HIV-1 infected women in Europe

A thesis presented for the degree of

Doctor of Philosophy

University of London

Simona Fiore

Institute of Child Health

University College London

2005

HIV-1 infected women in Europe

Abstract

This thesis aims to describe the wider impact of HIV infection on reproductive choices and pregnancy outcomes in HIV-infected women in Europe.

Characteristics of 403 HIV-infected women enrolled in a survey on reproductive choices are described. There was no evidence to suggest HIV-infected women have problems to conceive; maternal well-being, an uninfected partner and not having children yet were strongly associated with being pregnant.

Results from a laboratory-based descriptive study, including 57 pregnant women provide a biological explanation for HAART-associated premature delivery in HIV-infected women; cytokine patterns (IL2 and IL10) were analysed over three trimesters of pregnancy and in relation to gestational age at delivery.

Intrauterine growth (femur length, head and abdominal circumference) of infants born to 316 HIV infected mothers, compared to an uninfected population is reported. The average z-score of head circumference and femur length in HIV-infected women was below the reference (32th centile and 15th centile respectively), but the average z-score for abdominal circumference differs only marginally (49th centile).

In order to add one more piece of information in relation to maternal treatment and gestational age at delivery, birth-weight from a large European cohort was analysed and compared to the British standard. Mean z-scores decreased from -0.10 (46th centile), -0.13 (45th centile) and -0.30 (37th centile) throughout gestation indicating that children born to HIV infected mothers became smaller towards the end of pregnancy, and premature delivery in HAART-treated mothers was not associated to fetal distress.

Complications after delivery according to infection status of the mother and mode of delivery, were reported from a total of 250 HIV-status matched pairs delivered vaginally and from 158 by elective caesarean section. HIV-infected women suffered an increased risk of minor complications, but major complications only occurred in the caesarean section arm.

HIV-1 infected women in Europe

Title page

Abstract

Contents

List of figures

List of tables

List of abbreviations and explanations

Appendices

Acknowledgments

CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDICES

I   Collaborators

II   Data collection forms: survey on reproductive choices and outcome

III   Data collection forms: matched-case-controls studies on complications after delivery

IV   List of publications arising from the research

LIST OF ABBREVIATIONS AND EXPLANATIONS

HIV/AIDS: the human immunodeficiency virus (HIV) is a frequently mutating retrovirus that attacks the human immune system and which has been shown to cause acquired immune deficiency syndrome (AIDS).

LAV: The first AIDS cases were described in 1981. HIV was discovered and identified as the agent for AIDS by Luc Montagnier of France and Robert Gallo of the United States in 1983-1984. At the time, the virus was called Human T-Lymphotropic Virus type III (HTLV-III) or Lymphadenopathy-Associated Virus (LAV). In 1986, the genome of the virus was cloned and sequenced. The name HIV has been in use since 1986.

CCR5/ CXCR4: CCR5 is the chemokine receptor which HIV uses as a coreceptor to gain entry into macrophages. Certain strains of HIV target macrophages rather than **T cells**, because their envelope protein is configured such that it works best with CCR5 as a coreceptor - these strains are called macrophage-tropic. (Interestingly, macrophage-tropic strains tend not to be able to induce **syncytia**).

CCR5 is perhaps the most important of the known **coreceptors** for HIV, since the most commonly transmitted strains of HIV are strains that bind to CCR5 - so-called "**R5**" strains. CXCR4 (aka "fusin") and CXCR5 are both **chemokine** receptors. That is, they are proteins normally embedded in the membrane of a cell, which respond to the presence of **chemokines** outside the cell to trigger some kind of response within the cell.

HIV-1 is able to use either CXCR4 or CXCR5 as a co-receptor (**CD4** being the main receptor) to facilitate **binding** and entry into **T cells**.

About 10% of all Europeans carry a polymorphism of CCR5. People with this mutation (a 32 base pair deletion) have a very low risk of acquiring infection.

PCR: Polymerase Chain Reaction (PCR) is a biological molecular method for amplifying DNA without using a living organism.

RT: Reverse transcriptase is an enzyme used by all retroviruses and retrotransposons that transcribes the genetic information from the virus or retrotransposon from RNA into DNA, which can integrate into the host DNA. Eukaryotes with linear DNA uses a variant of reverse transcriptase, called telomerase, with the RNA template contained in the enzyme itself. The enzyme collectively referred to as reverse transcriptase generally includes an RNA-dependent DNA polymerase and a DNA-dependent DNA polymerase, which work together to perform transcription in the reverse of the standard direction. Usually, transcription only runs from DNA to RNA, catalyzed by RNA polymerase. In addition to the transcription function, retroviral reverse transcriptase carries a RNase domain, which belongs to the RNase H family.

RT PCR: Reverse transcriptase is commonly used in the field of research to be able to apply the polymerase chain reaction technique to RNA. The classical PCR technique can only be applied to DNA strands, but with the help of reverse transcriptase, RNA can be transcribed into DNA making PCR analysis of RNA molecules possible. The technique is collectively called: Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Reverse transcriptase is also used to create cDNA libraries from mRNA. Since HIV uses reverse transcriptase, together with integrase, to infect human DNA with viral DNA, reverse transcriptase inhibitors are used to prevent this.

PBMC: peripheral blood mononuclear cells.

RPMI 1640: Liquid classic cell culture media

Immune cells: Pathogens (viruses or bacteria) that escape antibody detection can enter and infect cells. The surface of infected cells changes, and this change is recognized by T cells. Cytotoxic T cells kill infected cells, preventing these cells from producing more pathogens. Cytotoxic T cells must interact with **Helper T cells** (Th) to regulate destruction of infected cells. Dendritic cells must activate CD4+ helper T cells before our bodies can produce B cells secreting pathogen specific antibodies or **cytotoxic T cells** (CTL) to destroy infected cells.

Natural Killers (NK) cells accumulate mostly in "secondary lymphoid tissues" - the tonsils, lymph nodes and spleen - after emerging from the bone marrow. There, the natural killer cells await activation (probably after stimulation by sentinel dendritic cells) before they react in two distinct modes. In one mode, they promptly secrete cytokines, chemical messenger proteins, which modulate emerging T and B immune cell responses. In the other, they become potent killers of tumors and virus-infected cells.
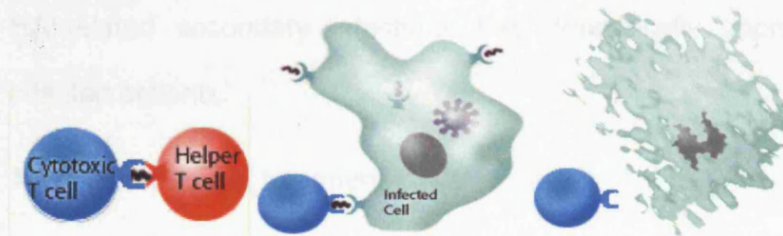
PHA: Phytohemoagglutinin induces cell activity , it can activates lymphocytes or different cell lines (ex hematopoietic cells).

ENV: "envelope" the proteins derived from env are a surface (gp120) and a transmembrane gp41 protein . They are located at the outer part of the virus particle and enable the virus to attach to and to fuse with the target cells to initiate the infectious cycle. The gene-product has a knoblike structure.

ELISA: (HIV enzyme immunoassay)

λ λ λ Patient serum which contains antibodies. If the patient is HIV+, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate.
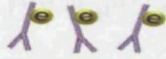
Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies.

Chromogen or substrate which changes color when cleaved by the enzyme attached to the second antibody.
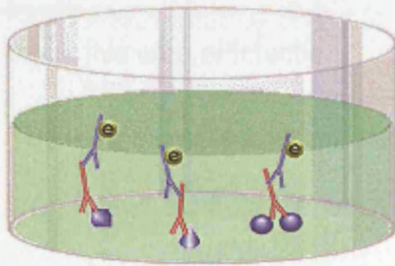
Positive Elisa test

HAART: highly active anti-retroviral therapy with a combination of drugs results in a dramatic reduction in viral levels. HAART coupled with improved treatments of HIV-related secondary infections has dramatically improved survival for HIV infected patients.

ART: Antiretroviral treatment
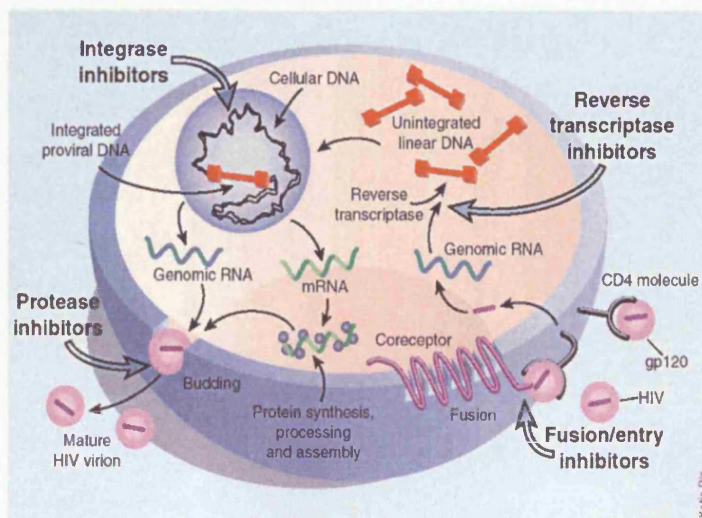
HIV binds to CD4 cell surface molecules, entry into the cell also requires binding to co-receptors CXCR4 and CCR5). This step can be inhibited by fusion/entry inhibitors.

HIV is uncoated inside the cell and reverse transcriptase copies genomic RNA into DNA, making errors at a frequence of about one per replication cycle. **Reverse transcriptase inhibitors** were the first class of HIV inhibitors to be used as drugs.

Viral DNA can integrate into host DNA and become a part of the cellular genome. This step makes the infection irreversible, and may mean that eliminating the virus from an infected individual is not possible. Integrase inhibitors are designed to block this step of infection.

The virus uses cellular machinery to synthesize viral proteins. Several of these are long amino acid chains which must be cleaved by a specific viral protease before new viral particles can become active. **Protease inhibitors** block viral maturation at this step.

ACKNOWLEDGMENTS

Chapter 1   Women and HIV infection: an overview

"Women are increasingly affected, now making up nearly half of the 37.2 million adults living with HIV worldwide. The virus is spreading into the female population because it is more readily transmitted from men to women in sexual contact. The lower status of women and girls in many regions and their vulnerability to sexual exploitation by men are also cited as factors in their susceptibility to infection."

"Strategies to address gender inequalities are urgently needed if we want a realistic chance at turning back the epidemic,"

Source: AIDS Epidemic Update 2004, the annual report by the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO).

1.1 Introduction

The rapid spread of the human immunodeficiency virus (HIV) across populations has been facilitated by the health-related and lifestyle changes which have taken place over the last three-quarters of a century. This started with the discovery and widespread use of penicillin and other antibiotics which provided a cure for most common sexually transmitted diseases, thereby changing the perception of sexual risk substantially (Browne 1970; WHO 1972). The use and increasing efficacy of contraceptives has also been key; the use of condoms started to become common amongst soldiers in the Second World War. Subsequently, the availability of hormonal contraceptives from the 1960s has had an additional major impact on sexual practice; with markedly decreased risk of pregnancy. Lifestyles have also changed substantially with easier long distance travelling allowing for more social migration and sexual mixing (Stigum, 1994; Lydie, 2004; Lurie, 2003)

The first evidence of AIDS in the developed world was related to groups of individuals who shared a common exposure risk (CDC, 1985). In the United States, sexually active homosexual men were among the first to be discovered with manifestations (Pneumocystis carinii pneumonia) of what is now known to have been HIV disease, followed by recipients of blood or blood products, injecting drug users, and ultimately children born to infected mothers.

The first cases of AIDS in children were reported in 1982 (Thomas 1984) and by the end of 1995, there were more than an estimated 1.5 million children with AIDS worldwide. The findings of three female half-siblings who had clinical and laboratory evidence for AIDS, whose mother had abnormal T-cell immunity strongly suggested vertical transmission of HIV during the perinatal period (Cowan 1984). However, although the presence of HIV in children and then their mothers was recognized fairly early on in the epidemic, it took longer, and prospective follow-up

studies of HIV infected pregnant women and their children, to understand that not all children born to HIV infected women are themselves infected. The HIV epidemic among children is closely linked to that in women, since the vast majority of paediatric infections are the result of vertical transmission from mother to infant. In order to estimate the number of children at risk of perinatally acquired infection, knowledge of both the prevalence of infection among women of childbearing age, and the rate of vertical transmission is required.

Women represent an increasing proportion of reported AIDS cases in Europe, and worldwide; now representing 47% of all young adults living with HIV and AIDS; this proportion had been steadily increasing over time (UNAIDS, 2004) (Figure 1.1). The characteristics of the HIV-infected women have changed since the start of the epidemic. HIV-infected women in Europe are now more likely to have acquired their infection heterosexually than through injecting drug use; they are having their children at increasingly older ages and are more likely to know that they are HIV-infected when they become pregnant.

In developing countries, the AIDS epidemic manifested itself quite differently, both because the HIV-related signs and symptoms were harder to identify because of other competing causes of morbidity and mortality and because the epidemic did not seem to be limited to "high-risk" groups and, instead, was more generalized . By the end of 2004, nearly 37 million people worldwide were living infected with HIV, of whom about 17.6 million were women; out of 610 thousand HIV-infected people living in Central and Western Europe, 160 thousand are women. (UNAIDS,2004). Globally, there are currently two HIV-infected women for every three infected men but it is expected that the number of new infections among women will equal those among men (Stover, 2004). However, the male-to-female ratio varies between countries: approximately equal numbers of male and female AIDS cases are already reported from sub-Saharan Africa, with an excess of

female cases (Figure 1.2) reported from some areas such as Uganda and in younger age groups. (Population Reference Bureau, 2003).

Figure 1.1: Percent of adults (15–49) living with HIV who are female, 1985–2004(Source UNAIDS/WHO, 2004)
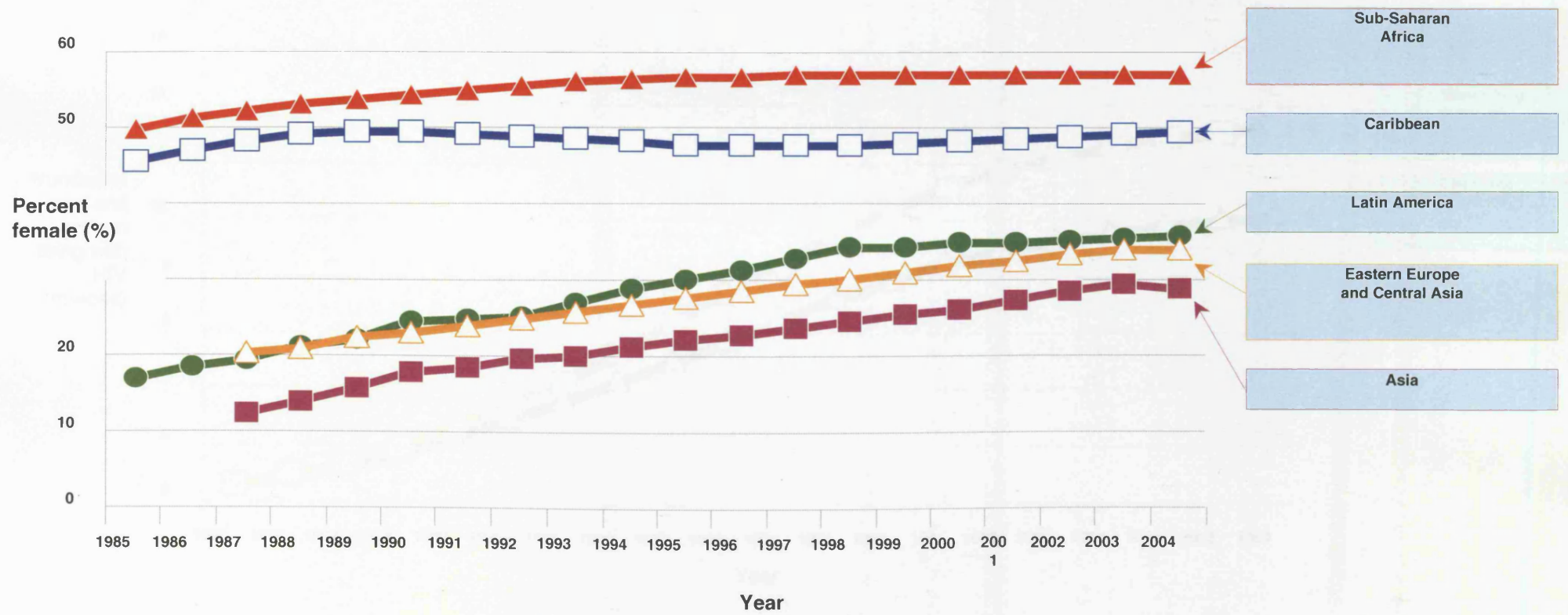
Figure 1.2: Number of women and men living with HIV in sub-Saharan Africa, 1985–2004 (Source: UNAIDS/WHO, 2004)
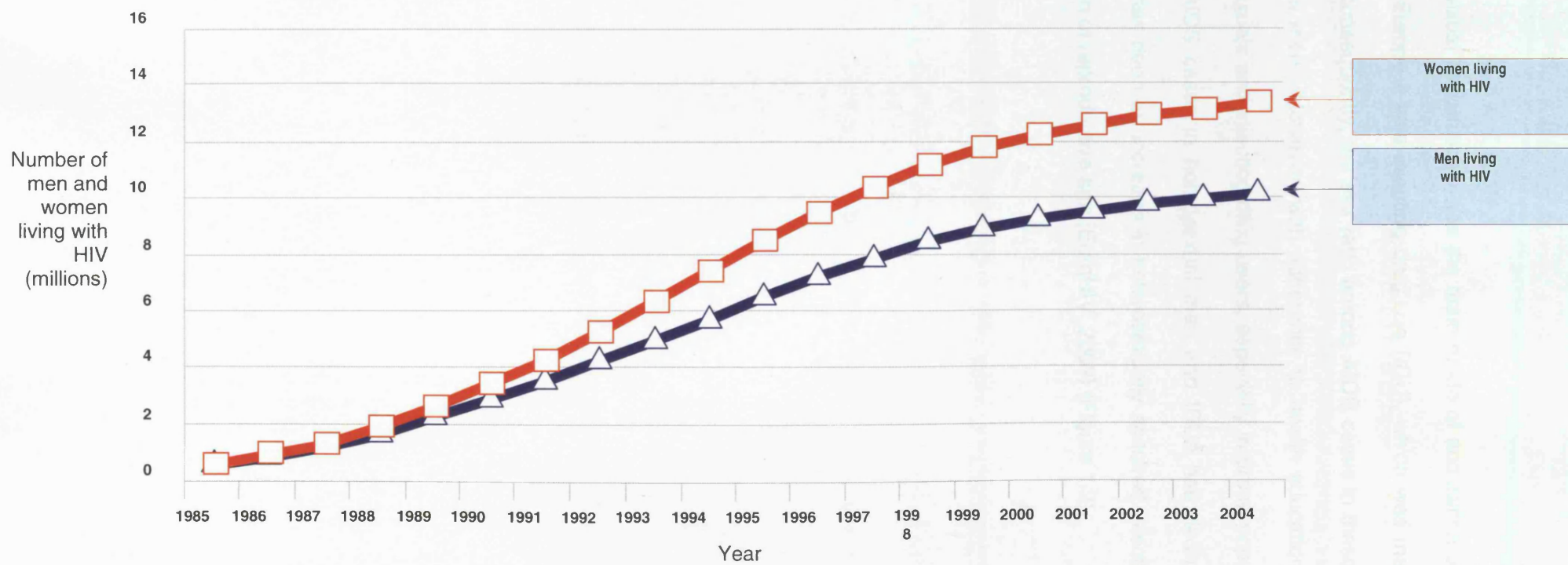
Initially, homosexual transmission was the main mode of acquisition of HIV in the USA, whilst in Europe it was injecting drug use (IDU), which was mainly a male phenomenon; consequently, the sex ratio among AIDS cases in these areas was skewed towards men. However, with adherence to health education messages among homosexuals and injecting drug users, especially in urban populations, the proportion of AIDS cases in homosexual men and IDUs has declined and, in parallel, there has been an increase in heterosexually acquired infections among men and women of reproductive age (EuroHIV, 2003) (Figure 1.3).

Figure 1.3: HIV infections newly diagnosed by transmission group 1994-2003, Western Europe (Source: EuroHIV)
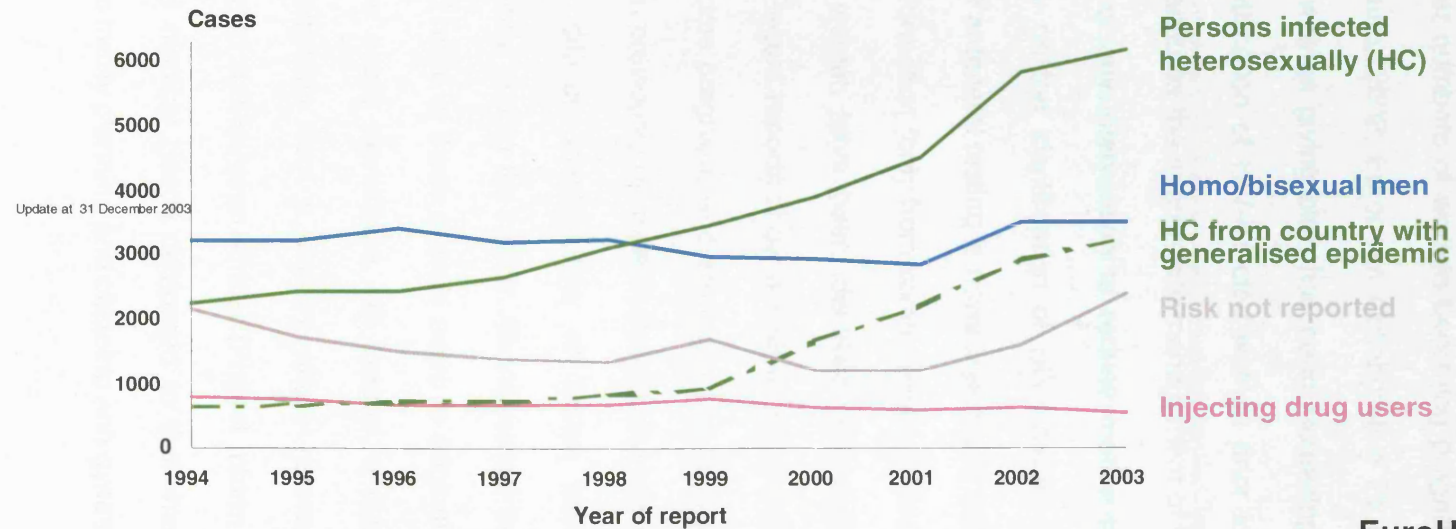
Belgium   Denmark, Finland, Germany, Greece, Iceland
Luxembourg, Norway, Sweden, Switzerland, United Kingdom

1.2   Epidemiology of HIV infection in women

Changes in the therapeutic management of HIV disease, in particular, the increasing use of combinations of at least three antiretroviral drugs since 1996/7, have resulted in a significant improvement in AIDS-free survival (Mocroft, 2003) with larger numbers of women becoming pregnant while on combination therapy (Dorenbaum, 2002; European Collaborative Study, 2005) The number of HIV-infected women giving birth has been increasing constantly in the past decade. The identification of HIV-infected women prior to or during pregnancy or before delivery ensures the appropriate management of the woman and her child and the success of any intervention to reduce mother-to-child transmission will depend ultimately on the identification of HIV-infected women during pregnancy. The uptake of antenatal testing is nowadays highly variable across Europe and there is partial information (only from some countries) available to quantify how many HIV-infected women have been identified during pregnancy (Figure 1.4) (EuroHIV, 2003). Recent reports show a shift in the characteristics of HIV-infected women who become pregnant, who are now most likely to be heterosexually infected and older than previously (European Collaborative Study 2005). This may lead both to a more difficult identification of these women through selective testing programmes, and to the higher risk of vertical transmission, despite the fact that more and more of these women seem to originate from African countries and are thus more easily identifiable. In Europe, a substantial number of HIV-infected women originate from a country where heterosexual transmission is frequent (mainly from sub-Saharan Africa) (Project Inform 2001; Fleischman 2004) having additional medical needs compared to their male counterparts, such as those relating to family planning and obstetric and gynaecological services.

Figure 1.4: HIV prevalence surveys and routine diagnostic testing among pregnant women: HIV prevalence per 10,000, WHO European Region, 2000 (Source: EuroHIV)
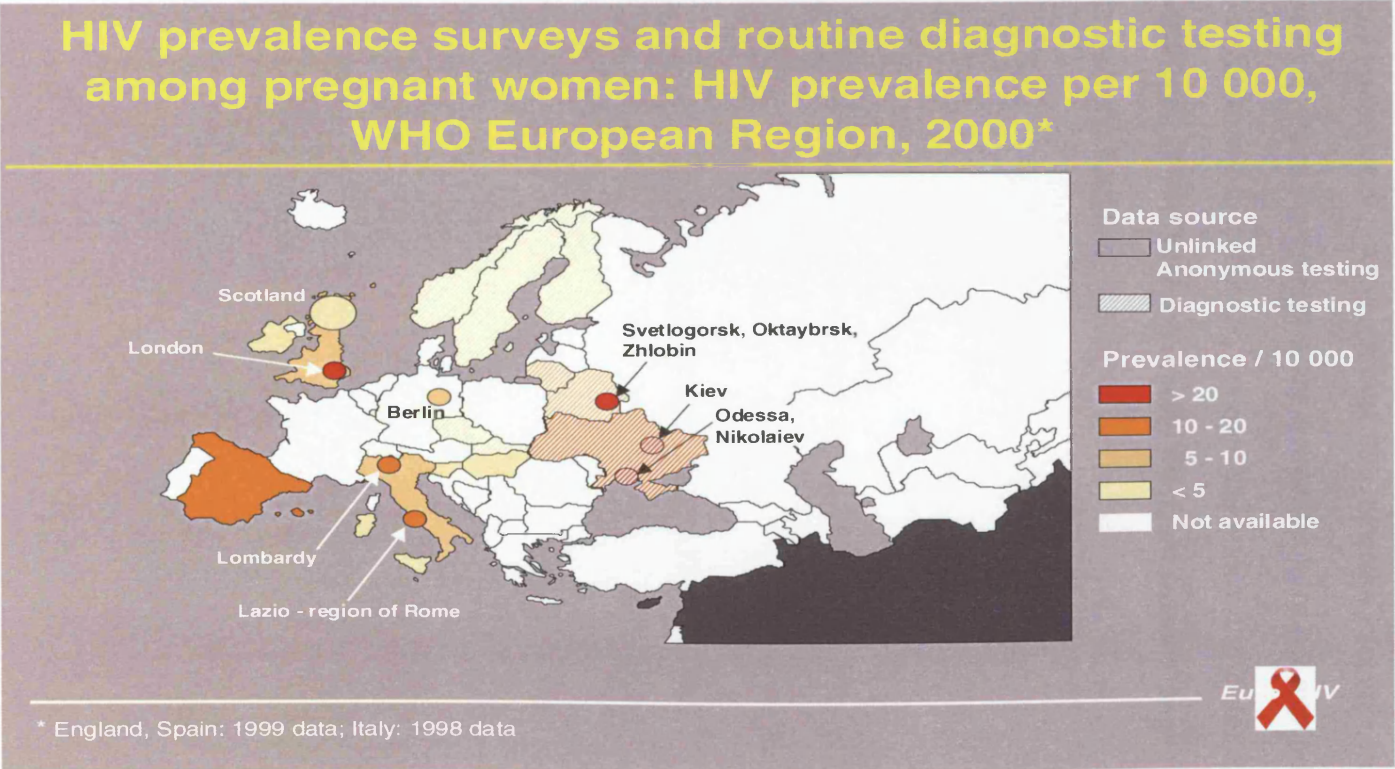
The use of interventions to reduce mother-to-child transmission on a population basis has been shown to have resulted in a decline in the number of new paediatric HIV infections in developed countries (Dorenbaum et al, 2002; Simpson et al, 2000; Mandelbrot et al 2001; Cooper et al, 2002) Despite this, nearly all the estimated 710,000 new HIV infections in children each year globally still result from mother-to-child transmission. Although over 90% of these infections occur in sub-Saharan countries where interventions are not available or not applied, a number of infants are still at risk of becoming congenitally infected in Europe, where the estimated prevalence of HIV infection among pregnant women ranges from 2.6 to less than 0.2 per 1000 livebirths.

1.3 HIV acquisition in women

Transmission of HIV infection takes place through three main routes: sexual contact with an infected person, parenteral exposure to infected blood or blood products, and vertical transmission from mother to child.

The demographic characteristics of those newly infected with HIV are changing, and the proportion of incident and prevalent cases that are in women is increasing. Twenty percent of HIV-infected individuals in North America and Europe are female (UNAIDS 2004).

Women could be biologically more vulnerable to heterosexual acquisition of HIV infection than men, and for a long time the rate of male-to-female transmission has been considered nearly double that of female-to-male transmission of HIV. The risk per single receptive intercourse appears to be approximately 0.2–1%, but there are many factors which may increase this risk (Nicolosi, 1994, De Vincenzi, 1994, Vernazza, 1999). Furthermore, some women at risk of acquisition of infection are unable to require consistent male condom use, which provides effective protection against sexually transmitted diseases, including HIV.

Among European women with HIV (new infections), sexual transmission constitutes 40% of reported cases (EuroHIV, 2003). However, AIDS cases are not indicative of current trends of HIV infection in the general population, especially now that widespread use of effective antiretroviral therapy has made HIV a more chronic disease; information on trends on of newly HIV infected individuals represents better the current status of the epidemic.

Parenteral transmission includes that in illicit drug users exposed to infected blood, recipients of contaminated blood transfusions or other blood products, such as factor VIII used by haemophiliacs and patients infected in health care settings where there is inadequate sterilization of injecting equipment. HIV can spread very

rapidly in illicit drug users, reflecting the efficiency of parenteral transmission. Information campaigns and needle exchanges have been relatively successful in Western Europe in reducing the infection rate through this mode, as evidenced by reports of stabilisation or even declines in the incidence of infection among IDUs. However, the increasing incidence of HIV infection among IDUs in Eastern Europe, especially Poland and the Ukraine, is of concern (UNAIDS, 2004). Many HIV-infected female IDUs also have sexual risk factors for HIV acquisition, including IDU sexual partners and the exchange of sex for drugs or money. It is therefore difficult to determine by which route women with high risk injecting and sexual behaviours acquired their HIV infection.

Vertical transmission will be discussed in Chapter 2. Other routes of transmission are of theoretical concern in the household setting, where close contact may occur between parent and child, or siblings, although epidemiological evidence for horizontal transmission within families is lacking (Shuster, 2005).

1.4 Factors facilitating HIV acquisition

Transmission of HIV infection can be influenced by several factors, including the quantity and infectivity of the virus, and the characteristics of the HIV-infected host.

There is an association between the quantity of virus transmitted and the risk of acquiring HIV infection (Roques, 1993; Royce, 1997). Several studies have found that HIV-infected persons may be more likely to transmit the infection when viral replication is high, both during the initial stage of infection (Palasanthiran, 1993) and at more advanced stages of HIV disease (Dyer 1998). People with high blood viral load are more likely to transmit HIV to recipients of blood, their sexual partners, and their offspring (Quinn, 2000; Vernazza, 1999). HIV has been quantified in semen (Coombs, 1998; Speck, 1999; Vernazza, 1997) and detected in female genital secretions (Ghys, 1997; Mostad, 1998), and virus in these

locations may facilitate transmission. However, the association between infectivity and disease stage is not absolute; HIV-infected women may transmit virus to a first-born child but not to a second-born child (De Martino, 1991), and temporal studies of semen from HIV-infected men demonstrate fluctuating viral titers over time. Viral load can be low in plasma but high in semen or genital secretions, explaining the possibility of transmission from mothers with undetetectable viral load to the fetus.

Factors that decrease viral titers, including antiretroviral therapy, may decrease but not eliminate the risk of HIV transmission (Kovacs, 2001; Fiore, 2003; Garcia-Bujalance, 2004). Individuals receiving antiretroviral therapy have also shown reduced rates of HIV transmission to their sex partners (Musicco, 1994). Several studies have suggested that antiretroviral treatment reduces detection of HIV in female genital secretions (Cu Uvin, 1998) and the concentration of HIV in semen (Gilliam, 1997; Gupta, 1997), but most data are on the effect of antiretroviral therapy on plasma viral load.

Factors that increase the risk of exposure to blood, such as genital ulcer disease (Serwadda, 2003; Plummer, 1991), trauma during sexual contact, and menstruation of an HIV-infected woman during sexual contact (European Study Group, 1992; Kalichman, 2004) may all increase the risk of transmission.

The method of contraception also affects the likelihood of HIV transmission (Plummer, 1997). There is overwhelming evidence that the correct and consistent use of latex condoms protects both men and women against HIV. However, because of methodological difficulties in studies of contraceptive use and HIV transmission, it remains unclear whether the use of hormonal contraceptives, IDUs, and spermicides alters the risk of HIV transmission.

Similarly, characteristics of the uninfected individual may increase the likelihood of infection for a given exposure to HIV. There is increasing evidence that genetic or immunological factors of the host may protect against HIV infection. For example, individuals who are homozygous for a nullallele of CCR5 are relatively resistant to sexually transmitted infection with HIV, indicating an important, though not absolute, rôle for this receptor in viral transmission (Lucotte, 1998).

1.5 Natural history of HIV infection

HIV is a retrovirus belonging to the lentivirus family and was initially known as lymphadenopathy-associated virus (LAV) and human T-cell lymphotropic virus type III (HTLV-III). REF Cells expressing the surface antigen complex CD4, the main receptor for HIV, are the major targets for the virus, the most important being the central regulatory cells of the immune system, the T-helper lymphocytes (CD4 cells). HIV enters the target cells and integrates into the nucleus following reverse transcription of viral RNA to DNA. Viral replication may then take place, although some infected cells remain in a latent state of infection and continue to circulate in the body as viral reservoirs.

Cell death in a variety of human cell lines results from HIV infection. T-helper lymphocytes (also known as CD4 cells) are a major target of viral infection, and circulating CD4 cells are killed off in peripheral blood in the majority of infected individuals not taking antiretroviral therapy. Severe CD4 cell depletion is not common in individuals free of HIV infection and is usually associated with severe illnesses, for example chemotherapy-induced leukopenia (Aldrich, 2000).

Individuals infected with HIV who do not receive antiretroviral therapy suffer immunological damage and loss of an effective immune response to specific opportunistic pathogens and tumours and will ultimately become symptomatic of, and be classified with, AIDS. There is a classification process that is used to

standardize clinical evaluation, clinical interventions and therapies. In the developed world, the system for classifying HIV infection and AIDS which is most commonly used is that published by the United States Centers for Disease Control and Prevention in 1993 (CDC, 1993).

Table 1.1: The 1993 Revised CDC Classification System for HIV Infection			
	Clinical categories*		
	A	B	C
CD4+ T cell categories (cells/cu mm)	Asymptomatic, acute (primary) HIV or PGL**	Symptomatic, not A or C	AIDS Indicator
(1) >=500	A1	B1	C1
(2) 200-499	A2	B2	C2
(3) <200	A3	B3	C3
* Persons in categories A3, B3, C1, C2, and C3 have AIDS under the 1993 surveillance case definition.			
** PGL = persistent generalized lymphadenopathy. Clinical Category A includes acute (primary) HIV infection.			

A large number of laboratory tests have been evaluated as prognostic indicators for disease progression in HIV infection. Generally, tests can be divided into three main groups: (i) measures of HIV replication (specific to HIV infection), (ii) measures of immune function (relatively specific to HIV infection), and (iii) measures of inflammation (generally not specific to HIV infection). HIV RNA quantification, performed on fresh or frozen plasma or serum, is a powerful and accurate prognostic indicator for progression of HIV infection and is uniquely useful in determining response to antiretroviral therapy (Saag, 1996). In general, the best measures of prognosis and staging include combinations of HIV RNA level and CD4 cell count. (Vlahov, 1998; Cozzi-Lepri, 1998, CDC, 2001). The use of a combination of prognostic indices can enhance the accuracy of the prediction.

1.6 Gender effect

For some years there has been debate over a potential gender effect on the natural history of HIV and patient outcome, especially after starting antiretroviral therapy. However, although in the majority of cases gender has not been found to be associated with outcome (Nicastri, 2005; Mocroft, 2003), in the pre-HAART era some studies suggested possible advantages for male patients (Lundreg, 1994; Reeves, 1998; Farzadedgan, 1998; Anastos, 2000), though others suggested that women might do better (Prins, 1999; Buira, 1992;Junghans, 1999; Evans, 1997).

The impact of gender on the manifestations and progression of HIV disease is still being investigated. Initially, it appeared that women progressed more rapidly than men, presenting with a different spectrum of opportunistic conditions. However, when controlling for the tendency of women to have reduced access to healthcare resulting in more advanced disease at HIV diagnosis, gender-based differences appeared to be less marked (Chaissson, 1995, Cozzi-Lepri, 1994). Better measures of viral activity, though, have once again raised the issue of the effect of gender on the progression of the disease. This has resulted in a renewed interest on the impact of gender, hormones, and demographic factors on the progression of HIV infection.

In general, the predictors of the rate of HIV disease progression and survival among women are the same as in men. CD4 cell count depletion and higher HIV RNA level are strong predictors of progression and survival in women. Recent results suggest that RNA levels can be 30% lower in women than men in the same CD4 cell count category. Similar results occurred in analyses restricted to people with a primary infection or when HIV culture was used to quantify cell-associated infectious units rather than RNA assays for viremia (Sterling, 1999). Generally, lower levels of circulating HIV RNA suggest lower steady-state levels of cell-associated infectious unit, and should be associated with better outcome.

However, several recent studies suggest that the lower HIV RNA level does not provide benefit to women. Comparing men and women with a similar HIV viremia, women experienced more rapid CD4 cell depletion and faster progression to AIDS and death, even when race and age are taken into consideration (Farzadegan, 1998). In vertically infected children there are distinctive differences by gender, which are however less marked than those by race (ECS, 2004). In most studies women have shorter duration of infection prior to AIDS and death than men, but these differences tend to disappear when CD4 cell count and drug use are taken into consideration (Alioum, 1998; Santoro-Lopes, 1998).

1.7 The HAART Era

A significant decline in mortality has been noted in countries that are able to provide highly active antiretroviral therapy (HAART) (Mocroft, 2003; Palella, 1998; Egger, 1997; Mocroft, 2000). The death rate across Europe dropped rapidly, and within 2 years of the widespread availability of HAART, the number of deaths was less than a fifth of those before HAART. This followed the results of a multitude of clinical trials that demonstrated the clinical and virological benefits of HAART (Bartlett, 1996; Collier, 1996; Hammer, 1997).

The initial success associated with HAART might not have continued, and high levels of treatment failure have been reported, associated mainly with serious adverse events, emergence of drug resistance, difficulties in maintenance of long-term adherence, and the few types of drugs available. Adherence to medication regimens, bioavailability of medications and inadequate dosing can trigger the emergence of antiretroviral resistance (Hirsch, 2003). In addition to drug resistance, clinical treatment failure is also associated with the rapidity of progression of the disease. The introduction and continued use of HAART over the past six years has resulted in very low morbidity and mortality rates across Europe, suggesting that limitations of current treatment, including potential adverse

effects of long-term HAART and problems with compliance, have not yet affected the clinical success of HAART in the population (Mocroft, 2003).

The continued fall in morbidity and mortality in the late-HAART treatment era could relate to increased experience of treatment with HAART, better understanding and management of complicated drug regimens and toxicities, and of the rôle of drug resistance. Additionally, the availability of new drugs such as efavirenz, abacavir, and kaletra, and use of HAART without previous antiretroviral treatment (i.e., treatment naive), or with antiretrovirals not previously given, might all have contributed to improved long-term adherence and subsequent survival.

Nonetheless, clinical progression continues to occur among recipients of HAART, particularly among persons who received antiretroviral treatment before initiation of HAART (Ledergerber, 1999), which is associated with drug resistance.

The reductions in morbidity and gains in survival in HIV patients that have been demonstrated in many developed countries do not extend to developing countries in which the majority of HIV cases worldwide occur. It is in resource-poor countries where efforts still need to be made to make antiretroviral therapy widely available and to provide continuity of care and resources for assessment of drug efficacy and tolerance.

1.8 Key points

- Health related lifestyle changes and use of antibiotics for the cure of sexually transmitted disease have changed the perception of sexual risk over the last century, decreasing the risk of adverse pregnancy outcome.

- It was in the 1970s that wider dissemination of HIV occurred with the first cases of AIDS appearing in the 1980s.

- Nearly 37 million people world-wide are estimated to living with HIV infected with HIV, of whom about 17.6 million are women; out of 610 thousand people living in Central and Western Europe, 160 thousand are women.

- Globally, there are currently two HIV-infected women for every three infected men but it is expected that the number of new infections among women will equal those among men shortly.

- Women who become pregnant, are now most likely heterosexually infected and older than was previously the case.

- Changes in the therapeutic management of HIV disease has resulted in a significant improvement in AIDS-free survival, with increasing numbers of women becoming pregnant while on combination therapy.

- Cell death in a variety of human cell lines results from HIV infection and a large number of laboratory tests have been evaluated as prognostic indicators in HIV infection.

Chapter 2 Women and HIV infection: reproductive health, childbearing choices and outcome

"Life is a fatal sexually transmitted disease!"

Source: Prof RV Short, Dept Of Obstetrics and Gynaecology, Prof Of Zoology; "Reproduction in Mammals, Fertility, AIDS and HIV"

Royal Women's Hospital, Monash University of Melbourne, 1997.

2.1 Introduction

Good standards of reproductive health enable women to practise and enjoy sexual relationships in safety, to regulate their own fertility, reproduce safely and to bring pregnancies to a successful outcome REFS. In order for this to occur, women require a health infrastructure that enables access to safe contraceptives, obstetrical, ante-natal and post-natal care, abortion, and appropriate services to prevent and treat diseases of the reproductive system. HIV-infected women have special needs in sexual and reproductive health, including information and services to protect their own health as well as to reduce the risk of mother-to-child HIV transmission.

Reproductive counselling to individuals with HIV might motivate them to ask for reproductive care in order to limit the risk of infection for their partners, to limit the risk of toxicity for the fetus and to reduce the vertical transmission rate. The best way to avoid perinatal transmission of HIV is the protection of women from infection.

2.2 HIV and Reproduction

Results from studies in Africa, as well as in developed countries, have suggested that HIV may have an adverse effect on fertility in both HIV symptomatic and asymptomatic women (Gregson, 1999; Blair, 2004; Degrees, 1997). However, some European reports suggest that the incidence of pregnancy decreased with HIV progression but that pregnancies after HIV diagnosis appear to be related largely to social and cultural attitudes (van Benthem, 2000). In a cross-sectional study in Uganda, the likelihood of a recognized pregnancy was lower in HIV-infected women than in HIV-uninfected women and lowest in women who were symptomatic from HIV or were coinfected with syphilis (Gray, 1998) A prospective study in South Africa found that pregnancy rates were 12% lower in HIV infected

women and that HIV could only explain a small part of the fertility decline (Camlin, 2004).

On the other hand, in a US prospective cohort study, women with HIV were more likely to conceive than at-risk uninfected women, but pregnancy outcomes were similar (Massad, 2004). With the widespread use of HAART there are anecdotal reports of HIV-infected women living in Europe more likely to want to become pregnant (Watts, 2004). The potential increase in pregnancy rates during the era of widespread use of highly active antiretroviral therapy highlights the need for prevention treatment (Blair, 2004). HAART can be very effective in terms of prolonged life and quality of life, but it may also have effects on fertility and fecundability. The use of combination therapy can affect the desire for parenthood, either impacting on the biological ability and/or on the personal desire of maternity. In the HIV-infected population, as in the general population, there are a number of other factors that have an impact of the desire of maternity, for example parity or ethnicity. In a recent report, pregnancy rates were compared before and after HIV diagnosis by geographical origin (sub-Saharan Africa versus European) among 533 HIV-infected women in France (Forquet, 2001). Among women of African origin, pregnancy incidence and birth rates increased after HIV diagnosis, in women with less than two live births at diagnosis. Among women of European origin, the incidence of pregnancy and terminations after HIV diagnosis were three times lower than in women of African origin. These results suggest that fertility plans reflect culture rather than knowledge of HIV diagnosis.

Most studies to date examining the impact of pregnancy on HIV disease have been small but have not shown significant differences in HIV progression or survival between pregnant women and non-pregnant women with HIV infection (Brettle, 1995; French, 1998). A meta-analysis of seven prospective cohort studies found no overall significant differences in death, HIV disease progression, progression to

an AIDS-defining illness, or fall in CD4 count to below 200/mm^3 between cases and controls (French 1998). Repeat pregnancies do not appear to have a significant effect on the course of HIV disease (Minkoff, 2003). Overall progression rates of HIV disease are similar in men and women, suggesting indirectly a limited effect of pregnancy on HIV progression.

2.3   HIV and gynaecological problems

HIV-infected women commonly suffer from gynaecological problems but insufficient data have been collected and analyzed in order to establish whether they are significantly different from the general population. It has been reported that women infected with the human immunodeficiency virus are significantly more likely to have prevalent and incident gynecologic disorders but not disorders related to risk taking (sexually transmitted diseases). The latter disorders increased in women with CD4(+) cell counts >500 cells/mm3 (Minkoff, 1999). It has also been reported that only 9% of 67 women were admitted to an inpatient AIDS service with a primary gynaecological problem, but 83% had coexisting gynaecological disease when evaluated (Frankel, 1997).

Although there have been few epidemiological observations on gynaecological problems in HIV-infected women, we can assume that certain symptoms can be directly related to HIV disease, others to HIV drug treatment and others still can be specifically related to the woman – irrespective of her HIV status. We can expect that some sexually transmitted infections may be more present in this group of women who acquired HIV in a heterosexual context (Lurie 2003).

HPV infection and cervical dysplasia are more common in HIV-1–infected women than in other women (Duerr, 2000; Palesky, 1999; Cu-Uvin, 1999; Massad, 2001). Concomitant genital infections are common in HIV-1–infected women, and their effects on the natural history of HPV infection have not been well studied. Bacterial

vaginosis were present in 42% of HIV-1 infected women at the time of enrollment into the Women's Interagency HIV Study (Greenblatt, 1999), and appear to be more persistent in HIV-1infected women than in other women (Jamieson, 2001). Given the higher prevalence of HPV infection and frequent concomitant genital infections in HIV-1infected women than in other women, any potential associations between HPV infection and other genital infections could be accentuated, especially in women with severe immunosuppression (Watts, 2005)

Gynaecological problems are directly related to reproductive health because most of HIV-infected women are in their prime reproductive years. With the widespread use of antiretroviral therapy, which has a positive impact on life expectancy and quality of life, these problems will be ever more present. It is likely that antiretroviral therapies might impact on the functionality of the reproductive system – directly causing menstrual irregularities or ovarian dysfunction (Clark, 2001).

2.4 Contraception

To date only condoms have been shown to reduce the risk of transmission of HIV. The options to avoid a pregnancy available to HIV-infected women are no different from those available to non-infected women, which are condoms, hormonal contraception, intrauterine devices and spermicides. After condoms, hormonal contraception is the second most commonly used contraceptive method; there is, however, some concern that its use may be associated with an increased risk of HIV acquisition.

Hormonal contraception can interact with antiretroviral medication resulting in either a decreased efficacy of the contraception or increased or decreased concentrations of the coadministered drug (CDC, 1998). Much evidence exists that that users of hormonal contraceptives are at increased risk of genital tract shedding either directly or indirectly through increasing the risk of cervical ectopy (Wang,

2004; Mostad, 1998; Mostad, 2000). Indeed, we hypothesize that epithelial changes secondary to the use of hormonal contraception may increase susceptibility and vulnerability to HIV (Mostad, 1998; Plummer 1998).

The use of intrauterine devices has been shown to increase the risk of HIV infection and to be associated with a series of side effects that exist amongst HIV-uninfected women also. These side effects include increased menstrual duration and foreign body inflammatory reaction, which can, on their own, play an important rôle in increasing the risk of infection with HIV. A worsening of drug-induced anaemia, common in women taking HIV medication, can also result from these side effects.

In *in vitro* studies, spermicides have been shown to be able to kill HIV and other sexually transmitted agents/pathogens such as Chlamydia and gonorrhoea. Some studies have evaluated if regular use of spermicide may have a protective effect on HIV transmission. A UNAIDS trial raised concern that spermicide might not only not be protective but might increase the risk of HIV transmission (Altman, 2000). The use of spermicide is known to result in increased genital ulcers, which may act as an increased risk factor for HIV transmission.

The consistent use of condoms provides the best-known protection against sexual transmission of HIV and should be emphasized for all HIV-infected and at-risk women to decrease risk of HIV transmission/acquisition and transmission/acquisition of other STDs. Other barrier contraceptive methods provide limited STD protection and have not been shown to offer significant protection against HIV transmission. Some authors suggest a sizeable proportion of women do not use any form of contraception independent of their desire to conceive. This could be linked to their social environment – family pressure or less access to healthcare and lack of knowledge of the options available to them.

Existing studies are still inconclusive as to whether the use of hormonal contraception could be a safe option for preventing pregnancies in HIV-infected women.

2.5   Choices regarding childbearing

Historically, the medical community has considered HIV a serious barrier to reproduction.   In 1994 the Ethics Committee of the American Society for Reproductive Medicine expressed concern about potential transmission of the virus to an uninfected partner or to the couple's offspring. It suggested that physicians counsel couples about the option of adoption, or not having children (Ethics Committee of the ASRM, 1994).

In relation to HIV-discordant couples however, over the past 10 years the introduction of potent antiretroviral therapy has resulted in HIV-infected patients living longer, healthier lives.   Many health care providers and HIV-infected people now view HIV infection as a manageable chronic illness and seek to maintain normal lives. This would likely include a 'normal' desire for children.

The desire by HIV-infected women to conceive has not been a topic of research for a long time. Similar to the general population, HIV-affected couples desire to have children.   Chen found that 28% to 29% of 1421 HIV-infected adults surveyed wanted to have children sometime in their lives (Chen, 2001). In a retrospective study, HIV-infected subjects cited raising children as a way to give purpose to life. In addition, many HIV-infected women reported pregnancy and childbirth as a way to regain their sense of womanhood and sexuality, often making childbearing a high personal priority.   In view of these data, health care professionals now balance concerns about the risks of HIV transmission with the patients' desire to have children.

Also, interventions including antiretroviral therapy, elective caesarean section and refraining from breast feeding, have dramatically decreased the risk of vertical transmission to 2% or less (Dorenbaum, 2002; Simpson, 1997; Mandelbrot, 2001;Cooper, 2002). As a consequence of these changes in the management of HIV infection, the CDC amended its previous recommendations in 2001, bringing to the forefront the importance of reproductive issues, stating that HIV-infected pregnant women should receive information about all reproductive options and that reproductive counselling should be nondirective and supportive of the patient's decision (CDC, 2001).

Medical and social conditions of couples infected with HIV vary widely. A serodiscordant couple is defined as one HIV-infected and one HIV-uninfected partner. A seroconcordant couple is defined by two HIV-infected partners. Serodiscordant couples face many obstacles to considering reproduction, specifically the risk of HIV transmission between partners and transmission to the child. Men and women are each at risk of transmission from their HIV-infected partners. Studies indicate that although the risk of heterosexual transmission is relatively low, rate of male-to-female transmission per contact is significantly greater (0.001) than the risk of female-to-male transmission (<0.001). These probabilities increase with higher plasma HIV-1 RNA levels in the HIV-infected partner and the presence of other sexually transmitted diseases in either partner. A meta-analysis conducted by Davis and Weller in 1999 reported 0.9 seroconversions per 100 person-years of observation among participants who reported always using condoms, compared with a seroconversion rate of 6.7 per 100 person-years of observation among those who reported never using condoms. This analysis suggests that consistent use of condoms provides an 85% reduction in HIV transmission risk, compared with no use of condoms (Davis, 1999). Eighty percent of HIV affected couples surveyed that had previously conceived had

engaged in unprotected intercourse to achieve pregnancy. Despite transmission risks with unprotected sexual intercourse, HIV-discordant couples are often willing to practice unsafe sexual intercourse in order to conceive (Mandelbrot, 1997). Cusick and Rhodes found that HIV-affected couples associated condom use with the "early stages" of a relationship, and that encouraging continued safer sex seemed to threaten love and intimacy as the relationship matured (Rhodes, 2002). Reports from interviews with 60 people with HIV and 40 of their caregivers suggested that HIV-infected individuals thought seeking a seroconcordant partner was an acceptable alternative to celibacy However, the threat of horizontal and vertical transmission of a drug-resistant strain of HIV still necessitates safe sex.

Some research suggests that the use of assisted reproductive techniques decreases the chance of sexual transmission to almost negligible rates, and thus provides HIV-infected couples with the possibility of a pregnancy while minimizing the number of "unprotected" exposures necessary to conceive. Serodiscordant couples in which the woman is HIV-infected have significant concerns about HIV transmission to the child and transmission to the HIV-uninfected man. Artificial insemination and self-insemination can be used to decrease the risk of HIV transmission to the male partner. Autoinsemination is available to HIV-infected women as they can inseminate themselves with freshly ejaculated semen using a syringe (without the needle) or a disposable pipette.

Reproductive options are available to serodiscordant and seroconcordant couples and there is anecdotal evidence to suggest that HIV-infected women may now positively choose to become pregnant and that those who do become pregnant are less likely to have this pregnancy terminated, because their own disease is well managed and interventions to reduce the risk of vertical transmission are available.

2.6 HIV and Pregnancy

Pregnancy involves genetically disparate individuals and, in the context of HIV infection, the coexistence of an uninfected fetus in an infected mother. Furthermore, these two different individuals have different pharmacokinetic characteristics. Therefore, a pregnant woman infected with HIV cannot be simply considered an infected adult when planning her antiviral treatment.

The issue of antiretroviral treatment during the nine months of pregnancy should be put in the context of the long-term progression of HIV infection, in order to balance the maternal benefits with the exposure of the fetus to potentially toxic drugs for its entire prenatal life. As well as the risks and benefits for the mother and for the child, pharmacological treatment during pregnancy needs to be adjusted to the physiological modifications that take place in the pregnant woman. Many parameters influencing drug pharmacokinetics are modified during gestation, starting from the first weeks of amenorrhoea. There is a change in salivary pH, delayed gastric emptying and reduced bowel transit, marked increase of plasmatic mass and kidney filtration rate, decreased albumin and increased globulin concentrations. Both the trophoblast and fetal liver can metabolise drugs and amniotic fluid can also become a significant drug reservoir. The pharmacokinetic of drugs that have a short half-life, a narrow therapeutic window or are highly liposoluble (thus easily crossing the placental layers) can be significantly changed during pregnancy.

During pregnancy, cell-mediated immunity is reduced, with an accompanying decline in the T-cell helper-suppresser ratio. On the basis of case reports of women dying of AIDS in pregnancy or shortly after delivery and of the immunosuppressive effect of HIV, it was initially hypothesized that pregnancy in HIV-infected women could lead to an accelerated decline in CD4 cell count and to progression of disease.

However, prospective studies have found no convincing evidence that pregnancy accelerates the progression of HIV disease, while natural history studies have found gender effect for progression or overall survival, but not providing any evidence for the existence of a pregnancy effect on disease. Other studies have reported stable levels of HIV activity during pregnancy, in terms of viral load and CD4 cell percentage.

In both HIV-positive and HIV-negative women there is a decline in absolute CD4 cell counts in pregnancy, which is thought secondary to hemodilution; which is in line with the finding that the percentage of CD4 cells remains relatively stable. (European Collaborative Study, 1997; Miotti, 1992). When comparing changes in CD4 count/percentage over time, there is no difference between HIV-infected pregnant and non pregnant women (Tuomala, 1997), suggesting that pregnancy does not accelerate decline in CD4 cells. Adverse pregnancy outcomes may occur secondary to underlying disease processes (or their treatment), as well as for unknown reasons. Recent data suggest that HIV, especially when more advanced, may result in increases in certain pregnancy complications. Furthermore, concerns have been raised that antiretroviral treatment itself may increase some adverse outcomes in pregnancy (Brocklehurst, 1998).

2.7 Mother to child transmission

Mother-to-child, or vertical, transmission of HIV-1 can occur during pregnancy, around the time of delivery or postnatally, through breastfeeding. Without prophylactic interventions, including avoidance of breastfeeding, vertical transmission rates range from 15-40% (Working group on mother to child transmission 1995, ECS 1996). Maternal plasma HIV RNA level is the best individual predictor of MTCT risk, and other factors increasing risk include AIDS, primary infection, vaginal delivery, low CD4 count, and prematurity (Kuhn, 1999; European Collaborative Study, 1999; Mandelbrot, 1996; International Perinatal HIV

Group, 2001,Newell 1998,Thorne 2004). Breastfeeding is associated with an approximate doubling of overall MTCT risk, with the risk remaining as long as breastfeeding continues.(Nduati 2000).

In Western Europe where interventions to reduce mother to child transmission are nowadays available, including antiretroviral prophylaxis, elective caesarean section and refraining from breastfeeding, the risk of transmission of HIV from the mother to the fetus can be substantially reduced to less than 2% (Dorenbaum, 2002; Simpson 1997; Mandelbrot, 2001; Cooper, 2002). Table 2.1 illustrates the use of interventions for prevention of mother to child transmission in Europe since the beginning of the epidemic.

Table 2.1 **Interventions for the prevention of vertical transmission in Europe** (Thorne 2005)

Pre-1994	• No antiretroviral prophylaxis available for PMTCT • MTCT rates in Europe around 15-18% (Gabiano 1992, ECS ...)

1994	• Results of the ACTG 076 placebo-controlled clinical trial (Connor 1994)
	  - three-part regimen of zidovudine monotherapy
	  - antenatal oral zidovudine started at between 14 and 34 weeks gestation
	  - intravenous zidovudine during labour
	  - neonatal oral zidovudine for 6 weeks after delivery
	  - MTCT rate of 8.3% in the intervention arm versus 25.5% in the placebo arm at 18 months (no breastfeeding)

1994-1996	• Observational evidence for protective effect of elective caesarean section in MTCT (ECS 1994)
1997	• Rapid uptake of zidovudine monotherapy for PMTCT (Mayaux 1997) and elective caesarean section (ECS 1998)
1999	• HAART becomes the standard of care for adults requiring treatment, although at this time had only limited use in pregnancy in Europe
2002	• Results of the mode of delivery randomised trial (The European Mode of Delivery Collaboration 1999) and meta-analysis (The International Perinatal HIV group.1999) - elective CS before labour and before rupture of membranes is associated with a two-thirds reduction in MTCT risk

2002-2005	• Results of the PACTG 316 placebo-controlled trial (Dorenbaum A 2002)
	  - Single dose nevirapine intrapartum and to infant versus placebo in addition to non-study ART (antenatal and neonatal)
	  - MTCT rate of 1.4% in nevirapine arm versus 1.6% in placebo arm
	  - single dose nevirapine did not confer an additional protective effect in this population
	• European consensus guidelines on pregnancy and HIV infection updated (Newell 2002)
	
	• MTCT rates of less than 2% in non-breastfeeding populations (ECS 2005)
	• Antenatal HAART use widespread in Europe (ECS 2005)

It has been suggested that exposure to antiretroviral therapy in utero or early life could have an adverse effect on the infant in the medium- to long-term, but, although this effect is poorly quantified, it is likely to be relatively rare.

Since the mid 1990s, potent and effective antiretroviral therapy (HAART) to delay progression of disease in HIV-infected adults has become the standard of care. Such regimens are now usually applied before serious disease has developed, and an increasing number of HIV-infected adults are receiving complex antiretroviral regimens. There is a lack of information on the impact of antiretroviral therapy before, during and after pregnancy or the impact of caesarean section delivery on disease progression of HIV-infected women. The management of pregnancy in HIV-infected women, which allows for the optimum care of both woman and child, is becoming increasingly complicated.

Knowledge about timing and risk factors has determined approaches to interventions to reduce the risk of vertical transmission (Table 2.2). Based on indirect evidence it is now generally accepted that, in the absence of breastfeeding, about 75% of vertically infected infants acquire their infection around the time of delivery, while in breastfeeding populations this is about 50% (Rouzioux, 1995). Risk factors for vertical transmission include indicators of progression of maternal disease, such as viral load and clinical disease, and obstetrical and neonatal factors such as prematurity, mode of delivery and breastfeeding. Prophylactic antiretroviral therapy aims to reduce the viral load in the mother, which remains the stronger predictor of perinatal transmission, and thus prevent transmission. Elective caesarean section delivery avoids contact of the foetus with contaminated maternal secretions, microtransfusions between maternal ad fetal compartment and avoids possible transmission through ascending infections after rupture of membranes. Refraining from breastfeeding reduces exposure to potentially infectious milk. Approaches to reduce postnatal transmission in settings where bottle feeding is unacceptable

include early weaning and exclusive breastfeeding as well as antiretrovirals for brestfeeding mothers.

Table 2.2 MTCT risk factors in the HAART era based on 4500 mother-child pairs (Source: European Collaborative Study 2004)		
	Unadjusted Odds Ratio	**Adjusted OR (95% CI) n=1200**
Viral load: <1000 copies/ml ≥10,000	1.00 2.30	1.00 2.20 (1.19-4.07)
ART: None Mono/dual HAART	1.00 0.29 0.09	1.00 0.37 (0.18-0.78) 0.11 (0.03-0.40)
MoD: Vaginal Emergency CS Elective CS	1.00 0.48 0.24	1.00 0.53 (0.23-1.26) 0.18 (0.08-0.38)
Gestation: Term Premature	1.00 1.25	1.00 2.09 (1.18-3.69)

*multivariable analyses adjusting for RNA VL, ART, mode of delivery and gestational age at delivery

2.8 Prophylactic anti-retroviral therapy

After the positive results from the American-French trial ACTG076 (Connor 1994), which showed that zidovudine administered antenatally, during labour and neonatally reduced vertical transmission risk by over two-thirds in a non-breastfeeding population, trials were set up to investigate shorter, easier and cheaper zidovudine regimens to be effective in both breastfeeding and non-breastfeeding populations. Results from these trials confirm the substantial effect of zidovudine in reducing the risk of vertical transmission (Shaffer 1999; Guay 1999; Wade 1998). A recent issue is the fact that increasing numbers of HIV-infected women are becoming pregnant while on combination therapy for their own disease progression (Figure 2.1). Observational studies have confirmed a substantial reduction in vertical transmission risk associated with the combination regimens, emphasising in this case the dual nature of vertical transmission prophylaxis and optimal treatment of the mother's HIV infection.

Figure 2.1: Antenatal ART use (Source: European Collaborative Study 2004)
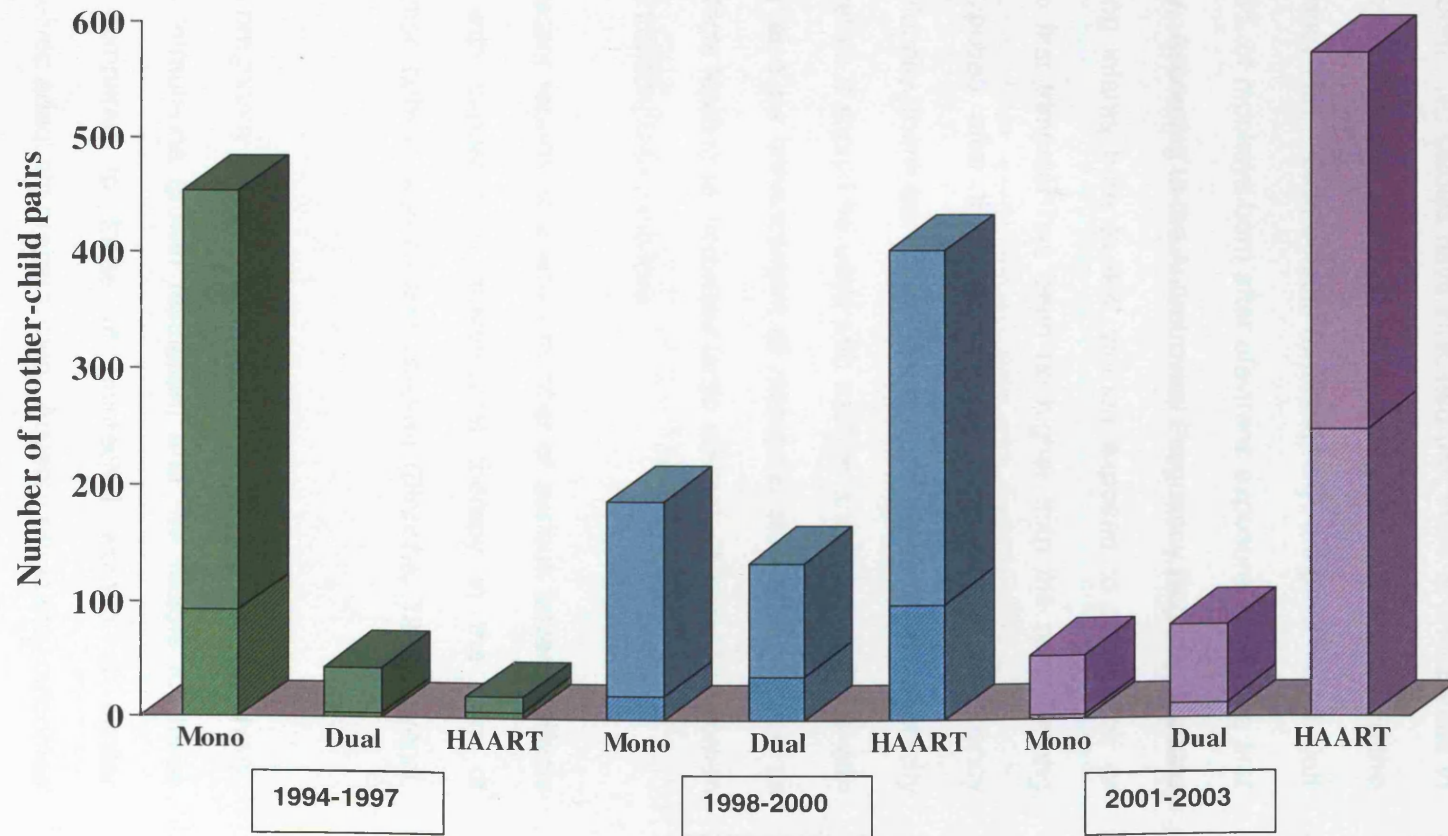
HIV-infected women may worry about the effects that antiretroviral drugs will have on the pregnancy and the child. Pregnancy requires unique considerations, including the possible need to alter dosage as a result of physiologic changes associated with pregnancy and the potential for adverse short or long-term effects on the fetus and newborn. No studies have indicated that there is an increase in birth defects related to HIV infection; however, efavirenz is not recommended in the early stages of pregnancy REF. Birth defects (anencephaly, anopthalmia, or cleft palate) occurred in 15% of monkeys born after efavirenz exposure during the first trimester of pregnancy. According to the Antiretroviral Pregnancy Registry, the rate of birth defects among infants born to 400 women exposed to zidovudine or lamivudine during the first trimester has been no higher than the rates among infants who were exposed after the first trimester (Antiretroviral Pregnancy Registry, 2004) Additionally, there are no well-controlled studies of teratogenicity using nevirapine, therefore it should be used with caution and in accordance with its label. There may also be enhancement of metabolic side effects, such as mitochondrial toxic effects leading to increased lactic acidosis in pregnant women on nucleoside reverse transcriptase inhibitors.

Of concern are the recent reports of a small number of serious adverse effects possibly associated with exposure to antiretroviral therapy in the form of mitochondrial dysfunction both in women and children (Blanche, 1999; Lorenzi, 1998).

High rates of adverse pregnancy outcome have been described (preterm delivery, low birth weight and intrauterine growth restriction) and risk factors for these adverse outcomes, compared to those of uninfected women of similar race/ethnicity who received adequate prenatal care. Adverse pregnancy outcomes have been associated with HIV disease stage, but in most of these studies, only a small percentage of women were receiving ART (Lambert, 2000). HIV-associated

risk factors may become less or more important in women receiving ART. In a recent analysis of European data it was shown that HIV-infected pregnant women treated with two or more anti-retroviral drugs are at a nearly three-fold increased risk of premature delivery compared to those on no or monotherapy (European Collaborative Study 2000). This association was especially pronounced when treatment included protease-inhibitors (PI) and when started before or in early pregnancy.

An increased rate of prematurity (<37 weeks) associated with use of PI was first reported in a report in 1998 from a Swiss study, of 30 PI-exposed infants, which also reported two cases of intracerebral haemorrhage and extrahepatic biliary atresia. The rate of prematurity in these 30 infants was significantly higher than that among infants exposed to zidovudine (33% versus 17%).

Data from two cohort studies were merged, the European Collaborative Study and the Swiss Mother+Child HIV Cohort Study, involving nearly 4000 mother-child pairs, and the use of antenatal antiretroviral therapy (with a PI) was associated with a 2.5 times greater risk of premature delivery compared with no treatment, after adjustment for maternal CD4 count and use of illicit drugs. A dose-response relationship was suggested by the finding that risk of premature delivery was greatest for those women who started HAART before pregnancy or during the first trimester of pregnancy.

Prematurity has not been identified as an adverse effect of antiretroviral therapy in pregnancy by several large studies, including a meta-analysis of over 3000 mother-child pairs enrolled in observational studies in the USA (The Perinatal Safety Review Working Group, 2000). An immunological mechanism may be behind the association between shorter duration of pregnancy and exposure to HAART (see Chapter 3).

2.9 Mode of delivery

A consistent body of evidence from observational studies (Dominguez, 2003; Rowland, 2001), a meta-analysis (The International Perinatal HIV Group, 1999) and a randomized trial (The European Mode of Delivery Collaboration, 1999) indicate that elective caesarean section before onset of labour and rupture of membranes significantly reduces the risk of transmission compared to both vaginal and emergency caesarean section deliveries.

Caesarean section avoids the passage of the fetus in the birth canal where HIV, present in cervical secretions, can infect the fetal ocular and buccal mucosas. When the procedure is performed before labour, it can also impede the passage of maternal blood in the fetal circulation through placental breaks occurring with contractions. According to these two possible ways of protection against HIV infection, caesareans can be divided into those performed prior to labour, which can interfere with both the bloodborne and the transmucosal routes of infection, and procedures carried out during labour when only the latter mechanism of fetal contamination can be avoided.

In the European mode of delivery trial a total of 436 women were randomized to either vaginal or caesarean section delivery. The trial results confirm findings from earlier observational studies that vaginal delivery is associated with a more than two-fold increased risk of vertical transmission of HIV infection, independent of the use of prophylactic antiretroviral therapy. The transmission rate in the trial was 1.8% in women allocated to caesarean section compared with 10.5% in those randomized to vaginal delivery.

It has been reported that the risk of post-partum complications in HIV-infected women may be higher than in uninfected women, especially after caesarean section (Semprini, 1995, Marcollet, 2002; Rodriguez 2001). In the light of the low

risk of vertical transmission associated with the use of antiretroviral therapy, the assessment of the risk of post-partum morbidity associated with elective caesarean section or vaginal delivery has gained in importance. It has recently been suggested that in women successfully treated with highly active antiretroviral therapy with a low (<1000 copies/ml) or undetectable viral load near the time of delivery the potential side effects of caesarean section delivery may no longer outweigh the benefits of reduced vertical transmission risk (ECS 1999; Ioannidis, 2001).

With increasing numbers of pregnant HIV-infected women receiving antiretroviral prophylaxis and having an elective caesarean section delivery, more information has become available on the interaction between these interventions. In the French Perinatal Cohort Study, multivariate analysis of 902 mother-child pairs receiving zidovudine prophylaxis indicated a five-fold reduction in transmission risk following elective caesarean section compared with vaginal delivery, with a vertical transmission rate in women exposed to both interventions of 0.8%. Similarly, the Swiss Neonatal HIV Study Group reported a 0% transmission rate in 31 infants born to women receiving both interventions, compared with 8% after elective caesarean section without zidovudine (7/86), 17% (4/24) after zidovudine alone and 20% (55/271) after no intervention.

2.10   Policy and practice

Although prevention of mother-to-child transmission of HIV is a success in developed country settings, with vertical transmission rates of less than 2% reported, programmes need a coherent approach to include the identification and monitoring of infected women from before pregnancy, as well as antenatal and post-natal management. As many European centres only see a few pregnant-infected women annually, and given the rapidly changing field of HIV therapy, consensus expert guidelines are important in guiding practice at a local level. The

European consensus on the management of pregnancy and HIV infection, stressed the need not only to offer, but also to recommend, antenatal HIV testing as an integral component of antenatal care (European Collaborative Study, 2003). The first step to effective prevention of mother-to-child transmission is identification of infected women.

Management of pregnant HIV-infected women, or those wishing to become pregnant, is predominantly multidisciplinary in approach. This allows for sharing of expertise so that the woman receives optimum care, regarding her own HIV disease, reducing vertical transmission risk and the pregnancy and delivery per se.

2.11   Key points

- Reproductive counselling to individuals infected with HIV might motivate them to ask for reproductive care.

- HIV may adverse affect fertility outcome but it does not appear to impact on HIV disease.

- The use of HAART is associated with an increase in pregnancy rates in HIV infected women as it may affect the desire of maternity due to its effectiveness in prolonging life and improving quality of life.

- ART may affect fertility and fecundability (biologic ability to become pregnant).

- Gynaecological problems are related to reproductive health because HIV-infected women are mostly in their reproductive years.

- Contraceptive options available to HIV-infected women are no different from those available to non-infected women.

- Desire to conceive in the HIV population is an important topic of research.

- In Western Europe where interventions are available the risk of vertical transmission is today less than two percent.

- There are reports of adverse events associated with exposure to ART, in particular premature delivery in women treated with HAART.

- Elective caesarean section significantly reduces the risk of mother to child transmission, but the risk of post partum complications needs evaluation in the HAART era.

Chapter 3 Immunological aspects of pregnancy in HIV-infected women

59

During a normal pregnancy there is an increase in the production of Type 2 cytokines, accompanied by a decrease in the production of Type 1 cytokines, resulting in a Type 1 to Type 2 shift. Indeed, it has been suggested that the absence of Type 2 cytokines may adversely affect pregnancy outcomes with an increased risk for example of spontaneous abortions. HAART induces a shift towards a Type1 environment and therefore, it is predictable that it affects this balance.

.

Why were you not rejected by your mother?

Source: Dr Gil More Reproductive Immunology, Yale University

Immunology of pregnancy: the Th1/Th2 paradigm

A key development in our understanding of immune responses has been the description of the T helper 1/T helper 2 paradigm (Th1/Th2) which helps explain how the immune system directs responses to different types of pathogens and stimuli. The two major subsets of CD4 T helper cells, Th1 and Th2, have different patterns of cytokine production and different rôles in immune responses. Each subset induces functions that are effective at handling certain types of pathogens, but can be ineffective, or even pathological if made in response to other types of pathogens (Mosmann, 1996, Coffman, 1999) (Figure 3.1). Th1 cells secrete IFN (interferon), TNF (Tumor-necrosis factor), and IL-2 (Interleukine 2). Some of these cytokines like TNFhave cytolytic effects contributing to cellular immunity and are generally considered to be Th1-like or Th1-associated cytokines (Yui J 1994). Th1-type cytokines activate macrophages and cell-mediated reactions important in resistance to infection with intracellular pathogens, and in cytotoxic and delayed-type hypersensitivity (DTH) reactions (Figure 3.2).

Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13; Th2-type cytokines encourage vigorous antibody production and are, therefore, commonly found in association with strong antibody responses that are important in combating infections with extracellular organisms. Th1 and Th2 cells are mutually inhibitory; IL-10, a product of Th2 cells, inhibits the development of Th1 cells by acting on antigen-presenting cells, whereas IFN, a product of Th1 cells, prevents the activation of Th2 cells. The CD4 Th-mediated response is certainly more than just Th1 and Th2, but these represent two extremely polarized forms that play important rôles in immune responses.

Other cytokine patterns also exist; Th3 cells are TGF(transforming growth factor) - secreting CD4 cells that do not secrete IL-2, IFN , IL-4 or IL-10 and appear to be a

unique T cell subset that includes the mucosal T helper function and the down-regulation of Th1 and other immune cells. Since several cell types besides T helper cells contribute to an overall Th1 or Th2 cytokine pattern, it has been suggested that these responses should instead be described as type 1 or type 2. Which type of reactivity, type 1 or type 2, is activated first may influence the subsequent outcome; if a particular T cell subset is activated first or preferentially in a response, it can suppress the development of the other subset. The overall effect is that certain responses are dominated by either humoral (type 2) or cell-mediated (type 1) immunity.

Figure 3.1: Humoral and cellular response (Source: Trabattoni & Clerici 2003)
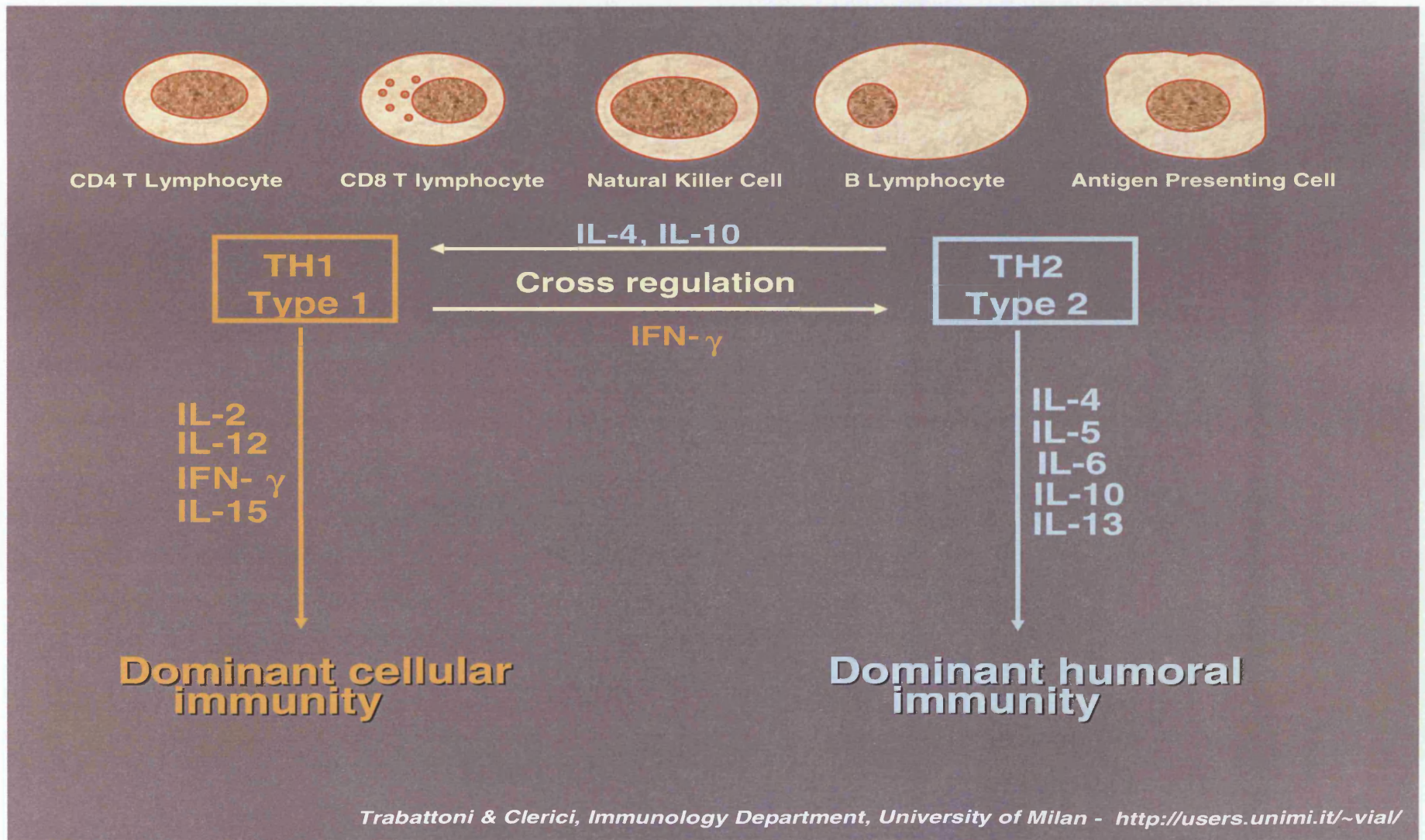
Figure 3.2: Cellular immune responses mediated by cytotoxic T lymphocytes (CTL) (Source: Trabattoni & Clerici 2003)
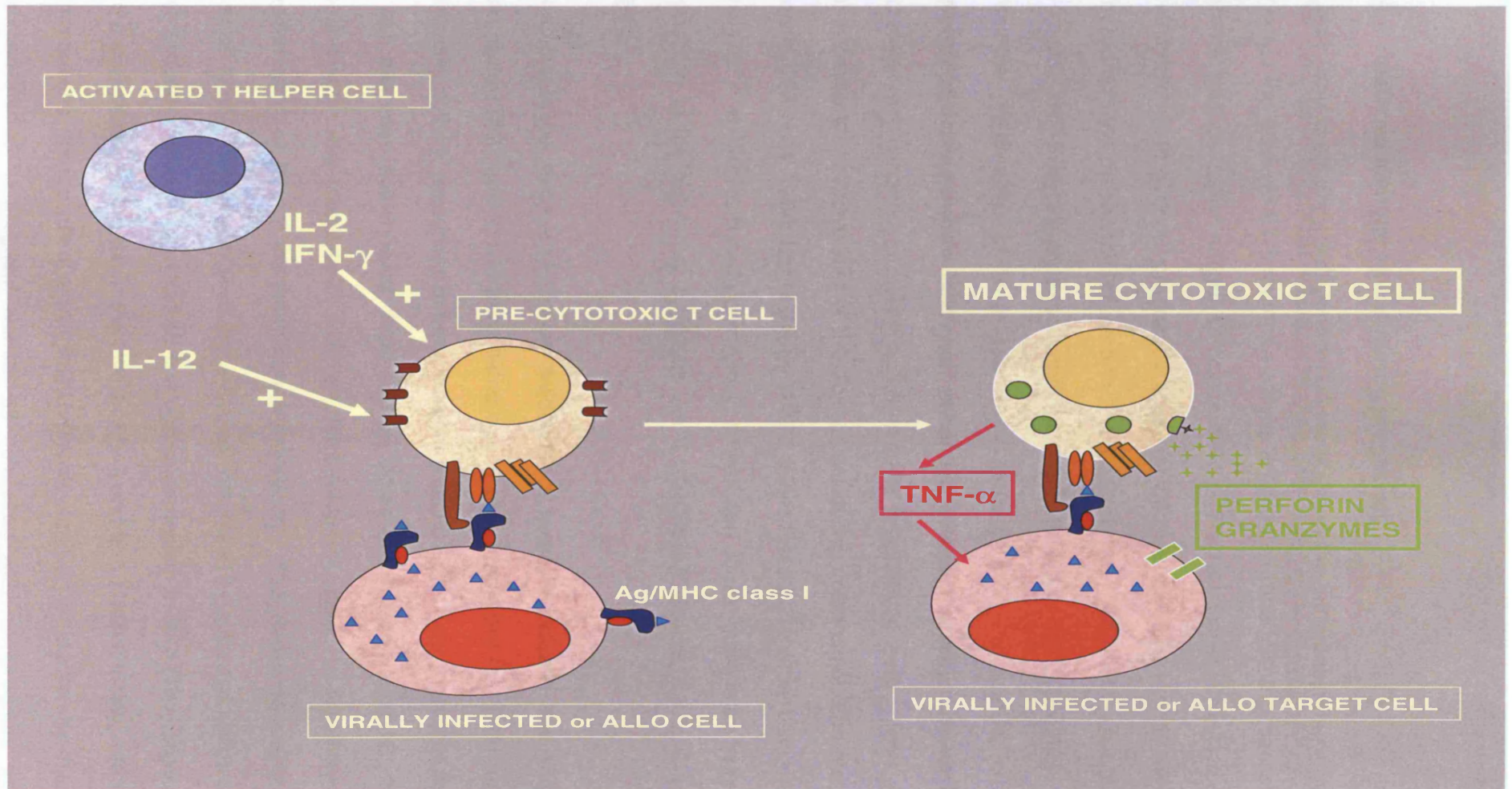
3.1 Maternal immune status during pregnancy

A successful pregnancy is dependent on the ability of the maternal immune system to retain the fetus, which immunologically is an allograft, due to the possession of paternal transplantation antigen (Piccinini, 1996). The feto-placental unit produces Th-2 cytokines throughout pregnancy; these inhibit maternal Th-1 activity, which in turn protects the pregnancy (Wegmann, 1993, Marzi, 1996). Maternal generation of IL-2, and TNF-β is directly associated with compromise of the pregnancy. IL-2 promotes proliferation of large granular lymphocytes in the uterus, creating lymphokine-activated killer cells with enhanced cytotoxic activity. *In vitro* IL-2 increases the lymphocyte ability to kill cultured trophoblast cells. This would compromise the invasiveness of the trophoblast during implantation and placental growth (Reinhard, 1998, Raghupathy, 1997). While there is rather less information available in relation to human pregnancy, it has been shown there is an increased production of the regulatory cytokines like IL-10 (Th2) (Chaouat, 1999, Chaouat, 1995).

Experiments on mice and clinical evidence in humans indicate that humoral responses (Th2, type 2) are potentiated during pregnancy, but that DTH, natural killer (NK) activity, responses to intracellular infections (Th1, type 1) and the course of cell mediated autoimmune disorders are ameliorated (Russell, 1997). These observations are consistent with a dampening of type 1 reactivity and augmentation of type 2 immunity during pregnancy. Pregnancy seems to shift towards type 2. Dudley showed that cytokine responses by activated lymphocytes from pregnant mice are marked by a progressive decline in IL-2 (Th1) production and a concomitant increase in the levels of IL-4 and IL-6 (Th2) (Dudley, 1993). In humans there are reports of significantly higher IL-10 (Th2) production by mitogen-activated PBMC in pregnant women as compared with non-pregnant women (Hanna, 2000). Using a sensitive on-line quantitative RTPCR (Reverse

transcription polymerase chain reaction) Kruse reported a significantly reduced IL-2 and IFN (Th1) mRNA expression during normal human pregnancy (Kruse, 2000). Type 1/type 2 ratios revealed a shift to a pronounced type 2 status. Using flow cytometric techniques on a single cell level significantly increased IL-4 (Th2)-producing CD4 and CD8 T cells were demonstrated in normal pregnant women as compared to non-pregnant women. In contrast, IFN - and IL-2-producing CD4 and CD8 T cells were significantly reduced in pregnancy, which is suggestive of a type 2 shift in pregnancy (Ragupathy, 1997). The Th-2 and T regulatory cytokines have additional critical functions in stimulating cell growth and differentiation. Thus there is a fine balance of cytokine production between mother, placenta, and the fetus, orchestrating a down-regulation of maternal immune responses to feto-paternal antigens, at the same time as encouraging normal fetal growth and immunological responsiveness.

3.2 Cytokines and pregnancy failure

The activation of some forms of maternal cellular immunity is potentially hazardous for fetal development. Indeed, pregnancy is not nearly as successful as one might think; the actual rate of early pregnancy loss after implantation may be as high as 31% (Hill, 1992). Spontaneous abortion, defined as a clinically detectable pregnancy loss prior to 20 weeks of gestation, is one of the most common complications of pregnancy with approximately one in every four pregnant women undergoing one or more pregnancy losses (Clark, 1999). About 40–60% of recurrent spontaneous abortions (RSA), defined as the occurrence of three or more pregnancy losses before the 20th week of gestation, are attributable to the so-called 'known' causes such as chromosomal anomalies, endocrinologic abnormalities, infections, anatomic problems and humoral factors, with as much as 60% relegated to 'unknown' or 'unexplained' etiology (Prigoshin, 2004).

The existence of such a large proportion of cases with unidentified etiologies has in part fuelled interest in the investigation of possible immunologic etiologies of pregnancy failure. Cellular immunity mediated by effector cells and/or their cytokines has been shown to have significant deleterious effects on the conceptus (Makhseed, 1999, Raghupathy, 2000). TNF, IFN and IL-2 (Th1) figure prominently among cytokines that are particularly detrimental to the survival of the conceptus. The administration of TNF, IFN and IL-2 into pregnant mice causes abortions while anti-TNF antibodies have been shown to reduce resorption rates in a murine model of natural, immunologically mediated abortion (Tangri, 1993). TNF and IFN inhibit outgrowth of human trophoblast cells *in vitro* and synergistically stimulate the programmed death of human primary villous trophoblast cells (Haimovici, 1991, Hill, 1995). *In vitro* stimulation of maternal spleen cells with placentas of mice prone to immunologically-mediated spontaneous fetal resorption results in the secretion of high levels of TNF, IFN and IL-2; these cytokines together fit the type 1 cytokine profile. It was not long before this intriguing association between type 1 cytokines and pregnancy failure was noticed that researchers observed interesting connections between successful pregnancy and type 2 immunity, leading to Wegmann and colleagues putting forward the hypothesis that the conceptus protects itself by secreting type 2 cytokines which down-regulate harmful type 1 cytokines (Wegmann, 1993).

Studies on animals have shown that the injection of type 1 cytokines results in abortions, thus a causal relationship has been established in animal models, while in human studies what we have available at present are indications of a similar correlation between the type of maternal immune reactivity and pregnancy outcome. However, studies on human pregnancy loss have not yet conclusively proven that type 1-reactivity can indeed be a cause of pregnancy failure. One study that comes closer towards a causal relationship is the one by Clerici's group

in which cytokine production was tested 1–2 weeks before any pathology could be detected (Marzi, 1996). Their study on 40 women with a normal pregnancy and five women with spontaneous abortions showed a decreased production of IL-4 and IL-10 (type 2) and increased production of IFN- and IL-2 (type 1) by antigen-stimulated PBMC in women with spontaneous abortions. Interestingly, the PBMC in this study were stimulated with influenza virus and with allogenic PBMC, implying that the type 2 bias seen in pregnancy may be generalized to any antigenic stimulus.

3.3   Type 1 immunity induction during pregnancy

Genetic alterations of molecular mechanisms have been shown to be directly involved in the expression of IL-4 or IL-12 and deficient regulation by Th1-inhibiting cytokines such as IL-4 or IL-10. (Mosmann, 1996, Coffman, 1999). Is it possible, then, that some women have a genetically determined tendency to produce higher levels of certain type 1 cytokines? The predominance of a given cytokine in the microenvironment at the time of antigen presentation is an important factor in driving naive CD4 T cells toward Th1- or Th2-dominated populations. IFN and IL-12 promote Th1 differentiation whereas IL-4 is the dominant factor in determining Th2 polarization. IL-12 is produced by several cell types, particularly by macrophages, in response to microbial products. Thus, certain infectious diseases, even sub-clinical ones, may result in enhanced IL-12 production causing an overall shift towards type 1. IFN, synthesized by NK cells in response to IL-12 and to viral infections, also enhances Th1 development. Authors refers to the conceptus as an 'innocent bystander' when the mother must combat an infection with a vigorous Th1- type proinflammatory cellular immune response (Clark, 1999). IL-12 may play a key Th1-inducing rôle in the cascade of events that provoke type 1 bias in pregnancy. Women with a history of recurrent abortion have been shown to have significantly elevated levels of IL-12 compared to women with a normal

pregnancy. IL-12 stimulates the release of IFN by decidual large granular lymphocytes and this effect is amplified by decidual macrophages (Marzusch, 1997).

Hormones may also contribute to the differentiation of Th cells or in favouring the shifting of already differentiated Th cells from one to another cytokine profile; for example, relaxin, a hormone produced by the corpus luteum, favours the development of human Th cells producing type 1 cytokines (Piccinni, 1996). A lack of suppression at the maternal–fetal interface may induce a type 1 shift (Michel, 1999). Supernatant fluids from decidual cultures were shown by Clark's group to inhibit lymphocyte proliferation in subsequent *in vitro* assays whereas supernatants from cultured abortion tissue had a reduced inhibitory capacity.

3.4 Mechanisms of type 1 immunity-mediated damage

The trophoblast is resistant to killing by cytotoxic T lymphocytes, conventional NK cells and conventional macrophages. At the same time increased plasma and uterine NK activity has been linked to abortion, and increased NK activity in the blood has been shown to be predictive of recurrent miscarriages. While direct cell-mediated lysis is unlikely to cause trophoblast damage, it has been proposed that NK cells, like activated Th1 cells, could release cytokines deleterious to the trophoblast. Direct effects of type 1 cytokines may include the apoptosis of trophoblast cells by TNF and IFN, inhibition of secretion of the growth stimulating GM-CSF from the uterine epithelium and perhaps most importantly the upregulation of procoagulant factors (Clark, 1998; Clark 1999).

It should be pointed out that in pregnancy some type 1 cytokines are not necessarily bad all the time; perhaps depending on their time of expression, stage of gestation and relative concentrations they may serve as saviours. TNF when administered systemically or when induced in the placenta as part of an anti-

parasite cellular response damages pregnancy, can also stimulate the programmed cell death of human trophoblasts (Krishnan, 1996; Yui, 1994) . However, TNF is produced by normal gestational tissues and has been proposed to be a pivotal factor in gestation (Hunt, 1996). We have to reconcile these seemingly disparate observations, keeping in mind that cytokines are pluripotent, their functions could depend on the relative concentrations of different cytokines, their activities could be mitigated by receptors and antagonists and they could have stage-dependent functions that may seem incongruent.

Cytokines, hormones and other molecules are likely to play critical rôles in directing the immune reactivity towards a type 2-bias and then maintaining it in that fashion. The Th2-inducing effect of IL-4 predominates over other cytokines, so that if IL-4 levels reach a necessary threshold, differentiation of the Th cells into the Th2 phenotype occurs. Recently IL-6 has been shown to polarize naive Th cells to effector Th2 cells by inducing the initial production of IL-4 in CD4 T cells IL-10 is probably one of the most critical cytokines responsible for maintaining a type 2-bias once established; it interferes with antigen presentation, down-regulates cytokine production by Th1 cells and directly or indirectly inhibits NK responses. Interestingly enough, the cytotrophoblast and syncytiotrophoblast have been shown to produce IL-10 preferentially in a gestational age-dependent manner (Hanna, 2000). Evidence for a possibly crucial rôle for IL-10 came from the studies of Chaouat in which the administration of IL-10 to abortion-prone mice was shown to reverse the high rate of fetal resorption in these mice (Chaout, 1995). Likewise, Rivera and colleagues showed that the increased uterine TNF, release of nitric oxide and apoptosis of uterine epithelia that followed administration of LPS were all normalized upon injection of IL-10 (Rivera, 1998). Concurrently IL-10 attenuated the LPS-induced fetal death rate and growth restriction, leading these researchers

to conclude that IL-10 produced in the placenta may play vital rôles in counteracting deleterious inflammatory cytokines.

Progesterone has been shown to favour the development of human T cells producing type 2 cytokines and Piccinni proposes that progesterone may therefore be responsible, at least in part, for a type 1/type 2 switch at the maternal–fetal interface (Piccinni, 1996). Progesterone has been shown to follow another interesting route towards influencing the Th1/Th2 balance: in an extensive series of studies Szekeres-Bartho and colleagues have demonstrated that in the presence of progesterone, lymphocytes from pregnant females produce an immunomodulatory protein, the progesterone-induced blocking factor (PIBF) which inhibits several Th1-type responses *in vitro* and prevents resorptions induced by transfer of spleen cells with high NK activity (Szekeres-Bartho, 1990). In the presence of PIBF, murine splenocytes activated with mitogen produce significantly higher levels of IL-10 and IL-4, bringing about a Th1/Th2 shift. In pregnancy, priming and maturation of T cells occurs in an environment that is gradually progesterone-enriched (Szekeres-Bartho, 1996). This would result in T-cell priming, activation and differentiation in the presence of lower concentrations of IL-2 and IFN and higher concentrations of IL-4 and IL-10, an environment that has been shown to favour the development of type 2 T cells.

Progesterone and relaxin appear to be hormones with opposite functions but produced by the same tissue (Szekeres-Bartho,1992). Predominance of progesterone at the beginning of pregnancy is needed to orientate the local immune response towards a type 2 pattern, with type 2 cytokines down-regulating a potentially harmful type 1 response and preventing premature fetal rejection. On the other hand, relaxin may be useful in maintaining and restoring a Th1-oriented response that is required for delivery (Sherwood, 1994). An inappropriate local imbalance in the concentration of the two hormones in favour of relaxin in early

gestation might impair the type 2 shift. Data emerging from several laboratories are beginning to indicate that type 1 cytokines along with a few others are probably also involved in causing pregnancy complications of other kinds — pre-eclampsia or preterm labour (Arosio, 2004; Madazli ,2003; El-Shazly, 2004).

3.5 Cytokines and HIV

During HIV infection a shift from Type 1 to Type 2, even if not antigen specific, indicates progression of HIV disease (Shearer, 1998, Clerici, 2000; Clerici, 2002) (Figure 3.3), and HIV therapy specifically aims to reverse this shift. It has been suggested that an imbalance in the Th1-type and Th2-type responses contributes to the immune dysregulation associated with HIV infection, and resistance to HIV infection and/or progression to AIDS is dependent on a Th1-->Th2 dominance In support of this hypothesis, it has been reported that progression to AIDS is characterized by loss of IL-2 and IFN-gamma production concomitant with increases in IL-4 and IL-10 (Vasilescu, 2003).

Biological and molecular studies of HIV-1 have demonstrated that HIV is highly heterogeneous. In particular, HIV primary isolates display *in vitro* distinct biological properties, amongst which the rate of viral replication (slow/low or rapid/high), and ability to induce a cytopathic effect (nonsyncytium-inducing or syncytium-inducing) differentiate diverse viral strains. The prevalence and the emergence of distinct phenotypic variants appear to be correlated with different stages of infection. Thus, the asymptomatic phase is characterized by the inability to isolate HIV in culture or by the presence of slowly replicating nonsyncytium-inducing (NSI) isolates. In contrast, highly replicating syncytium-inducing (SI) variants emerge in 50% to 60% of HIV-infected individuals in the progression of HIV infection; the emergence of these viral variants is related to rapid CD4 T-cell depletion and progression to AIDS. Progression of HIV infection is also associated with impairment of cell-mediated immunity (CMI), which has both *in vivo* and *in vitro* correlates. Thus, the

inability to develop delayed type hypersensitivity reactions (DTH) to ubiquitous antigens precedes and heralds the development of opportunistic infections. Analogously, defective production of the CMI-inducing cytokines interleukin IL-2, IL-12, and gamma interferon (IFN-y) and augmented production of type 2 cytokines IL-4, IL-5, IL-6, and IL-l0 are observed in HIV infection and have been proposed as an *in vitro* immunologic marker of progression in HIV individuals.

The introduction of different antiretroviral drugs used in combination results in the suppression of viral replication and a degree of immune reconstitution. Immune reconstitution in HAART-treated individuals is incomplete because increases in CD4 cell counts are usually only partial, and the HIV-specific immune response is only marginally ameliorated in these patients. Some authors suggest that an improvement in CD4 T cell counts and a reduction in HIV plasma viraemia in chronically HIV-infected individuals are early HAART associated events, whereas the modulation of antigen-specific responses and cytokine production are later events that occur after a prolonged period of therapy. IL-2 and IFN production is increased in long-term HAART treated individuals (2 years of therapy) (Figure 3.4). HAART fails to restore HIV-specific cytotoxic T lymphocytes (CTL) and the activity of HIV-specific CTL decreases after HAART-associated suppression of HIV. CTL are essential in controlling HIV infections. Reduced CTL activity in HAART patients could be secondary to an impaired production of type 1 cytokines or to a decreased antigenic burden.

Figure 3.3: Kinetics of HIV (Source: Trabattoni & Clerici 2002)
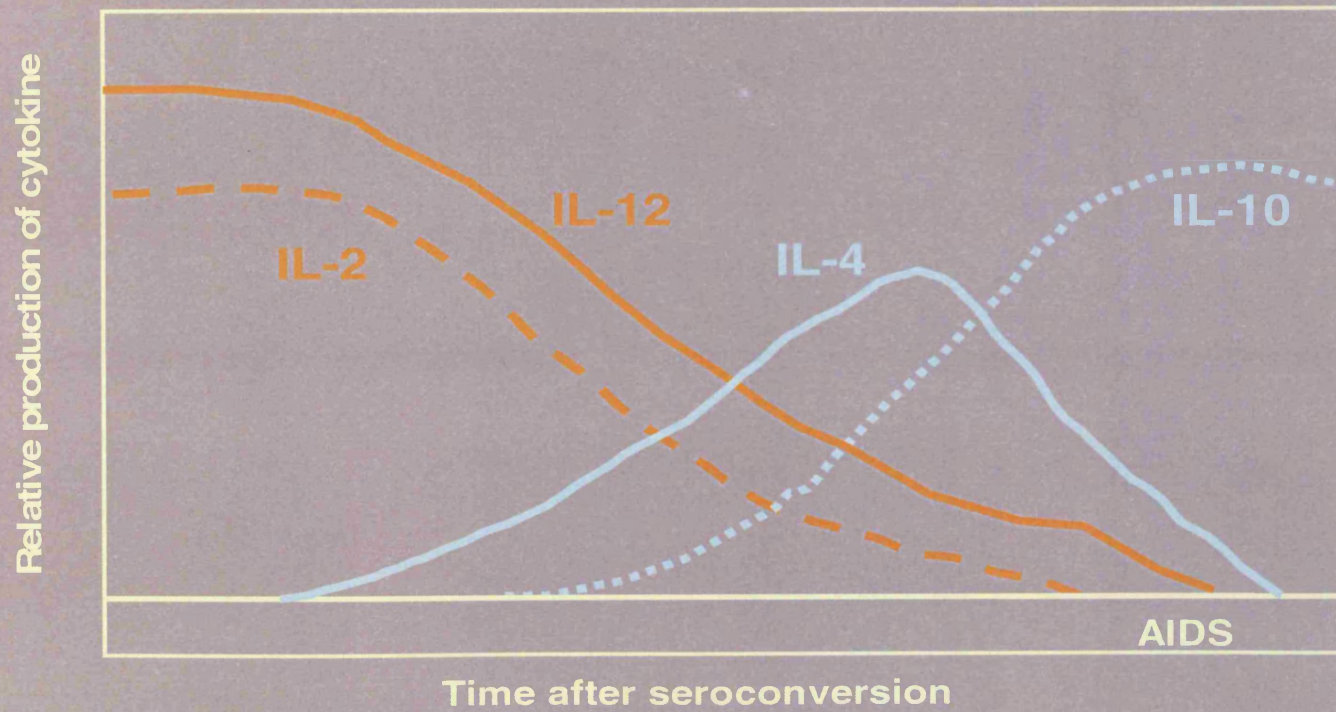
Figure 3.4: Immune reconstruction in HAART-treated patients (Source: Clerici et al 2002)

A Two Year Immunovirological Follow-up of Antiretroviral-Treated and Antiretroviral-Naive Chronically HIV Infected Patients - M. Clerici, et al AIDS 16:1676, 2002
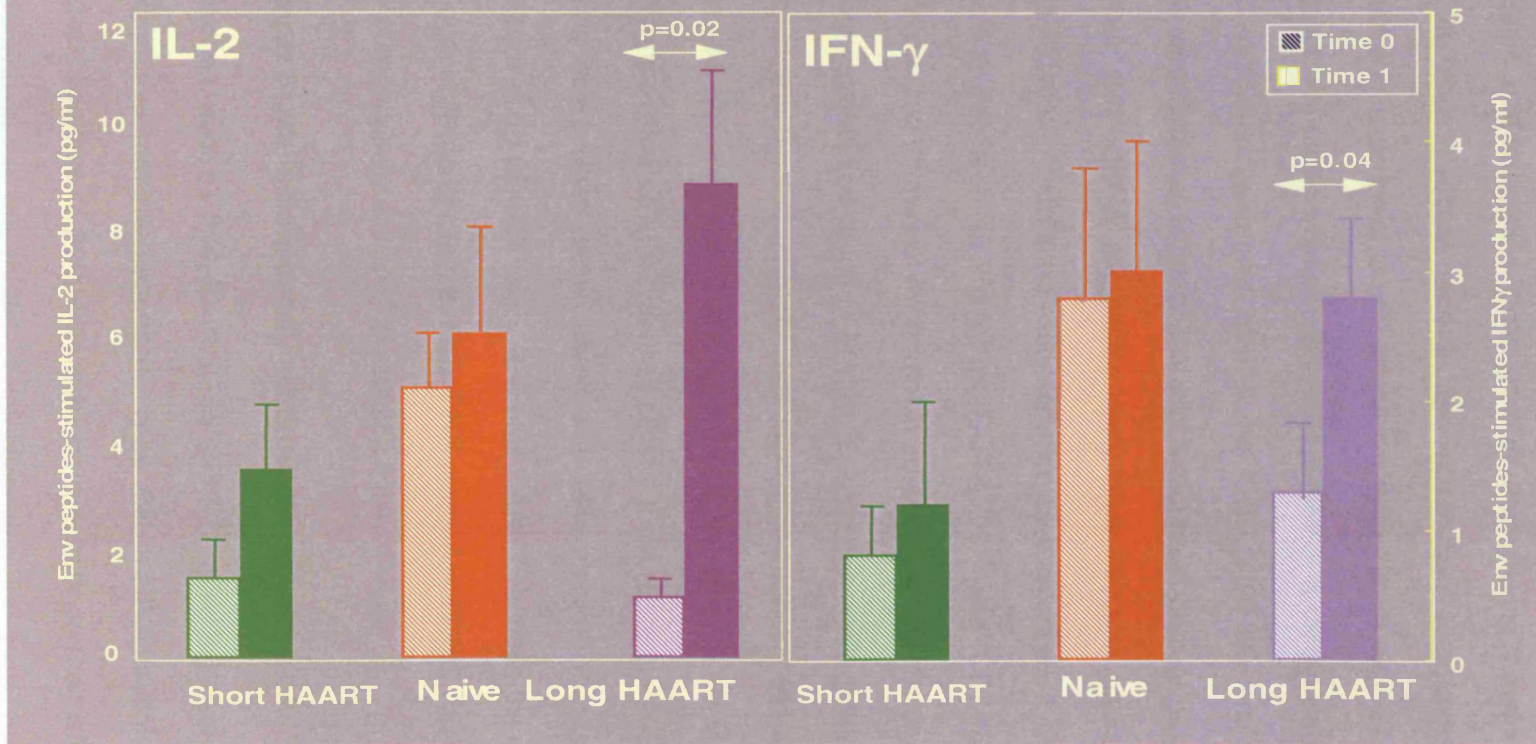
3.6   IL-2: an example of key pro-inflammatory cytokines in HIV infection

IL-2 is a central regulator of T cell function. IL-2 induces proliferation and activation of both CD4+ and CD8+ T cells, potentiates the cytotoxicity of CD8+ T lymphocytes and NK cells and stimulates B cell function, thus playing a major rôle in the containment of viral infections and in the elimination of intracellular organisms. IL-2 production is deficient in HIV-infected individuals as well as after *in vitro* infection of peripheral blood mononuclear cells (PBMC), a defect that has been correlated to an increased production of IL-4 and IL-10. IL-2 expression in lymph nodes is barely detectable at all stages of HIV infection in both adults and children. In macaques, IL-2 mRNA expression early after infection has been correlated with SIV replication. Increased administration of exogenous IL-2 has been shown to prevent depletion of immature CD4+CD8+ and CD5+CD1+ thymocytes in the HIV-infected thymus of mice implanted with human fetal thymus and liver tissues without increasing viral load. *In vitro*, IL-2 strongly synergized with IL-4, causing viral production from *in vitro* infected mature thymocytes (an effect associated with increased expression of CCR5 and CXCR4). Unique among all cytokines, IL-2 is currently evaluated in a phase III clinical trials (Emery, 2002) for its potential therapeutic effect when administered with anti-retroviral therapy to increase the levels of circulating CD4+ T cells. IL-2 plus HAART reduced the state of immune activation and, in some studies, the HIV DNA content of PBMC, likely as a consequence of the numerical expansion of circulating CD4+ T cells.

3.7   IL-10: an example of anti-inflammatory cytokines in HIV infection

Anti-inflammatory cytokines, such as IL-4, IL-10, IL-13 and TGF downregulate both the innate and the adaptative immune responses. Their rôle in HIV infection has not been well understood because of frequently conflicting results on their effects on HIV replication depending on the model of infection and the experimental conditions.

IL-10 is a cytokine mostly secreted by Th2 cells which inhibits the production of all proinflammatory cytokines and chemokines and the expression of costimulatory molecules, thus shutting-off T cell activation. IL-10 has been found to upregulate *in vitro* CXCR4, although not affecting the efficiency of viral transmission. Depending on the concentration of IL-10, both inhibitory and inductive effects on *in vitro* HIV replication have been observed. Inhibition of viral replication was correlated with the prevention of the synthesis and release of endogenous TNF and IL-6. However, lower concentrations of IL-10 resulted in the enhancement of HIV replication, an effect that has been associated with the cooperation with the released TNF and IL-6. However, others have shown that IL-10 inhibits *in vitro* HIV infection in macrophages that elevated IL-10 levels have been found, particularly in lymph nodes of HIV-infected individuals, while progression to AIDS has been correlated with an IL-10 promoter variant.

3.8 Key Points

- The two major subsets of CD4 T helper cells, Th1 and Th2, have different patterns of cytokine production and different rôles in immune responses.

- Hormones in pregnancy may contribute to the differentiation of Th cellsom one cytokine profile to another.

- Pregnancy results in a shift towards type 2 immunity and progesterone may be responsible for a type 1/type 2 switch at the maternal–fetal interface.

- Type 1-reactivity can indeed be a cause of pregnancy failure and administration of IL-10 (Type-2) to abortion-prone mice was shown to reverse it.

- During HIV infection a shift to Type 2 immunity, even if not antigen specific, indicates progression of HIV disease.

- Improvement in Type 1 function is dependent on the presence of a sufficient number of CD4+ T cells. HIV-infected people successfully treated with anti-retroviral therapy show increased Type 1 functions and a rise in the number of CD4 cells.

- The introduction of different antiretroviral drugs used in combination results in the suppression of viral replication and a degree of immune reconstitution.

- Type 1 cytokine (IL-2) production is increased in long-term HAART individuals (2 years of therapy).

- No adverse effect of HIV on pregnancy outcome, but in HAART treated women a three-fold increased risk of premature delivery has been reported.

Chapter 4 Aims, objectives and methods

4.1 Aim

The aim of this thesis is to analyse the wider impact of HIV infection on reproductive choices and on pregnancy outcomes in HIV-infected women in Europe.

4.2 Objectives

The main objectives are:

— to analyse the impact of HIV infection on couples and their reproductive choices;

— to assess the interaction between HIV infection, antiretroviral therapy and fetal outcome, in terms of intrauterine fetal growth and birth weight; and

— to quantify the association between HIV infection, antiretroviral therapy and maternal outcome in terms of mode of delivery and maternal post partum complications.

4.3 Structure of the thesis

The overall focus of the research presented in this thesis is the reproductive and sexual health of HIV-infected women as well as the wider impact of HIV infection on reproductive choices and pregnancy outcomes. Chapters 1 and 2 provide an introduction to aspects relating to HIV infection in women living in Europe and on mother-to-child transmission. Chapter 3 focuses more on the description of the immunology of pregnancy. This chapter reports data sources and methods for two different research project areas (Figure 4.1):

Survey on reproductive choices of HIV-infected women living Europe in which the following issues are addressed:

• dealing with an HIV diagnosis;

- exploring sexual and reproductive health;

- knowledge of the risk of transmitting HIV;

- choices in childbearing; and

- time to conceive.

Immune response, HIV and outcome of pregnancies, which addresses:

- pattern of cytokines over pregnancy and association with pregnancy duration;

- intrauterine fetal growth;

- birth weight and gestational age at delivery; and

- maternal complications after delivery.

Chapter 5 reports results from the survey on reproductive choices (Project 1) and chapter 6 explores the possible mechanism underlying the association between HAART and premature delivery and pattern of cytokines in HIV-infected women (Project 2). Chapter 7 summarizes some of the broad conclusions of the project in terms of intrauterine fetal growth, birth weight and gestational age at delivery. Chapter 8 shows the analyses of post partum complications. Chapter 9 presents overall conclusions and identifies research gaps.

Figure 4.1 Thesis Outline
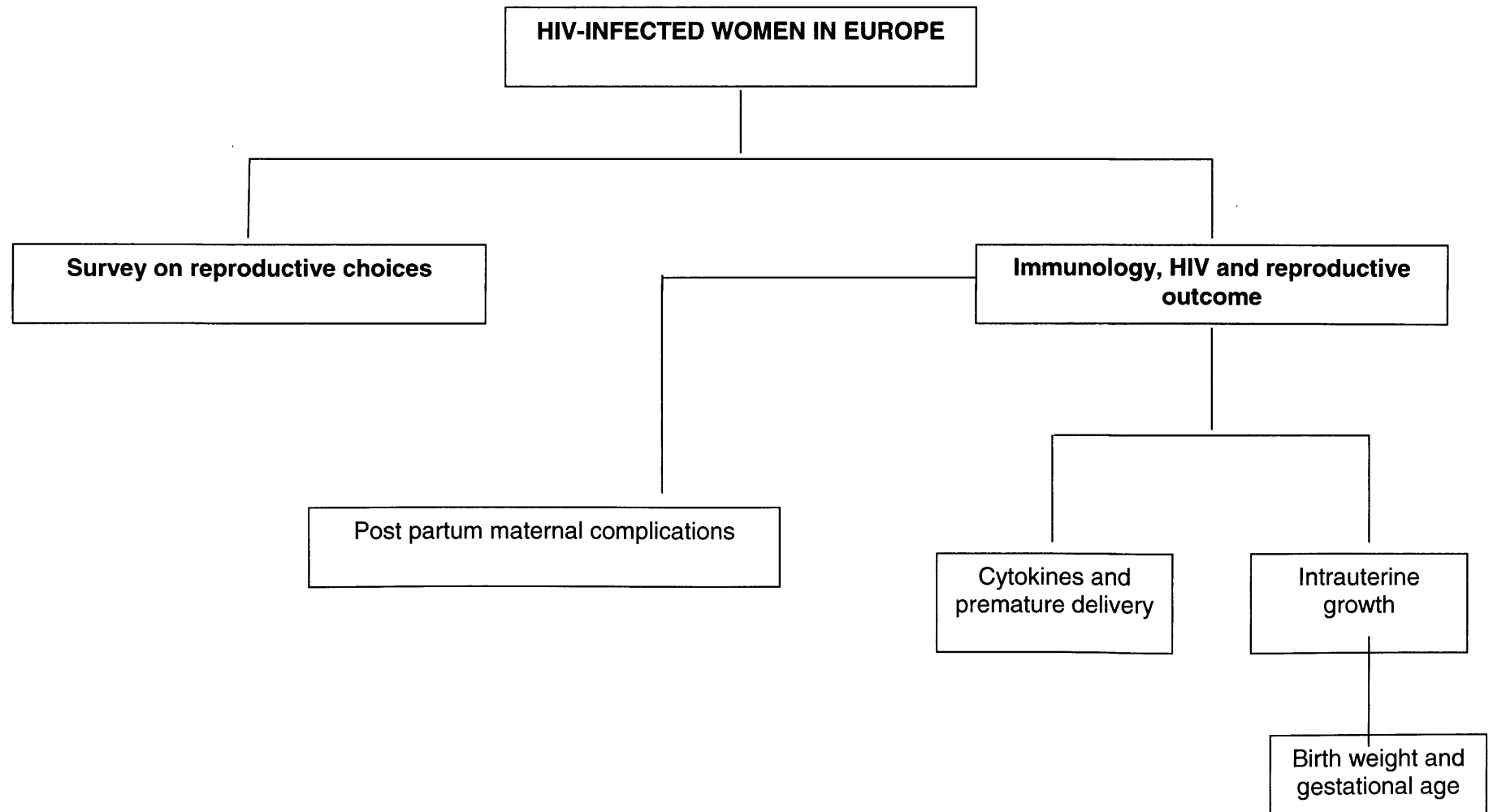
4.4 Development of different projects under the ECS umbrella.

The ECS is a birth cohort study, in which infants born to HIV-1 infected women enrolled in pregnancy are prospectively followed according to standard clinical and laboratory protocols. Pregnant women are screened for HIV infection within standard antenatal care and those infected are invited to enroll; pregnant women identified as HIV-infected from before pregnancy are also invited to participate. Informed consent is obtained before enrolment, according to local guidelines and local ethics approval has been granted. The ECS was set up in 1985 to determine the rate of mother-to-child transmission of HIV, to identify risk factors associated with mother-to-child transmission and to investigate the natural history of paediatric HIV disease; centres from Spain, Italy, the United Kingdom, Germany and Belgium have participated since the study started, with centres from Sweden (1986), the Netherlands (1987), Poland (1989), Denmark (1995) and Ukraine (2000) joining subsequently. In addition to epidemiological analyses, the data reported to the ECS provides the opportunity to investigate more specific, clinically based topics and where appropriate ECS also responds to clinical concerns by initiating or supporting ad-hoc additional research projects .

The ECS involves now 25 centres from European countries (Appendix I). Pregnant HIV-infected women are enrolled in the study and their children followed from birth according to standard clinical and laboratory protocols. Women having spontaneous abortions, or terminations of pregnancy are not included in the study. Obstetricians and/or paediatricians at enrolment and at each subsequent follow-up visit complete a range of data collection forms. These forms are then returned to the co-ordinating centre in London, where the data is coded and entered into an Access database. Data checking programmes have been in operation since the study started to verify the quality of the data received, which is also checked manually during the processes of coding and entry. There is constant liaison with the key physicians in each centre

to address any data queries and to maintain enrolment. In addition to the routine data quality control, specific checking of the relevant data was carried out prior to the analyses presented here.

Between 1998 and 2004, 19 clinicians in obstetric centres in Italy, Spain, Sweden, UK, Belgium, Germany, Switzerland, Holland and Denmark were asked to join different projects under the ECS umbrella and obtained site-specific research ethics committee approvals.

The project of this thesis involved HIV-infected pregnant women and also HIV-infected non-pregnant women and HIV-uninfected pregnant controls; setting up the clinical network meant using the ECS infrastructure and the obstetric ECS arm, but also involving additional clinicians – mainly infectious disease specialists and immunologists in different centres .

4.5   The researcher's rôle

As this work was carried out within the framework of a large and on-going prospective study, it is necessary to clarify my rôle in the research. With regard to the ECS, I had limited involvement in the data, questionnaires and data entry, but liaised with obstetricians regarding data queries specific to the projects detailed in this study. I performed the specific analysis related to birth weight and gestational age at delivery on the ECS data in order to complete my research on intrauterine growth. In relation to the ECS, I also collaborated in the preparation of various manuscripts for publication (Appendix IV).

All the different projects were nested within the large ECS study. Figure 4.2 shows data sources within different research areas.

I was the lead researcher in the reproductive survey, designing the questionnaire and being responsible for the survey administration (correspondence, distribution

of questionnaires and reminders and so forth). I created the database, analyzed results and I will prepare the manuscript for publication. I was the lead researcher on the project relating to the "Immune response and pregnancy outcome" collaborating with Prof Mario Clerici in Milan. I was responsible for selection of clinical cases and blood samples collection, organization of laboratory materials and administration, (the organization of the provisioning of the laboratory and payment therefore).

A biologist researcher performed cytokine *in vitro* production testing and I spent a fair amount of time helping in setting up methods and preparing PBMCs from fresh samples. I created the database and entered all the data and sought statistical advice in order to discuss different statistical options and programmes. I also prepared a manuscript for publication (Appendix IV).

Data for two different projects on maternal complications after delivery and on the fetal intrauterine growth were all retrospectively collected. Although I was the key researcher involved, data relating to uninfected controls were collected by different networks and used within this study. I was responsible for administration, designing the questionnaires, data collection and management (database formation and data entry). I also prepared a manuscript for publication (Appendix IV).
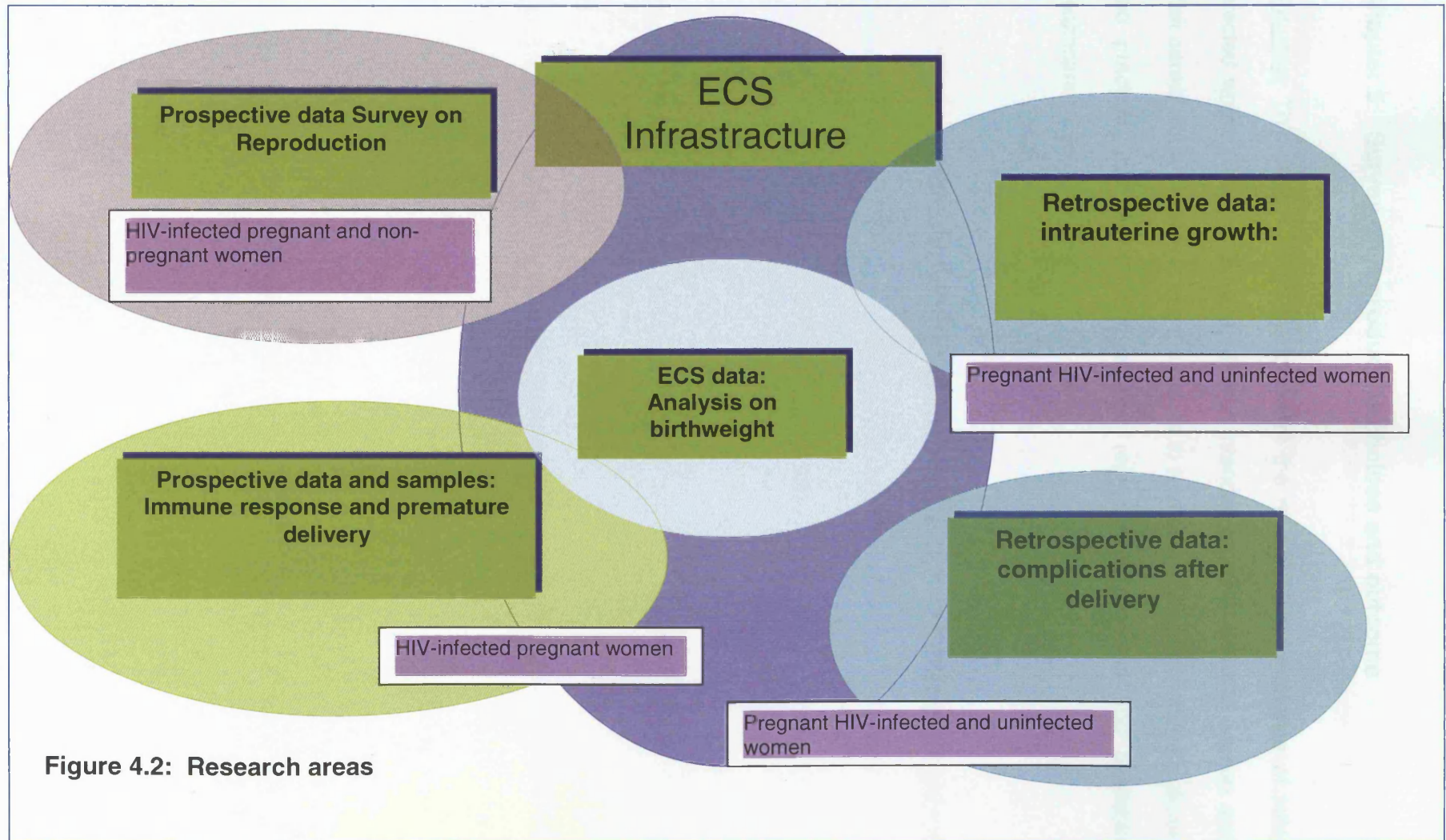
Figure 4.2: Research areas

Chapter 5   Survey on reproductive choices and outcome

Summary This project aimed to document the reproductive experience of HIV-infected women living in Europe. It was intended that HIV-infected women and their carers would be able to use the results to advocate change in national policies and practices that would improve their reproductive outcome and available healthcare choices.

5.1   Methods

(i)   Objective

To explore the impact HIV infection on women's sexual and reproductive decisions and choices by investigating:

—   factors affecting decision to become pregnancy;

—   their relationships and experience with HIV in relation to their desire of maternity;

—   their ability to conceive as measured by self-reported length of time to conceive;

—   their obstetrical history, contraception and reproductive health problems; and

—   their experiences of reproductive counselling.

(ii)   Study Design and data source

This survey was carried out to obtain information on social, demographic and basic reproductive health characteristics of HIV-infected women living in Europe. The chosen method was a self-completed anonymous questionnaire to be completed by HIV-infected women attending an obstetric and gynaecologic unit or outpatient infectious disease unit. The aims of the survey were described on the front page of the questionnaire. Clinicians working in major centres of HIV expertise (many of which were participating in the European Collaborative Study or national equivalents Appendix I), were asked to identify women infected with HIV infection and to discuss with them the rationale and the aims of the project. The questionnaire was sent to clinicians and, where required, we asked them to translate it into the national language. A pilot phase including 100 women was carried out in order to develop the questions on sexual health and health care through practice interviews. The

questionnaire was finalized on the basis of the experience from the pilot. During a training period, clinicians were instructed how to ask women to participate and the meaning and importance of confidentiality was discussed. The final version of the questionnaire consisted of 47 multiple choice and semi-structured questions (Appendix II). Completion of the form was taken as consent to participate; non-participation did not affect their care. HIV infected pregnant women were also enrolled in the ongoing ECS study.

(iii)   Study population

Four hundred and three women in 5 European countries were interviewed between July 2003 and July 2004. It is not possible to calculate the response rate because HIV infected women were left free to pick up their own copy from the reception desk. The women were asked questions about their reproductive health experiences since their HIV diagnosis, starting with the circumstances of their diagnosis, their health, sexual relationships, and decisions about reproduction and the counselling received and time taken to conceive. All the women interviewed knew that they were HIV-infected. Ten per cent of the women interviewed were pregnant and had known their HIV status for over a year. Hundred women were diagnosed over 10 years ago.

(iv)   Statistical Analysis

Close-ended questions resulted mainly in quantitative analyses. Access 2000 was used for data entry and data management and for statistical analyses. Univariable and multivariable logistic regression and survival analyses (Kaplan-Meier) were performed using STATA software.

5.2   Clinical characteristics

Four hundred and three HIV-infected women were responded from 5 European countries (Table 5.1) but some of these women did not complete the

questionnaires in full and some of the results are therefore not based on the full

403 questionnaires. The majority of these women (96%) reported to be in a long-

standing relationship with a median duration of 74 months. Most women were not

pregnant (282/403) at the time of completion of the questionnaire.

Table 5.1 European centres participating

Centre	Women enrolled
Barcelona (Spain)	65
Milan, Naples, Turin (Italy)	91
Odessa (Ukraine)	63
Paris (France)	73
Warsaw (Poland)	111
Total	**403**

Table 5.2: Socio-demographic characteristics of respondents, by pregnancy status			
Country of birth	**Not pregnant**	**Pregnant**	**Total**
North Africa	4 (2%)	2 (2%)	6 (2%)
Sub-Saharan Africa	22 (12%)	12 (13%)	34 (12 %)
Europe	153 (84%)	75 (8.3%)	228(84%)
Other	4 (2%)	1 (1%)	5 (2 %)
Total (missing 130)	183 (100%)	90 (100%)	273 (100%)
chi2(3) = 0.4647 Pr = 0.927			
Age	**Not pregnant**	**Pregnant**	**Total**
21-29	71 (30%)	24 (22%)	95 (27%)
30-35	51 (21%)	36 (33%)	87 (25%)
36-40	81 (34%)	41 (38%)	122 (35%)
40-53	36 (15%)	8 (7%)	44 (13%)
Total	239 (100%)	109 (100%)	348 (100%)
chi2(3) = 0.65 Pr = 0.1			
Profession	**Not pregnant**	**Pregnant**	**Total**
Manual	15 (13%)	15 (27%)	30 (18%)
Nonprof/non man	55 (48%)	18 (33%)	73 (43%)
Professional	25 (22%)	7 (13%)	32 (19%)
Housewife/ unemployed	20 (17%)	15 (27%)	35 (20%)
Total	115 (100%)	55 (100%)	170 (100%)
chi2(3) = 9.6138 Pr = 0.022			
Sexual orientation	**Not pregnant**	**Pregnant**	**Total**
Heterosexual	174 (76%)	90 (83%)	264 (78%)
Homosexual	51 (22%)	18 (17%)	69 (20%)
Bisexual	5 (2%)	0	5 (2%)
Total	230 (100%)	108 (100%)	338 (100%)
chi2(2) = 3.9948 Pr = 0.136			

Table 5.2: Socio-demographic characteristics of respondents, by pregnancy status			
Marital Status	Not pregnant	Pregnant	Total
Cohabiting	101 (42%)	38 (35%)	139 (40%)
Divorced	7 (3%)	5 (4%)	12 (4%)
Married	87 (37%)	57 (52%)	144 (40%)
Separated	14 (6%)	2 (2%)	16 (5%)
Single	4 (2%)	2 (2%)	6 (2%)
Widowed	13 (5%)	1 (1%)	14 (4%)
Other	12 (5%)	4 (4%)	16 (%5)
Total	238 (100%)	109 (100%)	347 (100%)
chi2(6) = 12.9183 Pr = 0. 044			

5.2.1   HIV diagnosis

Seventy percent (280/403) of the women had a positive result when first tested for HIV. Reasons for testing were, in most of cases, related to health problems or the result of having an HIV-infected partner (Figure 5.1). Overall, heterosexual infection was reported as being the predominant mode of acquisition of infection (190/344 - 55%) with 39% of these women (74/190) infected by their current partner, and 48% (92/190) by a previous HIV-infected partner. Illicit drug use was still an important mode of acquisition (28%) but the majority of such women had had their first positive HIV test before 1996 (Table 5.3).
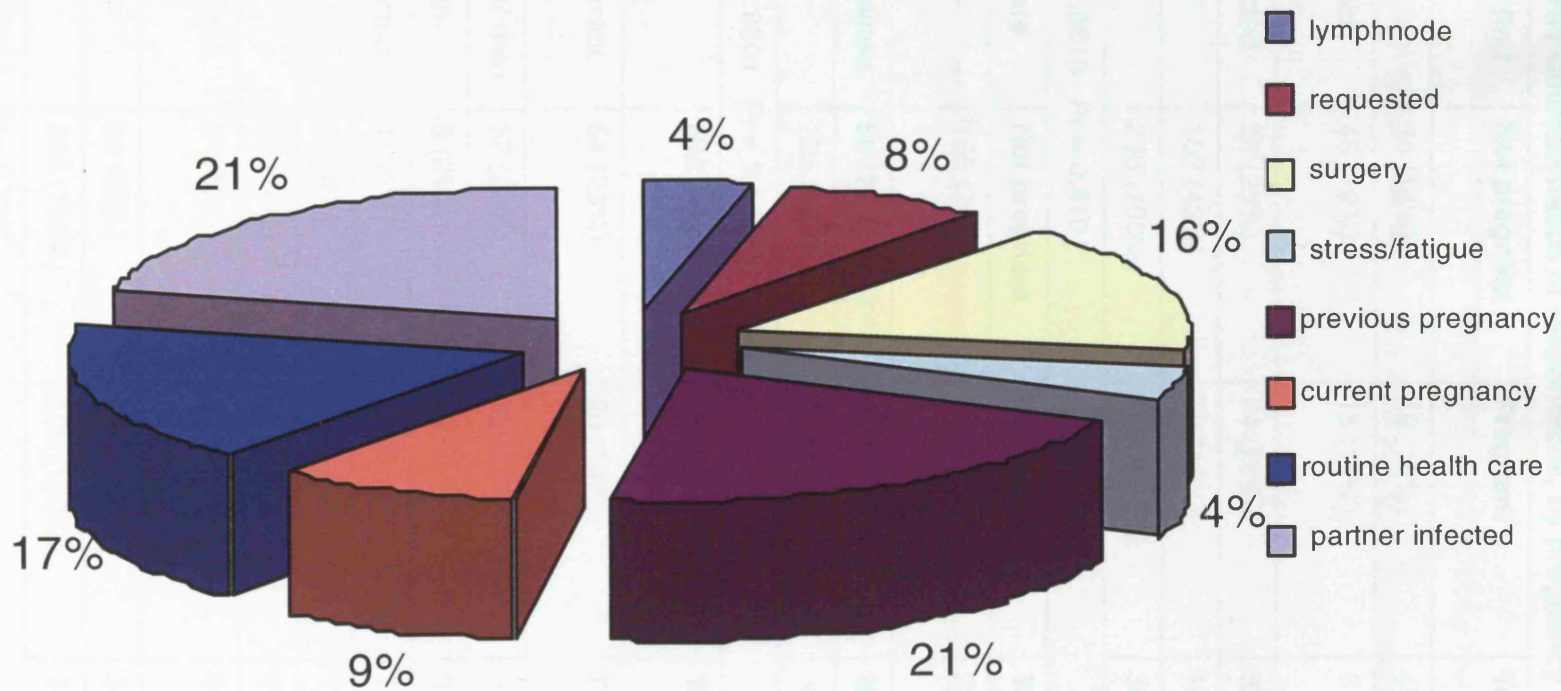
Figure 5.1: Reasons for testing reported by 399 respondents

Legend:
- lymphnode
- requested
- surgery
- stress/fatigue
- previous pregnancy
- current pregnancy
- routine health care
- partner infected

Percentages shown: 4%, 8%, 16%, 4%, 21%, 9%, 17%, 21%

Table 5.3: HIV Characteristics of respondents, by pregnancy status			
Reason for first testing	**Not pregnant**	**Pregnant**	**Total**
Pregnancy	30 (13%)	19 (18%)	49 (14%)
Routine health care	46 (19%)	15 (14%)	61 (18%)
partner infected	58 (25%)	24 (22%)	82 (24%)
Other	102 (43%)	50 (46%)	152 (44%)
Total	236 (100%)	108 (100%)	344 (100%)
chi2(3) = 2.8816 Pr = 0.410			
Tested before	**Not pregnant**	**Pregnant**	**Total**
No previous testing	165 (74%)	68 (67%)	233 (72%)
Previously tested	58 (26%)	34 (33%)	92 (28%)
Total	223 (100%)	102 (100%)	325 (100%)
chi2(1) = 1.8501 Pr = 0.174			
Mode of acquisition	**Not pregnant**	**Pregnant**	**Total**
Sex with current partner	54 (23%)	20 (18%)	74 (21%)
Sex with HIV man	57 (24%)	35 (32%)	92 (27%)
Sex IDU man	6 (2%)	3 (3%)	9 (3%)
Sex IDU partner	11 (5%)	4 (4%)	15 (5%)
Blood	10 (4%)	2 (2%)	12 (3%)
IDU	69 (30%)	28 (26%)	97 (28%)
High prevalence country	5 (2%)	3 (3%)	8 (2%)
Do not know	24 (10%)	13 (12%)	37 (11%)
Total	236 (100%)	108 (100%)	344 (100%)
chi2(7) = 4.5903 Pr = 0.710			

Table 5.3: HIV Characteristics of respondents, by pregnancy status			
HIV drugs	**Not pregnant**	**Pregnant**	**Total**
No Drugs	63 (27%)	19 (17%)	82 (24%)
Monotherapy	9 (4%)	18 (17%)	27 (8%)
Double therapy	54 (23%)	28 (26%)	82 (24%)
HAART	108 (46%)	44 (40%)	152 (44%)
Total	234 (100%)	109 (100%)	343 (100%)
chi2(3) = 18.7353 Pr = 0.000			
HIV symptoms	**Not pregnant**	**Pregnant**	**Total**
No symptoms	92 (51%)	68 (77%)	160 (59%)
Mild symptoms	24 (13%)	16 (18%)	40 (15%)
Severe symptoms	65 (36%)	4 (5%)	69 (26%)
Total	181 (100%)	88 (100%)	269 (100%)
Pearson chi2(2) = 30.6370 Pr = 0.000			

5.2.2   Relationship

Table 5.4 shows characteristics of the women's relationships in relation to their pregnancy status. Seventy per cent of the women (283) were currently cohabiting or married and 118 had a previous child with the current partner. Only 308 responded to the question about the HIV status of their partner: 145 (47%) had an HIV-uninfected partner, 123 (40%) had an HIV-infected partner, and 40 (13%) did not know whether or not their partner was HIV-infected. Fourteen women (5%) stated that their partners were not aware of their HIV status and six of these women were pregnant.

Half of the women with a current HIV-infected partner (72/123) were diagnosed with HIV after their partners had been. The majority (209/283 – 74%) of the long standing couples (married +cohabiting) couples had children together or were expecting a baby at the time of completion of the questionnaire.

Table 5.4: Characteristics of respondents relationships, by pregnancy status			
Partner aware	**Not pregnant**	**Pregnant**	**Total**
No	8 (4%)	6 (6%)	14 (5%)
Yes	180 (96%)	96 (94%)	276 (95%)
Total	188 (100%)	102 (100%)	290 (100%)
chi2(1) = 0.3810 Pr = 0.537			
HIV status partner	**Not pregnant**	**Pregnant**	**Total**
Uninfected	83 (47%)	62 (68%)	145 (54%)
Infected	94 (53%)	29 (32%)	123 (46%)
Total	177 (100%)	91 (100%)	268 (100%)
chi2(1) = 10.9183 Pr = 0.001			
First to be identified	**Not pregnant**	**Pregnant**	**Total**
Woman	37 (43%)	14 (47%)	51 (44%)
Her partner	49 (57%)	16 (53%)	65 (56%)
Total	86 (100%)	30 (100%)	116 (100%)
chi2(1) = 0.1198 Pr = 0.729			

5.2.3 Reproductive health problems

One quarter of women described their current HIV symptoms as "severe", but most of the women reported to be asymptomatic. Most of the women recalled the date that they commenced medication and the type of medication that they took. Eighty-two women (20%) did not use any antiretroviral drug at the time of the interviews (Table 5.2) and only 152 women were on combination therapy including three or more drugs. Of the 152 (44%) women on combination therapy, 110 (110/152 – 72%) had switched drugs and the reason recalled for this was pregnancy (20%) or decline in CD4 count/rising titers of HIV viremia.

All the health conditions women described in their interviews were self-defined and were in terms of symptoms rather than of named diseases. Symptoms were mostly mentioned as the reasons for testing (fatigue, lymph nodes, surgery, etc) and in the section of the questionnaire exploring gynaecologic problems.

Over a third of women in the survey reported genital tract infections to be the most common gynaecological problem. Many women interviewed described symptoms of the reproductive tract disorder (for example: fibroids, ovarian cysts, abdominal pain) but only non-pregnant women complained about irregular menstrual periods in relation to their HIV infection status (35/227 – 15%).

5.2.4 Fertility

One hundred and twenty-one (30%) respondents were pregnant when they took part in the survey. Very few women reported their profession and within these, housewives, unemployed and women having a manual job were more likely to be pregnant (Table 5.2). Sixty women (60/121 – 49%) planned their pregnancy and 24 women (21%) despite not having not planned their pregnancies declared themselves wanting a pregnancy. Of the 60 women who reported to have planned the pregnancy, 35 (58%) women conceived in less than six months, with nine taking only one month. For the 24 women who were desirous of a pregnancy but who were not specifically planning it, the time taken to conceive is irrelevant but it can be assumed that they did not have any difficulty in conceiving. Thirty-seven women had an undesired pregnancy but intended to take the pregnancy to term. Figure 5.2 shows the distribution of planning status of pregnancy according to living children. Around 97 pregnant women were aware of the HIV infection status of their partner and 64 (66%) of these reported to be in a relationship with an uninfected man. Out of 64 couples, 22 avoided the risk of infecting the uninfected partner by means of autoinsemination.
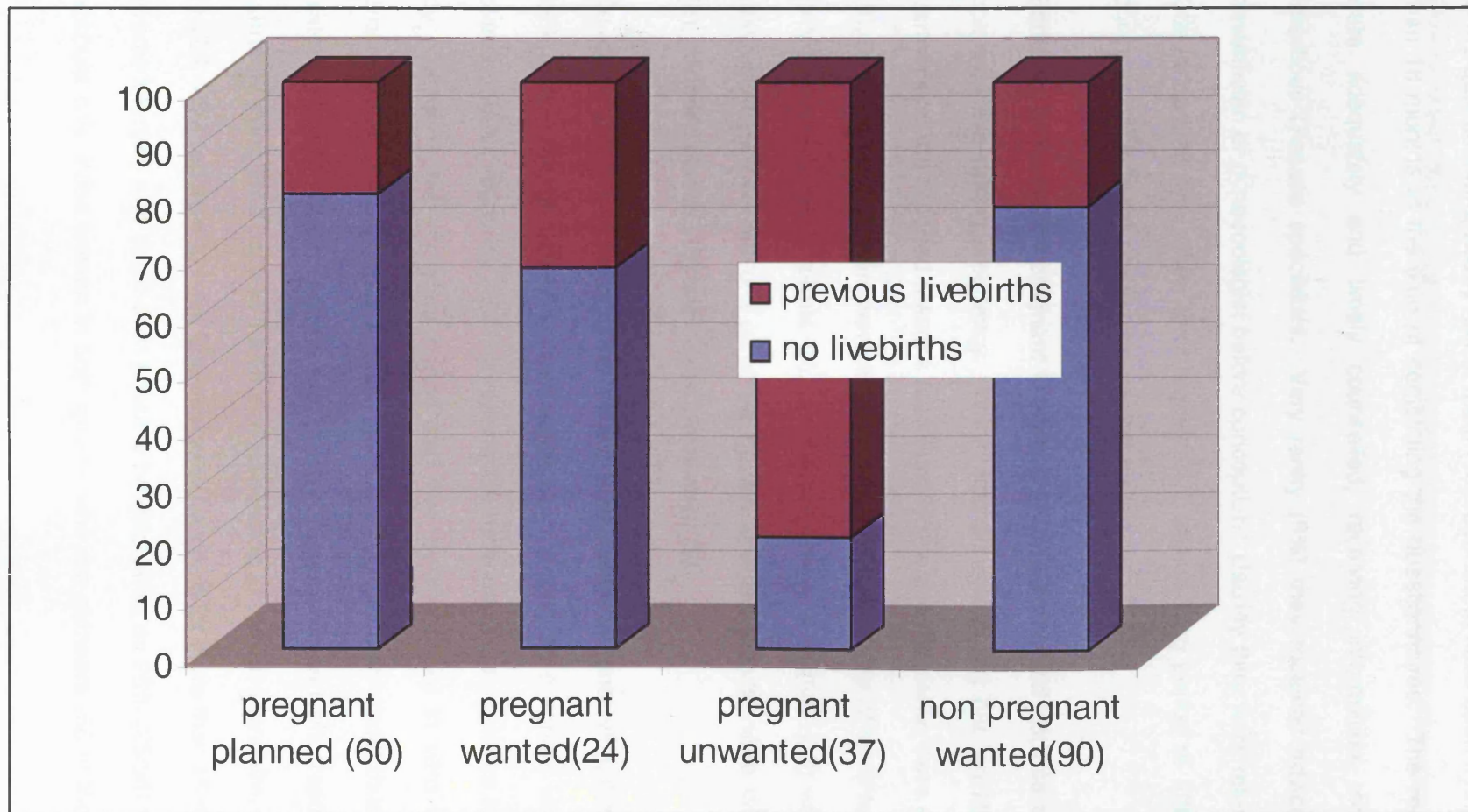
Figure 5.2: Distributions of planning status of pregnancies according to living children

Amongst the 226 non pregnant women, 39% (90/226) expressed themselves to desire a baby but only 34 women were actually trying for a baby when responding to the questionnaire; nearly half of them (15) reported to have been trying for more than 18 months at the time of completing the questionnaires. The respondents were adequately and timely counselled, receiving information mostly from Infectious Disease specialists. Very rarely (5%) they received advice from an obstetrician or gynaecologist before conception. Usually they were referred to the Obstetrics Unit only when they requested it after a long period of attempting to conceive or when the pregnancy was confirmed.

Forty-five HIV-infected pregnant women (46%) said they did not use any barrier contraceptive during pregnancy. Within this sub-group, 22 had an HIV-infected partner and the reported reasons for failure to use a prophylactic were abstinence (9), because both partners were already infected (4), because of low infectivity due to the woman's low viraemia (9). In the sub-group of women (23) who had an uninfected partner, reasons given for not using a prophylactic were latex allergy (8), partner's choice (10) and sexual abstinence (5).

To assess the size of the problem relating to difficulties in conceiving, it is helpful to look at the two groups of women separately: among all pregnant women who planned their pregnancy (60) five reported to have needed assistance to conceive by monitoring the ovulation period and five by means of in vitro fertilisation programmes. An estimated infertility rate in this population would thus be about sixteen percent (10/60). Among the 34 non pregnant women who reported to be currently trying for a baby at the time of responding to the questionnaire, nearly half (15/34 44%) reported to have been trying to conceive for more than 18 months. An overall infertility rate could then possibly be calculated as 26% (25/94) taking into account only those women in both groups who are planners (60 in the pregnant

group and 34 in the non pregnant women), and who had problems (10 in the pregnant group and 15 in the second group).

5.2.5 Obstetrical history and contraception

HIV-infected women reported their previous obstetric experience in relation to their current partner and their awareness of their own HIV status. Most of the women (275/403) had been pregnant before. Whilst 160 (58%) of these women had had a previous successful outcome, 48 (17%) women had had a previous miscarriage and 67 (25%) women had had a voluntary termination. Figure 5.3 shows the interval between a previous event and the current pregnancy and shows that there is little difference in the time between those women having had a previous negative event (voluntary termination, extrauterine pregnancies and miscarriages) and those having had a successful pregnancy (p=0.6679).

Compared to non-pregnant women, pregnant women were more likely to have an uninfected partner (p 0.002) and to have had a previous unsuccessful pregnancy (p 0.0171). However, this was not the case for the 22 African women who were the small group of women with three, four or five children.

Of the 67 women who had opted for a voluntary termination in the past, approximately half (33) reported to have done so as a result of their awareness of their HIV status. Twenty-six of these women were pregnant when they completed the questionnaire and 14 had changed partners since the termination.

We asked the respondents to provide information on the history of contraceptive methods that they had adopted. This information is shown in Figure 5.4.

Figure 5.3: Interval between any obstetrical event and the current pregnancy

Kaplan-Meier survival estimates, by previous livebirth

Long rank test p=0.66

analysis time

days

previouslivebirth = 0   previouslivebirth = 1

Figure 5.4: Distributions of current contraceptive methods

Figure 5.4: Distributions of current contraceptive methods

Chi square=7.19
p=0.007

Chi square=6.7
p=0.09

Chi square=0.02
p=0.89

Chi square=0.12
p=0.72

- pregnant
- non pregnant

no use — 48, 11
condom — 101, 51
pill — 28, 26
IUD — 6, 3
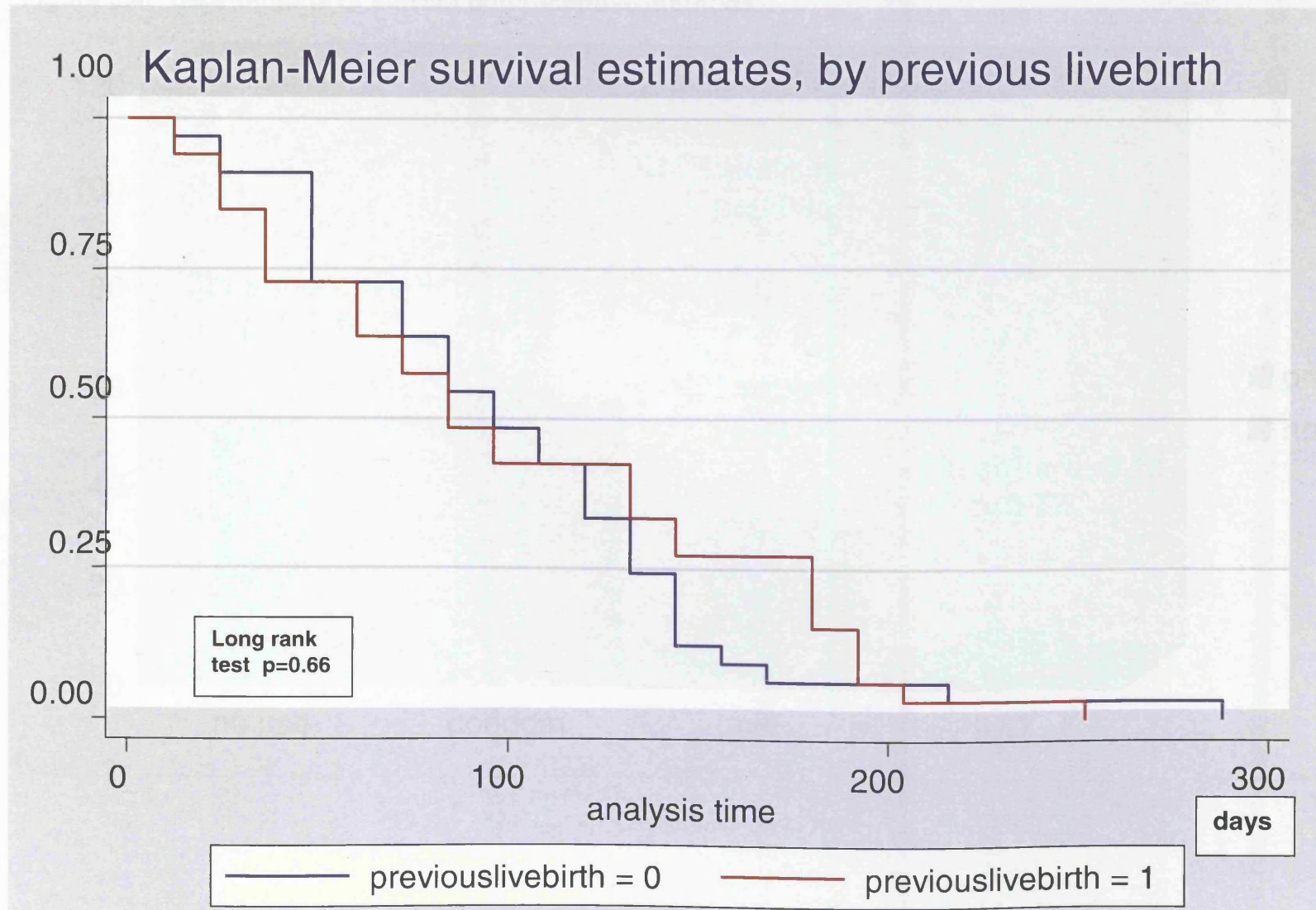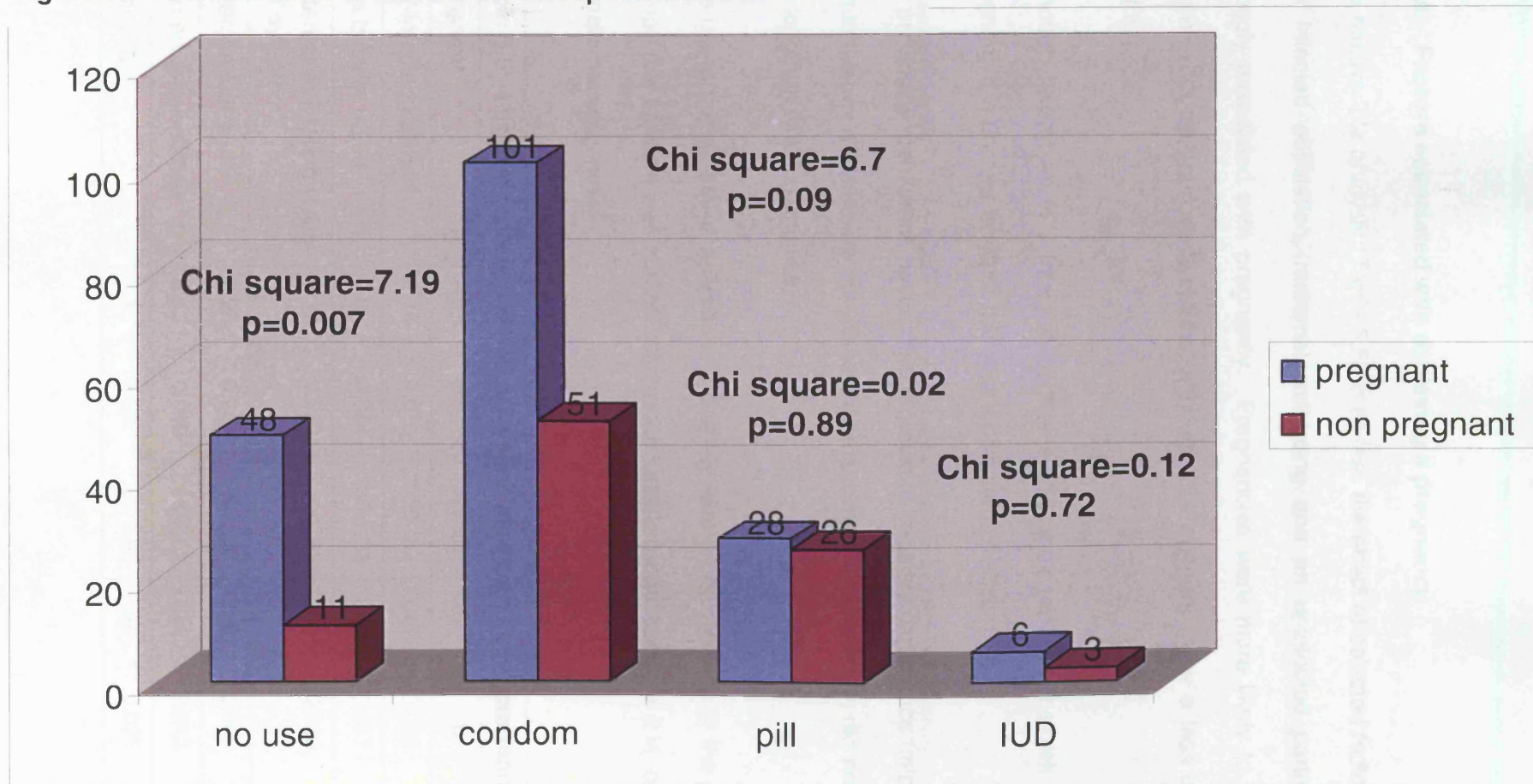
5.2.6 Factors associated with achieving a pregnancy

In a multivarible analysis (Table 5.5) to assess the effect of selected factors in the HIV infected population, maternal well-being and an uninfected partner were strongly associated with pregnancy. Pregnancies were more likely to occur in women with no previous livebirths, although this appears to be a less important factor.

Although having an HIV infected partner significantly reduced the risk of being pregnant, it is unclear whether this was a conscious decision.

As previously mentioned, having had a previous negative experience (miscarriage, termination or extrauterine pregnancy) or a successful pregnancy, did not change the length of time to conceive.

The use of highly active antiretroviral therapy was more common in the pregnant group; this variable was not included in the logistic model because it is correlated to maternal well-being.

Table 5.5: Logistic regression: factors associated with being pregnant						
Pregnant	**AOR**	**p**	**95%CI**	**OR**	**p**	**95% CI**
HIV-infected partner	0.44	0.010	.23-.82	0.46	0.003	.28-.76
One baby at home	0.55	0.064	.30-1.03	0.53	0.017	.31-.89
Maternal well-being (Any HIV symptom)	0.38	0.032	.16-.92	0.33	0.000	.14-.52
Heterosexual route	0.97	0.06	.89-1.07	0.99	0.09	.93-1.06
Married or cohabiting	0.83	0.090	.68-1.02	0.94	0.043	.82-1.08
Profession	1.1	0.30	.69-1.96	0.94	0.025	.82-1.08

5.3 Conclusions

All women had access to antiretroviral treatment to control their disease and to reduce the risk of HIV transmission to their infants. The study showed that most women are found to be infected on the basis of their first HIV blood test and showed poor knowledge of HIV transmission or risk before diagnosis with HIV.

Reproductive counselling after their HIV diagnosis was rarely provided by obstetricians but was commonly provided by infectious disease specialists and had had a positive impact on those women who had benefited from such counselling.

These results do not indicate a general difficulty in achieving conception with most of these HIV-infected women having short time to conceive.

The findings of this survey do not necessarily reflect the views or experience of young women, as only about 5 per cent of participants were under 25 years of age. Furthermore all women who participated in the study attended an Obstetric and Gynaecology Unit, and may thus be better informed and more confident about living with HIV than women in the general HIV infected population.

5.4 Key points

- The majority of HIV-infected women were in a long-standing relationship at the time of the survey.

- Heterosexual infection was the leading mode of acquisition with 39% of heterosexually infected women infected by their current partner.

- One quarter of women interviewed reported to be suffering severe (self-defined) HIV symptoms.

- One quarter of women did not use any antiretroviral drug.

- Genital tract infections was the most common gynaecological problem.

- Non-pregnant women (15%) reported irregular menstrual periods since they were HIV-infected.

- Thirty percent of the respondents were pregnant, half of whom reported to have specifically planned the pregnancy.

- Pregnancies were more likely to occur in women with no previous livebirths and in those with an uninfected partner.

- Time to conceive was less than six months in 35/60 pregnant women, but was more than 18 months for 15/34 non-pregnant women who were currently trying for a baby.

- Contraception was widely used with around 20% women having used hormonal methods.

Chapter 6   Immunology of HIV and outcome of pregnancy

Summary Antiviral therapy-associated modulation of type 1 and type 2 cytokines is associated with a negative outcome of pregnancy in HIV-infected women. HIV-infected, HAART-treated pregnant women are at increased risk of premature delivery. We investigated the mechanism of this phenomenon analysing IL-2 and IL-10 production by PBMCs in HIV-infected, HAART-treated women followed longitudinally during the course of pregnancy.

6.1 Methods

(i) Objective

To understand the mechanism that could explain the association between immunological changes induced by antiretrovirals and maintenance of pregnancy.

(ii) Study design and data source

Prospectively collected data and experimental laboratory-based analysis. HIV infected pregnant women enrolled in this project were part of the ongoing longitudinal ECS study; samples were only collected in two main centres close to the laboratory (Milan, Turin) given the need to use fresh samples for the preparation of peripheral blood mononuclear cells (PBMCs). The size of the study was determined by the limited availability of funding .

(iii) Study population

Between January 2001 and December 2002, 57 HIV-infected women were identified, and followed during pregnancy according to local practice in two main Italian maternity centres. Full ethics approval was obtained at centre level and maternal clinical and laboratory information collected during pregnancy, and at the time of delivery. All women were followed according to a standard clinical and laboratory protocol. At delivery, neonatal details were recorded. All women gave written consent for the use of the samples for research purposes. The final analyses included 49 women who were tested three times during gestation (first, second and third trimester); with production of type 1 (IL-2) and type 2 (IL-10) cytokines by PBMCs evaluated in the presence or absence of stimulation (basic value, unspecific phytohaemogglutinin-PHA response and HIV specific-ENV response). Levels of cytokines thus measured were related to the use of anti-retroviral therapy, CD4 count, plasma RNA viral load and delivery details, including premature delivery.

Treatment administration for each woman was categorized as ever/never HAART at any point during gestation and premature delivery was defined as birth before 37 weeks of gestation.

Mothers were screened at the third trimester visit for Group B streptococcus (GBS) by lower vagina and rectum swab, for bacterial vaginosis by vaginal swab, for HPV and Chlamydia trachomatis by cervical swab, and for Mycoplasma hominis by DNA PCR assay on cervical and urethral swab. Exclusion criteria were hepatitis B and C co-infection, multiparous, multifetal gestation, previous surgery on the uterine cervix, maternal smoking and illicit drug use as risk factors for premature delivery.

Whole blood was collected by venipucture in Vacutainer tubes containing EDTA (Becton Dickinson Co., Rutherford, NJ). The samples were taken in the first, second and either the third trimester of pregnancy or when the woman presented in labour. Peripheral blood mononuclear cells (PBMCs) were separated on lymphocyte separation medium (Organon Teknika Corp., Durham, NC) and washed twice in PBS, and the number of viable leukocytes was determined by trypan blue exclusion and a hemocytometer. The samples were kept at room temperature and were analysed within 24 hours. We adopted methodology used to assess the functioning of the immune system in HIV infection (Clerici, 2002).

(iv)   In vitro cytokine production

PBMCs were resuspended at 3x106/ml in RPMI 1640 and were incubated in the presence or absence of PHA or ENV at 37°C in a moist, 7% CO_2 atmosphere. Supernatants were harvested after 48 hours for PHA stimulation or after five days for ENV. Production of type 1 (IL-2) and type 2 (IL-10) cytokines by PBMCs was evaluated with commercial ELISA kits (ENDOGEN). All test kits were used following the procedures suggested by the manufacturer. Cytokine production was

calculated from a standard curve of the corresponding recombinant human cytokine in each case.

(v)   Statistical analysis

To represent the individual trends of the repeated cytokine measurements within women over gestation, slopes for IL2-Pha, IL2-Env, IL10-Pha and IL10-Env levels were estimated by fitting linear regression lines through available measurements across gestation for each woman. Median slope values were compared by pre-term birth status and treatment group, using Wilcoxon rank sum tests. Regression analyses were used to quantify the association between cytokine slope values with pre-term delivery status. Extension to multiple regression allowing for treatment was used to clarify whether the relationships of cytokine slope value and pre-term delivery status could be explained by a treatment effect. Analyses were performed using STATA 7 statistical software.

6.2   Clinical characteristics of women enrolled

Fifty-seven women were initially involved in this study. The final analysis involved 747 blood samples (249 per trimester) from 49 women, with 147 visits. Problems with blood collection led to the exclusion of four patients from the analyses. A further four patients were excluded due to GBS positivity (n= 2) and severe intrauterine growth restriction (n=2).

Baseline characteristics of women by use of antiretroviral therapy are shown in Table 6.1. Twenty-six women were on HAART, most (22/26, 85%) of whom received a PI. The immunological status of mothers did not differ by treatment received, as CD4 cell count and RNA viral load at enrolment were similar in both groups. All women were primiparous and all tested negative for STDs (bacterial vaginosis, HCV, HBV, HPV, Chlamydia trachomatis, Mycoplasma hominis). Thirty-three women (67%) delivered by elective caesarean section at term; two women

delivered vaginally (27 weeks and 24 weeks) and 14 (29%) had an emergency caesarean section due to premature labour before the planned date for the elective procedure.

Preterm delivery (delivery before 37 gestational weeks) was more likely to occur in women receiving HAART (X^2=4.5 p=0.03) than in those not receiving HAART and occurred before 34 weeks in eight out of the 16 cases. Babies born to HAART treated women had a significantly lower mean birth weight (p=0.004) than those born to women not treated with HAART. No infants were subsequently diagnosed as being HIV-infected

.

6.3 Effectiveness of HAART

During the course of pregnancy, all women on HAART showed a progressive increase in their CD4 cell count and a decrease in the level of RNA viremia. The median CD4 cell count increased from 438 cells per ml (range 100-1187) during the first trimester to 467 cells per ml (range 84-14390) during the second trimester and 448 cells per ml (range 141-1177) at delivery. The median RNA viral load decreased from 19,589 copies per ml (range 50-190,000) during the first trimester, to 6,158 (range 50-34,775) in the second trimester and 2007 copies per ml (range 50-15,000) at delivery. The immune status of these women was relatively intact and the RNA viremia was modest, with about half of them having undetectable viremia at birth. Those women in the never HAART category showed a steady level of CD4 and of RNA viremia .

Table 6.1: Clinical characteristics of 49 HIV-infected pregnant women by use of HAART			
	Non-HAART (nr 23)	HAART (nr 26)	P
Maternal age in years (mean~, SD)	29 (3,4)	28 (4,1)	0.16
Median gestational age at delivery (range)	37(34-39)	36 (24-39)	0.04
Preterm delivery	4/23	12/26	0.03
Mean birth weight in gr (SD)	2680 (674)	2514 (366)	0.02
Median CD4 cell count at entry (range)	400 (178-850)	439 (84-1093)	0.60
Median RNA VL at entry (range)	13718 (max 190,000)	19589 (max 34,775)	0.12
Median CD4 cell count at delivery (range)	423 (158-950)	467 (141-1177)	0.50
Median RNA VL at delivery (range)	10158 (max 180,000)	2007 (max 50,15000)	0.23

~ mean and median did not differ

6.4 Cytokines slopes over pregnancy

Production of Type 1 (IL-2) and Type 2 (IL-10) by PBMCs by trimester of pregnancy is shown in Figures 6.1 and 6.2. It is noteworthy that IL-10 patterns over pregnancy are less sharp than those of IL 2 ones, but the scale for IL-10 is 5 times that for IL-2, which dulls the response seen. Therefore slopes were calculated to provide a similar unit in response for IL-2 and IL-10. Trends of IL-2 (PHA and env-stimulated) and IL-10 (PHA and env-stimulated) measurements within women over gestation are shown in Figure 6.3. There were no statistically

significant differences by prematurity in the median values for slopes of any of the cytokines and only env-stimulated IL-10 production differed significantly by treatment group (p=0.043). However, Figure 6.3 shows that the slope of PHA-stimulated IL-2 is positively associated with a preterm delivery (p=0.008) and with treatment although the latter association did not reach statistical significance probably due to lack of power with small numbers. Env-stimulated IL-10 was negatively associated (decrease over gestation) with preterm delivery (p=0.34) and with treatment (p=0.007). Values are shown in the table following the figure.

IL-2 values

Figure 6.2: Production of Type 2 (IL-10) cytokines by PBMCs by trimester of pregnancy

IL-10 values

Figure 6.3: Cytokine slopes over pregnancy (values of the slopes are shown in the following table)

(a) by pre-term delivery
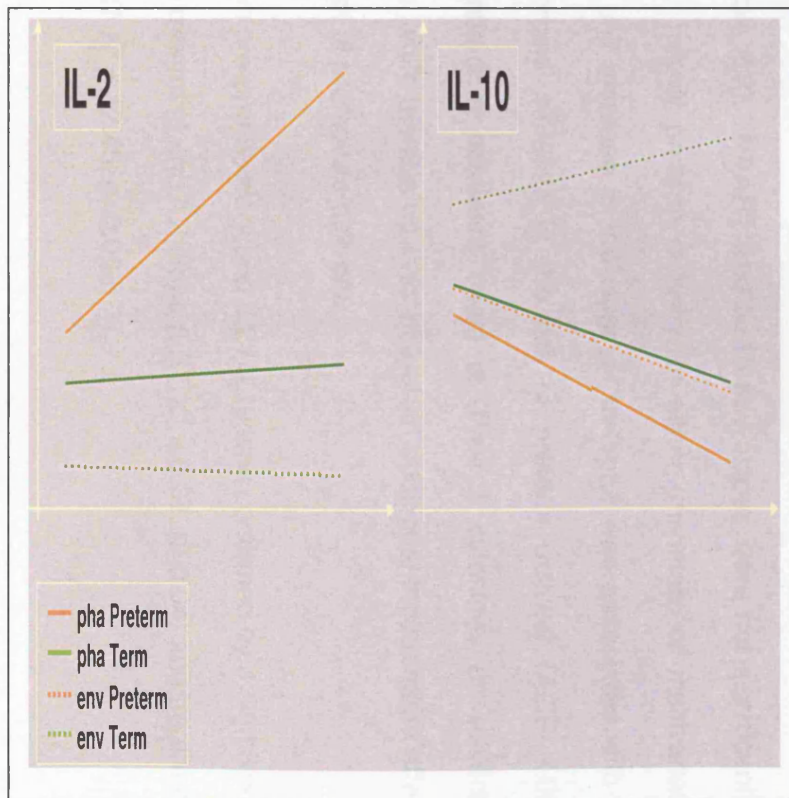
(b) by treatment
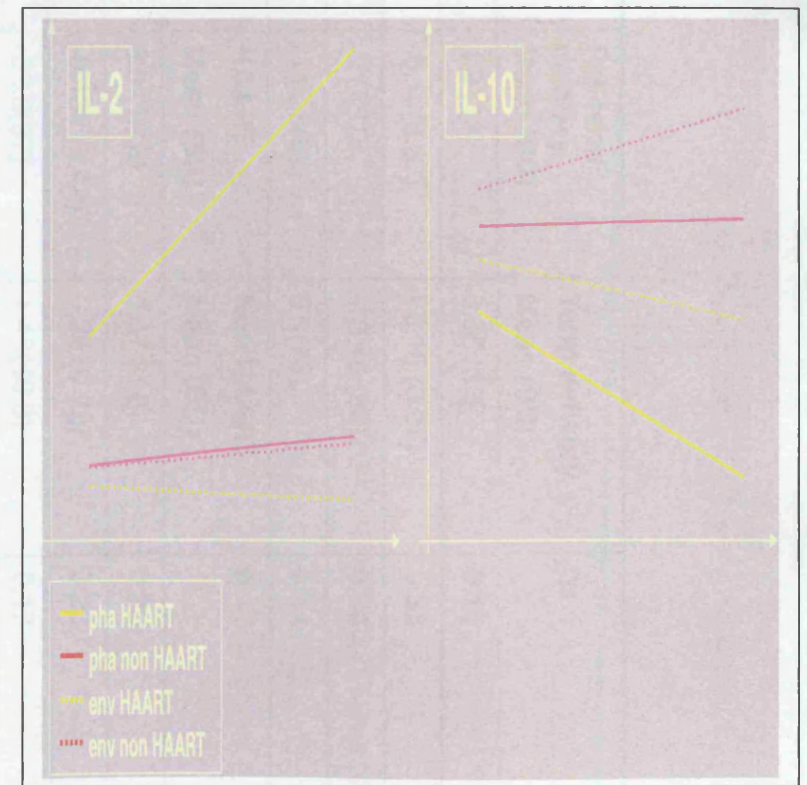
Values for figure 6.3a	Pre-term delivery Mean (SD)	Term delivery Mean (SD)	*p
IL2-pha	4.9 (13)	0.36 (3.8)	0.10
IL2-env	-0.14(0.37)	- 0.15 (0.67)	0.33
IL10-pha	-21 (51)	-13.46 (83)	0.30
IL10-env	-14.35(85)	9.1(73)	0.34
Values for figure 6.3b	HAART Mean (SD)	Non HAART Mean (SD)	p
IL2-pha	3.0 (10.6)	0.31 (3.0)	0.23
IL2-env	-0.132 (0.42)	0.26 (0.72)	0.23
IL10-pha	-33.8(97)	1.49(43.8)	0.17
IL10-env	-12.2 (85)	16.6 (66.7)	0.043

To assess the independent contribution of cytokine slopes and HAART on the risk of premature delivery, all three variables were included in a regression analysis (Figure 6.4 and Table 6.2). HAART and IL-10 env slopes were not significantly associated with the risk of preterm delivery, in either univariate or multivariate analysis, but each unit increase in the slope of IL-2-pha was associated with a significant eight percent increase in the risk of preterm delivery (AOR 1.08, p=0.05). This confirms a dominant effect of Type 1 cytokines on adverse pregnancy outcome, while there is no evidence of an additional independent effect of HAART on the risk of premature delivery.

The relation between preterm delivery and IL2 / IL10 was confirmed by a significant interaction between treatment and IL2 slope (LRT X^2 = 6.37, p=0.04) and treatment and IL-10 slope (LRT X^2 = 12.45, p=0.006).

Figure 6.4

Regression analyses : slope values of PHA-IL-2 and ENV-IL-10 over gestational age by delivery

PHA-IL2=0.36+(4.55)*(delivery=0/1) p=0.008

ENV-IL10=9.1+ (-23.54)*(delivery=0/1) p=0.07

- IL2-PHA-pre
- IL2-PHA-term
- IL10-ENV-pre
- IL10-ENV-term

Slope values

Gestation weeks
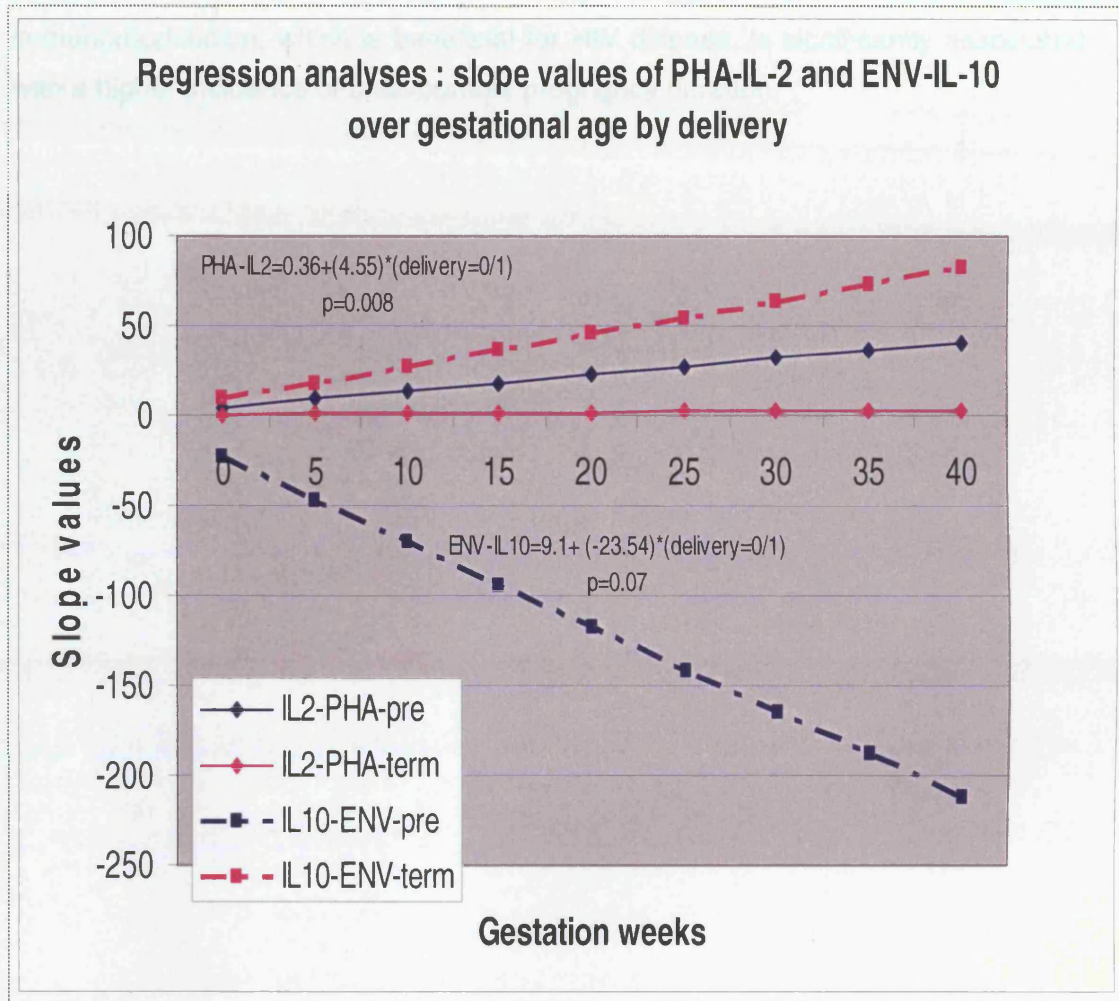
Table 6.2: Risk of pre-term delivery by cytokine slopes over pregnancy allowing for HAART

	OR (95 %CI),p	AOR (95% CI),p
No treatment	1	1
HAART	1.27 (.52-3.0) 0.59	96 (0.37-2.52) 0.95
IL2-pha	1.07(1.00-1.15) 0.032	1.08(1.0-1.17) 0.05
IL10-env	0.99 (.99-1.00) 0.174	0.99 (0.99-1.00) 0.58

6.5 Conclusions

Results from this descriptive study suggest that HAART-induced immunomodulation, which is beneficial for HIV disease, is significantly associated with a higher incidence of unfavourable pregnancy duration.

6.6 Key points

- Preterm delivery (delivery before 37 gestational weeks) was more likely to occur in women receiving HAART (X^2=4.5 p=0.03) than in those not receiving HAART

- During the course of pregnancy, all women on HAART showed a progressive increase in their CD4 cell count and a decrease in the level of RNA viremia

- Slopes were calculated to provide a similar unit in response for IL-2 and IL-10 as the scale for IL-10 is 5 times that for IL-2.

- There were no statistically significant differences by prematurity in the median values for slopes of any of the cytokines and only env-stimulated IL-10 production differed significantly by treatment group (p=0.043).

- Env-stimulated IL-10 was negatively associated (decrease over gestation) with preterm delivery (p=0.34) and with treatment (p=0.007).

- To assess the independent contribution of cytokine slopes and HAART on the risk of premature delivery, all three variables were included in a regression analysis

- HAART and IL-10 env slopes were not significantly associated with the risk of preterm delivery, in either univariate or multivariate analysis, but each unit increase in the slope of IL-2-pha was associated with a significant eight percent increase in the risk of preterm delivery (AOR 1.08, p=0.05).

- This confirms a dominant effect of Type 1 cytokines on adverse pregnancy outcome.

Chapter 7   HIV infection affecting fetal intrauterine growth

Summary Infants born to HIV infected mothers reportedly are of lower birth weight than those of HIV uninfected mothers. To analyse intrauterine growth profiles of infants born to HIV infected mothers allowing for antiretroviral therapy, in comparison to a healthy, uninfected population we prospectively collected data(head circumference, femur length, abdominal circumference) for 316 HIV infected women on intrauterine parameters.

From the large ECS population, birth weight was analysed and compared to the British standard to add one more piece of information to the debate on maternal treatment and gestational age at delivery

7.1   Methods

(i)   Objectives

To investigate whether HIV infection in a pregnant woman affects the growth of her fetus, we analyzed intrauterine growth profiles of infants born to HIV-infected mothers compared to a healthy, uninfected population.

(ii)   Study design and data source

Retrospective and prospective observational studies. Clinicians who participate in the ECS longitudinal study, were asked to collect data, where feasible, on ultrasound intrauterine longitudinal measurements. The sample size was determined on the availability of data.

(iii)   Study population

Data were collected on 316 HIV-infected pregnant women who were referred to four obstetrical reference centres in Italy between 1990 and 1999. In each centre, after giving consent, women were prospectively monitored according to a standard protocol. Information collected included current antiretroviral treatment, CD4 cell count, and injecting drug use (IDU) history. IDU was classified into never, ex and current IDU on the basis of self-report. Delivery and neonatal characteristics included mode of delivery, gestational age, gender, birth weight, presence of congenital abnormalities and neonatal complications. Laboratory tests, including serology and CD4 cell count measurements, were carried out locally. Maternal CD4 cell counts nearest the time of delivery were used. Maternal HIV RNA was available only for a few women, and could thus not be used in further analyses. Mothers enrolled were asymptomatic and all were classified in stage A of disease according to the CDC classification. Antiretroviral therapy prophylaxis to reduce mother-to-child transmission was not an option for 127 patients who delivered before 1995.

Perinatal outcome was recorded in terms of birth weight, gestational age at delivery, premature delivery (before 37 weeks of gestation), and presence of congenital anomalies. Small for gestational age fetuses (SGA) were defined on the basis of an abdominal circumference below the 10th centile for gestation. A child was classified as infected after the detection of virus or antigen in at least two blood samples or persistence of antibody beyond 18 months of age; a child was presumed uninfected if at least two blood samples were antibody-negative and if no virus or antigen was ever identified.

Fetal growth was assessed by ultrasound measurements of femur, head and abdominal circumference and serial measurements were used to model individual growth shape (Hooper, 2002; Todros, 1987). High quality sonographic scanners, with 3.5-5.0 MHz convex transducers, were used in all centres. Measurements procedures were the same in all centres and followed international standards: head circumference was measured on a fronto-occipital transverse section at the level of the upper third of the talami, the abdomen was measured on transverse section at the mid-portion of intrahepatic umbilical vein, the femur length (FL) was measured from the greater trochanter to the lateral condyle.

The number of examinations varied depending on individual obstetric management and we included infected mothers-to-be with a minimum of two longitudinal ultrasound assessments in order to evaluate the longitudinal growth curve for each fetus.

Nine hundred ultrasound measurements of fetuses born to HIV-infected mothers were compared to a uninfected control group of 3000 cross–sectional measurements in 1200 uneventful pregnancies delivered at term during the same years in the same geographical area.

(iv)   Statistical analysis

All calculations were done in S-Plus2000. The LMS method developed by Cole and Green was used to obtain centiles for the reference data set (Pinehiro, 2000; Cole, 1992) Z-scores were calculated with respect to the standard from the reference data set for measurements relating to fetuses of infected mothers using linear interpolation on the centiles. Fitted linear mixed effects models to the z-scores were used as a function of gestational age adjusting for birth weight, gestational age at delivery, CD4 count at delivery, gender, infection status of the child, and maternal antiretroviral treatment in order to assess the differences in fetal growth patterns between babies born to HIV-infected and uninfected mothers. Random effects for the intercept and the slope of gestational age for each fetus were included to allow for unobserved variables such as smoking or social background.

All reported t test values are two-tailed and univariate comparisons for categorized variables were tested with the χ^2 test.

7.2   Maternal characteristics and perinatal outcome

Baseline characteristics of 316 HIV-infected mothers are summarized in Table 7.1: 127 (40%) women were not exposed to ART, 110 (35%) received Zidovudine (ZDV) prophylaxis and 79 (35%) combination therapy in order to reduce vertical transmission. Few women (15/189-8%) were on therapy before pregnancy and initiation of antiretroviral therapy in pregnancy was intended to prevent vertical transmission. Of the 174 women who started antiretroviral therapy during pregnancy, 109 (63%) received zidovudine monotherapy, and 65 (37%) double or triple therapy. Women treated during pregnancy with combination therapy were older on average than women who received no treatment (t= -4.164, p<.0.001) or who were on ZDV monotherapy (t= --2.81 p=.005).

Virtually all women (308/316) were white Caucasian, seven were of African and one of other origin and overall 125 (39%) women had a history of illicit drug use (but were not current users), with a small percentage in the combination therapy group. Information on CD4 cell count was available for all women; 32 (10%) women were HIV symptomatic with a CD4 count less than 200 cells/ml. There were no significant differences between the three treatment groups in stage of HIV disease (X^2= 6.13 p= 0.19), or CD4 cell count near the time of delivery (t= -0.09 p=.92). Women who were not treated were more likely to report a previous history of IDU than treated women (X^2= 38.54, p<0.001).

Elective caesarean section was the mode of delivery for 28% of the patients (33/127) in the no treatment group, for 59% (65/110) in the mono therapy group and for 86% (68/79) in the combination therapy group (X^2= 73.44 p< 0.001); this trend reflects changing policies relating to the management of HIV-infected pregnant women over the period.

Table 7.1: Baseline characteristics of 316 HIV infected mothers by antiretroviral treatment

	No therapy	AZT	Combination
Number of patients	127	110	79
Median maternal age (SD)	28.6±4.6	29.1±5.1	31.3±4.4
Ethnicity			
Caucasian origin (%)	123 (97%)	108(98%)	77(97%)
African origin	3	2	2
Others	1	0	0
Mode of acquisition (%)			
Past IDU	75 (59%)	34 (31%)	16 (20%)
Heterosexual risk	45 (35%)	72 (65%)	59 (74%)
Others	7 (6%)	4 (4%)	4 (6%)
Stage of disease (%)			
Asymtomatic	70 (55%)	66 (60%)	36 (46%)
Symtomatic and > 200 CD4	41 (32%)	35 (32%)	36 (46%)
Symtomatic and < 200 CD4	16 (13%)	9 (8%)	7 (8%)
Lymphocyte (cell/ml)			
median CD4 (SD)	490 (226)	466 (277)	493 (228)
median CD8 (SD)	813 (286)	851 (350)	846 (321)
Patients on therapy before pregnancy	---	1 (1%)	14 (18 %)
Gestational age at delivery *(Median; range in weeks)*	39; 29-42	38; 33-43	38;29-41
Birthweight (g) (Mean ±SD)	2998 (482)	2915 (466)	2990 (401)
Small for gestational age (< 10th percentile) **	16 (12.5%)	13 (12%)	9 (11.5%)

* classified as CDC classification **classified by clinicians according to a weight distribution, allowing for gestational age

There were no statistically significant differences in mean birth weight (t=1.3 9

p=0.18) or mean gestational age at delivery (t=1.366 p=0.17) overall, but infants

born to women who received combination treatment were on average delivered

nearly one week earlier than those in the no treatment group (t=2.48, p=.0136 Table 7.1).

The overall prematurity rate was 9%, higher in both the untreated (12%) and in the combination (13%) treatment group than in the group of women who received monotherapy only (6.4%), although the difference did not reach statistical significance (X^2 =1.58 p=0.452). Only two cases of severe prematurity occurred at 29 weeks of gestation. In the sub-set of 29 women with pre-term babies, there were no cases of small for gestational age , but in full-term deliveries, the association between maternal HIV and low birth weight was strong, although not statistically significant (OR 3.4, 95% CI 0.57-5.6).

Fourteen of 79 (18%) patients on combination therapy discontinued therapy soon after their positive pregnancy test, and started antiretroviral again around 14 weeks of gestations, earlier than those who started ZDV therapy for the first time, around 24 weeks in pregnancy (t=-8.648, p<0.001). There were a total of 6 maternal complications possibly associated with therapy, these included severe anaemia necessitating discontinuation of ZDV monotherapy at 32 weeks of gestation, thrombocytopenia at 36 weeks, hepatic cholestasis at 36 weeks, two emergency caesarean sections for placental abruptio at 33 and 37 weeks of gestation in the monotherapy treated group; and one case of placental abruptio at 29 weeks in a woman receiving combination therapy. No events were reported in the no treatment group.

Neonatal complications in infants of HIV-infected mothers who did not receive ART during pregnancy were rare and included one case of cerebral haemorrhage (caesarean section, birth-weight <2000 grams at 35 weeks of gestation, uninfected at follow-up) and one case of cardiac malformation (2/127-1,6% 95%CI 0.001-0.55). Among infants of the 110 infected women who received ZDV treatment

during pregnancy there was one case of respiratory distress (emergency caesarean section at 36 weeks of gestation, birth weight 2185 grams), one case of kidney calicopielectasy dilatation, one case of syndactily and one case of agenesis of the external ear (4/110-3,6% 95%CI 0.009-0.9). No cases were reported in women on combination therapy.

A total of 33 children born to 316 infected mothers were diagnosed to be HIV-infected (overall 10.4%, 95% CI 7.2-14.3); the rate of mother to child transmission was 24.4% (30/127, 95% CI 16-31) in the untreated group, where the majority of these women delivered vaginally (68%), 2.7% (3/110 95% CI 0.5-7.7) in the monotherapy group and zero (0/79 95% CI 0-4.6) in the combination treatment group (X^2 =39.79 p<0.001).

7.3   Intrauterine growth (z-score)

We used the 3000 measurements from 1200 uninfected women to construct the standards upon which the z-scores were based. Figure 7.1a to 7.1c show z-scores for head circumference (HC), abdominal circumference (AC) and femur length (FL) measurements adjusted by gender, maternal CD4 count and maternal treatment at delivery, and HIV-infection status of the child for all babies born to HIV mothers. Growth in all measures was significantly reduced (p<0.0001; p<0.001; p<0.001) in infants of HIV-infected mothers compared to the uninfected reference population, especially in the third trimester (slopes became negative around 30 gestational weeks, indicating increasingly diverging lines). Intrauterine growth of children subsequently diagnosed to be HIV-infected did not differ significantly from those of uninfected infants (p=0.64). Type of maternal antiretroviral treatment was not significantly associated with a different pattern of growth among babies born to HIV-infected mothers (p=0.20).

The average z-score of head circumference and femur length in HIV-infected women was substantially below the reference (-0.46 or 32th centile and -0.0997 or 15th centile respectively), but the average z-score for abdominal circumference differed only marginally from that for the uninfected reference population (-0.023 or 49th centile). To investigate within HIV-infected women the effect of maternal and HIV related variables we fitted a regression model, which included a random effect at mother level to allow for the effect on growth of unobserved variables such as smoking or social background. The inclusion of a random effect improved the fit of the model, but only marginally affected the magnitude of the associations of interest. Table 7.2 shows the coefficients for these variables and their p-values from a model including gestational age, gender, maternal ART and CD4 counts and infant's infection status as well as a random effect. The negative coefficients for gestational age indicate that fetuses become smaller with respect to the standard as they near delivery, with the smallest effect for abdominal circumference. Maternal treatment did not show any association with intrauterine fetal growth, neither maternal CD4 did, probably due to the small sample size. (Figure 7.2)

Figure 7.1(a): Fetal Head Circumference z-scores for babies born to HIV infected mothers, by gestational age.
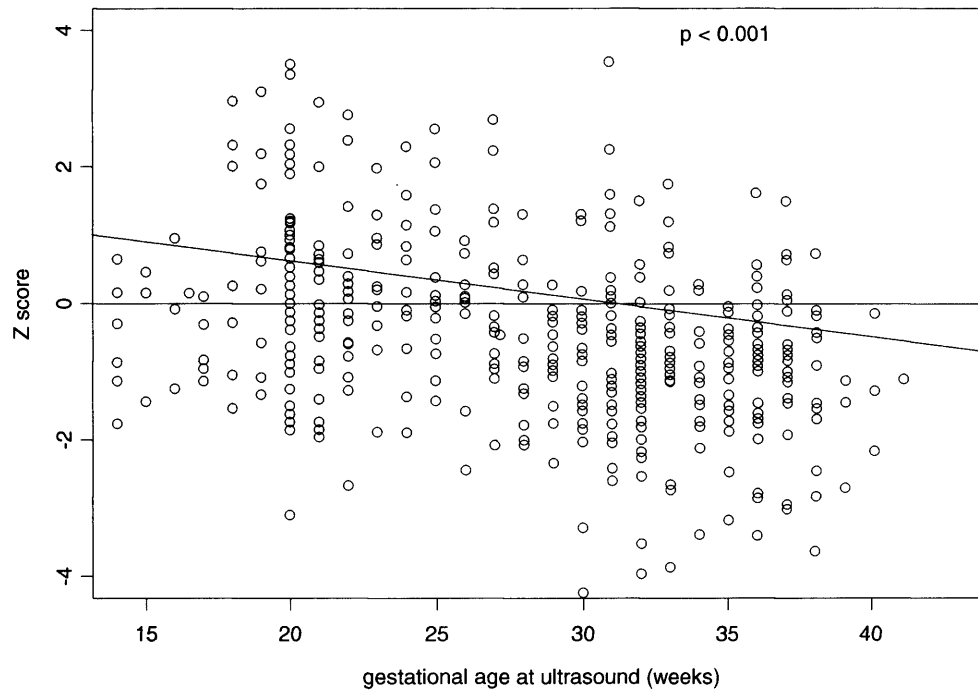
(Empty Dots = individual z-score for each fetus with respect to the uninfected mothers' standard. Continuous line = regression line for Z scores of HIV infected mothers allowing for repeated measurements.)

Figure 7.1(b): Fetal Abdominal Circumference z-scores for babies born to HIV-infected mothers by gestational age
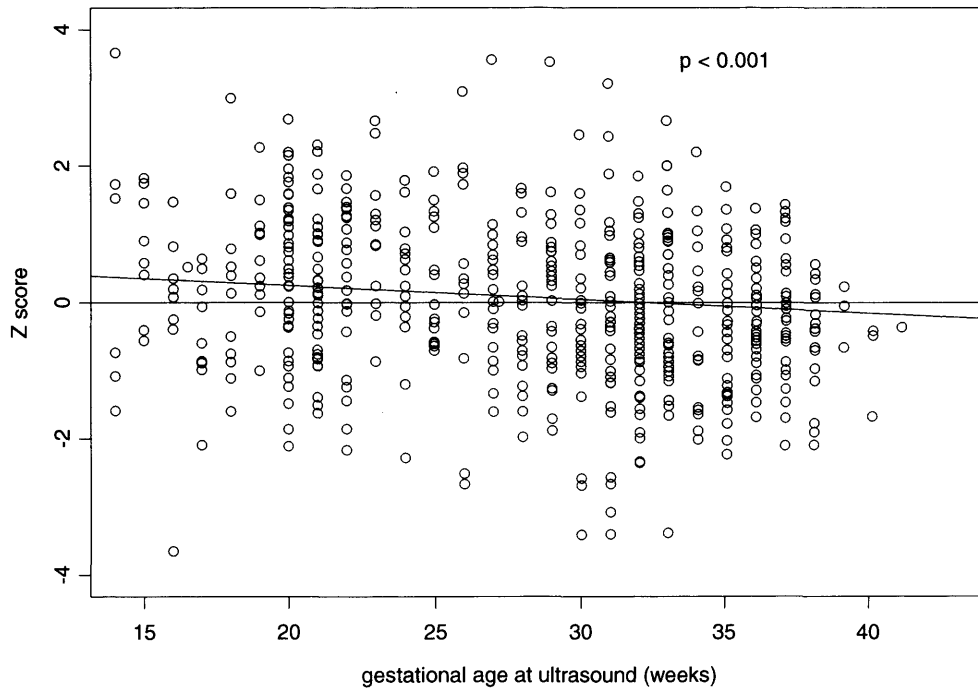
(Empty Dots = individual z-score for each fetus with respect to the uninfected mothers' standard. Continuous line = regression line for Z scores of HIV infected mothers allowing for repeated measurements.)

Figure 7.1(c): Fetal Femur Length z-scores for babies born to HIV infected mothers, by gestational age
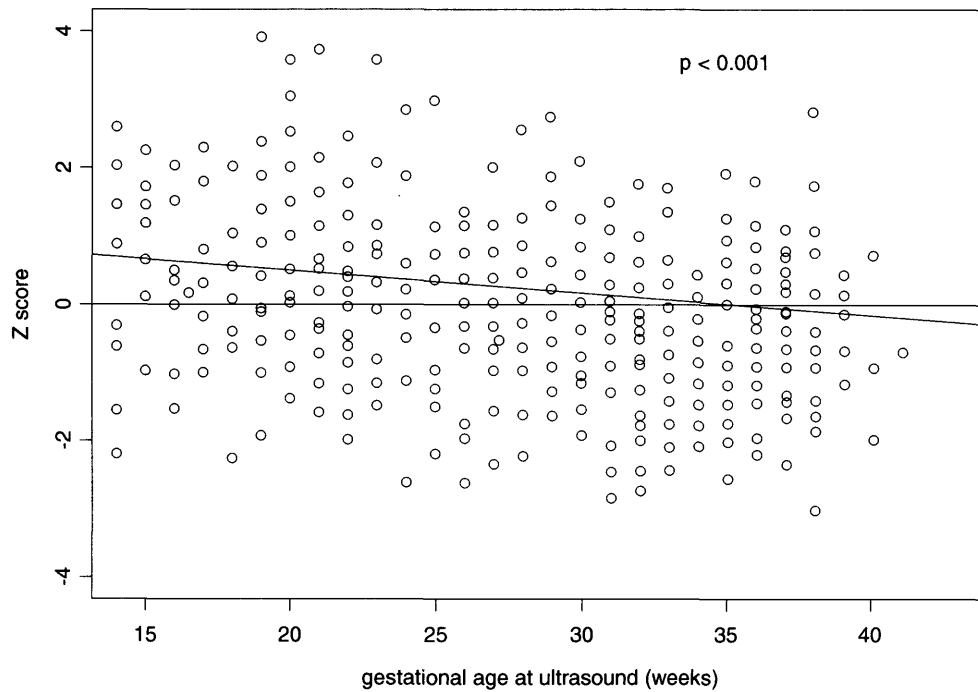
(Empty Dots = individual z-score for each fetus with respect to the uninfected mothers' standard. Continuous line = regression line for Z scores of HIV infected mothers allowing for repeated measurements.)

Table 7.2: Association between Z-scores of fetuses of HIV infected mothers and selected maternal or infant variables; multivariable analysis, including random effect to allow for unobserved variables

	Head Circumference Coeff (p)	Abdominal Circumference Coeff (p)	Femur Length Coeff (p)
Gestational age (wks)	-0.05 (p<.0001)	-0.02 (p<.0022)	-0.03 (p<.0001)
Gender	0.27 (p=0.08)	0.18 (p=0.17)	0.28 (p=0.32)
Congenital HIV Infection	-0.17 (p=0.68)	0.45 (p=0.22)	0.22 (0.54)
Maternal Treatment	0.15 (p=0.39)	-0.13 (p=0.41)	-0.22 (p=0.16)
Maternal CD4	-0.28 (p=0.39)	-0.15 (p=0.60)	0.23 (p=0.41)

Figure 7.2 Head Circumference for all babies born to HIV infected mothers stratified for maternal treatment
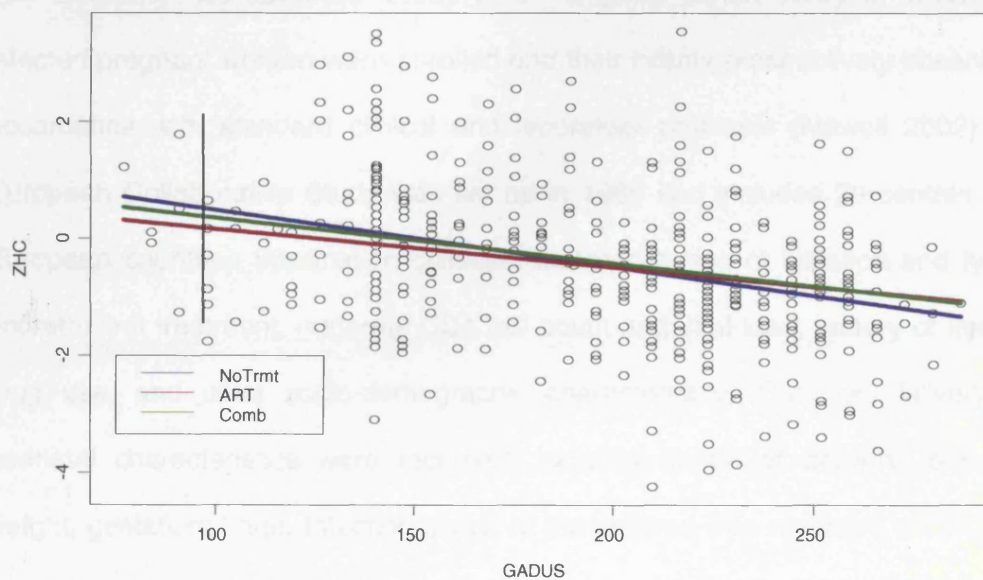
(Empty Dots = individual z-score for each fetus with respect to the uninfected mothers' standard. Continuous line = regression line for Z scores of HIV infected mothers allowing for repeated measurements.)

7.4 Birth weight and duration of pregnancy

7.4.1 Methods

(i) Objective

To investigate further whether the birth weight of infants born to HIV-infected women differs from those born to HIV uninfected mothers allowing for gestational age. This is to understand whether preterm babies were appropriate for gestational age or whether there was evidence for restricted intrauterine growth and whether this varied by HAART exposure.

(ii) Study design and data source

Prospective cohort study. Data used came from the ongoing longitudinal ECS study.

(iii) Study population

The European Collaborative Study is an ongoing cohort study in which HIV-infected pregnant women were enrolled and their infants prospectively observed in accordance with standard clinical and laboratory protocols (Newell 2002). The European Collaborative Study was set up in 1985 and includes 29 centres in 10 European countries Information collected included timing of initiation and type of antiretroviral treatment, maternal CD4 cell count and viral load, history of injection drug use, and other socio-demographic characteristics. Data on delivery and neonatal characteristics were recorded, including mode of delivery, sex, birth weight, gestational age. Infection status of the children was recorded according to standard protocol. Gestational age at delivery was defined as delivery before 34 weeks of gestation; between 34-37 and after 37 weeks of gestation; gestational age was confirmed by ultrasonography, with data reported to the nearest completed week. Mean z scores for birth weight for 5968 babies were calculated allowing for gender of the baby, gestational age at delivery, maternal ART

treatment and HIV infectious status of the children, compared to the British standard.

(iv) Statistical Analyses

Data entry was performed using Access 2000 (Microsoft), and analyses were made using SAS statistical software, version 8.02 (SAS Institute). Z-scores for birth weight, with reference to the 1990 British Growth Standard were calculated using the Growth program excel add-in (version 1.12, Institute of Child Health, London, 2002-3). Ordinary least squares (OLS) regression models were used to investigate the effect of maternal characteristics, and ART use during pregnancy on these z-scores (Weisberg, 1985).

Mean z scores for birth weight for 5968 babies were calculated allowing for gender of the baby, gestational age at delivery, maternal ART treatment and HIV infectious status of the children, compared to the British standard.

7.4.2 Z-scores for birthweight from the large ECS cohort (5968 babies)

A multivariable model using data from the ECS cohort (5968 mother-child pairs) and adjusting for maternal ART treatment IDU in pregnancy, infectious status of the infants and gestational category was created. (Table 7.3)

Table 7.3 Multivariable model: z-scores for birthweight from the large ECS cohort (5968 babies)

	Coeff	p-value	Adjusted Coeff	p-value
Intercept			-0.20	<0.001
Use of IDU				
None/ex-user	-0.66	<0.01	0.0	
Current user	-0.49	<0.001	-0.41	<0.001
Infection status				
Negative	-0.27	<0.001	0.0	
Positive	-0.27	<0.001	-0.13	<0.01
Antenatal ART exposure:				
None	0.48	<0.001	0.0	
Mono	0.35	<0.001	0.20	<0.001
Dual therapy	0.42	<0.001	0.27	<0.001
HAART	0.43	<0.001	0.19	<0.001
Gestational age at delivery				
<34 weeks	0.044	0.582	0.0	
34 to 37 weeks	-0.12	0.182	-0.168	0.05
>37 weeks	-0.39	<0.001	-0.44	<0.001

Maternal IDU during pregnancy reduced z-score for birth weight to a significant extent (mean z-score for IDU users -0.55 (30th centile) and for non IDU users -0.0042 (49th) and future HIV infective status of the child affected only marginally the size at birth (mean z-score -0.022, 41th centile).

The mean z-score for an infant not exposed to antenatal ART or maternal IDU in utero was -0.63 (indicating that the birthweight of this infant lay between the 25th and 50th centile). Infant exposed to antenatal monotherapy or double therapy or HAART were heavier, with estimated mean z-scores respectively of of -0.156 (43th centile),-0.124 (45th centile) -0.07 (47th centile) (Figure 7.3).

Categorising gestational age at delivery into three main categories (less than 34 week, between 34-37 weeks, and above 37 weeks), mean z-scores progressively decreased from -0.10 (46th centile), -0.13 (45th centile) and to -0.30 (37th centile) indicating that children born to HIV infected mothers when compared to their standard, become statistically different (smaller) towards the end of pregnancy (Figure 7.4), after controlling for maternal antiretroviral treatment and use of IDU during pregnancy.

Figure 7.3

Box-plot for z-scores of birthweight by antenatal ART use
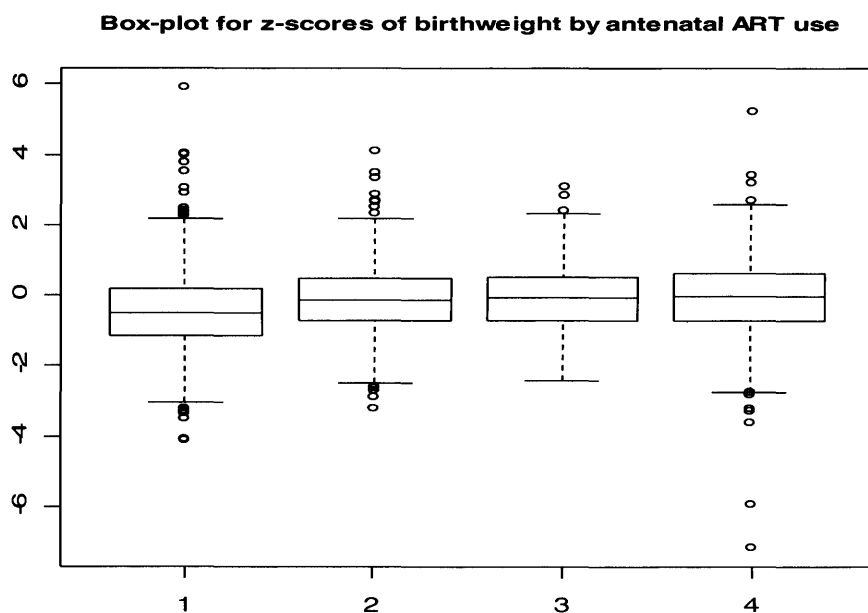
Y- axis: Z scores for birthweight (mean)

X-axis: Antriretroviral therapy

1=no therapy   2=monotherapy   3 =combination therapy   4=HAART

Figure 7.4
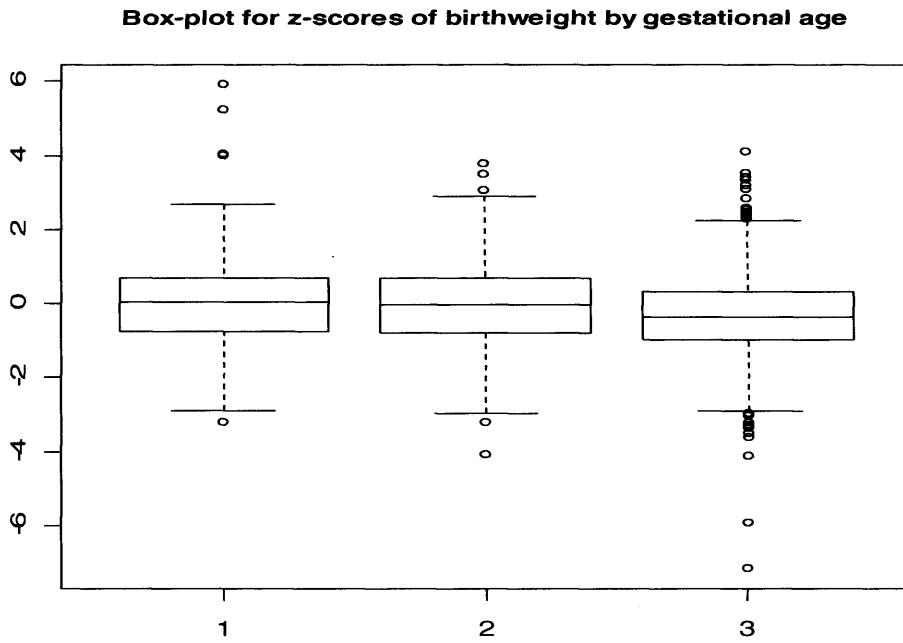
Box-plot for z-scores of birthweight by gestational age

Y-axis

Z scores for birthweight (mean)

X-axis: Gestational age

1= Gestational age<34 weeks

2=Gestational age between 35 and 37 weeks

3= Gestational age >38 weeks

Figure 7.5

Z-score for birthweight at different levels of treatment and gestational age
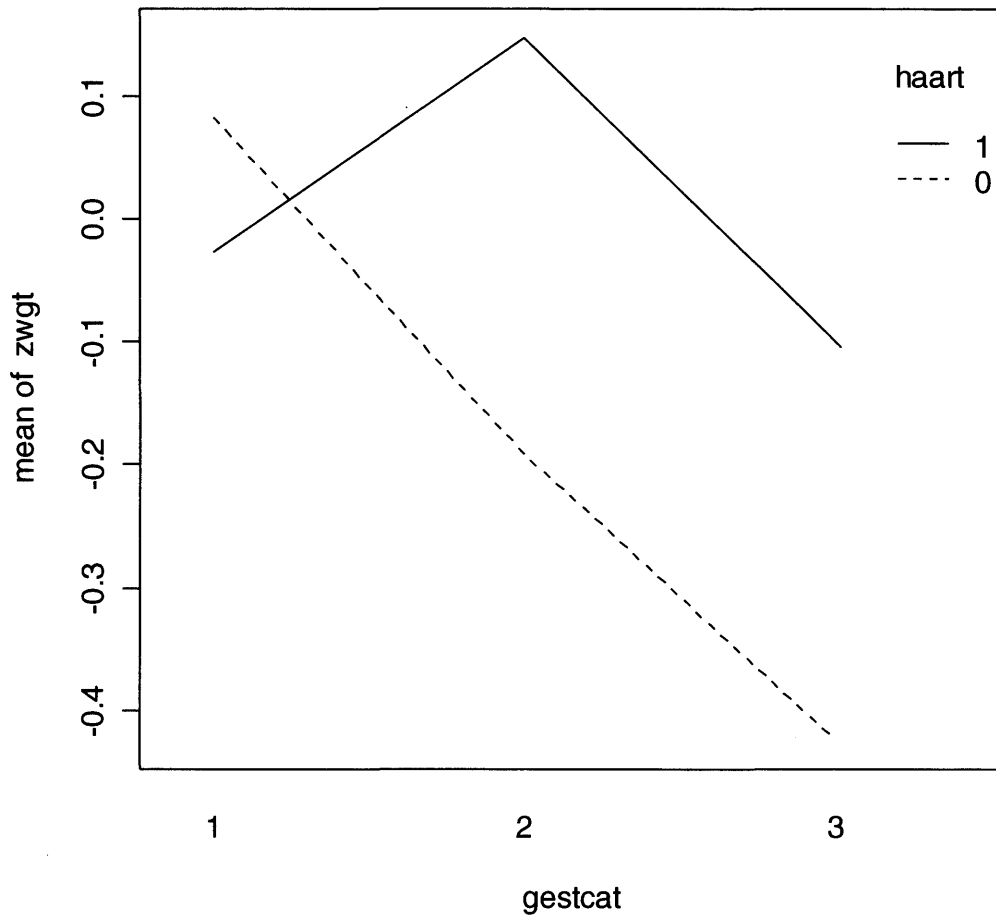
Y-axis

Z scores for birth weight (mean)

X-axis: Gestational age

1= Gestational age<34 weeks

2=Gestational age between 35 and 37 weeks

3= Gestational age >38 weeks

There was a significant joint effect of HAART and gestational age on birthweight (p value for the interaction terms 0.020) with the effect being concentrated on children born after 35 weeks of gestation with similar coefficients for the other variables included in the multivariable analyses than seen previously without inclusion of the interaction term.

The birthweight of babies born prematurely was more appropriate for gestational age than that of infants born at term, suggesting that babies in utero tend to slow their growth pattern proportionally with their gestational age. (Figure 7.5)
This would lead to the conclusions that premature delivery when occurring in HAART treated women is likely to be due to somehow toxicity on the fetal membranes and not due to fetal distress.

7.5   Key points

- Growth was significantly reduced (p<0.0001; p<0.001; p<0.001) in infants of HIV-infected mothers compared to the uninfected reference population, especially in the third trimester (slopes became negative around 30 gestational weeks, based on   Z- scores for head circumference (HC), abdominal circumference (AC) and femur length (FL) measurements adjusted by gender, maternal CD4 count and maternal treatment at delivery.

- The average z-score of head circumference and femur length in HIV-infected women was substantially below the reference (-0.46-32th centile and -0.0997-15th centile respectively), but the average z-score for abdominal circumference differed only marginally from that for the uninfected reference population (-0.023-49th centile).

- Maternal treatment did not show any association with intrauterine fetal growth, neither maternal CD4 did, probably due to the small sample size.

- Comparing birthweight of babies born to HIV infected mothers to the British Standard, categorising gestational age at delivery mean z-scores progressively decreases indicating that children born to HIV infected mothers when compared to their standard, become statistically different (smaller) towards the end of pregnancy.

- Infant exposed to antenatal monotherapy or double therapy or HAART were heavier.

- Babies born prematurely are more appropriate for gestational age, suggesting that babies in utero tend to slow their growth pattern proportionally with their gestational age.

Chapter 8 Maternal post partum complications in relation to mode of delivery

_Summary_To inform the debate on the use of elective caesarean section (CS) delivery in HIV-infected women, we investigated the occurrence of clinical events in the immediate post-partum period in women delivering in 13 European centres. We analysed data from two matched-case-controls studies (HIV infected women matched to HIV uninfected women) on spontaneous deliveries and elective caesarean section.

8.1 Methods

(i) Objectives

To determine whether the surgery involved in delivery by elective caesarean section is related to post-partum complications in HIV-infected mothers.

(ii) Study design and data sources

Two case-control studies, one for vaginal delivery and one for caesarean section, HIV-infected women (cases) were matched with HIV-uninfected controls. HIV infected women were enrolled in the ECS longitudinal study. Clinicians were asked, where possible, to collect extra data on post partum complications. Sample size to detect the clinically important difference (at 5% level; 95%CI) between the two groups was calculated using Epinfo program for case-control studies. Assumptions for this calculation included random sampling from the population and a doubling of the rate of complications in the HIV infected group (OR= 2) with 80% power.

(iii) Study Population

Data on HIV-infected women delivering in one of 13 European HIV reference centres in Italy, Spain, Sweden, Poland and Ukraine from 1992 to 2002 were collected retrospectively from the medical notes.(Appendix III) Most of these sites were already participating in the European Collaborative Study.   Local ethics permission was obtained.

In a case-control approach involving two studies, one for vaginal delivery and one for caesarean section, HIV-infected women were matched with HIV-uninfected controls. Controls were selected as the first uninfected woman delivering after the index case on the birth register of the same unit, without a history of current injecting drug abuse. Cases and controls were matched by age, ethnicity and parity, for being admitted to the delivery unit with active labour in the spontaneous delivery arm, or for having

received antibiotics during labour or during caesarean section. Women admitted for medical induction of labour or for emergency caesarean section were not included in the study. (NIH, 1981; Watts, 2000) Post-partum haemorrhage requiring blood transfusion or surgical treatment, pneumonia or pleural effusion, peritonitis, sepsis, diffuse intravascular coagulation and thromboembolism were considered major complications. Anaemia not requiring transfusion (less than 10g%), fever in excess of 38°C after twenty four hours from delivery, wound infection, curettage of the uterine cavity, endometritis and urinary tract infection were considered minor complications.

Recorded clinical parameters for HIV infection included CD4 lymphocyte count in late pregnancy, use of antiretroviral therapy, and clinical symptoms. Routine measurements of HIV RNA viral load in HIV-infected women was only introduced in these centres in recent years and this information was thus not available for the majority of cases.

(iv)   Statistical analysis

Statistical significance of differences between means was evaluated by Student's t test, differences between proportions were evaluated by χ^2 test in HIV-infected women and uninfected women separately.   The analysis of post-partum complications and HIV infection used conditional logistic regression analysis for the two different matched case-control studies: (a) vaginal delivery, and (b) caesarean section. All analyses were carried out using a commercial software package (STATA 7).

8.2   Clinical characteristics

A total of 250 matched pairs were delivered vaginally and 158 by elective caesarean section. Table 8.1 shows baseline characteristics of HIV-infected and -uninfected women by mode of delivery and the prevalence of post-partum complications in the

four groups. The overall complication rate was 29.2% (119/408) among HIV-infected women and 19.3% (79/408) for the HIV-uninfected group (OR 1.7, 95% CI 1.22-2.40, p=.0001). Among the 316 women delivered by caesarean section, 42.7% (135) experienced complications, as did 12.7% (63) of the 500 women who delivered vaginally (OR 5.1, 95% CI 3.6-7.42, p=.001).

Within mode of delivery group, infected and uninfected women did not differ significantly in terms of age, height, birth weight or neonatal outcome. Hospital stay was longer for mothers delivered by caesarean section, regardless of infection status (Table 8.1). Maternal weight at delivery for HIV-uninfected mothers was higher for those who delivered by caesarean section compared with those in the vaginal delivery group.

Amongst the HIV-infected women, those in the caesarean section arm were more likely to be on antiretroviral therapy (ART) during pregnancy (44 (27.8%) not treated, 28 (17.7%) on monotherapy and 86 (54.4%) on combination therapy) than those in the vaginal arm (44 (17.6%) on monotherapy, 206 (82.4%) not treated) (p<0.001) reflecting the time periods these women delivered in, and changes in clinical management of HIV-infected pregnant women. Only a small proportion of infected women in both arms were severely immunosuppressed with a CD4 cell count of less than 200 cells per ml: 18/250 (7.2%) in the vaginal arm and 21/158 (13.3%) in the caesarean arm (p=0.1).

Table 8.1: Maternal characteristics according to HIV infection and prevalence of post-partum complications by mode of delivery						
	HIV-infected women			**HIV-uninfected women**		
Number: 816	Vaginal delivery (250)	Caesarean section (158)	P value	Vaginal delivery (250)	Caesarean section (158)	P value
Age yrs (mean, SD)	26.71 (3.95)	29.00 (6.944)	0.60	25.89 (3.6)	29.6 (7.8)	< 0.001
Weight kg (mean, SD)	67.85 (11.44)	65.8 (10.52)	0.90	63.23 (9.3)	72.00 (12.93)	< 0.001
Height cm (mean, SD)	164.80 (7.19)	163.90 (7.40)	0.98	163.55 (6.8)	162.66 (7.3)	0.10
Gestational age in weeks (mean, SD)	38(1.6)	37.88 (1.39)	0.97	39 (1.2)	38.54(1.5)	< 0.001
Blood loss >500 ml n (%)	6 (2.43)	44 (28.35)	OR=15 (6-42)	8 (3.2)	36 (23.0)	OR=8.93 (3.8-21)
Minor complications n (%) Yes No	 42 (16.8) 208 (83.2)	 77(48.7) 81 (51.3)	 OR= 0.34 (0.25-0.47)	 21(8.4) 229(91.6)	 58 (36.7) 100 (63.3)	 OR=0.239 (0.14-0.36)
Major complications n (%) Yes No	 0 250 (100)	 5 (3.2) 153 (96.1)	 OR= 8.4 (0.92-18.2)	 0 250 (100)	 1(1) 157 (99)	 NA
Mean hospital stay (days) (SD)	5.4 (3.3)	7.4 (3.7)	< 0.001	5.3 (3.2)	8.7 (5.9)	< 0.001

8.3   Complications after vaginal delivery

The following analyses refer to the 250 matched pairs delivered vaginally. All women delivered between the 37th and the 41st week of gestation. In 183 (73.2%) pairs membranes were intact when admitted to the delivery unit; while in the remaining 67 (26.8%) pairs the membranes were ruptured at admission (for 35 pairs for more than 12 hours). Eight (3%) pairs received Amoxicillin 2 gram intramuscularly as prophylaxis for bacterial infection. Episiotomy was performed in 147 pairs (in 92 as a mediolateral and in 55 as a median perineal incision). Only three uninfected patients required manual removal of the placenta.

There were no major complications in this group of either HIV-infected or in uninfected women. In matched analysis post-partum fever was the only minor complication for which HIV-infected women were at significantly higher risk compared to uninfected women (OR 4.5, 95% CI 1.55-13.07, p=.001). Table 8.2 shows the correlation between fever and episiotomy in the two groups of women. Although there was a trend towards increased risk of endometritis in infected women, this did not reach statistical significance (OR 4.4, 95% CI 0.44-12 p=0.3). In multivariate analysis to assess the effect of selected risk factors on the incidence of complications in these infected women, including history of illicit drug use, CD4 lymphocyte count, episiotomy, only episiotomy, and in particular a medio-lateral incision (p=0.022), was associated with a significant increase of puerperal fever [z=- 3.19 (SE 0.312 P>z=0.001 (95% CI -1.61 -0.38)

Table 8.2 Fever and episiotomy

	HIV-positive Mothers	HIV-negative mothers	P value
Median episiotomy	4/55	1/55	Ns
Mediolateral episiotomy	9/92	1/92	0.02 (OR 9.8)
No episiotomy	5/63	2/63	Ns

8.4 Complications after caesarean section delivery

All 158 matched pairs in this group were delivered between the 37th and the 39th week of gestation, all had intact membranes when admitted to the hospital and none were in labour. All pairs received prophylactic antibiotics during the surgical procedure. Median duration of the procedure was 45 minutes (min 22 - max 90) and a total of 26 women (16%) were assisted by a senior surgeon (consultant or head of department) at delivery. Clinical indications for caesarean section delivery included reduction of vertical transmission for all infected cases and for the uninfected controls: breech presentation (73), macrosomia (19), maternal hepatitis C infection (8), maternal human papillomavirus infection (5), maternal HELLP syndrome (10), uterine abnormalities (26), obesity (7) and choice (10). Wound drainage was used once in four HIV-infected and two uninfected women for less than 3 days and a bladder catheter was used for less than 24 hours in 201 (80%) patients: 85 (42%) cases and 116 (57%) controls. The method of caesarean section was reported as conventional for all patients and spinal anaesthesia was used in 13% (32) of the HIV-infected cases.

There were five major complications in the HIV-infected cases and only one among the controls (Table 8.2). All five HIV-infected cases with major complications were on ART during pregnancy. Overall, infected women were at higher risk of both minor

(OR 1.51, 95% CI 1.22-2.41, p=.001) and major complications (OR 5.1, 95% CI 0.58-45.0) compared with uninfected women. Anaemia not requiring blood transfusion was the most prevalent complication regardless of infection status but was significantly more frequent in the infected cases [z=- 2.18 (SE 0.27) P>z=0.029 (95% CI −1.1 −0.6)]. With the exception of anaemia there were no significant differences between cases and controls in relation to minor complications [z=- 1.1 (SE 0.83) P>z=0.273 (95% CI −2.55 −0.72)].

Among anaemic HIV-infected women there was a strong association between excessive blood loss during surgery and post-partum anaemia (OR=2.7, 95% CI 1.47 - 4.9), irrespective of the seniority of the surgeon. Anaemia was strongly associated with both the use of antiretroviral therapy during pregnancy (OR=3.1, 95% CI 2.7-6.9), and a low CD4 count (OR=2.9, 95% CI 2.1-9.1).

Table 8.3: Post-partum complications, by mode of delivery and infection status					
	Vaginal delivery 250 matched pairs			Caesarean section 158 matched pairs	
	HIV infected 250	HIV uninfected 250		HIV infected 158	HIV uninfected 158
Minor complications					
Fever > 38°	18			3	4
Anaemia not requiring transfusion	7	6		64	49
Urinary tract infections	3	3		4	1
Endometritis	11	5		2	4
Haematoma	1	1		0	0
Wound gapping	1	1		3	0
Wound infection	1	1		1	0
Total	*42*	*21*		*77^*	*58^*

Table 8.3: Post-partum complications, by mode of delivery and infection status					
Major complications					
Peritonitis	0	0		3	1
Pneumonia	0	0		2	0
Sepsis	0	0		0	0
Thrombosis	0	0		0	0
Diffuse intravascular dissemination	0	0		0	0
Subileus/ileus	0	0		0	0
Haemorrhage	0	0		0	0
Total	*0*	*0*		*5*	*1*

8.5 Conclusions

Table 8.3 shows complications after delivery according to infectious status of the mother and mode of delivery. Results indicate that fever was a recurrent post-partum complication for those women who delivered vaginally and anaemia for those who delivered by caesarean section. Overall HIV-infected women suffered an increased risk of minor complications. Major complications were more likely to occur in the caesarean arm than in the vaginal delivery arm

8.6   Key points

- Two separate matched case–control studies (vaginal and elective CS deliveries) among infected women (cases) and uninfected controls delivering between 1992 and 2002.

- The prevalence of minor and major post-partum complications was assessed overall for infected and uninfected women; within mode of delivery group (vaginal/cesarean section) the complication rates of infected cases were compared with uninfected controls in a matched analysis.

- Overall complication rates were 29.2% (119 of 408) for HIV-infected women, 19.4% (79 of 408) for uninfected women, 42.7% (135 of 316) for CS deliveries and 12.6% (63 of 500) for vaginal deliveries.

- There were no major complications in women delivering vaginally; but, compared with controls, HIV-infected women were at increased risk of puerperal fever [odds ratio (OR), 4.5; 95% confidence interval (CI),1.55–13.07), especially after medio-lateral episiotomy.

- In the CS group, there were six major complications (five among cases, one control) (OR, 5.1; 95% CI, 0.58-45) and cases had an increased risk of minor complications (OR, 1.51; 95% CI, 1.22–2.41) compared with controls, mainly anaemia not requiring blood transfusion

- HIV-infected pregnant women are at increased risk of post-partum complications regardless of mode of delivery

- Modification of clinical practice, particularly use of prophylactic antibiotics, would reduce this risk.

Chapter 9 Childbearing in HIV infected women in Europe (discussion and recommendations)

9.1 Introduction

The era of combination therapy with highly active drugs has resulted in HIV infected individuals living longer and healthier lives (Mocroft 2003; Vittinghoff 1999). HIV infection is now classified as a chronic disease in the Western world because of a significant increase in the quality and expectancy of life with the introduction of highly active antiretroviral therapy. This, along with the developments in preventing vertical transmission and partner infection, has made it necessary to open the discussion on the reproductive desires and rights of HIV infected individuals and to provide clinicians involved in their care with recommendations.

The studies presented in this thesis focused on HIV-infected women's reproductive and sexual health as assessed by themselves as well as the wider impact of HIV and treatment on their reproductive choices and pregnancy outcomes. Analysis of data from several cross-sectional and longitudinal studies on reproductive outcomes of HIV infected women was preceded by a cross-sectional survey of reproductive choices in relation to relationships, their partners, desire of parenthood and previous obstetric experience. Results from the reported studies explore three different aspects of reproductive behaviour in HIV infected women, confirming a strong desire for motherhood, suggesting a small interaction between HIV and fecundability and highlighting the importance of pregnancy management.

The studies reported here complement each other and provide further evidence to inform guidelines and recommendations on the management of HIV infected childbearing women in Europe.

9.2 Reproductive choices

Most studies examining the impact of HIV and antiretroviral treatment on desire of parenthood and reproductive choices have been on a population rather than an individual basis (Gray 1998; Degrees 1997; Forquet 2001). To date, little has been discussed in the literature regarding the various factors that could influence the reproductive options of HIV-infected women.

Reproductive decision making in women infected with HIV is likely to be influenced by a complex array of factors, including demographic and situational variables, psychological patterns, locus of decision making, counselling techniques, access to care, and the attitude of health care providers.

Previous studies have been limited to discussing the decision of whether or not to become pregnant and to describing women feeling in relation to this. (Wesley, 1996). Three major themes emerged from an American survey on 25 HIV infected women: (1) motherhood was viewed as a joy and a means of meeting a woman's own needs, (2) concerns about their children's well-being, and (3) the minor role that HIV infection played in their lives. Women reported negative reactions to providers who focused exclusively on their HIV status, and not on the need to view the women's lives as a whole. (Wesley, 1996)

A similar suggestion of the importance of culture over HIV infection came from a European study, in which the incidence of pregnancy and terminations after HIV diagnosis were three times lower in white European than in women of African origin. (van Benthem 2000)

It is likely that far more HIV infected women in Europe are now considering to become pregnant, although a London-based study, published in 1996, comparing rates of reproductive events before and after HIV diagnosis in a cohort of women with HIV infection, concluded that diagnosis of HIV infection in women had a

substantial impact in reducing live-birth rates (women aged 20-34 years, the age-adjusted live-birth rate fell by 44% , reflecting an increase in termination rate). (Stephenson, AIDS 1996)

However, since that time, HAART has became widely available and interventions to reduce mother to child transmission have proven to be effective. Results from the survey reported in this thesis indicate that desire of parenthood was associated to maternal well being as having no HIV symptoms and the knowledge that the risk of mother to child transmission can be decreased with appropriate interventions. Pregnancies were more likely to occur in women with no previous livebirths and in those in a long standing relationship with an uninfected partner.

These findings are in line with results from a large survey conducted in America including 1,421 HIV-infected adults who were part of the HIV Cost and Services Utilization Study focusing on fertility desires and intentions of HIV infected men and women (Chen 2001). HIV-positive individuals who desired children or expected children were reported to be younger, to have fewer children and to report higher ratings of physical functioning or overall health than their counterparts who did not desire children. Women whose partner's HIV status was known were significantly less likely to desire children but were significantly more likely to expect children in the future than were women whose partner's HIV status was unknown (Chen, 2001).

Regardless of women's pregnancy experiences or intentions, reproductive decision-making themes in the survey presented here included: the perceived risk of vertical transmission; beliefs about vertical transmission risk reduction strategies, desire for motherhood, the HIV infection status of their partners, attitudes of health care providers; and the impact of the mother's health and longevity on the child.

Desire for children was higher among HIV discordant couples where the male was uninfected, thereby suggesting that the HIV status of the male partner is an important determinant to a couple's reproductive practice, as important as the health condition of the woman.

Women who reported not to want any children after their diagnosis did not mention vertical transmission risk as the reason, and most of these women already had children or were not in a relationship. Those who became pregnant or desired children after their diagnosis seemed more confident in the efficacy of risk reduction strategies and often did not already have children.

Women who wanted children or were pregnant, were more likely to have a long-standing relationship, be married or cohabiting, were heterosexually infected and older than non-pregnant HIV women. Time taken to conceive suggested that overall, conception was not a problem for most women and prevention of viral transmission from an infected woman to an uninfected man relied often on timed self-insemination using quills. A number of women failed to conceive, needing fertility advice and treatment as one or both may have impaired fertility, although fertility remains a medical concept related to both partners, and comparison with normal rates is difficult as most of the available data are on birth rates in populations (Thackway 1997).

These data, confirm previous findings on couples infected with HIV and their reproductive needs. Couples infected with HIV are actively seeking reproductive assistance for different reasons depending on which partner is infected and which has a fertility problem. When the woman is HIV infected or both the partners are, they are likely to need help when they fail to conceive spontaneously or by self insemination.

The issue of reproductive care in HIV infected individuals still poses ethical dilemmas and practical implications for the couples and the carers. In couples who have already tried to conceive unsuccessfully, medical intervention permits the conception of a child who is at risk of acquiring HIV, even with optimal reproductive care. It can be debated whether the couple's desire to have a child justifies medical intervention that involves the potential risk of infection for the the child.

In the past few years a number of clinics in several European countries have started to offer reproductive services for couples with HIV infection (AE SEmprini Personal Comunication 2005 on Creathe Network-Centers for Reproductive Assistance To HIV couples in Europe), although methods and procedures for reproductive counselling or fertility care are not yet standardised.

The impact of living with HIV can be observed in the awareness of the couple and their adoption of safer sexual practices. Both the pregnant and non-pregnant women in the survey reported to use condoms regularly, although condom use was higher in serodiscordant couples. Sexually transmitted infections were reported to be recurrent gynaecological problem. This confirms results from a large French study (Heard 2004) and emphasizes that reproductive counselling is very important and should include a discussion on reproductive issues, HIV and other sexually transmitted infections in relation to the partner's HIV infection status.

Although this is a cross-sectional survey and could not take into account changes in management over past few years, results suggest that in these days knowledge of HIV infection does not influence the desire for children or the decision regarding pregnancy in HIV-infected women living in Europe .

The fact that many HIV-infected adults desire and expect to have children has important implications for the prevention of vertical and heterosexual transmission of HIV, the need for counselling to facilitate informed decision-making about

childbearing. The availability of treatment and improvement in the quality of life, have given women living with HIV greater autonomy with respect to reproductive choices.

The second part of this study focused on reproductive outcomes and specifically on the interaction between antiretroviral therapy and pregnancy duration, intrauterine fetal growth and neonatal birthweight and post partum complications

9.3 Immunology of HIV and outcome of pregnancy

HIV disease and pregnancy both stimulate a Type 2 cytokine response and inhibit the production of Type 1 cytokines. The present study has focused on the secretion of Type 1 (IL-2) and Type 2 (IL-10) cytokines by peripheral blood lymphocytes over the course of pregnancy, to understand the association between HAART exposure and risk of premature delivery in HIV infected women (ECS 2000). We report decreasing levels of IL-10 (Type 2) between the beginning and the end of pregnancy, especially evident after HIV-specific stimulation, in HAART treated women delivering prematurely and a dominant significant effect of Type 1 (IL-2) cytokines on the risk of premature delivery. Results also suggest that the effect of HAART on the risk of prematurity is mediated through its effect on the cytokine environment, rather than through a direct toxic effect on the fetus or placenta.

An association between modulation of cytokine production and the likelihood to deliver healthy newborns at term has been shown in a number of studies (Marzi 1996; Raghupaty 1997). Increased Type 1 cytokine responses are seen in women with recurrent pregnancy losses and in pre-eclamptic patients. In successful pregnancies the maternal-fetal interface shows a higher expression of B7-H1, a costimulatory molecule associated with IL-10 production (Trabattoni 2003). In our population of HIV infected pregnant women delivering prematurely, trends of IL-10

and IL-2 were highly negatively correlated. In logistic regression, levels of non-specifically (pha) stimulated IL-2 was most strongly associated with the risk of premature delivery, while the effect of HAART was indirectly captured by the decrease in HIV specific (env) stimulated IL-10 slopes. IL-10 levels (Type 2) were consistently higher in the women delivering at term than in those delivering before 37 weeks, but the effect of IL-10 was not statistically significant in multivariate analyses, suggesting a secondary role of IL-10 in the mechanism of premature delivery, dependent on HAART and its link with IL-2.

While the bioactivity of different cytokines may not be quantitatively equivalent with considerably higher levels of IL-10 than of IL-2, the concentrations of these cytokines relative to each other are of importance because of the mutually inhibitory effects of Type 1 and Type 2 activity and therefore it is important to analyse the whole environment.

Although results are in agreement with the hypothesis that the association between HAART and increased risk of premature delivery is mediated by the changes in cytokine environment, the limited number of women in this study precluded further investigations and it was not possible to investigate whether different antiretroviral compounds (for example PI versus NNRTI) affect the duration of pregnancy in different ways, reflecting a possible different immuno modulating effect. Research is ongoing to investigate whether the effect is seen in local tissues like amniotic membranes or the decidual interface, in which immune cytokines may play an important role in the pre-apoptotic activation even when not secreted by circulating immune cells.

These preliminary data indicate the underlying mechanism for premature delivery in HIV HAART treated women, and highlight the need for careful consideration when initiating immunomodulating compounds during or before pregnancy.

9.4 Intrauterine fetal growth, duration of pregnancy and birth weight

There is lack of evidence to substantiate the suggestion that exposure to maternal HIV infection during fetal life may affect growth. Growth patterns in uninfected children who are born to mothers with HIV-1 infection have been described in detail previously beyond early childhood (European Collaborative Study 2003).

However, although poor growth and low birth weight have been reported in HIV-infected children, it is unclear what happens before birth and whether growth faltering is an independent HIV-related symptom or caused indirectly. To further investigate the effect of HIV and its treatment on the fetal environment, we analysed intrauterine growth of fetuses born to HIV-infected mothers as determined by head, abdomen circumference and femur length.

Results were based on data of HIV infected mothers delivering after 1990, and the changing epidemiological features of the HIV infected pregnant population during the past decade were reflected in the changes over time in use of antiretroviral therapy, mode of delivery and maternal mode of acquisition.

The intrauterine growth of fetuses born to HIV infected mothers as determined by head and abdomen circumference, was significantly reduced compared to that of fetuses born to HIV uninfected mothers.

Fetal growth was consistently lower in HIV infection than in normal controls obtained from the same population. Absence of skewness of birth weight, associated with the intrauterine growth curves, would confirm a genuine biological result.

To better understand the pattern of growth in children born to HIV infected mothers, this study also analysed size at birth (birthweight) of babies born to mothers enrolled in the same large prospective European cohort (ECS) and compared to the British standard (Cole, 1996).

Birthweight of babies born prematurely was more appropriate for gestational age than that of infants born at term, suggesting that babies in utero tend to slow their growth pattern proportionally with gestational age. Children who were premature following HAART exposure were heavier than those who were premature due to other causes, suggesting that premature delivery occurring in HAART treated women may be due to increasing toxicity on the fetal membranes and not due to fetal distress.

The damage to growth in fetal life was independent from the HIV infection status of the infant. The increased prematurity rate in infected women treated with combination ART has been noted before, and is probably related to labour initiation rather than fetal distress according to their appropriate birth weight for their gestational age. It is speculative to suggest that HIV infection can impair the prenatal environment, possibly through damage to placental cells and function and this progresses with time, affecting more intrauterine growth of full term babies. HAART can damage prenatal environment altering the cytokine balance and probably impacting on amniotic membranes, causing indirectly their rupture.

Our findings complement those from African studies, in which infants born to HIV-infected mothers have been shown to be of lower birth weight than those of HIV-uninfected mothers in providing for the first time evidence of impaired in utero growth in infants born to HIV-infected mothers that seems to be determined more by maternal HIV infection than by maternal antiretroviral therapy. (Arpadi 2000; Markson 1996).

Improved prenatal care could be offered to this women as it may offer protection against perinatal morbidity and mortality due to low-birth weight, although as Samuel Shwartz noted in 1932 "the role of prenatal care in the incidence of prematurity and perinatal mortality is neither well defined nor well established" (Shwartz 1932).

The cause of growth failure remains unknown and additional studies are needed to assess the possible interaction between maternal HIV infection and placental function. This study provides further insight on the interaction between HIV infection and pregnancy and completes one of the very few model of growth in the literature.

9.5 Maternal post partum complications in relation to mode of delivery

The aim of this project was to provide information on the comparative risks of complications associated with HIV infection and mode of delivery, to help inform decision-making by clinicians and women.

The overall prevalence of any post-partum complication here was five-fold higher in the caesarean section than in the vaginal delivery group, irrespective of HIV infection. However, most complications were minor, relating to anaemia and fever, with serious complications limited to women delivered by caesarean section. Consistent with previous reports, we found an increased risk of minor complications after elective caesarean section in infected women, albeit of a lesser magnitude (Semprini 1995, Marcollet 2002; Rodriguez 2001), but we also show increased risk after spontaneous vaginal delivery.

In previous studies among infected women, post-partum fever was associated with elective cesarean section delivery and immunosuppression. However, prevalence of fever was similar in infected and uninfected women delivered by electice caesarean section here; infected women were more likely to be on ART for long

periods, with restored immune function, and were also exposed to routine antibiotic prophylaxis. The infrequent use of antibiotic prophylaxis may partly explain the finding here that fever was common among infected women delivering vaginally. The association found between fever and medio-lateral episiotomy is not surprising given the extensive muscular and vascular damage associated with this procedure. Any delivery, but particularly abdominal delivery, results in blood loss, and a subsequent physiological fall in haemoglobin. In HIV-infected women, anaemia was also associated with ART use. Prevention of MTCT relies on the combination of elective caesarean section and antiretroviral prophylaxis, both of which are associated with anaemia (Newell 2002)

Women should participate in decisions regarding mode of delivery and be informed of potential risks and benefits. However, infected women opting for vaginal delivery should be informed of the 8-10% chance of needing an emergency caesarean section due to labour complications. This might expose the child to an increased MTCT risk and the mother to complications.

Paradoxically, infected women at greater theoretical risk of complications following caesarean section delivery, i.e. those with advanced disease, are also those who would benefit most regarding prevention of MTCT. Although antenatal HAART use is associated with a substantial decline in MTCT, elective caesarean section remains an independent intervention. Furthermore, it has been shown that elective caesarean section is cost-effective even when the MTCT rate among vaginal deliveries is low (ECS 2001).

A strategy of elective caesarean section for HIV-infected women in Europe does not appear to increase risk of serious maternal morbidity while it should be noted that vaginal delivery in infected women is also not risk-free. Results suggest the need to adopt precautions to reduce risk of infection after vaginal delivery

specifically for infected women, and of anaemia after caesarean section generally. HIV-infected women delivering vaginally requiring a medio-lateral episiotomy might benefit from antibiotic prophylaxis, which are already the standard of care during caesarean section procedures. Reducing risk of anaemia after caesarean delivery can be achieved by prenatal iron supplementation and by spinal rather than general anaesthesia (Andrews 92; Avidan 2002). Spinal anaesthesia, increasingly used, is associated with reduced blood loss and additionally reduces risk of post-surgical pneumonia.

9.6   Limitations and suggestions for future research

Mother to child transmission can be substantially reduced with maternal antiretroviral treatment and elective caesarean section. Whether there is benefit of elective caesarean section delivery in women who receive HAART is still debated, although it is shown that vaginal delivery is also not risk free in terms of post natal complications and increased risk of vertical transmission. Similarly, HAART use has been associated with premature birth and the understanding of the underlying mechanism in relation to intrauterine growth and birth weight of these children could the inform obstetric management and therapeutic options.

The majority of data presented are based on observational data and this remains a major limitation. Bias, like chance and confounding should always be considered as a possible alternative explanation of any observed statistical association, although this has always been taken into account in the analyses through the use of appropriate statistical methods, study design and conduct.

A limitation of the survey of HIV infected women was the potential for selection bias as subjects had more contact with an Obstetric and Gynaecological Unit, and thus they may be better informed, and a selected group

To strengthen work on cytokines it would have been helpful to investigate two important other Type 1 and type 2 cytokines, IFN-gamma and IL-4, particularly with respect to the mutually inhibitory effects of Type 1 and Type 2 activity, which are mainly exerted by IFN-gamma and IL4. But the cost of laboratory test forced us to choose only Il-2 and Il-10.

Future research could address the issue of HAART induced immunologic aspects of premature delivery, such as apoptosis in premature rupture of fetal membranes and the roles of cytokines in inducing the pre-apoptotic pathway, as a result of the use of antiretroviral treatment. Further research is needed in the area of placental functioning and HIV as suggested by the work on intrauterine growth and birth weight in newborn babies born to HIV infected mothers .

In summary, proposed components of a service for HIV infected women in Europe include the following:

(a)   Reproductive counselling

Done on a routine basis should include a discussion on reproductive issues, HIV and other sexually transmitted infections, partner HIV infection status.

Access to fertility advice and treatment should be easy to all couples infected with HIV; access to gynecologic and obstetric care, should be integrated into both AIDS clinics and drug treatment programs.

Although the use of contraception among HIV infected women was not an objective of this thesis, the literature review highlighted that despite significant advances in the development of new contraceptive technologies (ie transition from high-dose to low-dose combined oral contraceptives, from inert to copper- and levonorgestrel-releasing vaginal IUDs, combined injectable contraceptives, a combined hormonal patch and ring, progestogen-only injectables and implants) current policies and

health care practices within the HIV infected population are largely based on small, mostly unpublished studies (WHO, 2004). There is, thus, a need for more conclusive data on the safety and efficacy of contraceptive use in HIV-infected women, possible interactions with antiretroviral therapy, as well as data that describe the effects of patient education and counselling and closer follow-up on effective long-term contraception in HIV-infected women.

Prenatal counselling should be multidisciplinary, involving the infectious disease specialist and the obstetrician together at an earlier stage.

(b) Pregnancy

Need for intrauterine growth longitudinal evaluation, although the magnitude of growth failure remains small, as prenatal care can impact on weight at birth and on duration of pregnancy, reducing the morbidity and mortality risks.

Need for careful consideration when using immunomodulating compounds during pregnancy, in relation to the risk of premature delivery.

Spontaneous vaginal delivery requiring a medio-lateral episiotomy might benefit from antibiotic prophylaxis as standard of care. Reducing risk of anaemia after CS delivery can be achieved by prenatal iron supplementation and by spinal rather than general anaesthesia.

Chapter 10 References

1.   Agangi A, Thorne C, Newell ML; European Collaborative Study. (2005) Increasing likelihood of further live births in HIV-infected women in recent years. BJOG. 2005 Jul;112(7):881-8.

2.   Aldrich J, Gross R, Adler M, et al. (2000) The effect of acute severe illness on CD4+ lymphocyte counts in nonimmunocompromised patients. *Arch Intern Med* 160: 715-6

3.   Alioum A, Leroy V, Commenges D, Dabis F, Salamon R. Effect of gender, age, transmission category, and antiretroviral therapy on the progression of human immunodeficiency virus infection using multistate Markov models. Groupe d'Epidemiologie Clinique du SIDA en Aquitaine. Epidemiology. 1998 Nov;9(6):605-12.

4.   Altman LK. (2000).Hopes for anti-HIV treatment dashed. The New York Times on the Web, July 13, http://nytimes.gpass.com. (date assessed March 2005)

5.   Anastos K, Gange SJ, Lau B, et al.(2000) Association of race and gender with HIV-1 RNA levels and immunologic progression. J Acquir Immune Defic Syndr ; 24:218–26.

6.   Andrews WW, Ramin SM, Maberry MC, Shearer V, Black S, Wallace DH. (1992) Effect of type of anaesthesia on blood loss at elective repeat caesarean section. Am J Perinatol, 9: 197-200

7.   Antiretroviral Pregnancy Registry Steering Committee. Antiretroviral Pregnancy Registry Interim Report for 1st January 1989 to 31st January 2003. Available at http://www.apregistry.com/who.htm (date assessed March 2005)

8. Arosio B, Trabattoni D, Galimberti L, Bucciarelli P, Fasano F, Calabresi C, Cazzullo CL, Vergani C, Annoni G, Clerici M. (2004) Interleukin-10 and interleukin-6 gene polymorphisms as risk factors for Alzheimer's disease. Neurobiol Aging. Sep;25(8):1009-15

9. Arpadi SM. (2000) Growth failure in children with HIV infection J Acquir Immune Defic Syndr. Oct 1;25 Suppl 1:S37-42.

10. Avidan MS, Groves P, Blott M, Welch J, Leung T, Pozniak A, et al. (2002) Low complication rate associated with caesarean section under spinal anaesthesia for HIV-infected women on ART. Anesthesiology; 97: 320-324

11. Bartlett JA, Benoit SL, Johnson VA, Quinn JB, Sepulveda GE, Ehmann WC, Tsoukas C, Fallon MA, Self PL, Rubin M. (1996). Lamivudine plus zidovudine compared with zalcitabine plus zidovudine in patients with HIV infection. A randomized, double-blind, placebo-controlled trial. North American HIV Working Party. Ann Intern Med. Aug 1;125(3):161-72

12. Benki S, Mostad SB, Richardson BA, Mandaliya K, Kreiss JK, Overbaugh J.. (2004) Cyclic shedding of HIV-1 RNA in cervical secretions during the menstrual cycle J Infect Dis. Jun 15;189(12):2192-201

13. Blair JM, Hanson DL,Jones JI, Dworkin MS. (2004)Trends in pregnancy rates among women with human immunodeficiency virus. Obstet Gynaec Apr 103 (4):663-668..

14. Blanche S, Tardieu M, Rustin P, et al. (1999) Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues. Lancet 354: 1084.9,.

15. Brettle RP, Raab GM, Ross A, Fielding KL, Gore SM, Bird AG. (1995). HIV infection in women: immunological markers and the influence of pregnancy. AIDS 9: 1177.84.

16. Brocklehurst P, French R. (1998) The association between maternal HIV infection and perinatal outcome: a systematic review of the literature and meta-analysis. Br J Obstet Gynaecol 105: 836.48,.

17. Brown WJ, Donohue JF, Axnick NW, Blount JH, Ewen NH, Jones OG. (1970). Syphilis and other venereal diseases. Cambridge, MA: Harvard University Press,

18. Buira E, Gatell JM, Miro JM, et al. (1992) Influence of treatment with zidovudine (ZDV) on the long-term survival of AIDS patients. J Acquir Immune Defic Syndr Hum Retrovirol; 5:737–42

19. Bunders M, Thorne C, Newell ML; for the European Collaborative Study. (2005) Maternal and infant factors and lymphocyte, CD4 and CD8 cell counts in uninfected children of HIV-1-infected mothers. AIDS. Jul 1;19(10):1071-1079.

20. Camlin CS, Garenne M, Moultrie TA. (2004) Fertility trend and pattern in a rural area of South Africa in the context of HIV/AIDS. Afr J Reprod Health. Aug;8(2):38-54.

21. Cassens U, Gohde W, Kuling G, Groning A, Schlenke P, Lehman LG, Traore Y, Servais J, Henin Y, Reichelt D, Greve B. (2004) Simplified volumetric flow cytometry allows feasible and accurate determination of CD4 T lymphocytes in immunodeficient patients worldwide.Antivir Ther. Jun;9(3):395-405.

22. Centers for Disease Control 1982 Update on acquired immune deficiency syndrome (AIDS)--United States. MMWR Morb Mortal Wkly Rep. Sep 24;31(37):507-8, 513-4.

23. Centers for Disease Control and Prevention (2001) Guidelines for laboratory test result reporting of human immunodeficiency virus type 1 ribonucleic acid determination. Recommendations from a CDC working group. Centers for Disease Control. Centers for Disease Control and Prevention MMWR Recomm Rep. Nov 16;50(RR-20):1-12.

24. Centers for Disease Control and Prevention Centers for Disease Control and Prevention 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR R-17.

25. Centers for Disease Control and Prevention. 1998 Guidelines for treatment of sexually transmitted diseases. MMWR 47: 1.116.

26. Chaisson RE, Keruly JC, Moore RD. (1995) Race, sex drug use and progression of Human Immunodeficiency virus disease. N Engl J Med; 333:751–6.

27. Chaouat G, Cayol V, Mairovitz V, Dubanchet S (1999) Localization of the Th2 cytokines IL-3, IL-4, IL-10 at the murine fetomaternal interface during pregnancy, in Reproductive Immunology (Gupta SK, ed.) pp. 61–70. Narosa Publishing House, New Delhi

28. Chaouat G, Meliani AA, Martal J, Raghupathy R, Elliot J, Mosmann TR, Wegmann TG (1995) IL-10 prevents naturally occurring fetal loss in the CBA_DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vitro injection of IFN-J Immunol 154:4261–4270

29. Chen JL, Philips KA, Kanouse DE, Collins RL, Miu A Fertility desires and intentions of HIV-positive men and women.Fam Plann Perspect. 2001 Jul-Aug;33(4):144-52, 165

30. Clark DA, Arck PC, Chaouat G (1999) Why did your mother reject you? Immunogenetic determinants of the response to environmental selective pressure expressed at the uterine level. Am J Reprod Immunol 41:5–22

31. Clark DA, Chaouat G, Arck PC, Mittruecker HW, Levy GA (1998) Cytokine-dependent abortion in CBA_DBA/2 mice is mediated by the procoagulant fg12 prothrombinase. J Immunol 160:545–549

32. Clark DA, Ding JW, Chaouat G, Coulam CB, August C, Levy GA (1999) The

emerging role of immunoregulation of fibrinogen-related procoagulant fg12 in the success or spontaneous abortion of early pregnancy in mice and humans. Am J Reprod Immunol 42:37–43

33. Clark RA, Mulligan K, Stamenovic E, Chang B, Watts H, Andersen J, Squires K, Benson C. (2001) Frequency of anovulation and early menopause among women enrolled in selected adult AIDS clinical trials group studies. J Infect Dis. Nov 15;184(10):1325-7.

34. Clerici M (2002) A Two Years Immunovirologic Follow-up of Antiretroviral-Treated and Antiretroviral-Naive Chronically HIV Infected Patients - AIDS 16:1676,

35. Clerici M, Seminari E, Suter F, Castelli F, Pan A, Biasin M, Colombo F, Trabattoni D, Maggiolo F, Carosi G, Maserati R. (2004) Different immunologic profiles characterize HIV infection in highly active antiretroviral therapy-treated and antiretroviral-naive patients with undetectable viraemia. The Master Group. AIDS 2000 Jan 28;14(2):109-16 . Jul;52(1):45-52

36. Coffman RL, Romagnani S (1999) Redirection of Th1 and Th2 responses. Springer, Berlin

37. Cole TJ, Green PJ.(1992) Smoothing reference centile curves: the LMS method and penalized likelihood. Statistics in Medicine ; 11:1305-19

38. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, Jones M, Facey K, Whitacre C, McAuliffe VJ, Friedman HM, Merigan TC, Reichman RC, Hooper C, Corey L (1996).Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. N Engl J Med. 18;334(16):1011-7

39. Connor EM, Sperling RS, Gelber R, et al.(1994) Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment.

N.Eng.J.Med ; 331:1173-1180.

40.   Coombs RW, Speck CE, Hughes JP, et al.(1998) Association between culturable human immunodeficiency virus type 1 (HIV-1) in semen and HIV-1 RNA levels in semen and blood: evidence for compartmentalization of HIV-1 between semen and blood. J Infect Dis 177: 320.30..

41.   Cooper ER, Charurat M, Mofenson LM, et al. (2002) Combination antiretroviral strategies for the treatment of pregnant HIV-1–infected women and prevention of perinatal HIV-1 transmission. J Acquir Immune Defic Syndr; 29:484–94.

42.   Coulam CB, Goodman C, Roussev RG, Thomason EJ, Beaman KD (1995) Systemic CD56C cells can predict pregnancy outcome. Am J Reprod Immunol 33:40–46

43.   Cowan MJ, Hellmann D, Chudwin D, Wara DW, Chang RS, Ammann AJ. (1984). Maternal transmission of acquired immune deficiency syndrome. Pediatrics. Mar;73(3):382-6.

44.   Cozzi Lepri A, Katzenstein TL, Ullum H, et al. (1998) The relative prognostic value of plasma HIV RNA levels and CD4 lymphocyte counts in advanced HIV infection. AIDS; 12:1639–43

45.   Cozzi Lepri A, Pezzotti P, Dorrucci M, et al. (1994) HIV disease progression in 854 women and men infected through injecting drug use and heterosexual sex and followed for up to nine years from seroconversion. Italian Seroconversion Study. BMJ; 309:1537–42.

46.   Cu Uvin S, Caliendo AM, Reinert SE, et al. (1998).HIV-1 in the female genital tract and the effect of antiretroviral therapy. AIDS 12: 826-827,

47.   Cu-Uvin S, Hogan JW, Warren D, et al. (1999).Prevalence of lower genital tract infections among human immunodeficiency virus (HIV)-seropositive and high risk

HIVseronegative women. HIV Epidemiology Research Study Group. Clin Infect Dis 29: 1145.50,

48. Davis KR, Weller SC. (1999) The effectiveness of condoms in reducing heterosexual transmission of HIV. Fam Plann Perspect. Nov-Dec;31(6):272-9.

49. De Martino M, Tovo PA, Galli L, et al. (1991).. HIV-1 infection in perinatally exposed siblings and twins. The Italian Register for HIV Infection in Children. Arch Dis Child 66 1235.8,

50. De Vincenzi I. (1994) A longitudinal study of human immunodeficiency virus transmission by heterosexual partners. European Study Group on Heterosexual Transmission of HIV. N Engl J Med. Aug 11;331(6):341-6.

51. Degres du Loi LA, Msellati P, Yao A, Noba V, Viho I, Ramon R et al. (1999) Impaired fertility in HIV-1 infected women: a clinical based survey in Abidjan, Cote d'Ivoire. AIDS ;13(4):517-521.

52. Dominguez KL, Lindegren ML, D'Almada PJ, et al.(2003). Increasing trend of cesarean deliveries in HIV-infected women in the United States from 1994 to 2000. J Acquir Immune Defic Syndr; 33:232–8.

53. Dorenbaum A, Cunningham CK, Gelber RD, et al. (2002). Two-dose intrapartum/newborn nevirapine and standard antiretroviral therapy to reduce perinatal HIV transmission: a randomized trial. JAMA 288:189–98.

54. Dudley DJ, Chen C-L, Murray MD, Daynes RA, Araneo BA (1993) Adaptive immune responses during murine pregnancy: pregnancy-induced regulation of lymphokine production by activated T lymphocytes. Am J Obstet Gynecol 168:1155–1163

55. Duerr A, Jamieson D, Cu-Uvin S, et al. (2000) The effect of highly active antiretroviral therapyon Pap smear abnormalities. XIII International AIDS Conference, Durban, South Africa (AbstMoPeB2245), July 9.14,.

56. Dyer JR, Kazembe P, Vernazza PL, Gilliam BL, Maida M, Zimba D, Hoffman IF, Royce RA, Schock JL, Fiscus SA, Cohen MS, Eron JJ Jr. (1998) High levels of human immunodeficiency virus type 1 in blood and semen of seropositive men in sub-Saharan Africa. J Infect Dis. Jun;177(6):1742-6

57. Egger M , Hirschel B and Francioli P et al., (1997), Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: prospective multicentre study—Swiss HIV Cohort Study, BMJ 315 pp. 1194–1199

58. El-Shazly S, Makhseed M, Azizieh F, Raghupathy R. (2004). Increased expression of pro-inflammatory cytokines in placentas of women undergoing spontaneous preterm delivery or premature rupture of membranes.Am J Reprod Immunol Jul;52(1):45-52.

59. Emery S, Abrams DI, Cooper DA, Darbyshire JH, Lane HC, Lundgren JD, Neaton JD; ESPRIT (2002) The evaluation of subcutaneous proleukin (interleukin-2) in a randomized international trial: rationale, design, and methods of ESPRIT. Study Group Control Clin Trials. Apr;23(2):198-220.

60. Ethics Committee of the American Society for Reproductive Medicine (1994) Special considerations regarding human immunodeficiency virus and assisted reproductive technologies. Fertil Steril;62(Suppl 1):85S.

61. European Collaborative Study. (2003); Pregnancy-related changes in the longer-term management of HIV infected women in Europe. Eur J Obstet Gynecol Reprod Biol 111:3–8.

62. EuroHIV. HIV/AIDS Surveillance in Europe. (2004) End-year report 2003. Saint-Maurice: Institut de veille sanitaire. No. 70.

63. European Collaborative Study (2001) HIV-infected pregnant women and vertical transmission in Europe since 1986. AIDS.;15:761-70.

64. European Collaborative Study (2004) Gender and race do not alter early-life determinants of clinical disease progression in HIV-1 vertically infected children. AIDS. Feb 20;18(3):509-16

65. European Collaborative Study (2005). Mother to child transmission of HIV infection in the era of HAART. Clinical Infectious Disease;458-465

66. European Collaborative Study and the Swiss Mother and Child HIV Cohort Study. (2000) Combination antiretroviral therapy and duration of pregnancy. AIDS; 14:2913–20.

67. European Collaborative Study. (1999); Maternal viral load and vertical transmission of f HIV-1: an important factor but not the only one. AIDS 13:1377–85.

68. European Collaborative Study (2003) Height, weight, and growth in children born to mothers with HIV-1 infection in Europe. Pediatrics. Jan;111(1):e52-60

69. European Collaborative Study (1996). Vertical transmission of HIV-1: maternal immune status and obstetric factors. AIDS; 10:1675-1681.

70. European Collaborative Study (1994). Caesarean section and risk of vertical transmission of HIV-1 infection. Lancet; 343:1464-1467.

71. European Collaborative Study (1998). Therapeutic and other interventions to reduce the risk of mother-to-child transmission of HIV-1 in Europe. Br J Obstet Gynaecol; 105:704-709.

72. European Study Group (1992). Comparison of female to male and male to female transmission of HIV in 563 stable couples. European Study Group on Heterosexual Transmission of HIV. Br Med J 304: 809.13,.

73. Evans JS, Nims T, Cooley J, et al. (1997) Serum levels of virus burden in early-stage human immunodeficiency virus type 1 disease in women. J Infect Dis;

175:795–800

74. Farzadegan H, Hoover DR, Astemborski J, et al.(1998) Sex differences in HIV-1 viral load and progression to AIDS. Lancet; 352:1510–14

75. Fiore J, Suligoi B, Saracino A, et al. (2003) Correlates of HIV-1 shedding in cervicovaginal secretions and effects of antiretroviral therapies. AIDS; 17:2169–76.

76. Fleischman J (2004). Breaking the cycle: ensuring equitable access to HIV treatment for women and girls. Washington, Center for Strategic and International Studies. at http://www.csis.org/africa/ (date assessed March 2005)

77. Forquet F, Le Chenadec J, Mayaux MJ, Meyer L (2001); Reproductive behaviour of HIV-infected women living in France, according to geographical origin. 15(16);2193-96

78. Frankel RE, Selwyn PA, Mezger J, Andrews S. (1997) High prevalence of gynecologic disease among hospitalized women with human immunodeficiency virus infection. Clin Infect Dis 25: 706.12,

79. French R, Brocklehurst P (1998). The effect of pregnancy on survival in women infected with HIV: a systematic review of the literature and meta-analysis. Br J Obstet Gynaecol 105: 827.35,.

80. Gabiano C, Tovo PA, de Martino M, et al (1992). Mother-to-child transmission of Human Immunodeficiency Virus Type 1: Risk of infection and correlates of transmission. Pediatr ; 90:369-374.

81. Garcia-Bujalance S, Ruiz G, De Guevara CL, et al. (2004);Quantitation of human immunodeficiency virus type 1 RNA loads in cervicovaginal secretions in pregnant women and relationship between viral loads in the genital tract and blood. Eur J Clin Microbiol Infect Dis 23:111–5.

82. Garcia-Bujalance S, Ruiz G, De Guevara CL, Pena JM, Bates I, Vazquez JJ,

Gutierrez A. (2004) Quantitation of human immunodeficiency virus type 1 RNA loads in cervicovaginal secretions in pregnant women and relationship between viral loads in the genital tract and blood. Eur J Clin Microbiol Infect Dis. Feb;23(2):111-5

83. Gilliam BL, Dyer JR, Fiscus SA, et al. (1997).Effects of reverse transcriptase inhibitor therapy on the HIV-1 viral burden in semen. J Acquir Immune Defic Syndr Hum Retrovirol 15: 54.60,

84. GM Shearer, M Clerici.(1998) Cytokine profiles in HIV Type 1 Disease and protection Aids Hum Ret 14:2;

85. Gray RH, Wawer MJ, Serwadda D, Sewankambo N, Li C, Wabwire-Mangen F, Paxton L, Kiwanuka N, Kigozi G, Konde-Lule J, Quinn TC, Gaydos CA, McNairn D. (1998) Population-based study of fertility in women with HIV-1 infection in Uganda. Lancet. Jan 10;351(9096):98-103

86. Greenblatt RM, Bacchetti P, Barkan S, et al (1999).. Lower genital tract infections among HIV infected and high-risk uninfected women: findings of the Womens Interagency HIV Study (WIHS). Sex Transm Dis 26: 143.51,

87. Gregson S, Zaba B, Garnett GP.(1999) Low fertility in women with HIV and the impact of the epidemic on orphanhood and early childhood mortality in sub-Saharan Africa. AIDS.;13 Suppl A:S249-57.)

88. Guay L, Musoke P, Fleming T, et al. (1999) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet;354:795-

89. Guilbert LJ (1996) There is a bias against type 1 (inflammatory) cytokine expression and function in pregnancy. J Reprod Immunol 32:105–110

90. Gupta P, Mellors J, Kingsley L, et al. (1997) High viral load in semen of human

immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. J Virol 71: 6271-.5,.

91.   Haimovici F, Hill JA, Anderson DJ (1991) The effects of soluble products of activated lymphocytes and macrophages on blastocyst implantation events in vitro. Biol Reprod 44:69–76

92.   Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, Eron JJ Jr, Feinberg JE, Balfour HH Jr, Deyton LR, Chodakewitz JA, Fischl MA. (1997) A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team N Engl J Med. Sep 11;337(11):725-33

93.   Hanna N, Hanna I, Hleb M, Wagner E, Dougherty Balkundi D, Padbury J, Sharma S (2000) Gestational agedependent expression of IL-10 and its receptor in human placental tissues and isolated cytotrophoblasts. J Immunol 164:5721–5728

94.   Heard I, Potard V, Costagliola D, Kazatchkine MD Contraceptive Use in HIV-Positive Women. J Acquir Immune Defic Syndr. 2004 Jun 1;36(2):714-720.

95.   Hill JA, Anderson DJ, Polgar K (1995) T helper 1-type cellular immunity to trophoblast in women with recurrent spontaneous abortions. J Am Med Assoc 273:1933–1958

96.   Hill JA, Polgar K, Harlow BL, Anderson DJ (1992) Evidence of embryo- and trophoblast-toxic cellular immune response(s) in women with recurrent spontaneous abortion. Am J Obstet Gynecol 166:1044–1050

97.   Hirsch MS, Brun-Vezinet F, Clotet B, Conway B, Kuritzkes DR, D'Aquila RT, Demeter LM, Hammer SM, Johnson VA, Loveday C, Mellors JW, Jacobsen DM,

Richman DD (2003). Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel. Clin Infect Dis. Jul 1;37(1):113-28.

98. Hooper PM, Mayes DC and Demianczuk N. A model for foetal growth and diagnosis of intrauterine growth restriction. Stats Med. 2002; 21:95-112

99. Hunt JS, Chen H-L, Miller L (1996) Tumor necrosis factors: pivotal components of pregnancy. Biol Reprod 54:554–562

100. Ioannidis JP, Tatsioni A, Abrams EJ, Bulterys M, Coombs RW, Goedert JJ, Korber BT, Mayaux MJ, Mofenson LM, Moye J Jr, Newell ML, Shapiro DE, Teglas JP, Thompson B, Wiener J.(2004) Maternal viral load and rate of disease progression among vertically HIV-1-infected children: an international meta-analysis.AIDS. Jan 2;18(1):99-108.

101. Ioannidis JP, Abrams EJ, Ammann A, Bulterys M, Goedert JJ, Gray L, et al. (2001) Perinatal transmission of human immunodeficiency virus type 1 by pregnant women with RNA virus loads <1000 copies/ml. J Infect Dis. 183:539-45.

102. Jamieson DJ, Duerr A, Klein RS, Paramsothy P, Brown W, Cu-Uvin S, Rompalo A, Sobel J. (2001) Longitudinal analysis of bacterial vaginosis: findings from the HIV epidemiology research study. Obstet Gynecol. Oct;98(4):656-63

103. John GC, Nduati RW, Mbori-Ngacha DA, Richardson BA, Panteleeff D, Mwatha A, Overbaugh J, Bwayo J, Ndinya-Achola JO, Kreiss JK (2001). Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission: association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. J Infect Dis. Jan 15;183(2):206-212.

104. Junghans C, Ledergerber B, Chan P, et al. (1999) Sex differences in HIV-1 viral load and progression to AIDS. Swiss HIV cohort study. Lancet; 353:589-

105. Kalichman SC, Simbayi LC. (2004) Sexual exposure to blood and increased risks for heterosexual HIV transmission in Cape Town, South Africa. Afr J Reprod Health. Aug;8(2):55-8.

106. Kovacs AAS, Wasserman SS, Wright DJ, et al.(2001) Determinants of HIV-1 shedding in the genital tract of women. Lancet ; 358:1593–601-32.

107. Krishnan L, Guilbert LJ, Wegmann TG, Belosevic M, Mosmann TR (1996) T helper 1 response against Leishmania major in pregnant C57BL/6 mice increases implantation failure and fetal resorptions. Correlation with increased IFN- and TNF and reduced IL-10 production by placental cells. J Immunol 156:653–659

108. Kruse N, Greif M, Moriabadi NF, Marx L, Toyka KV, Rieckmann (2000) Variations in cytokine mRNA expression during normal human pregnancy. Clin Exp Immunol 119:317–322

109. Kuhn L, Steketee RW, Weedon J, et al. (1999) Distinct risk factors for intrauterine and intrapartum human immunodeficiency virus transmission and consequences for disease progression in infected children. J Infect Dis; 179:52–8.

110. Lambert JS, Watts DH, Mofenson L, Stiehm ER, Harris DR, Bethel J, Whitehouse J, Jimenez E, Gandia J, Scott G, O'Sullivan MJ, Kovacs A, Stek A, Shearer WT, Hammill H, van Dyke R, Maupin R, Silio M, Fowler MG.(2000) Risk factors for preterm birth, low birth weight, and intrauterine growth retardation in infants born to HIV-infected pregnant women receiving zidovudine. Pediatric AIDS Clinical Trials Group 185 Team. AIDS. Jul 7;14(10):1389-99.

111. Lazzarin A, Saracco A, Musicco M, Nicolosi (1991) A.Man-to-woman sexual transmission of the human immunodeficiency virus. Risk factors related to sexual behavior, man's infectiousness, and woman's susceptibility. Italian Study Group on HIV Heterosexual Transmission. Arch Intern Med. Dec;151(12):2411-6.

112. Ledergerber B, Egger M and Opravil M et al., (1999), Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study, Lancet 353 : 863–868.

113. Lorenzi P, Spicher VM, Laubreau B, et al. (1998).Antiretroviral therapies in pregnancy: maternal,fetal, and neonatal effects. Swiss HIV Cohort Study, the Swiss Collaborative HIV and Pregnancy Study, and the Swiss Neonatal HIV Study. AIDS 12: F241-7,

114. Lucotte G, Mercier G. (1998) Distribution of the CCR5 gene 32-bp deletion in Europe.J Acquir Immune Defic Syndr Hum Retrovirol. Oct 1;19(2):174-7.

115. Lundgren JD, Pedersen C, Clumeck N, et al. (1994) Survival differences in European patients with AIDS, 1979–89. The AIDS in Europe Study Group. BMJ; 308:1068–73

116. Lurie M et al. (2003). Who infects whom? HIV-1 concordance and discordance among migrant and non-migrant couples in South Africa. AIDS, 17(15).

117. Lydié N et al. (2004). Mobility, sexual behaviour and HIV infection in an urban population in Cameroon. Journal of Acquired Immune Deficiency Syndrome, 35(1).

118. Madazli R, Aydin S, Uludag S, Vildan O, Tolun N. (2003) Maternal plasma levels of cytokines in normal and preeclamptic pregnancies and their relationship with diastolic blood pressure and fibronectin levels.Acta Obstet Gynecol Scand. Sep;82(9):797-802

119. Makhseed M, Raghupathy R, Azizieh F, Al-Azemi MMK, Hassan NA, Bandar (1999) Mitogen-induced cytokine responses of maternal peripheral blood lymphocytes indicate a differential Th-type bias in normal pregnancy and pregnancy failure. Am J Reprod Immunol 42:273–281

120. Mandelbrot L, Heard I, Henrion-Geant E, Henrion R (1997) Natural conception in

HIV-negative women with HIV-infected partners Lancet. Mar 22;349(9055):850-1

121. Mandelbrot L, Landreau A, Rekacewicz C, et al. (2001) Lamivudine-zidovudine combination for prevention of maternal-infant transmission of HIV-1. JAMA; 285:2083–93.

122. Mandelbrot L, Mayaux MJ, Bongain A, et al. (1996) Obstetric factors and mother-to-child transmission of human immunodeficiency virus type 1: the French perinatal cohorts. Am J Obstet Gynecol; 175:661–7.

123. Marcollet A, Goffinet F, Firtion G, Pannier E, Le Bret T, Brival ML et al (2002). Differences in post-partum morbidity in women who are infected with the human immunodeficiency virus after elective caesarean section delivery, emergency delivery or vaginal delivery. Am J Obstet Gynecol.; 186 :784-89

124. Markson LE, Turner BJ, Houchens R, Silverman NS, Cosler L, Takyi BK. (1996) Association of maternal HIV infection with low birth weight. J Acquir Immune Defic Syndr Hum Retrovirol.;13:227-34.

125. Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici F, Clerici M (1996) Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol 106:127–133

126. Marzusch K, Buchholz F, Ruck P, Handgretinger R, Geiselhart A, Engelmann L, Dietl J (1997) Interleukin-12- and interleukin-2-stimulated release of interferon-gamma by uterine CD56CC large granular lymphocytes is amplified by decidual macrophages. Hum Reprod 12:921–924

127. Massad LS, Ahdieh L, Benning L, Minkoff H, Greenblatt RM, Watts H, Miotti P, Anastos K, Moxley M, Muderspach LI, Melnick S (2001) Evolution of cervical abnormalities among women with HIV-1: evidence from surveillance cytology in the women's interagency HIV study. J Acquir Immune Defic

Syndr. Aug 15;27(5):432-42.

128. Massad LS, Springer G, Jacobson L, Watts H, Anastos K, Korn A, Cejtin H, Stek A, Young M, Schmidt J, Minkoff H. (2004) Pregnancy rates and predictors of conception, miscarriage and abortion in US women with HIV. AIDS. Jan 23;18(2):281-6.

129. Mayaux MJ, Teglas JP, Mandelbrot L, et al (1997). Acceptability and impact of zidovudine prevention on mother-to-child HIV-1 transmission in France. J Pediatr; 131:857-862.

130. Michel M, Underwood J, Clark DA, Mowbray JF, Beard RW (1989) Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion. Am J Obstet Gynecol 161:409–414

131. Minkoff H, Hershow R, Watts DH, Frederick M, Cheng I, Tuomala R, Pitt J, Zorrilla CD, Hammill H, Adeniyi-Jones SK, Thompson B. (2003) The relationship of pregnancy to human immunodeficiency virus disease progression. Am J Obstet Gynecol. Aug;189(2):552-9.

132. Minkoff HL, Eisenberger-Matityahu D, Feldman J, Burk R, Clarke L. (1999). Prevalence and incidence of gynecologic disorders among women infected with human immunodeficiency virus. Am J Obstet Gynecol 180: 824-36.

133. Miotti P, Liomba G, Dallabetta GA, Hoover DR, Chiphangwi JD, Saah AJ. (1992) T lymphocyte subsets during and after pregnancy: analysis in human Immunodeficiency virus type-1 infected and .uninfected Malawian others. J Infect Dis 165: 1116-19.

134. Mocroft A, KatlamaC and Johnson AM et al., (2000), AIDS across Europe, 1994–1998: The EuroSIDA study, Lancet 356 pp. 291–296

135. Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, Knysz

B, Dietrich M, Phillips AN, Lundgren JD; EuroSIDA study group. (2003) Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet. Jul 5;362(9377):22-9

136. Moore AL, Kirk O, Johnson AM, Katlama C, Blaxhult A, Dietrich M, Colebunders R, Chiesi A, Lungren JD, Phillips AN; EuroSIDA group. (2003) Virologic, immunologic, and clinical response to highly active antiretroviral therapy: the gender issue revisited. J Acquir Immune Defic Syndr. Apr 1;32(4):452-61

137. Mosmann TR, Sad S (1996) The expanding universe of T-cell subsets. Immunol Today 17:138–146

138. Mostad SB (1998) Prevalence and correlates of HIV type 1 shedding in the female genital tract. AIDS Res Hum Retroviruses. Apr;14 Suppl 1:S11-5

139. Mostad SB, Jackson S, Overbaugh J, et al. (1998) Cervical and vaginal shedding of human immunodeficiency virus type 1-infected cells throughout the menstrual cycle. J Infect Dis 178: 983.91,.

140. Mostad SB, Kreiss JK, Ryncarz AJ, et al. (2000) Cervical shedding of herpes simplex virus in human immunodeficiency virus-infected women: effects of hormonal contraception, pregnancy, and vitamin A deficiency. J Infect Dis 181: 58-63.

141. Mostad SB.(1998) Prevalence and correlates of HIV type 1 shedding in the female genital tract. AIDS Res Hum Retroviruses 14 (Suppl 1): S11-15,

142. Musicco M, Lazzarin A, Nicolosi A, et al. (1994).Antiretroviral treatment of men infected with human immunodeficiency virus type 1 reduces the incidence of heterosexual transmission. Italian Study Group on HIV Heterosexual Transmission. Arch Intern Med 154: 1971-6.

143. National Institutes of Health Consensus development task force statement on

caesarean childbirth. Am J Obstet Gynecol. 1981; 139 :902-9.

144. Nduati RW, John GC, Ngacha DA, et al.(2000) Effect of Breastfeeding and Formula Feeding on Transmission of HIV-1: a Randomised Clinical Trial. JAMA; 283(9):1167-1174.

145. Newell ML. (1998) Mechanisms and timing of mother-to-child transmission of HIV-1. AIDS ; 12:831-837.

146. Newell ML, Rogers MF. (2002). Pregnancy and HIV infection: a European consensus on management. AIDS 16:S1–18

147. Nicastri E, Angeletti C, Palmisano L, Sarmati L, Chiesi A, Geraci A, Andreoni M, Vella S; the Italian Antiretroviral Treatment Group (2005). Gender differences in clinical progression of HIV-1-infected individuals during long-term highly active antiretroviral therapy. AIDS. Apr 8;19(6):577-83

148. Nicolosi A, Correa Leite ML, Musicco M, Arici C, Gavazzeni G, Lazzarin A. (1994) The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. Epidemiology. Nov;5(6):570-5.

149. Nicolosi A, Musicco M, Saracco A, Lazzarin A. (1994) Risk factors for woman-to-man sexual transmission of the human immunodeficiency virus. Italian Study Group on HIV Heterosexual Transmission. J Acquir Immune Defic Syndr. Mar;7(3):296-300.

150. Palasanthiran P, Ziegler JB, Stewart GJ, et al. (1993) Breast-feeding during primary maternal human immunodeficiency virus infection and risk of transmission from mother to infant. J Infect Dis 167: 441-4..

151. Palefsky JM, Minkoff H, Kalish LA et al. (1999) Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high

risk HIV-negative women. J Natl Cancer Inst 91: 226.36.

152. Palella FJ, KM Delaney and AC Moorman et al. (1998), Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection, N Engl J Med 338 pp. 853–860.

153. Piccinni M-P, Romagnani S (1996) Regulation of fetal allograft survival by hormone-controlled Th1- and Th2-type cytokines. Immunol Res 15:141–150

154. Pinheiro JC; Bates DM. (2000) Mixed-Effects Models in S and S-Plus. New York, Springer-Verlag.

155. Plummer FA (1998). Heterosexual transmission of human immunodeficiency virus type 1(HIV): interactions of conventional sexually transmitted diseases, hormonal contraception and HIV-1. AIDS Res Human Retroviruses Suppl: S5-10..

156. Plummer FA, Simonsen JN, Cameron DW et al. (1991).Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. J Infect Dis 163: 233-9

157. Population Reference Bureau (2003). HIV/AIDS Demographics, Africa.

158. Prigoshin N, Tambutti M, Larriba J, Gogorza S, Testa R.(2004) Cytokine gene polymorphisms in recurrent pregnancy loss of unknown cause. Am J Reprod Immunol. Jul;52(1):36-41

159. Prins M, Robertson JR, Brettle RP, et al. (1999) Do gender differences in CD4 cell counts matter? AIDS; 13:2361–4.

160. Project Inform (2001). Women and AIDS at Twenty. Project Inform Perspective, August. Available at http://www.projinf.org/pub/33/women.html (date assessed March 2005)

161. Quinn TC, Wawer MJ, Sewankambo N, et al. (2000) Viral load and risk of

heterosexual transmission of HIV-1 among sexual partners. 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, Jan 30-Febr 2

162. Quinn TC. (1996) Global burden of the HIV pandemic. Lancet 348: 99-106..

163. Raghupathy R (1997) Th1-type immunity is incompatible with successful pregnancy. Immunol Today 18:478–482-9.

164. Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R (2000) Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Hum Reprod 15:713–18

165. Reeves GK, (1988) Overton SE. Preliminary survival analysis of UK AIDS data [letter]. Lancet, I:880

166. Reinhard G, Noll A, Schlebusch H, Mallmann P,Ruecker AV (1998) Shifts in the Th1/Th2 balanceduring human pregnancy correlate with apoptotic changes.Biochem Biophys Res Comm 245:933–38.

167. Rhodes T, Cusick L.. (2002) Accounting for unprotected sex: stories of agency and acceptability. Soc Sci Med. Jul;55(2):211-26

168. Rivera DL, Olister SM, Liu X, Thompson JH, Pennline K, Azuero R, Clark DA, Miller MJ (1998) Interleukin-10 attenuates experimental fetal growth restriction and demise. FASEB J 12:189–197

169. Rodriguez EJ, Spann C, Jamieson D, Lindsay M. (2001) Postoperative morbidity with caesarean delivery among human immunodeficiency virus-seropositive women. Am J Obstet Gynecol.; 184 :1108-11.

170. Roques P, Marce D, Courpotin C, et al. (1993) .Correlation between HIV provirus burden and in utero transmission. AIDS 7 (Suppl 2): S39-43.

171. Rouzioux C, Castgholia D,Burgod M et al. (1995) Estimated timing of mother to

child HIV-1 transmission by use of Mrkov model: the HIV infection in the newborn. French Collaborative Study Group. Am J Epidemiol ;142;1330-1337

172. Rowland BL, Vermillion ST, Soper DE.(2001)Scheduled cesarean delivery and the prevention of human immunodeficiency virus transmission: a survey of practicing obstetricians. Am J Obstet Gynecol; 185:

173. Royce RA, Sena A, Cates W Jr, and Cohen MS (1997). Sexual transmission of HIV. N Engl J Med 336: 1072.8,

174. Russell AS, Johnston C, Chew C, Maksymowych WP. (1997) Evidence for reduced Th1 function in normal pregnancy: a hypothesis for the remission of rheumatoid arthritis. J Rheumatol. Jun;24(6):1045-50

175. Saag MS, Holodniy M, Kurtizkes DR, et al. (1996).HIV viral load markers in clinical practice. Nature Med 2: 625-9.

176. Santoro-Lopes G, Harrison LH, Moulton LH, Lima LA, de Pinho AM, Hofer C, Schechter M. . (1998) Gender and survival after AIDS in Rio de Janeiro, Brazil. J Acquir Immune Defic Syndr Hum RetrovirolDec 1;19(4):403-7

177. Schuster MA, Beckett MK, Corona R, Zhou AJ. (2005) Hugs and kisses: HIV-infected parents' fears about contagion and the effects on parent-child interaction in a nationally representative sample. Arch Pediatr Adolesc Med. feb;159(2):173-9.

178. Semprini AE, Castagna C, Ravizza M, et al.(1995) The incidence of complications after cesarean section in 156 HIV-positive women. AIDS 9: 913-7.

179. Serwadda D, Gray RH, Sewankambo NK, Wabwire-Mangen F, Chen MZ, Quinn TC, Lutalo T, Kiwanuka N, Kigozi G, Nalugoda F, Meehan MP, Ashley Morrow R, Wawer MJ. (2003) Human immunodeficiency virus acquisition associated with genital ulcer disease and herpes simplex virus type 2 infection: a nested case-control study in Rakai, Uganda. J Infect Dis. Nov 15;188(10):1492-7.

180. Shaffer N, Chuachoowong R, Mock PA, et al. (1999) Short-course zidovudine for perinatal HIV-1 transmission in Bangkok, Thailand: a randomised controlled trial. Lancet;353:773-80.

181. Shah PN, Smith JR, Wells C, et al. (1994).Menstrual symptoms in women infected by the human immunodeficiency virus. Obstet Gynecol 83: 397-400.

182. Shaheen F, Sison AV, McIntosh L, Mukhtar M, and Pomerantz RJ. (1999) Analysis of HIV-1in the cervicovaginal secretions and blood of pregnant and nonpregnant women.J Hum Virol 2: 154.66,.

183. Sherwood, O.D., (1994) Relaxin. In: Knobil E Neill, E., Editor. (1994). The Physiology of Reproduction (2nd Edition ed.),, Raven Press, New York, pp. 861–1010.

184. Simpson BJ, Shapiro DE. (2000) Prospective cohort study of children born to HIV-infected mothers, 1985 through 1997: trends in the risk of vertical transmission, mortality and acquired immunodeficiency syndrome indicator diseases in the era before highly active antiretroviral therapy. Pediatr Infect Dis J; 19:618–24.

185. Speck CE, Coombs RW, Koutsky LA, et al. (1999) Risk factors for HIV-1 shedding in semen.Am J Epidemiol 150: 622-31.

186. Stephenson JM, Griffioen (1996) The effect of HIV diagnosis on reproductive experience. Study Group for the Medical Research Council Collaborative Study of Women with HIV AIDS. Dec;10(14):1683-7.

187. Sterling TR, Lyles CM, Vlahov D, et al. (1999). Sex differences in longitudinal human immunodeficiency virus type 1 RNA levels among seroconverters. J Infect Dis 180:666–72.

188. Stigum H, Falck W, Magnus P. (1994) The core group revisited: the effect of partner mixing and migration on the spread of gonorrhea, Chlamydia, and HIV. Math Biosci.

Mar;120(1):1-23

189. Stover J (2004) Projecting the demographic consequences of adult HIV prevalence trends: the Spectrum Projection Package. Sex Transm Infect. 80 Suppl 1:14-18.

190. Szekeres-Bartho J, Chaouat G (1990) Lymphocyte-derived, progesterone-induced blocking factor corrects resorption in a murine abortion system. Am J Reprod Immunol 23:26–28

191. Szekeres-Bartho J, Wegmann TG (1996) A progesterone dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol (31):81-95.

192. Szekeres-Bartho, J.(1992). Immunosuppression by Progesterone in Pregnancy, CRC Press, Boca Raton, FL.

193. Shwartz, Prenatal care, prematurity, and neonatal mortality.(1932) Am J Obstet Gynecol (83): 591–598.

194. Tangri S, Lin H, Wegmann TG, Raghupathy R (1993) Maternal anti-placental reactivity in natural, immunologicallymediated spontaneous fetal resorptions. J Immunol 152:4903–11

195. Thackway SV, Furner V, MiJch A,Coioper DA, Holland D,Martinez P et al. (1997) Fertility and reproductive choice in women with HIV-1 Infection. AIDS; 11(5):663-67.

196. The European Mode of Delivery Collaboration. (1999) Elective caesarean section versus vaginal delivery in preventing vertical HIV-1 transmission: a randomised clinical trial. Lancet;353:1035-39.

197. The International Perinatal HIV group. (1999) Mode of delivery and vertical transmission of HIV-1: a meta-analysis from fifteen prospective cohort studies. N.Eng.J.Med.;340:977-87.

198. The International Perinatal HIV Group. (2001) Duration of ruptured membranes and vertical transmission of HIV-1: a meta-analysis from fifteen prospective cohort studies. AIDS; 15:357–68.

199. The Perinatal Safety Review Working Group. (2000) Nucleoside exposure in the children of HIV-infected women receiving antiretroviral drugs: absence of clear evidence for mitochondrial disease in children who died under 5 years of age in five United States Cohorts. J Acq Immune Defic Syndr; 15:261-68.

200. Thomas PA, Jaffe HW, Spira TJ, Reiss R, Guerrero IC, Auerbach D. (1984) Unexplained immunodeficiency in children. A surveillance report. JAMA; Aug 3;252(5):639-44.

201. Thorne C, Newell ML.(2004) Prevention of mother-to-child transmission of HIV infection. Current Opinion in Infectious Diseases ; 17:247-252

202. Thorne C, Newell ML.(2005) Antiretroviral therapy and mother-to-child transmission of HIV . Hospital Pharmacy Newsletter July 2005.

203. Todros T, Ferrazzi E, Groli C et al. Fitting growth curves to head and abdomen measurements of the fetus: a multicentric study. J Clin Utrasound 1987.15: 95-105.

204. Trabattoni D, Saresella M, Biasin M, Boasso A, Piacentini L, Ferrante P, Dong H, Maserati R, Shearer GM, Chen L, Clerici M. (2003) B7-H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression Blood. Apr 1;101(7):2514-20.

205. Tuomala RE, Kalish LA, Zorilla C, Fox H, Shearer W, Landay A, Vermund SH, Landesman S, Burns D.Changes in total, CD4+, and CD8+ lymphocytes during pregnancy and 1 year postpartum in human immunodeficiency virus-infected women. The Women and Infants Transmission Study. Obstet Gynecol. 1997 Jun;89(6):967-74.

206. UNAIDS. 2004 report on the global HIV/AIDS epidemic : 4th global report.

207. van Benthem BH, de Vincenzi I, Delmas MC, Larsen C, van den Hoek A, Prins M (2000) Pregnancies before and after HIV diagnosis in a european cohort of HIV-infected women. European Study on the Natural History of HIV Infection in Women.. AIDS. Sep 29;14(14):2171-8.

208. Vasilescu A, Heath SC, Ivanova R, Hendel H, Do H, Mazoyer A, Khadivpour E, Goutalier FX, Khalili K, Rappaport J, Lathrop GM, Matsuda F, Zagury JF (2003) Genomic analysis of Th1-Th2 cytokine genes in an AIDS cohort: identification of IL4 and IL10 haplotypes associated with the disease progression. Genes Immun. Sep;4(6):441-9.

209. Vernazza PL, Eron JJ, Fiscus SA, and Cohen MS. (1999). Sexual transmission of HIV: infectiousness and prevention. AIDS 13: 155-66.

210. Vernazza PL, Gilliam BL, Dyer J, et al. (1997).Quantification of HIV in semen: correlation with antiviral treatment and immune status. AIDS 11: 987-93

211. Vince GS, Johnson PM (1996) Is there a Th2 bias in human pregnancy? J Reprod Immunol 32:101–04

212. Vittinghoff E, Scheer S, O'Malley P, Colfax G, Holmberg SD, Buchbinder SP. et al (1999) Combination antiretroviral therapy and recent declines in AIDS incidence and mortality. J Infect Dis.(179) :717-20

213. Vlahov D, Graham NMH, Hoover D, et al. (1998) Prognostic indicators for AIDS and infectious disease and death in HIV-infected injection drug users: plasma viral load and CD4 cell count. JAMA; 279:35–40

214. Wade NA, Birkhead GS, Warren BL, et al. (1998) Abbreviated regimens of zidovudine prophylaxis and perinatal transmission of the human immunodeficiency virus. N.Eng.J.Med.; 339: 1409-14.

215. Wang CC, McClelland RS, Overbaugh J, Reilly M, Panteleeff DD, Mandaliya K, Chohan B, Lavreys L, Ndinya-Achola J, Kreiss JK. (2004) The effect of hormonal contraception on genital tract shedding of HIV-1. AIDS. Jan 23;18(2):205-9

216. Watts DH, Balasubramanian R, Maupin RT, et al. (2004) Maternal toxicity and pregnancy complications in HIV-infected women receiving antiretroviral therapy: an analysis of the PACTG 316 Study. Am J Obstet Gynecol; 190:506–16

217. Watts DH, Fazarri M, Minkoff H, Hillier SL, Sha B, Glesby M, Levine AM, Burk R, Palefsky JM, Moxley M, Ahdieh-Grant L, Strickler HD.Effects of Bacterial (2005) Vaginosis and Other Genital Infections on the Natural History of Human Papillomavirus Infection in HIV-1-Infected and High-Risk HIV-1-Uninfected WomenJ Infect Dis. Apr 1;191(7):1129-39.

218. Watts DH, Lambert JS, Stiem ER, Bethel J R, Bethel J, Whitehouse J, et al. (2000) Complications according to mode of delivery among HIV infected women with CD4 lymphocyte count of <500/microL. Am J Obstet Gynecol.; 183:100-7

219. Wegmann TG, Lin H, Guilbert L, Mosmann TR (1993) Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today 14:353–356

220. Weisberg s(1985).Applied Linear regression 2nd E, John Wiley&Sons, New York.

221. Working Group on Mother-to-Child Transmission of HIV (1995). Rates of mother-to-child transmission of HIV-1 in Africa, America, and Europe: Results from 13 perinatal studies. J Acquir Immune Defic Syndr Hum Retrovirol 1995; 8:506-510.

222. World Health Organization. Reproductive Health and Research (2004). Medical eligibility criteria for contraception use, Third Edition.

223. World Health Organization (1982). Treponemal infections. Geneva, Switzerland: World Health Organization, Tech Report Series 674-

224. Yui J, Garcia-Lloret M, Wegmann TG, Guilbert L (1994) Cytotoxicity of tumor necrosis factor (TNF-alfa) and gammainterferon (IFN-gamma) against primary human placental trophoblasts. Placenta 15:819–28