However, this proportion varied according to what birth defects were included, the age by which birth defects were diagnosed, changes to sources of ascertainment, inclusion of terminations of pregnancy (TOPs), or reporting by cases rate (infants affected) or birth defect rate (individual birth defects). For example, Victoria includes birth defects diagnosed before 15 years of age in all live births, stillbirths, neonatal deaths and TOPs. Western Australia, on the other hand, includes birth defects diagnosed before 5 years of age and New South Wales includes cases diagnosed within the first year of life. These differences could give rise to variations in birth defect prevalence rates between the states (Merilyn, 2005). Taking all of these factors into consideration, the study reported, that 4.0% is an accurate population prevalence rate of birth defects in Victoria for 2003 (Merilyn, 2005).

There were 2205 babies born at, or after 20 weeks gestation with a birth defect and 339 TOPs before 20 weeks gestation with a birth defect. In 2003, there were 63 551 births in Victoria (including late TOPs at 20 weeks or more for any reason). This gives an overall birth defect prevalence rate of 400/10 000 or 4.0% (Merilyn, 2005).

Population based registry study of 839 521 births to mothers resident in five geographical areas of Britain during 1991–1999 yield 10 844 congenital anomalies, giving a total prevalence of 129 per 10 000 registered births (95% CI, 127-132) (Rankin et al., 2005).

This study reported a significant reduction in the total prevalence of all non-chromosomal anomalies combined (chi squared (x^2), 7.8; P, 0.005) from 83.8 per 10 000 births in 1991 to 81.2 per 10 000 in 1999. In contrary, there was a two-fold significant increase in the total prevalence of all chromosomal anomalies combined (x^2, 32.2; p, 0.00001) from 21.4 per 10 000 births in 1991 to 41.4 per 10 000 in 1999. The total prevalence for all neural tube defects declined from 15.6 per 10 000 births in 1991 to 12.9 per 10 000 in 1999 (x^2, 5.5; p, 0.02). However, the reduction in total prevalence of anencephaly and spina bifida did not reach significance (anencephaly: x^2, 1.8; p, 0.2; spina bifida: x^2, 2.6; p, 0.1). During the study period there was a trend towards an increase in the total prevalence of gastroschisis and a decrease in the prevalence of omphalocele, but neither of these reached statistical significance (gastroschisis: x^2, 2.9; p , 0.09; omphalocele:x^2, 1.2; p , 0.3) (Rankin et al., 2005).

The prevalence rates of some of the common birth defects have been ascertained in a black population in South Africa (Jenkins and Kromberg, 1982). The births of 29633 infants were studied retrospectively from hospital records. The commonest defect was found to be polydactyly (104/1000 births) and the other defects investigated were talipes equinovarus (155/1000 births), hydrocephalus (13/1000 births), spina bifida and anencephaly (78 and 40/1000 births respectively) and facial clefts (30/1000 births). These rates were compared with those found in other population groups in South Africa and, in some cases, in other African countries and in Negro groups in the USA and Canada (Jenkins and Kromberg, 1982). This study widely recognized that at least 2% of all infants born show some degree of congenital defect and that these defects are responsible for many deaths in the neonatal period. Nevertheless congenital defects tend to be neglected, either through lack of appreciation of the magnitude of the problem or because they often present as hopeless, incurable conditions (Jenkins and Kromberg, 1982).

1.1.4. Consequence (public health impact) of Birth Defects: Birth defects are a global problem; serious birth defects can be lethal which account for 20%-25% of perinatal mortality (Kim et al., 2012). For those who survive, these disorders can cause lifelong mental, physical, auditory or visual disability. Data from global report on birth defect (2006) shows, that at least 3.3 million children

under five years of age die from birth defects each year and an estimated 3.2 million of those who survive may be disabled for life.

The impact of birth defect is particularly severe in middle and low income countries where more than 94 percent of the births with serious birth defects and 95 percent of the deaths of these children occur (Global report on birth defect, 2006). However, in high income countries, about 30 percent of all children born with a serious birth defect of genetic or partially genetic origin die in infancy, another 30 percent can be treated and live with chronic disability and the remaining 40 percent can have their condition cured or largely ameliorated with treatment, primarily surgery (WHO, 1996; Christianson and Modell, 2004).

The proportions of births with birth defects as well as the absolute number of births are much higher in middle and low income countries than in high income countries. This is said because of sharp differences in maternal health and other significant risk factors, including poverty, a high percentage of older mothers and a greater frequency of consanguineous marriages (Global report on birth defect, 2006).

According to British birth defect prevalence study (2005) and WHO (2010), irrespective of definition, birth defects can cause spontaneous abortions and stillbirths and are one of the three major causes of death in the perinatal period in addition to premature birth and birth injury. In addition, birth defects are a significant but under recognized cause of mortality and disability among infants and children under five years of age. They can be life-threatening, result in long-term disability and negatively affect individuals, families, health-care systems and societies (Rankin et al., 2005; WHO, 2010). In figure, WHO (2010) estimates that some 260 000 deaths worldwide (about 7% of all neonatal deaths) were caused by congenital anomalies in 2004. Accordingly, they are most prominent as a cause of death in settings where overall mortality rates are lower, for example in the European region, where as many as 25% of neonatal deaths are due to congenital anomalies.

Birth defects are a serious public health problem in Michigan and across the nation which contribute significantly to childhood mortality, morbidity and long-term disability. The infant fatality rate for children born in 2006 with a reportable birth defect was 32.8 deaths per 1,000 infants with a birth defect. This compares to an infant death rate of 7.4 deaths per 1,000 live births for all resident infants born in Michigan for the same year. Recent analysis of MBDR surveillance data reveals that children with birth defects are at much greater risk of death due to causes other than a birth defect

(for example, accidental causes) (Copeland and Kirby, 2007; Birth defect prevalence and mortality in Michigan, 2011).

Moreover, the total mortality rate over ten years of life for those born in 1997 and reported to the MBDR was 59.6 deaths per 1,000 children with a birth defect compared to a rate of 10.5 deaths per 1,000 resident live births overall . This is higher than the 1 in 5 infant deaths usually attributed to birth defects based on death records alone and emphasizes the need for greater attention on the impact of birth defects as a cause of early childhood death (Birth defect prevalence and mortality in Michigan, 2011).

The mortality experienced by Michigan children with birth defects is appreciably higher than for children in general. Birth defects registry data indicate that the contribution of birth defects to infant and childhood fatality is more than twice of that indicated by cause of death data alone. The relative risk of death for children with birth defects is roughly five times that of other children. The elevated relative risk of death for children with birth defects is highest in children age one to two years old. Children with birth defects constitute 46% of the deaths in this age group, with relative risk of mortality that is six times the mortality rate of other children. Elevated mortality is experienced by children in the registry for all ages examined including through the age of 14 years (Birth defect prevalence and mortality in Michigan, 2011).

Population based registry study of 839 521 births to mothers resident in five geographical areas of Britain during 1991–1999 yields, the proportion of all stillbirths with a congenital anomaly as 10.5% (453 stillbirths). The proportion of pregnancies resulting in termination increased from 27% (289 cases) in 1991 to 34.7% (384 cases) in 1999, whereas the proportion of live births declined from 68.2% (730 cases) to 58.5% (648 cases) (Rankin et al., 2005).

1.1.5. Prevention: The ability to use comprehensive data on the incidence and types of birth defects affecting a particular countries children will lead to a better understanding of total health care and educational costs for this population; to plan and implement prevention and intervention strategies to reduce both the financial and emotional burden on families and society as a whole; and to see an improvement in the quality of life for affected children and their families.

Experience from high income countries shows that up to 70 percent of birth defects can either be prevented, or that affected children can be offered care that could be life saving or would reduce the

severity of disability (Global report on birth defect, 2006). These interventions include appropriate treatment, particularly surgery and prevention, especially before conception or in very early pregnancy. For example, in United States a remarkable 46 percent decline in infant mortality rates from birth defects over the period 1980 to 2001 have been reported (Koren et al., 1998) and much of this reduction can be attributed to improvements in diagnosis, care and prevention. Other high income countries have reported similar declines. On the other hand, limited data from low and middle income countries suggest that there has been little to no improvement in infant mortality rates from birth defects over the same general time period. Most middle and low income countries currently lack the comprehensive health services needed to reduce their toll of birth defects (Global report on birth defect, 2006).

For simplicity, preventive measures are grouped into three levels on the basis of timing of performing the activity and type of activities performed (CDC, 2005).

Primary prevention (preconceptional)*:* Seeks to ensure that individuals are born free of birth defects by being conceived normally and not being damaged in the early embryonic period which is the first eight weeks after conception when the mother is not aware she is pregnant. Services for the primary prevention of birth defects include basic reproductive health approaches, which should be part of established maternal, newborn and child health services. These include: family planning; optimizing women's diets; detecting, treating and preventing maternal infections; optimizing women's health through the control of such diseases as insulin dependent diabetes mellitus and epilepsy; distribution of educational materials and participation in the national birth defects prevention network, promoting birth defects prevention month (Birth defect prevalence and mortality in Michigan, 2011) and preconception screening for common recessive disorders (Global report on birth defect, 2006).

Examples of specific interventions include: folic acid supplementation to prevent neural tube defects and as research indicates at least half the cases of neural tube defects could be prevented if, women consumed sufficient folic acid before conception and during early pregnancy (MRC vitamin study research group, 1991; CDC, 2005); rubella immunization to prevent congenital rubella syndrome and programs to educate women of child-bearing age about the dangers of alcohol intake in early pregnancy (Global report on birth defect, 2006).

Secondary prevention (prevention during pregnancy)*:* Aims to reduce the number of children born with birth defects which, requires risk identification and management. This is achieved through medical genetic screening and prenatal diagnosis where birth defects are detected and the couple offered genetic counseling and therapeutic options. To make informed decisions affecting the outcome of pregnancy, parents need the best information available about their specific set of circumstances. This includes the diagnosis, if possible, affecting their fetus; the cause; the consequences for the fetus, available options for treatment and prognosis as far as this is available; and the risks for recurrence and whether this might be reduced (Global report on birth defect, 2006; WHO, 2010).

Secondary prevention requires prenatal diagnosis, which must be accompanied by genetic counseling that includes descriptions of the tests available with their scope and attendant risks. Some of the interventions and services related to this can raise ethical, legal and social issues and may have cost implications. Such services include diagnosis of birth defects, selective termination of pregnancy and the availability of counseling services. Minimally invasive screening methods are currently available, such as taking maternal blood for the measurement of several metabolites in the maternal serum. Abnormal levels of biochemical markers are associated with fetal structural defects such as Down syndrome, neural tube defects and open ventral wall defects. For example, alpha-fetoprotein screening of maternal serum is widely used to detect fetuses affected by neural tube defects (Erickson et al., 1974). The detection rate of congenital disorders in the first trimester through biochemical screening is improved when it is undertaken in tandem with ultrasound screening. Ultrasonography in the second trimester is useful to detect major structural defects (WHO, 2010).

Tertiary prevention (prevention following birth with the defect)*:* Is directed toward the early detection and cure and amelioration of problems once a child with a birth defect is born. Interventions include early recognition and diagnosis, including newborn screening if available, medical treatment of complications, surgical repair of congenital malformations such as cleft lip and palate and heart defects and offering neurodevelopment therapy programs to infants and children with disability. It also includes palliative care for children dying from the consequences of their birth defects. In middle and low income countries, as much as possible of the medical treatment, neurodevelopment therapy and palliative care needs to be managed in primary health care settings (WHO, 1985, 1999; Christianson et al., 2000; Christianson and Modell, 2004).

Generally training of physicians, nurses and allied health professionals in the causes and identification of birth defects; promotion of parent/patient support groups to promote research and attention to care and prevention; and education of the public and of policy makers, the media and other stakeholders about birth defects and the means for effective care and prevention strengthen all three levels of prevention.

Finally, with its emphasis on ensuring normal conception and early pregnancy primary prevention is the most important of all three levels and is both feasible and affordable, even for financially constrained health systems. At the end since the review is about birth defects in relation to anti HIV drugs, generally I will introduce about HIV/AIDS and next antiretroviral drugs.

1.2. Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV and AIDS)

The first cases of HIV/AIDS were reported in 1981 on clusters of gay men in New York and California who had been diagnosed with PCP or Kaposi's sarcoma (Global HIV/AIDS epidemic, 2011). HIV, the virus that causes AIDS, has become one of the world's most serious health and development challenges. Although cases have been reported in all regions of the world, almost all those living with HIV (97%) reside in low and middle income countries, particularly in sub-Saharan Africa (Global HIV/AIDS response, 2011). HIV not only affects the health of individuals, but it also impacts households, communities and the development and economic growth of nations (Global HIV/AIDS epidemic, 2011; Global HIV/AIDS response, 2011).

In 2011 according to the latest estimates from UNAIDS: globally approximately 34 million people were living with HIV at the end of 2010 versus 28.6 million in 2001 showing a 17% increase (Global HIV/AIDS Epidemic, 2011; Global HIV/AIDS response, 2011). New HIV infections have declined by more than 21% since their peak in 1997 and declined by 15% between 2001 and 2010 (Global HIV/AIDS response, 2011). Still, there were about 2.2 million new infections in 2011, 500 000 fewer than in 2001 (Together we will end AIDS, 2012). These trends reflect a combination of factors: the natural course of HIV epidemics, behavioral changes associated with greater awareness about the effects of the epidemics and with intensified prevention efforts and increasing coverage of antiretroviral therapy (Global HIV/AIDS response, 2011).

Globally, women constituted half (50%) of the adults (15 years and older) living with HIV in 2010 but more than half (59%) in sub-Saharan Africa (Global HIV/AIDS Epidemic, 2011; Global HIV/AIDS response, 2011).

Since the beginning of the epidemic nearly 30 million people have died of AIDS-related causes (Global HIV/AIDS Epidemic, 2011; Global HIV/AIDS response, 2011; UNAIDS, 2012), in 2011 the annual number of people dying from AIDS-related causes worldwide is steadily decreasing from a peak of 2.2 million in 2005 to an estimated 1.7 million (Together we will end AIDS, 2012).

Africa has claimed for at least 1 million AIDS lives annually since 1998. At end of 2010 about 22.9 million which is 67% of those living with HIV/AIDS globally are in Africa with adult prevalence rate of 5% though only about 12% of the world's population lives in the region (Global HIV/AIDS response, 2011; Phillips, 2011). In terms of mortality, the region represents about 79% of AIDS mortality globally (Edward et al., 2006), at end of 2010 the estimated 1.2 million people dying from AIDS related illnesses which is 29% less than the peaked death of 1.7 million in 2005 (Global HIV/AIDS response, 2011).

In Ethiopia according to 2011 Ethiopian demographic health survey the overall adult (age 15-49) HIV prevalence is 1.5 % (confidence interval 1.2-1.7 %) which is similar with 2005 Ethiopian demographic health survey that was 1.4 % (confidence interval 1.1-1.8 %). Peak HIV prevalence is occurred in Women (1.9 %) than men (1.0 %), in both Men and women (3.9 %), in age group of 30-34 women (3.7 %) and 35-39 of men (3%), in urban areas (4.2 %) than in rural areas (0.6 %), in employed (2.2 %) than unemployed (1.4 %), in those who attended secondary school (3.1 %) than those with less education or with more than a secondary school education, among regions highest in Gambela (6.5 %) and in Addis Ababa (5.2 %) (Central Statistical Agency, 2012). Moreover, in Ethiopia, there were more than 222,000 patients on antiretroviral treatment at the end of 2010 (Global HIV/AIDS response, 2011).

1.3. Antiretroviral Drugs

According to data from international HIV and AIDS charity (2012); antiretroviral drug treatment is the main type of treatment for HIV or AIDS. It is not a cure, but it can stop people from becoming ill for many years. The treatment consists of drugs that have to be taken every day for the rest of a person’s life.

The aim of antiretroviral treatment is to keep the amount of HIV in the body at a low level. This stops any weakening of the immune system and allows it to recover from any damage that HIV might have caused already. The drugs are often referred to as: Antiretrovirals, ARVs, anti-HIV or anti-AIDS drugs (Mobarak, 2011).

Taking two or more antiretroviral drugs at a time is called combination therapy. Taking a combination of three or more anti-HIV drugs is sometimes referred to as highly active antiretroviral therapy (HAART). There are more than 20 approved antiretroviral drugs by the American food and drug administration but not all are licensed or available in every country. Based on the mechanism of action to attack HIV, there are five groups of antiretroviral drugs (International HIV & AIDS charity, 2012).

Table 1: Five groups of antiretroviral drugs and actions on human immunodeficiency virus (adopted from international HIV & AIDS charity, 2012).

Antiretroviral drug class	Abbreviations	First approved to treat HIV	How they attack HIV
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors	NRTIs, nucleoside analogues, nukes	1987	NRTIs interfere with the action of an HIV protein called reverse transcriptase, which the virus needs to make new copies of itself.
Non-Nucleoside Reverse Transcriptase Inhibitors	NNRTIs, non-nucleosides, non-nukes	1997	NNRTIs also stop HIV from replicating within cells by inhibiting the reverse transcriptase protein.
Protease Inhibitors	PIs	1995	PIs inhibit protease, which is another protein involved in the HIV replication process.
Fusion or Entry Inhibitors		2003	Fusion or entry inhibitors prevent HIV from binding to or entering human immune cells.
Integrase Inhibitors		2007	Integrase inhibitors interfere with the integrase enzyme, which HIV needs to insert its genetic material into human cells.

Nucleoside Reverse Transcriptase Inhibitors (NRTIs): There are currently seven NRTIs that are licensed for HIV treatment: 3'-azido-2',3-dideoxythymidine (zidovudine, AZT); 2',3'dideoxycytidine

(zalcitabine, ddC); 2',3'-dideoxyinosine (didanosine, ddI); lamivudine, 3TC; stavudine, d4T; tenofovir, TDF and Abacavir, ABC. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs): There are 3 compounds that are licensed for HIV treatment: nevirapine, NVP; Efavirenz, EFV and Delvirdine, DVD. Protease Inhibitors (PIs): There are six PIs that are licensed for HIV treatment: Indinavir, IDV; Ritonavir, RTV(r); Nelfinavir, NFV; Lopinavir, LPV; Saquinavir, SQV and Amprenavir, APV; Atazanavir, ATV (Mobarak, 2011; International HIV & AIDS charity, 2012).

NRTIs and NNRTIs are available in most countries. Fusion/entry inhibitors and integrase inhibitors are usually only available in resource-rich countries. Protease inhibitors are generally less suitable for starting treatment in resource-limited settings due to the cost, number of pills which need to be taken, and the particular side effects caused by protease drugs (International HIV & AIDS charity, 2012).

The most common drug combination given to those beginning treatment consists of two NRTIs combined with either an NNRTI or a "boosted" protease inhibitor. Ritonavir (in small doses) is most commonly used as the booster; it enhances the effects of other protease inhibitors so they can be given in lower doses. An example of a common antiretroviral combination is the two NRTIs zidovudine and lamivudine, combined with the NNRTI efavirenz (Mobarak, 2011).

Some antiretroviral drugs have been combined into one pill, which is known as a ‘fixed dose combination’. This reduces the number of pills to be taken each day. The choice of drugs to take can depend on a number of factors, including the availability and price of drugs, the number of pills, the side effects of the drugs, the laboratory monitoring requirements and whether there are co-blister packs or fixed dose combinations available. Most people living with HIV in the developing world still have very limited access to antiretroviral treatment and often only receive treatment for the diseases that occur as a result of a weakened immune system. Such treatment has only short-term benefits because it does not address the underlying immune deficiency itself (International HIV & AIDS charity, 2012).

At the beginning of treatment, the combination of drugs that a person is given is called first line therapy. If after a while HIV becomes resistant to this combination, or if side effects are particularly bad, then a change to second line therapy is usually recommended. Second line therapy will ideally include a minimum of three new drugs, with at least one from a new class, in order to increase the likelihood of treatment success (International HIV & AIDS charity, 2012).

2. Objective

2.1. General objective

2.1.1. To review and present outcomes of antiretroviral drugs exposed pregnancy

2.2. Specific objective

2.2.1. To review and present outcomes of nucleoside reverse transcriptase exposed pregnancy

2.2.2. To review and present outcomes of non nucleoside reverse transcriptase exposed pregnancy

2.2.3. To review and present outcomes of protease inhibitor exposed pregnancy

3. Methodology

By reading different published Journals in the library and Internet website like: Hinnary, Google, yahoo, PubMed computer search to identify relevant articles that have been published.

4. Findings and Discussions of review of published researches with regard to the risk of birth defects associated to *in utero* exposure to ARV drugs

Now a day's antiretroviral therapies (ARTs) have become widely prescribed in pregnancy in the absence of proof of their safety (Mobarak, 2011). Particularly, there is inadequate fetal and neonatal safety data for non-nucleoside analogues and protease inhibitors (Fundaro et al., 2002; Santis et al 2002; Brogly et al., 2010). Assessment of drug safety in pregnancy is based on less rigorous evidence, such as reproductive toxicology studies in small mammals and non-human primates, retrospective case reports and pregnancy registry data (Chersich et al., 2006). The potential harm to the fetus from maternal ingestion of a specific drug not only depends on the drug itself, but also on the dose used, the gestational age at exposure, the duration of exposure, the interaction with other agents to which the fetus is exposed and to an unknown extent the genetic makeup of the mother and fetus (Beitune et al., 2004).

As reviewed below, there are various published studies which present conflicting data about association between ART and pregnancy outcomes. Generally, these studies may be organized into: experimental animal studies and human studies. Although animal studies suggest a possibility of congenital abnormalities with specific antiretrovirals, results from registries and cohort studies do not support the presence of congenital malformation associated with *in utero* ARTs exposure. The findings of the various studies, both in experimental animals and humans are reviewed below based on similar outcomes.

A. Gross congenital anomalies

***In vivo* animal studies:** All the NRTIs except ddI had preclinical animal studies that indicated potential fetal risk for *in vivo* teratogenicity and were classified as FDA pregnancy category C (Ayers, 1988; Stahlmann et al., 1988; Harris et al., 1990; Toltzis et al., 1994; Mobarak, 1995; Greene et al., 1990; Augernbraun and Minkoff, 1997; Birmingham, 2000). Didanosine (ddI) was classified as category B. Post implantation embryonic AZT exposure was associated with significantly increased pregnancy losses (resorptions and intrauterine deaths) in rats (Christmas et al.,1995) and it become incorporated into the DNA of the placenta and most fetal organs during short-term intravenous infusion in pregnant rhesus monkeys (Olivero et al., 1997). Studies conducted in rabbits and rats indicated an increased incidence of embryonic resorptions, both early and late, after exposure to AZT (Ayers, 1988; Stahlmann et al., 1988; Greene et al., 1990). In contrast early embryo death did not occur in rats or rabbits in teratological studies carried out by Applewhite-Black et al. (1998); However, pregnant rabbits given AZT during gestation days (GDs) 6-18 exhibited reduced weight gain, anemia and an increase in late fetal deaths. Exposure of pregnant mice (GDs 10-19) to AZT was found to alter offspring's sensorimotor and somatic maturation by affecting body weight gain and delaying the appearance of pole-grasping reflex, the latter effect was limited to male pups (Calamandrei et al., 2002). Significant levels of genetic damage were detected in erythrocytes of mouse pups treated with AZT and other nucleoside analogues *in utero* or postnatally (Von Tungeln et al., 2002; Bishop et al., 2004).

In primate studies, all the NRTIs seemed to cross the placenta, but ddI and ddC apparently showed significantly less placental transfer than did AZT, d4T, ABC and 3TC (Sandberg et al., 1995). A combined *in vivo* and *in vitro* comparative studies on the prenatal toxicity of five NRTIs in rat embryos resulted in similar abnormality patterns (head, neural tube, shape) and a wide range in embryotoxic potency (Klug et al., 1991).

The AZT was reported to be a strong transplacental carcinogen in mice where exposure to high concentrations of AZT *in utero* and for prolonged postnatal period, resulted in vaginal tumors in adult life, an increased incidence of hemangiosarcoma, mononuclear cell leukemia and hepatic carcinoma (Ayers et al., 1997; Olivero et al., 1997; Diwan et al., 1999; Walker et al., 2007). K-ras cancer gene mutations and lung carcinogenicity were found in male and female mice exposed transplacentally to NRTIs (Dunnick et al., 2007; Koujitani et al., 2008).

Zalcitabine was concentrated in the fetal kidney of the rhesus monkey and was presented in the brain at approximately 20% of fetal blood concentrations (Sandberg et al., 1995). Teratological studies were performed in mice exposed to up to 2000 mg/kg day. Doses of 400 mg/kg/day resulted in mild developmental toxicity, manifested as open eyelids and cleft palate. However, starting at 1000 mg/kg/day of ddC, an increased frequency of growth retardation, skeletal defects, micrognathia and bent long bones were observed (Harris et al., 1990).

Mobarak (1995) studied, the *in vivo* impacts of ddC on the embryonic development of NMRI strain of mice. Two ddC doses (600 mg and 1000 mg /kg b.wt.) were applied to pregnant mice by oral intubation for five consecutive days, from day 9-13 of gestation. Fetuses of mothers treated with the low dose exhibited haematoma formation on the limbs and skull, reduced body weight and length and 37.3% of fetuses exhibited external abnormalities (EAs). On the other hand, fetuses of mothers treated with the higher dose exhibited dramatic increase in the percentage (62.9%) and severity of EAs. Head and limb abnormalities ranging from mild to severe types were encountered in such instances. Amelia, phocomelia, shortness and twisting of digits and adactylia as well as tail defects were recorded with significant incidences comparable to the controls. On gestational day 18, fetuses of mothers treated with either low or high doses of ddC showed reduced body weight and length and mild EAs in percentages and severity lesser than those found in 14-day-old fetuses. Also maternally treated mice fetuses on gestational days 14 and 18 had designated delay and wide variations in the processes of chondrogenesis and ossification in most of their skeletal parts.

The effects of ddC on the developing forebrain and eyes of CD-1 mice fetuses were studied by Mobarak (2009). This study was conducted from May-2006 to January-2007 in Egypt. A single dose (800 mg/kg of ddC was orally administered to pregnant females from day 9 to day 13 of gestation. On GDs 14 and 18, fetuses from control as well as maternally-treated groups were examined for any EAs; their heads were then processed for studing histological and anatomical changes in the forebrains and eyes. The percentages of fetuses exhibited EAs on both GDs were shown in table 2, the percentages (58.3% and 43.4%) of EAs recorded on GD 14 and 18 were highly significant ($p<0.001$ and $p<0.01$,versus control), respectively. In both maternally-treated groups,the prevalent type of external abnormalities recorded following zalcitabine treatment was of the limb type; the four limbs showed variable degrees of mild malformations including size and length reduction, flexion and rotation. The abnormalities of the central nervous system (CNS) were represented by microcephaly and microphthalmia. However, the severe limb abnormalities such as amelia (complete absence of limb) and adactylia previously observed by Mobarak (1995) in NMRI mice fetuses were

not recorded in the present strain of mice which might represent a strain resistance to the severe teratogenic effects of ddC (Mobarak, 2009). On the other hand, mild to severe morphological variations and histological changes were observed in the forebrains and eyes of such fetuses incomparsion with those of the control ones.

Table 2: Percentages of malformed fetus (on GDs 14 and 18) of CD-1 mice treated with 800 mg ddC /kg from 9-13 days of gestation (Mobarak, 2009)

Animal groups	No.of fetuses examined on gd14	No.of fetuses examined on gd18	Percentage of malformed fetuses examined on gd 14	Percentage of malformed fetuses examined on gd 18
Control	28	22	3.5*	0.0
Maternally treated (800 mg/kg)	24	18	58.3***	43.4**

*p>0.05 and **p<0.01,***p<0.001 versus control (Student t-test)

Mobarak (2011) had investigated the impacts of ddC on the developing heart of 14-day-old NMRI mice fetuses.This study was conducted from March-1996 to January-1997 in egypt. Pregnant mice were treated with 0,600,or 1000 mg/kg ddC by oral gavage from day 9-13 of gestation. The results of this study were shown in table 3, among fetuses got the high dose treatment, 45.5% exhibited heart abnormalities in conjunction with spinal cord damage and the mean diameter (4.95±0.06mm) of the ventricle was significantly increased (p<0.05,versus control). In the control fetuses the heart of 14-day-old mice fetuses exhibited a completed structure of four chambers (two auricles and two ventricles), two major blood vessels (a bulbous arteriosus and a sinus venosus) and a well developed pericardium. Figure 1a showed the heart, with a broad ventricular apex, at the level of the pulmonary trunk and left auricle, also, the interventricular septum (figure 2a).

Table 3 : The effects of ddC on developing heart of 14-day-old mice fetuses as compared wth controls (Mobarak, 2011)

Animal groups	No. of fetuses Examined	Percentage of fetuses with abnormal heart	Mean diameter of ventricles±SD(mm)
Control	20	0.0	4.21±0.08
Treated (600 mg/kg)	20	10.0	4.29±0.1*
Treated (1000 mg/kg)	22	45.5	4.95±0.06**

*p>0.05 and** p<0.05 versus control (student t-test)

In the low dose ddC maternally treated fetuses, the general structure of the heart was either slightly affected or did not show any change relative to the controls. However, the higher ddC dose

resulted in ventricular defects which were the most frequent ones. These defects were represented by malformed ventricular apex (figure 1b), thinning out of ventricular wall, ventricular elongation, looseness of the interventricular septum and a decreased number of ventricular trabeculae (figure 2b). In addition, ventricular septal defect at the mesenchymal part that resulted in a direct communication between the two ventricles was observed in one fetuses. Excessive haemorrhage was also found in hearts of some fetuses. Extrathoracic ectopia (a phenomenon showed a part of the heart outside the chest protruding through a defected thoracic wall). In this case enlargement of the atrioventricular cushion, enlarged and damaged auricular and ventricular walls and excessive haemorrhage were observed (figure 3a,b). Among these fetuses, one fetus exhibited displacement of the heart (a condition called pericardia-diaphragmatic hernia) where the liver was seen in the left side of pleural space above the heart (figure 4b).

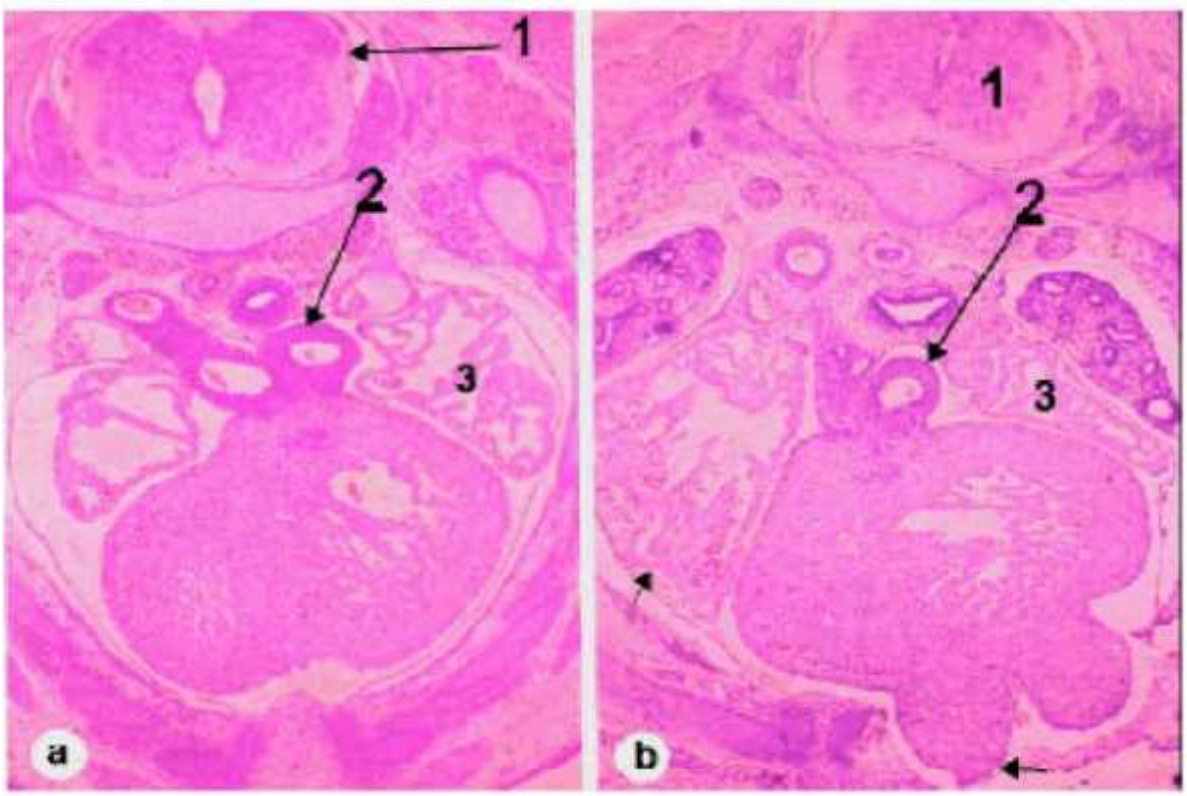

Figure 1: Transverse section of the heart of 14-day-old mice fetuses. (X40). (a) The heart of control fetus shows the pulmonary trunk(2), left auricle (3) and a broad apex of ventricles. Spinal cord (1) is also seen and (b) a similar section of fetus exposed to 1000mg of ddC showing defected heart and spinal cord. It exhibits haemorrhage in the pericardial cavity (short arrow), an abnormal ventricular apex (long arrow) and abdominal wall is damaged and opend. H and E stain. (Mobarak, 2011).

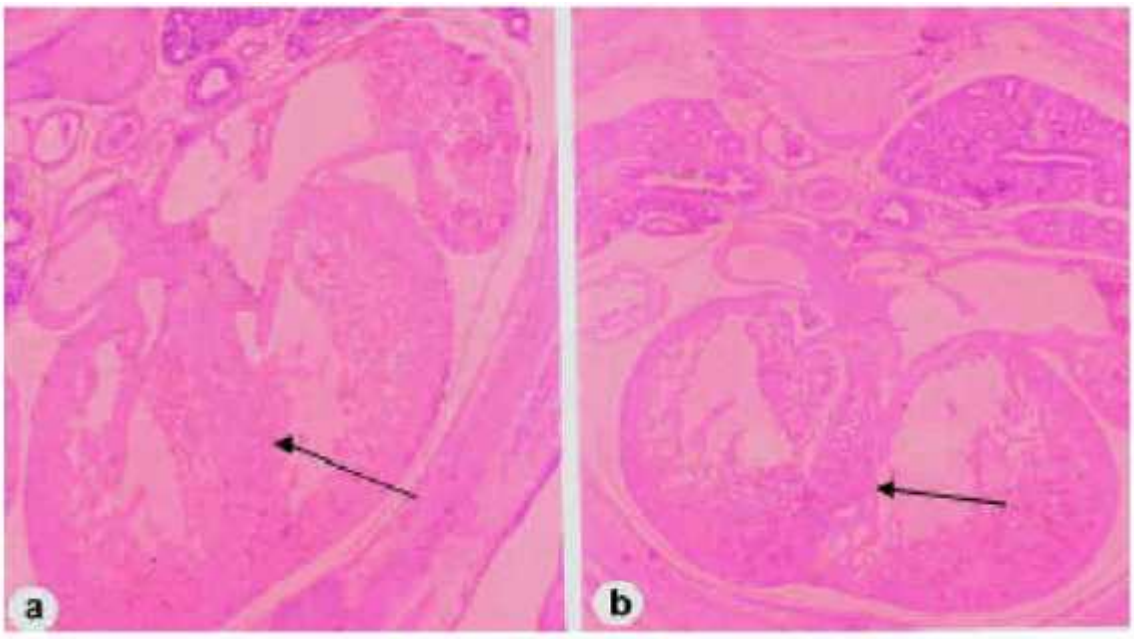

Figure 2: Photomicrographs of 14-day mice fetuses showing the heart. (X40). (a) A heart section of a control fetus and (b) a similar section of a fetus exposed to 1000 mg of ddC showing thinning of interventricular septum (arrow) and ventricular wall, while the trabeculae are widely spaced. H and E stain. (Mobarak, 2011).

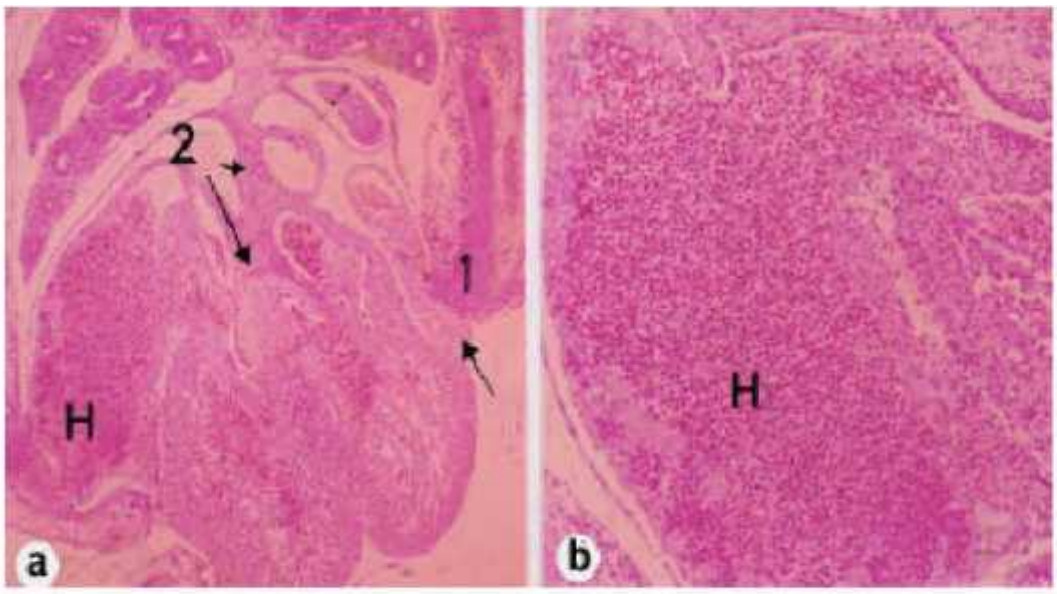

Figure 3: (a) Heart section of a 14 day-fetus exposed to 1000 mg ddC/kg displays extrathoracic ectopia, opened body wall (1), elongated atrioventricular cushion (2) and extensive haemorrhage (H). (X40) and (b) the rigth atrium of figure 3a magnified shows nucleated fetal red blood cells in extensive haemorrhage within the heart and pericardium. (X100). H and E stain. (Mobarak, 2011).

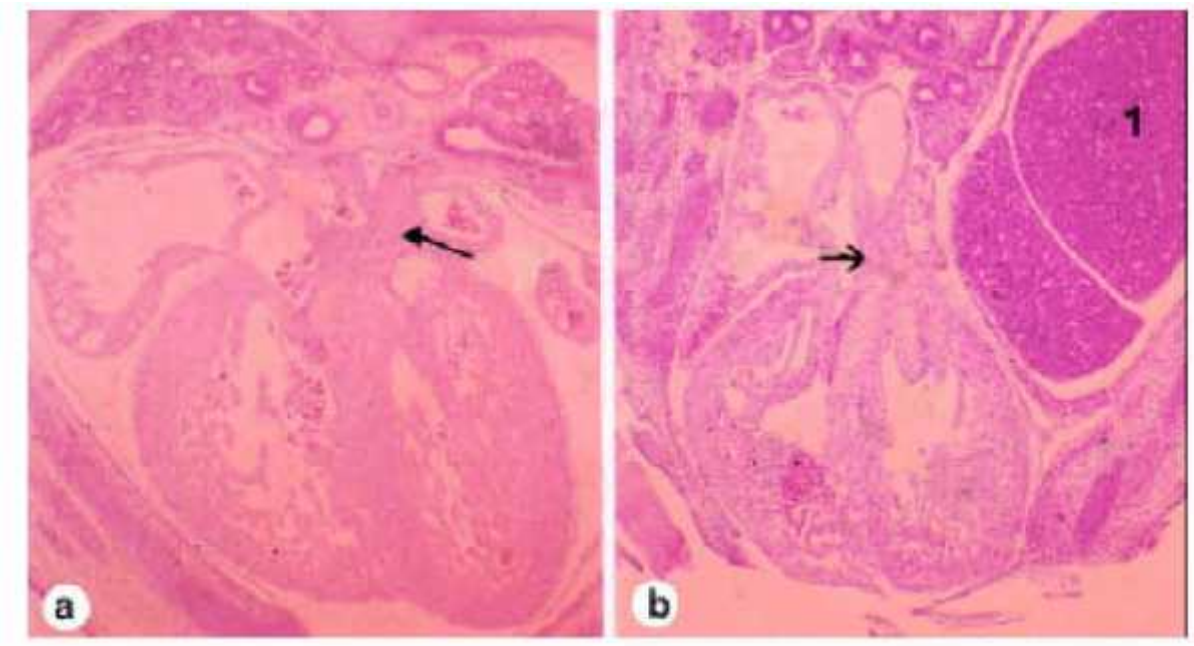

Figure 4: (a) A cross section of a control fetus displaying the heart at the level of the atrioventricular cushion (arrow). (X40) and (b) a similar section of a fetus, exposed to 1000 mg of ddC, displays pericardiaphragmatic hernia and the liver (1) is seen above the heart. H and E stain (Mobarak, 2011).

In a further study Mobarak (2011) investigated the damaging effect of ddC on the development of the spinal cord of 14-day-old (NMRI) mice fetuses. This study was conducted from March 1996 to January 1997 in Egypt. Pregnant mice were treated with 0,600,or 1000 mg/kg ddC by oral gavage, from day 9-13 of gestation. The results of this study were shown in table 4 and in the high dose treated animals, there was a rise in the percentage (59.1%) of fetuses exhibiting spinal cord damage as well as a significant incidence ($p<0.05$ versus control) in the decrease of spinal cord mean diameter. Spinal cord damage in conjuction with heart abnormalities was observed in 45.5% as mentioned above.

Table 4: The effects of ddC on the developing spinal cord of 14-day old mice fetuses as compared with control (Mobarak, 2011)

Animal groups	No.of fetuses Examined	Percentage of fetuses with abnormal spinal cord	Mean diameter of spinal cord±SD(mm)
Control	20	0.0	2.5±0.2
Treated (600 mg/kg)	20	5.0	2.4±0.04*
Treated (1000 mg/kg)	22	59.1	2.1±0.1**

*p>0.05 and** p<0.05 versus control (Student t-test)

The general structure of spinal cord in control fetuses revealed an outer marginal layer (white matter), an inner mantle layer (gray matter) and (an inverted pear-shaped) central neural canal (figure 5a). The spinal cord of fetuses of mothers fed the low dose did not show any change relative to the controls or slightly affected (figure 5b). However, the higher dose evoked severe changes in the spinal cord of the same age (14-day) fetuses. For example, the horn like structure was absent in the majority of spinal cords due to cellular disorganization of the mantle layer. Such malformations were seen most frequently in the lumbo-sacral and then cervical regions (figure 6a). The inverted pear-shaped structure of the normal neural canal was severely distorted and showed great morphological variation in all affected fetuses. In most cases it was more or less closed (figure 6b,c). Enlargemment of the neural canal associated with down-growths of the neuroepithelial cells was frequently observed (figure 7a). Cell death and necrosis were frequently seen in the dorsal and ventral horns and roof plate cells. In some fetuses, these lesions were accompanied by extensive cystic and hemorrhagic changes (figure 7).

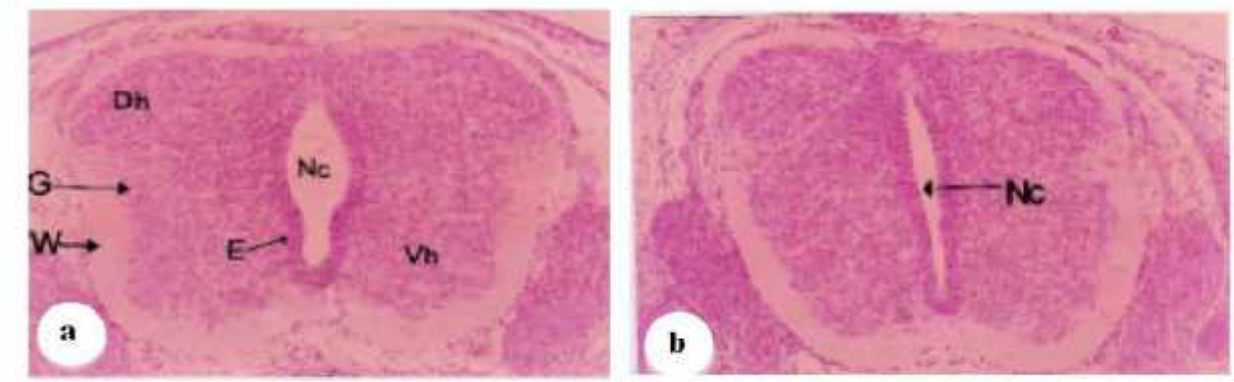

Figure 5: (a) A cross section of spinal cord of a control (14-day-old) fetus shows the Dorsal horn (Dh), Grey matter (G), White matter (W), Epenndymal cells (E), Neural canal (Nc) and Ventral horn (Vh). (X 100) and (b) a similar cross section of a spinal cord from a maternally treated (600 mg ddC/kg) fetus showing elongation of the neural canal. H and E stain. (Mobarak, 2011).

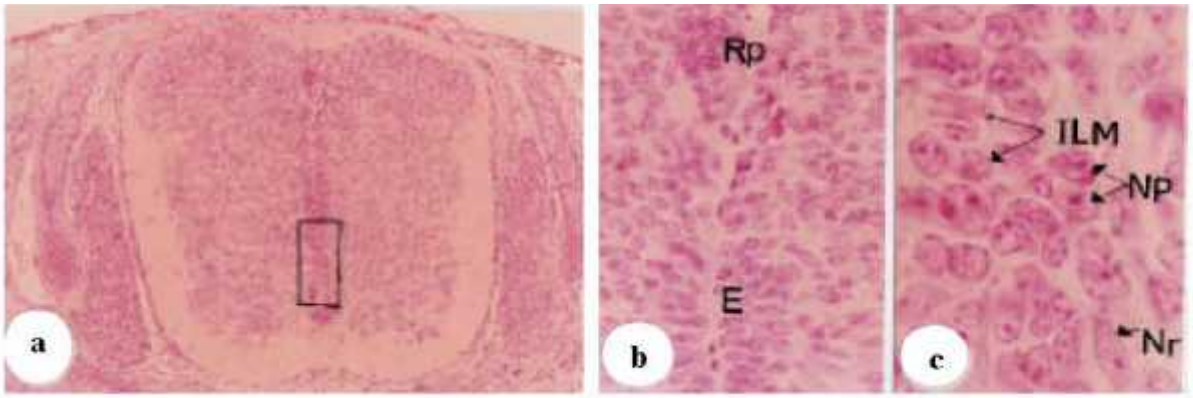

Figure 6: (a) Spinal cord section of a maternally treated (1000 mg ddC/kg) fetus shows absence of the neural canal, (b) higher magnification of mid dorsal part of figure 6a displays the Roof plate (Rp) and Ependymal cells (E) disorganized. (X400) and (c) a magnified part (in box) of figure 6a shows irregularity of ependymal cells, fusion of Inernal limiting Membrane (ILM), Neuroglia cells (Nr) and Neuroplasts (Np) are also seen. (X1000). H and E stain. (Mobarak, 2011).

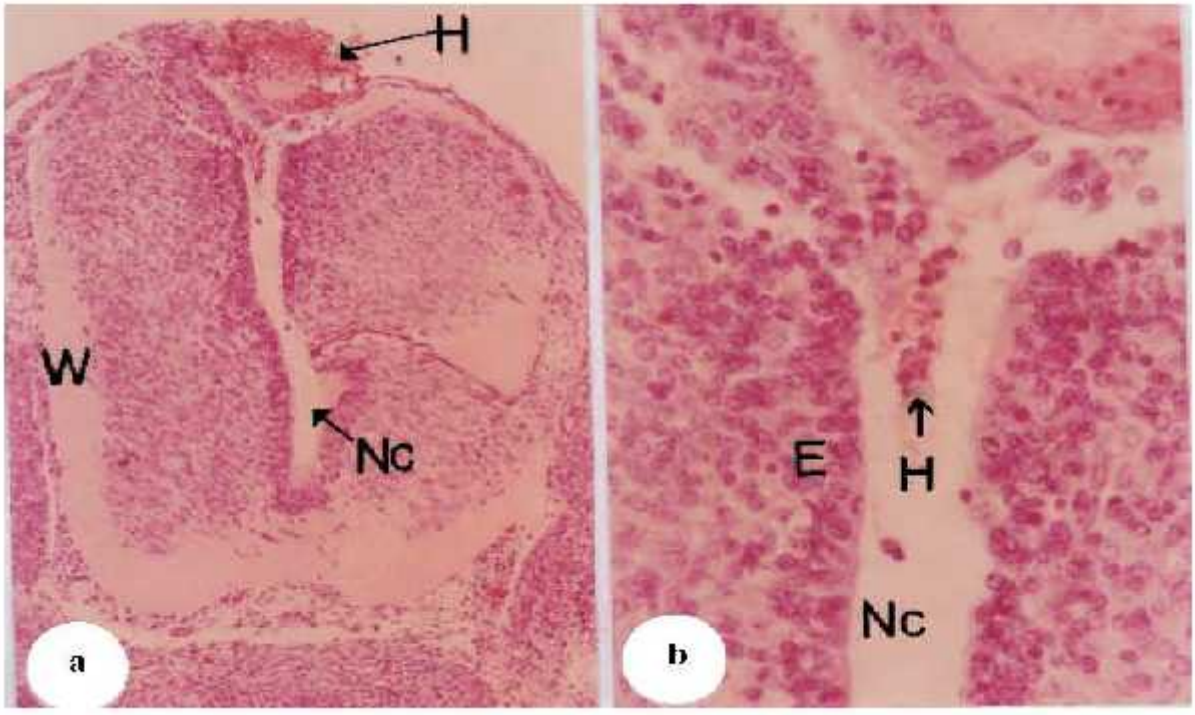

Figure 7: (a) Highly damaged spinal cord of a maternally treated (1000 mg ddC /kg) fetus, exhibiting extensive hemorrhage (H) and damaged neural canal (Nc). (X100) and (b) higher magnification of mid dorsal part of figure 7a displays extensive hemorrhage (H), Ependymal(E) cell death and necrosis, damaged Neural canal (Nc). (x400). H and E stain. (Mobarak, 2011).

Moreover, studies showed that high doses of tenofovir given to pregnant rhesus macaques (monkeys) caused bone demineralisation, impaired somatic growth and low levels of insulin-like

growth factor-1 in their infants (Tarantal et al., 2002; Van Rompay et al., 2004; Van Rompay et al., 2008). Renal tubular leak of phosphate resulted in bone demineralization and deformities in macaque infants given high dose tenofovir, although these effects were largely reversible once tenofovir was stopped (Van Rompay et al., 2004; Gibb et al., 2012).

Efavirenz, a non nucleoside analogue, is considered a potential teratogen on the basis of animal data and case reports (Nightingale, 1998; Fundaro et al., 2002; Santis et al., 2002; Brogly et al., 2010). Concerns of EFV-induced fetal effects began after a trial with cynomolgus monkeys (Nightingale, 1998). In the trial, monkeys were exposed to EFV throughout pregnancy at plasma drug concentrations similar to humans receiving 600 mg EFV per day. No major congenital malformations were observed in 20 control infant monkeys, but 3 of 20 EFV exposed infants had significant abnormalities (Nightingale, 1998). Anencephaly and unilateral anophthalmia were observed in one monkey infant, microphthalmia in another and cleft palate in a third. Similarly, Chersich et al. (2006) has been observed significant teratologic findings in studies with pregnant rabbits treated with EFV.

***In vitro* animal studies**: in two-cell embryos, harvested from unexposed females, exposed to low-concentration (1µm) of AZT in vitro for 24h, development beyond the blastocyst stage was inhibited (Toltzis et al., 1991). In contrast, drug exposure during *in vitro* blastocyst and post blastocyst development had resulted in little or no morphological toxicity. When embryonic or fetal mouse or human cells (from brain, limb buds, or different organ rudiments) were exposed to AZT or ddI *in vitro*, cytotoxicity and brain cells appearing slightly more sensitive to both nucleosides (Sieh et al., 1992). Hitchcock (1993) showed that the *in vitro* activity of ddI against the HIV was generally less potent than that of ddC. However, the potency of ddI was similar to or greater than that of AZT.

In vitro rat whole embryo culture system to assess the embryo toxicity of various nucleoside analogues namely: AZT, ddI and ddC and the HIV-1 protease inhibitor indinavir both alone and in combination was carried out by Fujinaga et al. (2000). The study concluded that AZT in combination with ddC resulted in severe growth retardation and morphologic abnormalities not seen with either agent singly. Comparsion of micronucleated cell frequencies induced by AZT alone or in combination with ddI suggested that ddI potentiates AZT-induced chromosomal damage following direct exposure (Phillips et al., 1991; Witt et al., 2004). In the cell cycle of human epitheliod carcinoma (HeLa) cells exposed to AZT and 3TC alone, AZT but not 3TC causes an arrest of cells in S phase with a consistent alteration in the expression of several cell cycle genes (Olivero et al.,

2005). AZT is incorporated into cellular DNA caused mutation and induced micronuclei, chromosomal aberrations, sister chromatid exchange, shortened telomeres and other genotoxic effects *in vitro* (Olivero, 2007).

Studies in humans on the risk of birth defects after ARV exposure are limited and varied, with some studies reassuring and others finding increased risks after specific exposures. In a French cohort three of ten infants born to women who became pregnant while receiving EFV had birth anomalies (Khuong-Josses et al., 2003). Similarly, cohorts of 2,202 HIV-exposed children in US were enrolled in the pediatric AIDS clinical trials group 219 and 219C protocols before one year of age. The study reported, prevalence of birth defects was higher in this cohort of HIV-exposed children than in other pediatric cohorts. There was no association with overall ARV exposure, but there were some associations with specific agents including efavirenz**.** The report were, 117 live-born children had birth defects for a prevalence of 5.3% (95% CI, 4.4- 6.3). Prevalence did not differ by HIV infection status or overall ARV exposure; rates were 4.8% (95% CI, 3.7- 6.1) and 5.8% (95% CI, 4.2- 7.8) in children without and with first trimester ARV exposure, respectively. The defect rate was higher among children with first trimester efavirenz exposure (5/32, 15.6%) versus children without first trimester efavirenz exposure [adjusted odds ratio (aOR), 4.31; 95% CI, 1.56-11.86]**.** The defects of these efavirenz exposed children included laryngiomalacia (n=1), meningomyelocele with arnold-chiari malformation Type II (n=1), hypospadias (n=1), varus feet and hyper tonicity of extremities (n=1) and cleft palate (n=1) (Brogly et al., 2010). Indeed, in humans four retrospective cases of central nervous system (CNS) defects in infants with first trimester exposure to EFV have been reported (three infants with meningomyelocele and one with a dandy-walker malformation) (Santis et al., 2002; Saitoh et al., 2005; Chersich et al., 2006). Thus, in light of animal studies and some human studies, there are concerns about links between efavirenz and birth defects specifically neural tube defects as suggested by Ford et al. (2010).

In contrast data from the antiretroviral pregnancy registry (APR) have been reassuring without overall increased risk of birth defects compared to population-based surveillance data or when comparing rates after first trimester exposure to those after ARV exposure only later in pregnancy (Antiretroviral Pregnancy Registry, 2011), although biases in reporting may occur. In prospective antiretroviral pregnancy registry based in the United States, birth defects were observed in 5 of 228 (2.2%; 95% CI, 0.7%-5.1%) live-born infants following first-trimester exposure to EFV and in 1 of 14 live births with second- or third-trimester exposure (Antiretroviral Pregnancy Registry, 2009). This prevalence of birth defects is comparable to the United States general population (3.1%; 95%

CI, 3.1%-3.2%) (Correa-Villasen et al., 2003). Similarly a sufficient number of live births have been monitored in the United States antiretroviral pregnancy registry to detect a two-fold increase in overall risk for birth defects following first-trimester exposure to EFV; no such increase has been detected (Antiretroviral Pregnancy Registry, 2002; Watts et al., 2004). Several features of the registry limit the ability to draw definitive conclusions. Of eligible woman-infant pairs, only about 15% are enrolled. It is unknown whether those not enrolled are at higher or lower risk of birth defects. Moreover, ascertainment of birth defects is not standardized, with varying use of diagnostic tests and level of expertise of reporting clinicians. The European Collaborative Study also collects data on pregnancy outcomes following ARV exposure during pregnancy. Thus far, 19 women in this study have become pregnant while receiving EFV-containing regimens, no congenital abnormalities were reported (0%; 95% CI: 0%- 17.6%) (Patel et al., 2005).

A similar observation was made in a study in South Africa where the proportion of congenital abnormalities was not significantly different between live births exposed to EFV during the first trimester (3.3%) and second/third trimester (2.6%) (Ekouevi et al., 2011). Indeed, Ford et al. (2010) concluded that there was no increased risk of overall birth defects between 35/1132 EFV-exposed women with live births compared to 289/7163 women with live births exposed to other antiretroviral drugs in the first trimester of pregnancy. This was also compatible with Bussmann et al. (2007) in which, increased risk of congenital abnormalities was not found with EFV-based ART compared to a non-EFV-based ART. Similarly a United Kingdom study covering a 17 year period on 3120 pregnancies and reported in 2009 (Townsend et al., 2009) showed no statistically significant association between the prevalence of congenital abnormalities and exposure to ART overall after adjustment for potential confounding factors: 3.4% (90 of 2657 pregnancies) in exposed pregnancies and 2.2% (10 of 463 pregnancies) in non exposed pregnancies (P, 0.166); prevalence was similar whether or not exposure occurred in the first trimester: 3.7% (20 of 541 pregnancies) after early exposure and 3.1% (80 of 2579 pregnancies) without early exposure (P, 0.476). There was also no significant association with type of ART in early pregnancy. Highly active antiretroviral therapy [HAART] vs. mono- or dual therapy, HAART with protease inhibitor and/or non nucleoside reverse transcriptase inhibitor like efaverinz. In sum, these findings indicate that any overall increase in risk for birth defects following exposure to EFV is likely to be low as suggested by Chersich et al. (2006).

A small study from England did not find an increased risk of birth defects with ARV exposure or folate antagonist exposure alone (n=34), but did find an increased risk of defects when the

combination of ARV therapy and folate antagonists (n=13), such as trimethoprim/sulfa were used in the first trimester (4% *v* 23.1%; OR, 7.10; 95% CI, 1.5-34.2) (Annemiek et al., 2001).

The pediatric AIDS clinical trials group 316 trial, studied infants born to HIV-infected women receiving nucleoside and protease inhibitor antiretroviral (ARV) therapy. The study reported, ARV use in early pregnancy was not associated with an increased risk of birth defects overall. However, found the possible association of ARV exposure with heart defects. Accordingly**,** birth defects were detected in 60/1414 (4.2%; 95% CI, 3.3–5.4%) infants including 30/636 (4.7%; 95% CI, 3.2–6.7%) with first trimester ARV exposure and 30/778 (3.9%; 95% CI, 2.6–5.5%) with exposure only after the first trimester (P, 0.51). Rates of classes of defects were similar between first trimester compared to later exposure groups except heart defects which occurred in 16 (2.5%; 95% CI, 1.4–4.1%) with first trimester ARV exposure and in six (0.8%; 95% CI, 0.3–1.7%) infants with later exposure (P, 0.02) (Watts et al., 2011). Similarly Brogly et al. (2010) found significantly more children with heart defects were exposed to zidovudine in the first trimester in unadjusted (OR, 2.11; 95% CI, 1.07-4.16) and adjusted models (OR, 2.04; 95% CI, 1.03- 4.05). An analysis of data from the pediatric AIDS clinical trials group (PACTG) study 185 suggested an increased risk of ventricular septal heart defects after zidovudine exposure in the first trimester (Watts et al., 2011). This study was unable to control for concomitant medications, such as trimethoprim/sulfa for pneumocystis pneumonia (PCP) prophylaxis, that were likely more common among women with first trimester exposure because of increased disease severity. In a study of both HIV infected and HIV-exposed, uninfected children compared to HIV and ARV unexposed controls, infants born to HIV-infected women were had lower fractional shortening at birth suggestive of left ventricular dysfunction, which persisted up to four months in HIV exposed but uninfected infants (Lipshultz et al., 2002). An analysis from the women and infants transmission study detected an increased risk of hypospadias after first trimester zidovudine exposure but again was unable to control for concomitant medication exposures (Watts et al., 2007). In contrast, protective effects of first trimester zidovudine exposure on musculoskeletal defects were detected in unadjusted (OR, 0.30; 95% CI, 0.10- 0.84) and adjusted models (OR, 0.24; 95% CI, 0.08- 0.69) (Brogly et al., 2010).

In a report of infant outcomes from the PACTG 076 study of zidovudine vs. placebo for prevention of maternal-to-child transmission, major defects were diagnosed among 36 (8.6%) of the 417 live born infants, however, in contrast to the above no ARV exposure occurred before the second trimester (Sperling et al., 1998). All women in PACTG 076 had CD4+ lymphocyte counts above 200 cells/μL, so were unlikely to be receiving trimethoprim/sulfa prophylaxis. Similarly, a large North

American study found the risk of congenital malformations with zidovudine exposure increased with later rather than first trimester exposure but was confounded by high rates of injecting drug, alcohol and tobacco use among the mothers (Newschaffer et al., 2000)

Prospective cohort study which was performed on 183 mother–child pairs in Germany and Austria reported the prevalence of congenital abnormalities was 3.3% (six of 183) other than PTD and LBW. Of the six infants with anomalies, one (with polydactyly) was exposed to monotherapy (zidovudine), two [one with hip dysplasia and one with patent ductus arteriosus (requiring intensive intervention)] were exposed to dual therapy (both 3TC and AZT), one (with a ventricular septal defect) was exposed to HAART without PI [3TC, stavudine (d4T), nevirapine (NVP) and Combivirs] and two (both with hypospadias) were exposed to HAART with PI [one mother received 3TC, d4T and nelfinavir (NFV) before pregnancy, switching to NFV, Combivirs and NVP during pregnancy and one mother received 3TC, d4T and NFV before pregnancy, but only NFV and AZT during pregnancy]; five infants had been exposed in the first trimester of pregnancy, two to PI-containing regimens (NFV) (Grosch-Woerner et al., 2008).

The rate of birth defects was higher in children exposed to lopinavir/ritonavir in the first trimester than in children unexposed to lopinavir/ritonavir in the first trimester. The association with lopanivir/ ritonavir was marginally significant (p, 0.07). The defects of the six lopinavir/ritonavir exposed children included hydronephrosis (n=1), supernumerary nipple and umbilical hernia (n=1), atrial septal defect (n=1), pyloric stenosis (n=2) and ventricular septal defect and hemangioma (n=1) (Brogly et al., 2010).

Recently, McArthur et al. (2009) reported twin preterm neonates with cardiac toxicity related to LPV/r. The twins were born preterm (32 weeks gestation) to an HIV-infected mother. One of them developed complete heart block and dilated cardiomiopathy, while the other developed mild bradycardia. Also Zareba et al. (2004) studies showed children exposed to HAART *in utero* are at an increased risk for cardiac abnormalities, peripheral and coronary artery disease.

Laubereau et al. (1998) were conducted observational study on a total of 37 HIV-infected pregnant women who have given birth to 30 children. All received RTI, which were combined with protease inhibitors in 16 cases. In those treated with two RTI with or without protease inhibitors, one or more adverse events occurred in 14 out of 30 babies. In newborns, one case of biliary malformation and one intracerebral haemorrhage in term baby are of concern.

B. Mitochondrial Dysfunction

Several studies have been published that demonstrate evidence of transient or prolonged mitochondrial compromise in offsprings of both laboratory animals and humans born to mothers receiving antiretroviral drugs during pregnancy (Alimenti et al., 2003; Poirier et al., 2003). There exists equivocal evidence of an etiologic association between *in utero* NRTI exposure and mitochondrial dysfunction (MD) in HIV-uninfected offsprings born to HIV-infected mothers (Brogly et al., 2007; Ciaranello et al., 2008; Foster and Lyall, 2008; Aldrovandi et al., 2009). The transplacental NRTI exposures induce significant similar mitochondrial DNA (mtDNA) depletion in umbilical cord endothelial cells taken from retroviral uninfected monkey infants and from human infants born to HIV-1-infected women (Divi et al., 2007a). After 1 year, the mtDNA levels in monkeys skeletal muscles had increased but remained significantly below normal (Divi et al., 2007b).

Animal studies have shown evidence of mitochondrial dysfunction in the offspring of animals treated with NRTIs. Monkey fetuses exposed *in utero* to the combination AZT plus 3TC sustain a higher level of drug-DNA incorporation and show evidence of more telomere damage than monkey fetuses exposed to AZT alone (Olivero et al., 2002). Incorporation of AZT into nuclear (Olivero et al., 1997, 1999, 2002) and mitochondrial (Olivero et al., 1997) DNA has been demonstrated in laboratory animals. Chronic AZT therapy has long been associated with the development of myopathy, cardiomyopathy, neuropathies, hepatic dysfunction, seizures and reduced respiratory chain activity resulting from mitochondrial toxicity and dysfunction (Dalakas et al., 1990; Mhiri et al., 1991; Lewis and Dalakas, 1995; Gerard et al., 2000).

Significant mitochondrial damage in heart muscle was demonstrated in 18-month-old mice prenatally exposed to AZT during gestation days 12–18, a period in which the last 40% of *in utero* development occurs (Walker et al., 2000). Similarly, significant MD was found in hearts and skeletal muscles of erythrocebus patas monkeys fetuses exposed *in utero* to doses of AZT equivalent to the normal human dose (Gerschenson et al., 2000; Gerschenson and Poirier, 2000; Ewings et al., 2000; Divi et al., 2005). The mtDNA quantity was substantially depleted (>50%) in heart, skeletal muscle, cerebellum and cerebrum from AZT plus 3TC-exposed monkey fetuses compared to unexposed controls (Gerschenson et al., 2004).

Zidovudine induced damage in nuclear DNA of mice exposed *in utero* and postnatally and mtDNA damage were observed in both human and mouse neonates following perinatal exposure to

AZT and AZT/3TC in combination (Chan et al., 2007). The temporal changes in cardiomyocyte and mitochondria at the light and electron microscope levels, in hearts of mice exposed transplacentally to commonly used NRTIs, showed that a subset of changes in cardiac mitochondria and myofibrils persisted and progressed months after transplacental exposure of female mouse to NRTIs; with combined AZT/3TC exposure yielding additive effects compared with either drug alone (Torres et al., 2010). A large French cohort study also reported mitochondrial damage in several infants with short-term perinatal exposures to AZT and other nucleoside analogues (Blanche et al., 1999; Barret et al., 2003; Bishop et al., 2004).

An experimental study reported mitochondrial damage that was revealed by morphometric and semiquantitative analysis of mouse pup cardiomyocytes following *in utero* and postnatal exposure to Zidovudine and Lamivudine. The study was performed by using a mouse model of human perinatal antiretroviral therapy to show the relationship between *in utero* nucleoside analogue exposure and mitochondrial integrity in cardiomyocytes**.** Pregnant CD-1 mice were treated twice daily with doses of 75 mg/kg AZT plus 37.5 mg/kg lamivudine throughout gestation and lactation; pups were exposed by direct gavage beginning postnatal day (PND) 4 and sacrificed on (PND) 28 (Bishop et al., 2004).

On PND 28, three female and three male mice were selected randomly and anesthetized by intraperitoneal injection of pentobarbital; hearts were excised swiftly and atrial and ventricular regions were transferred immediately into the fixative for electron microscopy, 3% glutaraldehyde buffered to pH 7.2 with 0.1M sodium cacodylate. Small pieces approximately 1 mm^3 were stored in fixative at 4 ^{0}C for 3 days, washed in 0.1M sodium cacodylate buffer, post fixed in cacodylate-buffered 1% OsO4 (Osmium tetroxide), rinsed in water, dehydrated through a series of graded ethanols and embedded in Polybed 812 epoxy resin. Semithin (1/2 µm) sections of ventricle stained with 1% toluidine blue were examined by light microscopy to locate regions containing longitudinal fibers. Ultrathin (90 nm) sections were cut from these regions, stained on-grid with 5% uranyl acetate and reynold's lead citrate and examined in electron microscope. Nine electron micrographs were taken for each animal (three blocks [regions]/animal, three random micrographs/block). Each micrograph was taken at 8350x and enlarged 3x to appear on the print at a magnification of 25,050x (Figure 8) (Bishop et al., 2004).

Semiquantitative morphological and statistical morphometrical analysis.

The scoring method used in this evaluation was adapted from Walker et al. (2000) and included four grades as follows: Grade 0, no evidence of cellular pathology or early autolysis, or an occasional mitochondrion with minimal loss of cristae while the remainder of mitochondria appear normal; Grade 1, discontinuous cristal membranes and/or partial loss of cristae and matrix material in a few mitochondria; Grade 2, multiple disruptions of the cristal membrane and substantial loss of cristae and matrix in approximately half of the mitochondria; Grade 3, fragmented cristal membranes and effacement of central architecture in a majority of the mitochondria.

Following visual assessment of mitochondrial damage in the micrographs, they conducted morphometric evaluations to quantitate the observed damage. All micrographs were scanned electronically. The area of ventricle analyzed for each micrograph was ~ 70μm^2. The numbers and areas of complete mitochondria were recorded. After converting these variables into a log scale, the mean area and mean number of mitochondria in the two groups (treated and control) were compared.

Semiquantitative Morphological Analysis

The mitochondrial damage observed in AZT/3TC-treated animals was typified by fragmented and missing cristae, which rendered matrices electronlucent and increased the spaces between remaining cristae (Figure 8B). Occasionally, unusual simple or whorled, lamellated membranes were observed within a mitochondrion and the double bounding membrane was ruptured; rarely, fusion between mitochondria appeared to be occurring. Damaged mitochondria appeared swollen, displacing adjacent sarcomeres. The results of semiquantitative evaluation of mitochondrial morphological pathology indicated that control animals (Figure 8A) generally exhibited mitochondrial alterations that ranged from grade 0 to 1, whereas mitochondria in treated animals showed alterations generally in the 1 to 2 grade range. The number and severity of damaged mitochondria appeared to be greater in females than males. Since variability existed from region to region and micrograph to micrograph within an individual animal, grading reflected the overall assessment of damage for an animal (Bishop et al., 2004).

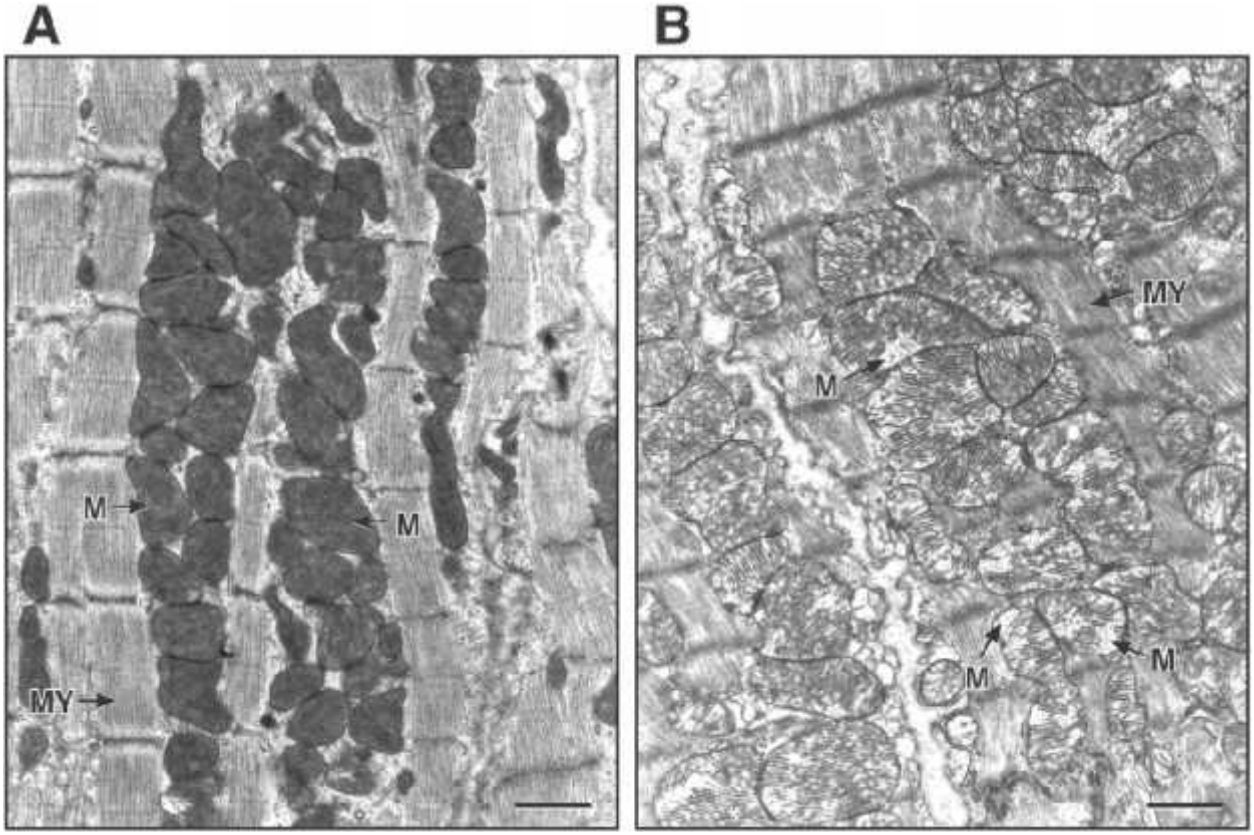

Figure 8: Electron micrographs of mitochondria from hearts of 28-day-old control (A) and AZT/3TC-treated pups (B). Each micrograph was taken at 8350x and enlarged 3x to appear on the study print at a magnification of 25,050x. M = mitochondrion, MY = myofibril; arrows indicate damage due to loss of cristae. (Bishop et al., 2004).

Some minimal mitochondrial damage, consisting chiefly of cristal breakage and metrical loss, was occasionally observed in control mice and interanimal variability was observed in both treated and control groups. They have, therefore conducted morphometric evaluations to quantitate the damage more precisely (Table 5). The data were analyzed after log-transformation.

Table 5: Morphometric analysis of mitochondria from 28-day-old control and zidovudine/ lamivudin-treated pupsa. (Bishop et al., 2004).

Mitochondrial area (μm^2)				Number of mitochondria (per picture)		
Group	Males	Females	Combined	Males	Females	Combined
Control	3	3	6	3	3	6
	.5112±.0286	.5108±.0225	.5111±.0171	47.11±2.34	45.37±2.65	46.24±1.65
AZT+3TC[b]	3	3	6	3	3	6
	.5625±.0288	.6101±.0229	.5862[c]±.0174	42.96±2.34	36.33±2.65	39.63[c]±1.65

[a]Data presented as mean ±standard error derived from the mixed effects model; three males and three females in each group (treated and control). Combined groups consisted of three males plus three females.

[b]150 mg/kg AZT +75mg/kg 3TC per day.

[c]P <0.05 versus matched control; statistical analysis was performed only for the combined data.

Morphometrical Statistical Analysis

Morphometrical analysis demonstrated that, for male and female mice combined, the mean area of mitochondria in the AZT/3TC treated group (0.5862 $\pm$ 0.0174 μm^2) was significantly greater than that of the control animals (0.5111$\pm$0.0171μm^2) (Table 5). The mean number of mitochondria in treated animals (39.63 $\pm$ 1.65 per picture) was significantly less than that in the controls (46.24 $\pm$ 1.65 per picture) (Table 5).

The fold-change in the mean area of mitochondria in AZT/ 3TC-treated animals was 121% (21% increase) for females, with 95% confidence limits ranging from 102% to 143% (increase of 2% to 43%); for males the fold-change in mean area was 108% (modest 8% increase), with 95% confidence limits ranging from 84% to 139% (decrease of 16% to increase of 39%). For treated males and females combined (Figure 9), the fold-change in mean area was 114% (14% increase), with 95% confidence limits ranging from 101% to 130% (1% to 30% increase). The fold-change in the mean number of mitochondria in AZT/ 3TC-treated animals was 83% (a 17% decrease) for females, with 95% confidence limits ranging from 67% to 103% (33% decrease to 3% increase); for males the fold-change in the mean number of mitochondria was 90% (decrease of 10%), with 95% confidence limits ranging from 72% to 111% (28% decrease to11% increase). For males and females combined (Figure 9) the fold-change in mean number was 86% (14% decrease), with the 95% confidence limits ranging from 76% to 97% (24% decrease to 3% decrease).

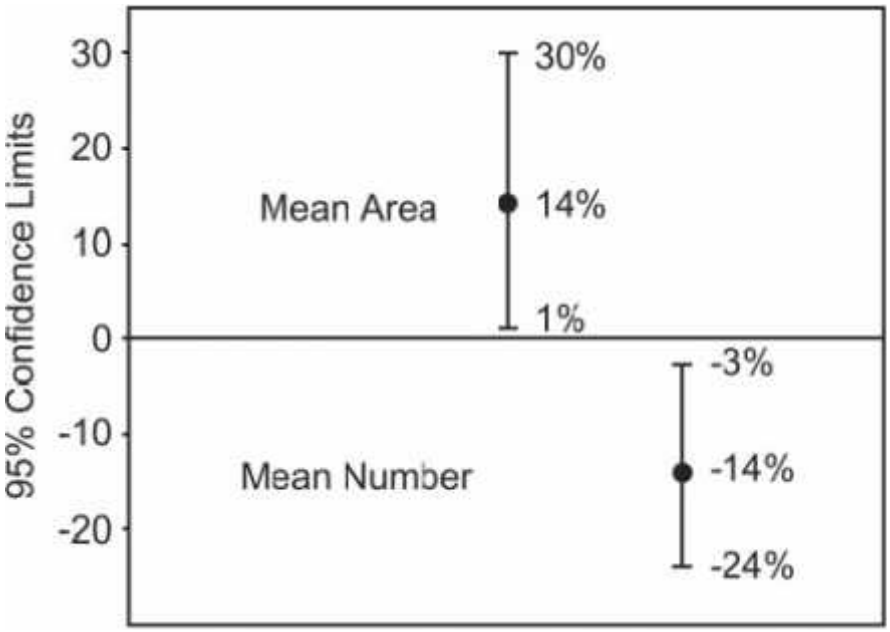

Figure 9: Estimated percent change in mean area and in mean number of mitochondria in AZT/3TC-treated mice relative to controls. The estimated percentage of change in mean area of mitochondria for the treated mice relative to the control mice is 14%, with 95% confidence limits of 1% and 30%. The estimated percentage of change in mean number of mitochondria for the treated mice relative to the controls is -14%, with 95%, confidence limits of _24% and _3%. Because neither confidence interval line intersects zero, the estimated percentages of change in mean area and mean number are both statistically significant at p, 0.05 (Bishop et al., 2004).

This data adds to the growing body of experimental and clinical evidence and for NRTI-induced damage to mitochondria in various animal and human tissues and indicate that not only chronic exposures, but also potentially short-term exposures during sensitive developmental periods, may be sufficient to induce adverse mitochondrial effects having possible long term health consequences (Bishop et al., 2004).

Similarly there are various human studies which report about mitochondrial dysfunction. In France, 8 uninfected children exposed to zidovudine or zidovudine and lamivudine were reported to have histologic evidence of mitochondrial dysfunction, 5 had neurologic symptoms and 2 with encephalopathy died (Blanche et al., 1999). In this prospective cohort, uninfected exposed children had a higher risk of neurologic syndromes associated with mitochondrial dysfunction than seen in the general population. In addition, Poirier et al. (2003) studied three groups of uninfected infants for markers of mitochondrial toxicity; those born to HIV-negative women (n=30) and those born to HIV infected women (n=20) who either received no antiretroviral therapy (n=10) or zidovudine during pregnancy (n =10). This study demonstrated that zidovudine causes depletion in mtDNA in infants

that can persist up to 2 years. Like in animals incorporation of AZT into nuclear (Olivero et al., 1997, 1999, 2002) and mitochondrial (Olivero et al., 1997) DNA has been demonstrated in human infants exposed *in utero* and mitochondrial DNA depletion was observed in cord blood cells and peripheral leukocytes of HIV-negative infants and children exposed *in utero* to AZT and 3TC (Poirier et al., 2003). Indeed, single-site, cross-sectional, controlled observational study of mitochondrial and apoptotic parameters in mononuclear cells from maternal peripheral blood and infant cord blood at delivery in 27 HIV-infected and treated pregnant women (HAART) and 35 uninfected control infants revealed, mitochondrial parameters were reduced in HAART exposed newborn; especially newborn mtDNA levels and fetal mitochondrial protein synthesis (38.6% and 13.6% reduced respectively, $P < 0.05$) (Hernandez et al., 2012).

Similarly, the study done on 1037 HIV-uninfected children born to HIV-infected women on NRTI therapy were enrolled in pediatric AIDS clinical trials group protocols 219/219C and revealed, first exposure to 3TC or AZT/3TC in the third trimester was associated with the occurrence of possible mitochondrial dysfunction (MD). Cases (means that has clinical manifestation of MD) (n = 20) were significantly more likely to be male and to be born in earlier years than non-cases (n = 1017). When adjusted for year of birth the odds of first exposure in the third trimester to 3TC (OR, 10.57; 95% CI, 1.93-75.61) and AZT/3TC (OR, 9.84; 95% CI, 1.77-71.68) were significantly higher among cases than non-cases (Brogly et al., 2007).

Moreover, a study was conducted to examine the mechanism of mitochondrial myocytotoxicity caused by long term administration of zidovudine in human immunodeficiency virus positive pregnant. According to the study, the effect of zidovudine was examined *in vitro* on human muscle in tissue culture. After 19 days, the zidovudine treated myotubes in tissue culture exhibited abnormal mitochondria characterized by proliferation (mean + or - standard deviation, 27.5 + or - 8 mitochondria/16 sq microns surface area, compared with 12.8 + or - 4 in the control cultures ($p < 0.001$), enlarged size, abnormal cristae and electron dense deposits in their matrix. The changes were partially reversible after zidovudine withdrawal (Lamperth et al., 1991). In contrast to the above human and animal studies, most large-scale surveillance studies have found no persistent treatment-related adverse effects in AZT-exposed children (Culnane et al., 1999; Hanson et al., 1999; The Perinatal Safety Review Working Group, 2000; Tuomala et al., 2002). Similarly, a large retrospective review of 5 cohorts in the United States failed to show clear evidence of clinically relevant mitochondrial diseases in nucleoside-exposed children who died before 5 years of age (Barret et al., 2003). The European Collaborative Study (2003) also showed no evidence of clinical

manifestations suggestive of mitochondrial abnormalities in children exposed to antiretroviral therapy *in utero*.

C. Hepatic and Hematologic parameters

Funk et al. (2007) reported, antiretroviral drugs exposure *in utero* has been associated with persistent decreases in lymphocytes, neutrophils and platelets as well as an increased risk of transient lactic acidaemia and anemia. Similarly ART exposure was associated with anemia in early life (p<.001) (European Collaborative Study, 2003). In contrast, data from follow-up of PACTG 076/219 uninfected infants through age six years did not indicate any differences in lymphocyte subsets compared with uninfected children who had received AZT and those who had received a placebo (Culnane et al., 1999). Among the uninfected PACTG 076/219 children, mean CD4 and CD8 lymphocyte percentages did not differ between treatment groups over time. There were no statistically significant differences between zidovudine and placebo groups either at each point or when analyzed on an overall basis (P, .53 for CD4; P, .38 for CD8).

Alimenti et al. (2003) prospectively measured plasma lactate in a group of 38 HIV negative infants exposed to antiretroviral therapy. Plasma lactate was elevated on at least one determination in 35/38 children followed over the first 6 months of life, with 10 having higher levels (5 mmol/L). The clinical significance of these findings is uncertain.

In addition, observational study was done on 22 HIV-infected women receiving antiretroviral therapy with ATV (atazanavir) 300 mg and ritonavir 100mg during pregnancy and their 23 HIV infants (including a twin pair). The study reported, three neonates had mildly elevated AST transaminase levels. Bilirubin concentrations at birth were significantly higher than maternal concentrations, with a median of 44 µm/L [range 24-129]; values on days 2-3 were 63 [8-212]. Five neonates had jaundice requiring phototherapy, without liver damage and recovered without sequelae. This was a direct effect of transplacental ATV on bilirubin metabolism in the fetus as suggested by Azria et al. (2011).

On the other hand a comparative cohort study in Brazil between 2002-2009 was conducted to describe laboratory abnormalities among HIV-infected women and their infants with standard and increased lopinavir/ritonavir (LPV/r) dosing during the third trimester of pregnancy. The study includes 164 women received LPV/r standard dosing [(798/198 or 800/200 mg/day) (group 1)] and 70 increased dosing [(> 800/200 mg/day) (group 2)]. The study reported, increased LPV/r dosing

during the third trimester of pregnancy appears to be safe for HIV-infected women and their infants. Infants born to mothers in group 1 had significantly higher mean ALT values at birth (p, 0.0006) and creatinine values at birth and at 6-12 weeks ($p < 0.0001$). The overall proportion of infants with Grade 3 or 4 adverse events was very low (≤ 1.9%) and there were no statistically significant differences in the proportions of infants in each group with Grade 3 or 4 adverse events related to ALT, AST, blood urea nitrogen (BUN), or creatinine (Peixoto et al., 2011). Similarly a cohort study in Spain revealed, no significant difference among the drugs (ARVs) used *in utero* for the four analyzed biochemical markers in the first three months of age (Ferna´ndezIbietaa et al., 2010).

D. Growth parameters

A study by Townsend et al. (2009) and Briand et al. (2009) have found length or head circumference z-scores were not associated with antiretroviral therapy exposure. Duration of therapy had no impact on fetal growth either.

Similarly, two clinical trials in Botswana the Mashi and Mma Bana retrospective comparative study, investigated growth patterns through six months of life of those *in utero* ARV-exposed infants. In total, 619 infants with *in utero* HAART exposure and 440 infants with *in utero* AZT exposure were included in the study. The study reported during birth, infants exposed to HAART *in utero* had significantly lower weight, length and weight-for-length normalized scores compared to those exposed to AZT *in utero*. However, weight differences were not apparent by 3 months of age and the incidence of wasting (defined as weight-for-length falling more than 2 standard deviations below norm) did not differ significantly between the two groups at six months of life. Infants exposed *in utero* to HAART had a mean birth weight z-score of −0.64 while the AZT exposed group had a birth weight z-score of −0.34 (P, <0.001). From the 3rd through 6th month of life, the mean change in the length for age z-score for both the HAART and AZT exposed infants was no longer statistically different (P, 0.08). According to the report wasting was present in 6.02% of the AZT exposed infants and 6.09% of the HAART exposed infants (P, 0.96) at 6 months of life. Stunting was present in 4.76% of the AZT exposed group and 4.70% of the HAART exposed infants (P, 0.96) at 6 months of life (Powis et al., 2011b). Gibb et al. (2012) was also observed the effect of *in utero* tenofovir containing ART exposure on infant growth in Uganda/Zimbabwe which compared routine laboratory monitoring versus clinically driven monitoring. No differences were observed between children with no tenofovir exposure versus ≥90% *in utero* exposure ($p>0.15$). Moreover, there was no evidence that *in utero* exposure to tenofovir affected growth after 2 years (p, 0.38) (Gibb et al., 2012) which was compatible with findings of Correa et al. (2007). In contrast the US pediatric

HIV/AIDS cohort study of 532 infants reported lower weight at 1 year among those exposed versus not exposed to tenofovir *in utero* (Siberry et al., 2012) although the effect was unrelated to duration of tenofovir exposure and was not present at birth. Similarly, animal studies showed opposite result (Tarantal et al., 2002; Van Rompay et al., 2004; Van Rompay et al., 2008).

Moreover, long-term follow-up of children exposed perinatally to zidovudine monotherapy in the pediatric AIDS clinical trial group 219/076 study has not indicated differences in growth up to the median age of 4.2 years (range: 3.2–5.6 years) (Culnane et al., 1999). The study found the mean age percentiles for uninfected zidovudine exposed group and placebo group followed similar curves, with normal mean weight and height. There were no observed differences between the groups through 208 weeks (4.2 years). Likewise, no treatment differences in head circumference were noted. Infant length/height, weight and head circumference measurements were converted to age and sex adjusted z scores using the centers for disease control and prevention/World Health Organization international growth standard based on the national center for health Statistics (Roche and Himes, 1980; Dibly et al., 1987; Culnane et al., 1999).

E. Neurodevelopment

A comparative prospective controlled cross-sectional study by Alimenti et al. (2006) in British Columbia, Canada were conducted to investigate the neurodevelopment on 39 HIV-uninfected children exposed to combination highly active antiretroviral therapy in pregnancy compared with 24 children not exposed to highly active antiretroviral therapy but with similar socioeconomic backgrounds. Children underwent standardized neurodevelopmental assessment at 18 to 36 months of age. The study showed that perinatal highly active antiretroviral therapy exposure is not significantly associated with altered neurodevelopment and behavior. All mean scores were lower for those in the highly active antiretroviral therapy exposed group than those in the control group (Bayley Mental Development Index: 85.4 vs 94.3; Bayley Psychomotor Development index: 93.4 vs 96.6; Vineland mean communication score: 90.1 vs 94.4; Vineland mean daily-living score: 91.2 vs 93.6; Vineland mean socialization score: 97.1 vs 98.4). However, when maternal substance use during pregnancy was controlled for, there were no significant differences between the groups in any domains assessed as shown in tables below.

Neurodevelopmental Measurements

The Bayley Scales of Infant Development-II (BSID-II) were used to assess cognitive, language and psychomotor functioning with the primary goal of identifying developmental delay. The BSID-II is

the most widely used scale for assessing development from infancy to 30 months. Results provide Mental Development Index (MDI) and Psychomotor Development Index (PDI) scores, with a mean of 100 and SD of 15.

The Vineland Adaptive Behavior Scales revised–survey form was used to assess the areas of communication, daily-living skills and socialization. This is a well standardized norm-based questionnaire based on semi-structured interview and has been used extensively to evaluate adaptive skills in young children. Results provide a score with a mean of 100 and SD of 15 for each of the domains.

In the study the 2 groups are compared by using analysis of covariance to allow for control of specific variables and on frequencies by using x^2 tests. In a second step, undertook exploratory analyses of factors that could impact neurodevelopment, such as the type and duration of exposure to other substances during pregnancy, the type and duration of exposure to antiretroviral agents, and the timing of exposure (from conception versus third trimester alone). Differences were considered significant for a P value of <.05.

Highly active antiretroviral therapy in pregnancy consisted of 2 nucleoside reverse transcriptase inhibitors for all 39 mothers combined with nevirapine in 22, a protease inhibitor in 13, both nevirapine and a protease inhibitor in 3, and 3 nucleosides in 1. Mean duration of the HAART exposure was 17.7 weeks (range: 1–40 weeks) with a median exposure to zidovudine of 15 weeks (range: 1–39 weeks), lamivudine of 17 weeks (range: 1–39 weeks), nevirapine of 12.5 weeks (range: 1–38 weeks), and Nelfinavir of 16.5 weeks (range: 5–35 weeks). Only 9.4% of the women had $\leq$4 weeks antiretroviral therapy during pregnancy. No significant relationships were observed between duration of HAART exposure and the dependent variables except for zidovudine duration, which was positively correlated with the Vineland socialization score (r, 0.42; P, .017).

Mean age at the time of neurodevelopmental assessment, for the HAART exposed group was 25.9 months (range: 18.1–35.8 months) and for the control group was 22.1 months (range: 17.8–32.8 months). All mean developmental scores on the BSID-II and Vineland scales were lower in the HAART-exposed group than the control group, as shown in Table 6.

Table 6: Comparison of neurodevelopment scores with HAART exposure (Alimenti et al., 2006)

	HAART exposed (n_39)	HAART unexposed (n_24)	P
BSID-II			
MDI	85.4 (17.2)	94.3 (15.1)	.041
PDI	93.4 (14.1)	96.6 (13.5)	—
Vineland			
Communication	90.1 (14.8)	94.4 (13.1)	—
Daily living	91.2 (13.4)	93.6 (8.3)	—
Socialization	97.1 (14.0)	98.4 (10.9)	—

Values are test score (SD) unless otherwise indicated. The difference in the SD portion expresses the magnitude of the score difference between groups in SD units.—indicates that the P value was not computed or not significant.

However, only the BSID-II MDI was significantly different. Although in the study it was anticipated that maternal substance use in pregnancy would be similar in the 2 groups, they found that it was actually disproportionately higher in the HAART-exposed group than in the control group (51% vs12%; P, .002). Maternal injection drug use was 46% in HAART- exposed children and 4% in control children (P< .001).

The substances used during pregnancy consisted of cocaine and/or heroin in 90% of the cases. It was noteworthy that 30% of the children in the HAART-exposed group had narcotic withdrawal syndrome, whereas none in the control group were affected (P, .002). Maternal cigarette use was higher in the HAART-exposed group than in the control group (59% vs 33%; P, .028) and alcohol use was not significantly different. Forty percent of the children who were exposed to substance use were also exposed to alcohol during the pregnancy. When maternal substance use during pregnancy was controlled for, none of the differences in neurodevelopment scores between the 2 groups was significant. Children who were exposed to maternal substance use (HAART-exposed and control groups combined) scored lower in all test components than children not exposed (Table 7). These differences reached statistical significance (all P values <.05) for all domains except communication skills.

Table 7: Comparison of neurodevelopment scores with illicit drug and alcohol use (Alimenti et al., 2006)

	Drug use (n-23)	No drug use (n-40)	P
BSID-II			
MDI	81.2 (18.3)	93.2 (14.4)	.031
PDI	89.5 (15.8)	97.5 (11.9)	.044
Vineland			
Communication	88.0 (14.2)	93.9 (14.0)	—
Daily-living skills	87.5 (12.5)	94.8 (10.4)	.024
Socialization	93.3 (12.7)	100.0 (12.3)	.047

—indicates that the P value was not computed or not significant.

An analysis of covariance was conducted to assess the differences between the 2 study groups controlling for maternal substance use. There were no significant differences between the groups on any of the mean developmental scores on the BSID-II and Vineland scales (all P values >.25). The adjusted group means are presented in Table 8.

Table 8: Mean neurodevelopment scores adjusted for maternal substance use (Alimenti et al., 2006)

	HAART exposed (n-39)	Control (n-24)
BSID-II		
MDI	86.9	91.9
PDI	94.5	94.7
Vineland		
Communication	90.9	93.2
Daily-living skills	92.3	91.8
Socialization	98.1	96.7

Values are test scores.

Subgroup analyses were also conducted for the HAART-exposed group, comparing those with maternal substance use to those with no maternal substance use and to those in the control group with no maternal substance use. It is important to note that there was no difference found between the latter 2 groups. For all outcome measures the means of the control group and the HAART-exposed group with no substance use were closer together than either was to the HAART-exposed group with substance exposure. Differences were noted between the HAART-exposed group with substance use and the control group in the BSID-II MDI result and also with the HAART-exposed group with no substance use in the vineland daily-living and socialization results. Statistical power is reduced when the group is subdivided. The results of these subgroup analyses are shown in Table 9.

Table 9: Neurodevelopment scores according to group and maternal substance use (Alimenti et al., 2006)

	HAART-exposed, drug use (n-20)	HAART-exposed, no drug use (n-19)	Control, no drug us (n-21)	P
BSID-II				
MDI	81.0 (17.8)	90.2 (15.5)	96.0 (13.1)	.012a
PDI	89.5 (15.4)	97.5 (11.7)	97.5 (12.2)	—
Vineland				
Communication	88.2 (14.3)	92.2 (15.5)	95.5 (12.6)	—
Daily-living skills	87.0 (12.1)	95.6 (13.5)	94.0 (6.8)	.044b
Socialization	92.3 (12.5)	102.2 (13.9)	98.1 (10.7)	.049b

Values are test score (SD).—indicates that the P value was not computed or not significant.
a HAART-exposed, drug use group scored significantly lower than the control, no drug use group.
b HAART-exposed, drug use group scored significantly lower than HAART-exposed, no-drug-use group.

In subgroup analysis, substance use had a greater impact on neurodevelopment than HAART exposure. Indeed, children exposed to HAART with no maternal substance use had equivalent neurodevelopment scores to control children exposed to neither HAART nor substance use in pregnancy. Overall, the study found that maternal substance use was a stronger predictor of a poor neurodevelopmental outcome than was HAART exposure as suggested by Alimenti et al. (2006). This finding was consistent with a study conducted by Culnane et al. (1999) to evaluate the long-term effects of *in utero* exposure to zidovudine vs placebo among a randomized cohort of uninfected children. Accordingly, there were no significant differences between groups for the Bayley MDI or PDI scores (P value for MDI scores, .24; for PDI scores, .84).

F. Other birth out comes (PTD, LBW, Intra-uterine Growth Retardation and Others)

Discrepant results on the risk of other birth outcomes in women infected with HIV treated with HAART have been reported. Cell culture studies have shown that PIs cause decreased insulin sensitivity by direct inhibition of the activity of the GLUT4 glucose transporter which are most abundant in muscle and adipose tissue. From this susceptible fetuses may exhibit impaired growth because of the action of PIs on GLUT4 activity **(**Chmait et al., 2002**).**

Prematurity occurring in pregnant women on ART has been attributed mainly to PI-based ART (Kourtis et al., 2007; Gibb et al., 2012). The 560 participants between 26 and 34 weeks gestation

were randomized to receive either NRTI based HAART (abacavir /zidovudine/ lamivudine coformulated as Trizivir twice daily) or PI based HAART (lopinavir/ritonavir with zidovudine/ lamivudine coformulated as Kaletra twice daily). According to the study, preterm delivery rates were higher among 267 women in the PI group than 263 women in the NRTI group (21.4% vs 11.8%, P, .003) regardless of gestational age at HAART initiation (Powis et al., 2011).

Similarly, the cohort study were carried out on 999 women who received antiretroviral therapy during pregnancy (monotherapy in 492, combination therapy without PI in 373 and combination therapy with a PI in 134) and 338 women who did not receive therapy. The study revealed, after adjustment for possible confounders, only combination therapy with a PI was associated with an increased risk of preterm delivery compared with any other combination (OR, 1.8; 95% CI, 1.1–3.0) (Cotter et al., 2006).

Moreover, prospective cohort study was carried out on 183 mother–child pairs from 13 centres in Germany and Austria delivering between 1995 and 2001. Of 183 mother–child pairs, 42% were exposed to antenatal monotherapy and 17% to dual therapy. Of the 75 women exposed to highly active antiretroviral therapy (HAART), 21 (28%) received protease inhibitor (PI) based HAART and the remaining 54 received non-nucleoside reverse transcriptase inhibitor based-HAART. In multivariable analysis (176 pregnancies), PI-based HAART exposure during pregnancy was associated with an increased risk of premature delivery (aOR, 3.40; 95% CI, 1.13–10.2; P, 0.029, compared with monotherapy) (Grosch-Woerner et al., 2008).

Similarly, in multivariate logistic regression analysis, use of protease inhibitor-based HAART regimen was associated with a 2-fold higher rate of preterm delivery compared with triple NRTI-based HAART (aOR, 2.02; CI, 1.25–3.27), after adjustment for self-reported maternal income (P, .02 for 3 df) (Powis et al., 2011a). Indeed, a French study explored whether LPV/r exposure during pregnancy was associated with adverse outcomes. For each HIV-infected woman, two uninfected women matched by age, parity and geographical origin were selected among patients delivering during the same period. The rate of preterm birth was higher among HIV-infected women exposed to LPV/r (21%) than among controls (10%) ($p < 0.01$) **(**Azria et al., 2009).

On the other hand, Townsend CL et al. (2007) revealed, HAART was associated with high rate of prematurity (< 37 weeks gestation) (14.1%, 476/3384) than in women on mono/dual therapy (10.1%, 107/1061), even after adjusting for ethnicity, maternal age, clinical status and injecting drug use as

the source of HIV acquisition (aOR, 1.51; 95% CI, 1.19-1.93; P, 0.001). Delivery at < 35 weeks was even more strongly associated with HAART (aOR, 2.34; 95% CI, 1.64-3.37; P < 0.001). The effect was the same whether or not HAART included a protease inhibitor as supported by Schulte et al. (2007). Similarly an analysis of combined data from the European Collaborative Study and the Swiss cohort study showed a significant association between the risk of premature delivery and combination therapy, without and with protease inhibitors (OR, 1.8 and 2.6, respectively), after adjustment for the maternal CD4+ cell count and the presence or absence of a history of parenteral drug use (European Collaborative Study and the Swiss, 2000). The rates of premature delivery in the European study were similar to those in Tuomala et al. (2002) study for women who received no therapy or monotherapy but were higher among women who received combination therapy without protease inhibitors and among those who received combination therapy with protease inhibitors (22 and 29%, respectively, as compared with 14 and 18%, respectively, in Tuomala et al. (2002) study. Tuomala et al. (2002) stated the reason for the differences between their results and those of the European study are unclear. However, the report showed neither Tuomala et al. (2002) nor the European investigators could control directly for the stage of maternal disease or the HIV-1 viral load factors that in some studies were associated with premature delivery as suggested by Newell et al. (1996). Similarly a multivariate analysis in European Collaborative Study of 1008 infants exposed to ART at any time, 906 (90%) were exposed antenatally, 840 (83%) neonatally and 750 (74%) both antenatally and neonatally showed, an association between prematurity and exposure to combination antiretroviral therapy in pregnancy, with a PI (OR, 4.14; 95% CI, 2.36-7.23) and without a PI (OR, 2.66; 95% CI, 1.52-4.67) (European Collaborative Study, 2003).

In addition, a retrospective Swiss study of 30 women with HIV-1 who had received combination antiretroviral therapy during pregnancy (with PI in 13 women and without PI in 17) showed that such treatment was associated with a 33 percent risk of premature delivery **(**Lorenzi et al., 1998). Contemporaneously, the pediatric AIDS clinical trials group (PACTG) observed that in phase 1 trials of nelfinavir, ritonavir, or indinavir given in combination with zidovudine and lamivudine during pregnancy, 4 of 11 deliveries (36 percent) occurred before 37 weeks of gestation (Tuomala et al., 2002).

Kowalska et al. (2003) was conducted study aimed to assess the effect of antiretroviral therapy on 102 HIV-1 infected pregnant women. The study reported, preterm deliveries were highly prevalent among women on HAART during pregnancy, especially when therapy was started before pregnancy or in the first trimester of pregnancy. Antiretroviral therapy was given to 81 (79.4%) patients, on

zidovudine monotherapy there were 35 (34.4%) women, on HAART with a protease inhibitor (PI) there were 18 (17.6%) patients and 28 (27.4%) patients on HAART with no PI regimen. Overall rate of preterm delivery at <37 weeks was estimated as 11.8% (12 out of 102). Premature delivery occurred in 16.7% of women on HAART with PI and in 17.9% of women on HAART without PI as compared to any case in patients not receiving ART. Combined therapy was started before pregnancy or during the first trimester in 62.5% of women who delivered prematurely versus 15.6% who gave birth at term. Similarly Kourtis et al. (2007) reported, the use of combination regimens before or early in pregnancy may slightly increase the risk of prematurity. The initiation of combination therapy before pregnancy or in the first trimester resulted in an OR of 1.71; 95% CI, 1.09-2.67) compared with therapy initiation in the second trimester and beyond. Contemporaneously Machado et al. (2009) revealed, pregnant women who were using ARVs preconception had higher rates of PTD (26.3% vs 17.7%; P, 0.09) as compared to with those treated after the first trimester. Moreover, Thorne et al. (2004) found a two-fold increased risk of premature delivery and a four-fold increased risk for severe premature delivery in women who started combination therapy pre conception. More recently, a 3.4-fold increase in the risk of PTD in patients treated with HAART was seen in a German/Austrian cohort where 52% of the patients were already being treated with HAART at conception (Grosch-Woerner et al., 2008).

Goldstein et al. (2000) noted that the women who received antepartum only AZT monotherapy had a high frequency of low birth weight infants (55.5%). Contemporaneously, women receiving HAART with PI were at a higher risk of delivering a baby with low birth weight (22.2% vs. 13.3% in the group of non-treated women) (Kowalska et al., 2003). Also the study conducted by Tuomala et al. (2002) showed, the association between combination therapy with protease inhibitors and an increased risk of very low birth weight. Seven of the women who received combination therapy with protease inhibitors (5%) had infants with very low birth weight as compared with nine women who received combination therapy without protease inhibitors (2%) (aOR, 3.56; 95% CI, 1.04 - 12.19). Goldstein et al. (2000) noted that the frequency of low birth weight infants was significantly higher in the PIs treated group (75%) compared to the no PIs (19.7%) (P, 0.033), which was compatible with the observation of Laubereau et al. (1998).

Townsend et al. (2007) reported in comparison with exposure to mono/dual therapy, exposure to HAART was associated with lower birth weight standardized for gestational age ($P < 0.001$) and an increased risk of stillbirth (aOR, 2.27; 95% CI, 0.96-5.41; P, 0.063). The effect was the same whether or not HAART included a protease inhibitor. Machado et al. (2009) also reported, pregnant

women who were using ARVs preconception had higher rates of LBW (33.3% vs 16.5%; P, 0.001) as compared to with those treated after the first trimester.

A study in Abidjan Côte d'Ivoire, West Africa was conducted on 326 HIV infected pregnant women, 175 women received short-course antiretroviral and 151 received HAART. On the basis of the study report the rate of LBW was 22.3% in the HAART group and 12.4% in the PMTCT group (P, 0.02). In the study multivariable logistic regression model analysis (n = 309), after adjustment on maternal CD4 cell count, WHO stage, age and maternal BMI (body mass index) showed, HAART initiated before pregnancy (aOR, 2.88; 95% CI, 1.10-7.51) and during pregnancy (aOR, 2.12; 95% CI, 1.15-4.65) were associated with LBW (Ekouevi et al., 2008)**.**

In contrast logistic regression analyses by Tuomala et al. (2005) on 2543 HIV infected pregnant women for controlling multiple covariates revealed use of ART containing AZT during early and late pregnancy was associated with decreased risk for PTD at <37 weeks (OR, 0.5; 95% CI, 0.3-0.8) and use of ART containing other NRTIs but not AZT during early and late pregnancy was associated with decreased risk for PTD at <32 weeks (OR, 0.3; 95% CI, 0.2-0.7). Similarly Newell et al. (1996) revealed, prophylactic zidovudine monotherapy was associated with a decreased risk of prematurity and low birth weight in a cohort of 2300 HIV-infected women which was compatible with findings of Grosch-Woerner et al. (2008). Moreover, a cohort study in Nigeria, on 249 HIV infected women who had received HAART early in pregnancy were compared to HIV positive pregnant women, who had not received antiretroviral drugs during antenatal period. The results of the study revealed pre term birth (25.0% vs. 9.8%, p, 0.005) were significantly higher among women with untreated-HIV infection in pregnancy compared with women who received HAART from early pregnancy (Biodun et al., 2011).

On the other hand, retrospective cohort study was performed in Canada, on 206 mother-infant pairs who had HIV and had been treated with HAART, of whom 176 had regimens containing protease inhibitors and a control group of 206 infants born to non-HIV infected mothers and thus not exposed to HAART. The study reported that at delivery, the weight, length and head circumference of the 176 infants exposed to protease inhibitors were similar to those of the control group. In addition the study reported that, in infants exposed to HAART the 10.6% rate of prematurity (11.1% with and 7.1% without protease inhibitors) was not significantly higher than that in the control group (7.8%). Moreover, the 9.9% rate for small for gestational age (9.8% with and 10.3% without protease inhibitors) was also not significantly higher than that in the control group (5.3%) (Carceller et al.,

2009). Indeed, in a large cohort of mothers and infants who received zidovudine and enrolled in PACTG 185, rates of preterm delivery and low birth weight were comparable to those of uninfected women (Cotter et al., 2006).

Similarly, prospective cohort study in Latin America and the Caribbean was done on 681 HIV-1 infected women receiving at least one antiretroviral drug [in order of increasing complexity: one or two nucleoside reverse transcriptase inhibitors (1-2 NRTI), two NRTI plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) (HAART/NNRTI), or two NRTI plus one PI (HAART/PI)] for at least 28 days during pregnancy, and who delivered live born, singleton infants with known birth weight and gestational age by 1 March 2005 (Szyld et al., 2006). The study showed, the incidence of LBW and PTD, respectively, was 9.6% and 7.4% (1-2 NRTI), 7.4% and 5.8% (HAART/NNRTI), and 16.7% and 10.6% (HAART/PI). There was no statistically significant increased risk of LBW (aOR, 1.5; 95% CI, 0.7-3.2) or preterm birth (aOR, 1.1; 95% CI, 0.5-2.8) among women who received HAART/PI compared with women receiving 1-2 NRTI. Indeed, Schulte et al. (2007) revealed triple ARV therapy was not associated with LBW**.** Highly active antiretroviral therapy (HAART) including a PI 133/1744 crude OR, 1.01; 95% CI, 0.78-1.30 and HAART; non PI 330/1744 crude OR, 1.12; 95% CI, 0.91-1.38.

Similarly, meta-analysis was done on thirteen prospective cohorts and one retrospective studies that has reported premature delivery for HIV-infected women treated with antiretroviral regimens during pregnancy. According to the analysis antiretroviral therapy during pregnancy did not increase the risk of premature delivery overall (OR, 1.01; 95%CI, 0.76-1.34). In subgroup analyses, compared with no therapy monotherapy (mostly zidovudine) conferred an (OR, 0.86; 95% CI, 0.73-1.01), whereas combination therapy conferred an (OR, 1.13; 95% CI, 0.79-1.63). The use of PI-containing combinations resulted in an OR for premature delivery of 1.24; 95% CI, 0.76-2.02, compared with combinations without PI (Kourtis et al., 2007). Also, data from a meta-analysis of 7 clinical studies in the United States on 2123 HIV-infected women who received some form of ART and 1143 women who did not receive any did not find an association between antiretroviral therapy and preterm delivery, low birth weight (<2500 g), or low Apgar scores. Both groups had similar rates of low Apgar scores, preterm birth and low birth weight infants (Tuomala et al., 2002). In addition, Cotter et al. (2006) revealed no differences in rates of low birth weight and stillbirth, regardless of ARV therapy.

Moreover, Patel et al. (2010) prospective cohort study on 777 HIV-infected pregnant women who were exposed to ARV with PI revealed, no association between use of combination ARV with PI during pregnancy and preterm birth which was also supported by Powis et al. (2011). Accordingly a total of 130 preterm births were observed. In adjusted analyses, combination ARV with PI was not significantly associated with preterm birth compared to ARV without PI (OR, 1.22; 95% CI, 0.70-2.12). Low birth weight results were similar (OR, 1.36; 95% CI, 0.76- 2.44).

A retrospective study in four HIV care centers participating to clinical trials and international cohort collaboration were conducted in Abidjan Côte d'Ivoire, West Africa between 2003 and 2009. All HIV-infected pregnant women who conceived on EFV or NVP-based antiretroviral therapy (ART) were included. From the study, no significant increased risk of unfavorable pregnancy outcome was reported. According to the study overall, 344 HIV infected pregnant women conceived while on ART (213 on EFV and 131 on NVP). Median age was 29 years and median CD4 count 217 cells/µl at ART initiation. The overall proportion was 6.7% for stillborn, 10.8% for PTD and 20.2% for LBW (Ekouevi et al., 2011).

In sum, the lower rates of PTD and LBW in Latin American cohorts when compared with those in Europe and Swiss could be related to a lower prevalence of illicit drug use and to the absence of women who did not receive ARVs during pregnancy in the studies as suggested by Machado et al. (2009).

5. Conclusion

Birth defects are structural and functional abnormalities present at birth, whether the abnormality is detected at the time of delivery or at a later time. The reported frequency of birth defect is 2%-3% of all births.

With its emphasis on ensuring normal conception and early pregnancy, primary prevention is the most important of all three levels (i.e. primary, secondary and tertiary levels of prevention) and is both feasible and affordable, even for financially constrained health systems.

Animal embryos exposed *in vivo* to antiretrovirals exhibited significantly increased pregnancy losses, drugs incorporation into the DNA of fetal organs, external abnormalities, skeletal defects, developmental toxicity, carcinogenicity, reduced weight, anemia, deaths and significant mitochondrial damage. In light of animal studies and some human studies, there are concerns about

links between efavirenz and birth defects specifically neural tube defects. In earlier human studies, management of AIDS positive pregnant women with antiretrovirals revealed exposure of their infants to such drugs with evidence of adverse events. However, recent publications present conflicting data about the associations between antiretrovirals and adverse pregnancy outcomes.

The predictive value of animal studies for humans may not always hold true; making it difficult for health workers to translate all animal risks into an assessment of teratogenic risk in their patients as suggested by some authors. Many associations have ultimately been shown to be false positive in humans and in some instances drug testing in animals has been negative and the drug subsequently shown to be teratogenic in humans. Of approximately 1,200 animal teratogens, only about 30 are known to be teratogenic in humans as suggested by Mills (1995). This could be because of many factors such as the dose and time of exposure. On the other hand, findings with animal studies should not also be ignored as they could give clues to serious teratogens that may lead to similar anomalies if conditions are met. Such a latter condition is of special interest with regard to the nucleosides *in utero* animal studies and non nucleoside, EFV *in utero* animal studies where abnormalities were observed in mice and primates at drug levels comparable to therapeutic ranges in humans and positive findings were detected in more than one animal species.

The benefits of antiretroviral therapy in a pregnant woman must be weighed against the risk for adverse events to the woman, fetus and newborn. In spite of the unquestionable benefits deriving from ARTs in perinatal transmission of HIV, an international agreement exists on the need to continuously reevaluate the risk benefit ratio of human exposure to ARTs during embryonic development**.** Thus, evaluation of the rates of and risks for birth defects after well-characterized exposure to ARV agents during pregnancy is needed to assist in selecting appropriate treatment regimens for pregnant and reproductive age women. As continued improvements for treatments that prevent mother-to-child transmission of HIV is sought**,** one must take advantage of experimental systems such as this CD-1 mouse, NMRI mouse model and other models to elucidate obscure biological side effects not detectable by standard clinical evaluation methods, which may substantially impact the long-term health of the exposed neonate.

6. Recommendation

Because of the increasingly frequent use of highly active antiretroviral therapy during pregnancy, ongoing efforts are needed to monitor any long-term effects of *in utero* exposure to multiple antiretroviral agents.

Since current studies about the risk of birth defect and ART exposure during pregnancy are contradict to each other it is impossible to draw conclusion. So that further larger studies are required to exclude an increased risk for specific congenital anomalies.

Since currently there is inadequate data with regard to teratogenic effects of non nucleoside analogues and protease inhibitors further studies are required to know about safety of the drugs.

In addition there exists no study with regard to birth defects in many countries, including Ethiopia and the study regarding the effects of ARV drugs on congenital anomalies should be broadened to include these countries.

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