SEXUALITY AND SEXUALLY TRANSMITTED DISEASES IN YOUNG WOMEN

by

Agneta Andersson-Ellström

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AKADEMISK AVHANDLING

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I. Andersson-Ellström A, Forssman L. Sexually transmitted diseases – Knowledge and attitudes among young people. J Adolesc Health 1991;12:1272-1276.

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VII. Andersson-Ellström A, Svennerholm B, Forssman L. Prevalence of antibodies to herpes simplex virus type 1 and 2, Epstein Barr virus and cytomegalovirus in teenage girls, with regard to sexuality. Scand J Infect Dis 1995;27:315-318.

ABSTRACT

The aims of this study were to investigate: (i) the level of knowledge and attitudes to sexuality and sexually transmitted diseases in young women, (ii) to relate these data to actual life-style and sexual behaviour, and (iii) to estimate the spread of sexually transmitted diseases (STD) in young women in relation to their sexual behaviour. In Paper I the knowledge and attitudes to STD and STD prevention were studied by a questionnaire in 18-19 year old students during a school lesson. Girls were found to be better informed than boys and the level of knowledge increased from 1986 to 1988, probably due to greater national attention to these problems. Paper II describes in a longitudinal 2 year study, the association between knowledge, attitudes and behaviour among 16-18 year old girls and demonstrates that a high level of knowledge not necessarily leads to adequate behaviour. Paper III emanates from the same group of 16-18 year old girls and compares life-style factors and continued sexual behaviour in relation to age of coitarche. An early coitarche was associated with an early menarche, big families and an early move from the parental home. Girls with an early coitarche had a more risky behaviour and had more STD:s and cervical atypias. In paper IV, women <25 year old coming for contraception advice were evaluated regarding cervical chlamydia trachomatis infection and cytological atypias. Twelve percent of the screened population were found to have a chlamydia infection and 3% had koilocytosis. Those with a positive test for chlamydia infection were followed for 15 months. During the follow up 27/32 women cumulatively had clinical or subclinical signs suggesting a genital papillomavirus infection and at the last visit 6/32 had an atypia. Paper VI describes the prevalence of HPV DNA in the cervix in 16 year old girls followed for 2 years, and shows no HPV DNA in virgins but a quick increase in cervical HPV DNA after coitarche and that occurrence was related to the number of sexual partners. Paper VI refers to the same girls and relates the occurrence of serum-antibodies against HPV16 and HPV33 to the existence of HPV DNA in the cervix. No virginal girl had antibodies and the correspondence between HPV16 antibodies and cervical HPV DNA was good. In paper VII the presence of antibodies against HSV-1, HSV-2, EBV and CMV in serum among the group of 16-18 year old girls was studied and it was concluded that the prevalence of antibodies against HSV-1 and EBV was significantly higher among sexually experienced girls.

Key words: adolescence; sexual behaviour; sexually transmitted diseases; knowledge and attitudes; coitarche; contraception; reproductive health; Chlamydia trachomatis; Herpes simplex virus; Epstein-Barr virus; Cytomegalovirus; Human papillomavirus; epidemiology; serology.

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From the Department of Obstetrics and Gynecology, East Hospital, University of Gothenburg, Gothenburg, Sweden

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by

AGNETA ANDERSSON-ELLSTRÖM

Centre for Public Health Research, County Council of Värmland, Karlstad, Sweden

In memory of my father

ABSTRACT

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CONTENTS

ABBREVIATIONS

AIDS:	Acquired immunodeficiency disease syndrome
ASCUS:	Atypical squamous cells of undetermined significance
CIN:	Cervical intraepithelial neoplasia
CMV:	Cytomegalovirus
CT:	Chlamydia trachomatis infection
CTP:	Chlamydia trachomatis positive
DFA:	Direct fluorescence antibody test
EBV:	Epstein-Barr virus
ELISA:	Enzyme-linked immunosorbent assay
FPYC:	Family planning clinic or Youth clinic (paper IV)
GPVI:	Genitoanal papillomavirus infection
HIV:	Human immunodeficiency virus
HPV:	Human papillomavirus
HSG:	Healthy school girls (papers II, III, V, VI, VII)
HSIL:	High-grade squamous intraepithelial lesion
HSV:	Herpes simplex virus
LSIL:	Low-grade squamous intraepithelial lesion
OC:	Oral contraceptives
RR:	Relative risk
P3HR-1:	An Epstein-Barr virus-carrying Burkitt lymphoma cell line
PCR:	Polymerase chain reaction
SQ:	School questionnaire group (paper I)
STD:	Sexually transmitted disease
VCA:	Virus capsid antigen
VAS:	Visual analouge scale

1. INTRODUCTION

1.1 Reproductive health

On a global level reproductive behaviour is an important health parameter, influencing both mortality and morbidity (Piot & Islam 1994). This is especially obvious today when the world-wide dissemination of the human immunodeficiency virus (HIV) threatens to wipe out whole populations. In addition, maternal mortality, and illness and death linked to illegal abortions, is still a problem of considerable dignity in many parts of the world (Bergström 1993).

The situation in Sweden is, however, more favourable. Maternal mortality is almost overcome, and appropriate legislation on abortion makes this procedure safe. HIV is still a rather uncommon infection, and infected children and teenagers are rare. The majority of our problems relate to other sexually transmitted infections such as chlamydia trachomatis, which affects fertility, human papillomavirus infections which causes cervical intraepithelial neoplasia, and herpes viruses which may seriously threaten the fetus and the new-born baby. There are also important psychological problems connected to reproductive health, influencing the identification process and well-being. Unconscious desires tend to inhibit logical thinking with regard to sexuality and make risk-taking behaviour more prevalent.

1.2 Adolescence

Several developmental issues are pertinent to adolescents (Elkind 1978), including psychosexual maturation, the development of hypothetical reasoning and conditions relating to risk-taking behaviour. Cates (1991), as well as Biro & Rosenthal (1992), have described this process regarding sexuality and sexually transmitted diseases. During early adolescense a transition in cognitive development from concrete operational to formal operational thinking enables the adolescent to use hypothetical reasoning. Formal operational thinking is believed to solidify at approximately 14 years of age but many adolescents may reach formal operational thinking at a later age. Hypothetical reasoning ability can be task-dependent and may for instance be less frequently applied to sexual behaviour than to school exercises. The onset of formal operational thinking produces egocentrism or self-centred thinking including the belief that natural laws do not apply to oneself ("personal fable"). There is also a tendency for adolescents to place him or herself in the centre of things surrounded by an

"imaginary audience", focusing attention on themselves. Volitional risk-taking behaviour has positive as well as negative effects and meets developmental needs. Sexual activity in adolescence may meet developmental needs such as autonomy, affiliation and identity (Irwin 1990). Protecting oneself from STD may be in conflict with meeting these developmental needs and these "costs" will exceed the current advantages.

1.3 Teenage sexuality

Reliable contraceptives have contributed to the acceptance of premarital and teenage sexuality. Age of coitarche differs in different countries due to cultural factors (Jones et al 1985). In Sweden the median age for sexual debut is some years earlier than in other European and American countries. In a large study among 15-25 year old women visiting family planning clinics in Gothenburg in 1988, the median age of coitarche was 16.5 years and 90% of the women had had their first coitus by 19 years of age (Ramstedt et al 1992). Riphagen & von Schultz (1989) and Gisecke et al (1992) have reported similar figures. The median age of female coitarche appears to have decreased by 2 years during the last 25 years (Zetterberg 1969; Klanger et al 1993), and probably has reached a steady state during the last decade. Nowadays girls tend to have their first intercourse at a younger age than boys (Gisecke et al 1992; Persson et al 1992a; Sundet et al 1992). Kontula & Haavio-Mannila (1994) found similar tendencies in Finland during the past few decades. In Denmark the age for coitarche corresponds to the Swedish figures (Wielandt & Boldsen 1989), in Norway the age for coitarche is a little higher (Kraft 1991; Sundet et al 1992). An early coitarche has been reported to be associated with continued risk behaviour (Greenberg et al 1992; Seidman et al 1994).

1.4 Sex education

Sweden was the first country in the world to establish compulsory sex education in its schools. The curriculum involves all grades and pays attention to contraception and the discussion of human and sexual relationships. Policy is based on the principle that attitudes are changed more effectively with openness and knowledge rather than with threats, edicts and repression (Sundström-Feigenberg 1988). In the spring of 1986 the Swedish National Board of Education requested schools to introduce teaching about HIV/AIDS and other sexually transmitted diseases (STD). During the following years information campaigns funded by the Swedish National Commission on AIDS have been

instigated and the media have drawn attention to the risks. Surveys in Sweden have shown widespread and good knowledge about STD and STD protection (Persson & Jarlbro 1992b). Brorsson & Herlitz (1988) found changes in knowledge and attitudes going on in Sweden, as well as Kegeles et al (1988) and Zimet et al (1993) did in the USA. Contrary to other studies questioning the use of appropriate behaviour (Tydén et al 1991a; Di Clemente et al 1993), Brorsson & Herlitz (1988) also reported changes in behaviour.

1.5 Contraception

Ever since oral contraception was introduced in the sixties, it has been the most used contraceptive method for teenagers in Sweden (Andersch & Milsom 1982; Brännström et al 1991; Milsom et al 1991; Larsson et al 1996). Changes in sexual habits and the transition from the use of condoms to the use of OCs assisted the transmission of genital infections. A substantial increase in gonorrhoea in the early seventies lead to campaigns to persuade people to use condoms. The discovery of the human immunodeficiency virus together with the high prevalence of chlamydia trachomatis infections led to new campaigns.

Several studies have indicated an increased use of condoms by young people (Tydén et al 1996; Larsson et al 1996). However, condoms are not always well accepted by teenagers, and an inconsistent use of condoms is reported to be a common phenomenon (Traeen 1994). Oral contraceptives have now been used for more than 30 years and several studies (Andersch & Milsom 1982; Milsom et al 1991; Larsson et al 1996) have described the connection between the womans' opinion of the possible health hazards of OC and its influence on compliance. In some countries even a female type of a condom has been introduced (Editorial 1993), but not in Sweden.

1.6 Sexually transmitted diseases, epidemiology

The annual number of gonorrhoea infections in Sweden declined from 40 000 in 1970 to 338 in 1994, probably due to efficient treatment and an effective contact tracing organization (Ramstedt et al 1985). It became possible to diagnose genital chlamydia trachomatis infections in the late seventies. Some parts of Sweden were quick to initiate testing. However, the county of Värmland, where this study was performed, began testing first in the middle of the eighties. Studies carried out in Family Planning Centres and Youth Clinics from various places in Sweden have reported prevalences of 10-20% in sexually active young women in the eighties (Örtqvist et al 1984; Rahm et al 1988). In Karlstad the

corresponding figures for women less than 25 years age were 11% in 1986-88 compared to 3% in 1990-1991. (Andersson-Ellström 1993). The decreasing prevalence was seen to coincide with the inclusion of chlamydia trachomatis infection in the Swedish STD-legislation, facilitating a better control of contact tracing. The age-specific rates of PID are highest for adolescents if appropriate adjustments are made for sexual activity (Bell & Holmes 1984; Weström 1980; Lidegaard & Helm 1990).

In spite of the declining prevalence of bacterial STD:s, sexually transmitted viral infections are still common (Evander et al 1995), indicating that the decrease in chlamydial infections for the most part was due to better diagnostics, contact tracing and treatment.

Human papillomavirus infection has been estimated to be the most common sexually transmitted disease in the Western world (von Krogh 1991). HPV is viewed as the primary cause of most cases of cervical intraepithelial neoplasia (Schiffman et al 1993) and is an important causal factor in most cases of cervical cancer (Munoz et al 1992). During the last few years non-sexual pathways for the transmission of genital HPV have been suggested (Sedlacec et al 1989; Jenison et al 1990; Pao et al 1993). A high prevalence of serum antibodies against various HPV16-derived antigens among healthy children (Jenison et al 1990) has been taken as evidence of prevalent non-sexual transmission of HPV16. However, Fairley et al (1992) and Rylander et al (1994) found no HPV16 in virginal girls, and several major studies found a close connection between genital HPV occurrence and sexual activity indicating that the major route of transmission is the sexual one (Ley et al 1991; Schneider & Koutsky 1992; Bauer et al 1993; Hildesheim et al 1993; Wheeler et al 1993). Common cancer-associated high-risk genitoanal HPV-types are types 16 and 18. HPV types 31, 33, 35, 39, 45, 51, 52 56 and 58 are also found in cancers. HPV types 6 and 11 are only very rarely found in cervical cancers (Lörincz 1992, Bosch 1995). Additional genitoanal types exist but have not been well characterized.

The Herpes group viruses, herpes simplex virus type 1 (HSV-1), HSV-2, cytomegalovirus (CMV) and Epstein-Barr virus (EBV), all have the characteristic ability to establish latency and are intermittently reactivated in the human host. Most infections are asymptomatic and the majority of the population acquires antibodies against both HSV, CMV and EBV. HSV-2 is predominantly sexually transmitted and HSV-1, EBV and CMV can also be identified in the genital mucosa (Gibson et al 1990; Sixbey et al 1986; Näher et al 1992; Ho 1990). HSV-2 has speculatively been suggested to play a role in the development of cancer as a contributing factor in the development of carcinoma of the cervix uteri (Rawls et al 1980; Rapp 1989). EBV is regularly associated with Burkitts lymfoma and nasopharyngeal carcinoma (Zur Hausen et al 1970; Nonoyama et

al 1973). The spread of the herpes group viruses has in a global and historical view been connected to socioeconomic conditions (Evans et al 1989; Nahmias et al 1990; Ho 1990). HIV infections are still rare in Sweden, but a further 260 persons were found to be HIV positive in 1994. Only a few of them were teenagers.

1.7 Development of new diagnostic techniques

1.7.1 Direct tests

Chlamydia trachomatis: Culture has been the "golden standard" for diagnosis, due to a 100% specificity. The sensitivity, however, is rather low, reported to be between 38-100%, and dependent on the specimen collection method, cell culture technique and the period of time since sampling, etc. (Kellog 1989). Other, less labour intensive tests, became available in the eighties'. Enzyme linked Immunoassay (EIA) is a simple analysis procedure and samples can be tested several days after sampling. The EIA test produced by Abbot (Chlamydiazyme, USA) has shown a rather good specificity (Lefebvre et al 1988; Svensson et al 1991) and a varying sensitivity from 75-95 % (Baselski et al 1987; Lefebvre et al 1988; Svensson et al 1991). To increase specificity, the EIA test is often verified by a direct fluorescence antibody test (DFA). By using the EIA as a screening method and DFA as a verification, one attains a less expensive and less labour-intensive method giving a sensitivity of 91% and by definition a 100% specificity (Svensson et al 1991). The current introduction of the PCR technique for chlamydial testing will probably further increase diagnostic reliability (de Barbeyrac et al 1994).

Human papilloma virus: Clinical signs of warts, colposcopic examination with acetic acid painting, and microscopical morphological examination for koilocytosis, were the only means of HPV diagnosis until the DNA detection methods became established. Koilocytosis is considered to be pathognomic of HPV infection (Meisels 1983; Schneider & Koutsky 1992). Colposcopy has a good sensitivity (Schneider et al 1988; Spitzer et al 1990) but the specificity of minor morphological abnormalities for the detection of subclinical HPV infection appears to be low (Schneider & Koutsky 1992). The most sensitive DNA detection method is the polymerase chain reaction (PCR) technique which requires only small fragments of the DNA chains. By using the thermo-stable enzyme Taq polymerase and specific primers, small amounts of HPV DNA can be multiplied by repetitive cycles of heat alterations. An alternative, somewhat less expensive and less sensitive hybrid capture human papillomavirus DNA

detection test has been launched commercially (Sun et al 1995).

Herpes viruses: In addition to virus isolation, DFA tests for herpes simplex viruses have been established. These tests are not impaired by long transport distances , but require puncture of intact blisters.

1.7.2 Serological tests

Antibodies: The development of more specific serological tests, often using ELISA techniques, makes it possible to determine different types of the same virus, e.g. HSV-1 can be differentiated from HSV-2 (Svennerholm et al 1984), and genital chlamydial infections from other types of chlamydial infections (Treharne et al 1977). Recently, ELISA tests, that measure type-specific infection with human papillomavirus have been developed (Dillner 1995). Antibodies against HPV16 capsids (Kirnbauer et al 1994) and HPV33 capsids (Andersson-Ellström et al 1994) have shown association with HPV DNA detectability and sexual behaviour.

2. AIMS OF THE STUDY

(i) To assess knowledge about STD and STD prevention among teenagers at the time close to their sexual debut.

(ii) To investigate the relationship between young girls' knowledge about STD and STD prevention, and their own sexual behaviour.

(iii) To study the association between biological, familial and social factors and the age of sexual debut, and to investigate continued risk behaviour in relation to age of sexual debut.

(iv) To study the prevalence of cervical Chlamydia trachomatis (CT) infections in young women visiting contraception clinics, and longitudinally re-examine chlamydia-positive women for 1 year regarding reinfections with CT or signs of human papillomavirus infections and cervical intraepithelial neoplasias.

(v) To study the prevalence and incidence of different types of HPV-DNA in the cervix and relate the occurrence to sexuality.

(vi) To study the prevalence and incidence of serum antibodies to papillomaviruses and herpesviruses.

(vii) To relate the prevalence of serum antibodies against human papillomaviruses to the occurrence of HPV DNA in the cervix and to sexuality.

3. MATERIAL

This thesis is based on studies in 3 groups, which investigate: 1) 18-19 year old male and female students (I); 2) women younger than 25 years of age (IV); and finally 3) 15-17 year old girls followed longitudinally for 2 years (II; III; V; VI; VII).

3.1 Paper I describes a study in 953, 18-19 year old boys and girls, completing their education in theoretical or practical upper secondary schools in Karlstad (a middle-sized Swedish city, 80.000 inhabitants) or in Torsby (a small town in the same county) in May 1986 or in May 1988 (referred to as the school questionnaire group - SQ.)

3.2 Paper IV describes a study in 323 women, <25 years, who visited the Gripen Family Planning Clinic or the Youth Clinic, Karlstad, between September 1986 and February 1987. Two hundred and ninety eight women had experience of coitus (the FPYC group). The median age of the women was 19 years (range 13-24), 55% being in the interval of 16-19 years, 4% were younger than 16 years. From this group of 298 women with coital experience, 32 of the 36 Chlamydia-positive women (median age 19, range 16-24) were followed for a median time of 15 months (range 9-22). These women will be referred to as the chlamydia trachomatis positive (CTP) group.

3.3 Papers II, III, V, VI and VII: All 168 girls younger than 18 years, starting their education in the health care program of an upper secondary school in Karlstad between September 1989 and September 1990, were invited to participate in a study about sexuality and sexually transmitted diseases. The 98 girls who accepted to participate (6 girls 15 years old, 78 girls 16, and 14 girls 17) were followed for 2 years. (Referred to as the healthy school girls - HSG, and when entering the study referred to as "16".)

4. METHODS

4.1 Design of the studies

4.1.1 The first study (I) took place in the class-room, where all pupils, without any preceding announcement, were given a questionnaire to complete during a lesson. The questionnaire comprised 19 multiple-choice and 3 open-ended questions, testing knowledge and attitudes about sexuality and sexually transmitted diseases. In 1988 an additional 1, open-ended question was added, evaluating the best sources of information.

There were no non-responders: All students answered the questionnaires, some of them passing over a few questions, as described in paper I.

4.1.2 The second study (IV) emanates from the family planning clinic and the youth clinic where women < 25 years, coming to a midwife for advice on contraception, were screened for cervical chlamydial infection (CT). The diagnosis was made by an enzyme immunoassay test (EIA, Chlamydiazyme, Abbot, USA) confirmed by a direct immunofluorescence test (DFA, MicroTrac, Syva, USA), as described below. The women were also screened for cytological changes by means of a Papanicolaou (PAP) smear, also described below. A face-to-face interview supplemented the investigation. Chlamydia-positive women (CTP), (median age 19 years, range 16-24) were followed by the author for 3 visits: before treatment, one month later, and one year after treatment. At the first visit, cultures for gonorrhoea were taken and colposcopy with acetic acid testing was performed. Treatment for the chlamydia trachomatis infection was given at the first visit with 200 mg doxycyklin followed by 100 mg for 8 days, and contacts were traced and treated (independent of the result of the test, if it was a steady partner). At follow-ups, cervical swabs for EIA and DFA were taken. At the first and third visit, colposcopy was performed and at the third visit another PAP-smear was taken. Biopsies were taken in some cases where colposcopic criteria indicated CIN or GPVI. No analysis has been performed on the 12 non-responders (4%).

4.1.3 The third study is a longitudinal study, performed on healthy schoolgirls (II; III; V; VI; VII), and comprising 2 years of gynecological health control and the possibility of contraceptive advice. The invitation was given by the author who visited the class-room, described the investigation, and asked girls to fill in a paper whether they wanted to participate in the study or not. Those who accepted to participate were called to the Youth Clinic every 6 months for a structured face-to-face interview. A total of 5 visits were made. At the first and

last visit the girls answered a self-administrated questionnaire about family situation, sexuality, knowledge and attitudes to contraceptives and STD. At every visit a blood sample was taken for serological tests. Twice, at the first or second visit, and at the last visit, a gynecological examination was performed and samples from the cervix were taken for cytology, chlamydia- and HPV-DNA direct tests in the same sequence as below. Girls were told to come for a virus isolation sample if genital blisters became apparent. The study was approved by the Ethics Committee, Örebro. Parents were informed about the study in meetings at school before the start of the study. In order to respect the integrity of the girls not choosing to participate, we have not performed any analysis of the non-responders (42%).

4.2 Diagnostic methods

4.2.1 Interview: All interviews were carried out according to a standardized questionnaire (II; III; IV; V; VI; VII). The questions were given in a similar and neutral way to all participants. Questions about sexual behaviour were limited to participants who reported sexual experience.

4.2.2 Questionnaires: The questionnaires comprised multiple choice and open-ended questions as mentioned above (I; II; III). In the questionnaires, testing knowledge and attitudes (I; II) a "don´t know" alternative was added, to decrease guesswork. The healthy school girls (III) were also requested to complete some visual analouge scales (VAS). The technique involves making a mark on a 10 centimetre long line, and the distance from the left end of the line to the indicated point was measured to describe their appreciation of themselves, their relationships to their families and friends, their mood and their worries about reproductive health.

4.2.3 Direct tests: The enzyme immunoassay tests for **chlamydia trachomatis** (Chlamydiazyme, Abbot, USA) were analysed at the Microbiological laboratory of the Central Hospital in Karlstad. To verify a positive or uncertain Chlamydiazyme test a direct fluorescence antibody test (Microtrac, Syva, USA) was used. Cultures for **Neisseria gonorrhoe** were performed using a modified Stuart transport medium (Gästrin & Kallings 1968) and tested in the for Scandinavia standardized way (Wilkinson 1977) at the Microbiological Clinic in Karlstad. For the analysis of **human papillomavirus deoxyribonucleic acid** (HPV-DNA), samples from the cervix were taken with a Cytobrush (Medscand, Sweden) and put in a test tube containing 96% ethanol for subsequent analysis with the polymerase chain reaction (PCR) method performed at the Stockholm

Central Microbiology Laboratory as described by Skyldberg et al in 1991. DNA sterility was observed throughout. Detection and typing of HPV DNA was performed by using the degenerated consensus-primer My 09-11 according to Manos and coworkers (1989) and type-specific HPV-primers (type 6/11, 16, 18, 31, 33). The procedure has been described in detail by Skyldberg et al 1995. HLA primers were used to check the adequacy of DNA extracted from the cytological samples by a routine procedure (Erlich & Bugawan 1989). **Papanicolau smears** were analysed at the Cytological laboratory of the Central Hospital in Karlstad. A re-screening of PAP-smears to determine the occurence of koilocytosis was performed on all 284 samples from the second study (IV) at the Cytology laboratory, Sahlgrenska University Hospital, Gothenburg by one investigator who had no access to the results of clinical examinations or previous cytological diagnosis. All positive samples from the third study (V) were re-evaluated by Björn Hagmar, Rikshospitalet, Oslo University.

4.2.4 Colposcopy: The colposcopic examination included a test with 5% acetic acid and was performed using the criteria described by Schneider et al in 1988.

4.2.5 Serological tests: The serological tests for herpes viruses (HSV-1, HSV-2, CMV, EBV) were evaluated at the Microbiological Clinic, Sahlgrenska University Hospital, Göteborg. HPV serology was evaluated at the Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm.

Herpes simplex virus: Serum was assayed in a direct enzyme-linked immunosorbent assay (ELISA), using a HSV type common antigen, containing envelope glycoproteins (Jeansson et al 1983) and the HSV-2 type-specific glycoprotein G (gG-2) antigen (Svennerholm et al 1984). Microtiter plates (NUNC Immunoplates, Denmark), coated with antigen, were incubated with serum samples in serial two dilutions starting at 1:100. For detection, horse-radish peroxidase conjugated antihuman IgG (Jackson USA) was used. By this method, antibodies against HSV-2 and indirectly against HSV-1, can be evaluated. Samples positive against HSV-type common antigen, which were gG-2-negative were regarded as HSV-1 positive. On the other hand, the procedure precluded reliable determination of HSV-1-positivity if HSV-2 antibodies were present.

Cytomegalovirus: Antibodies against CMV were determined by an ELISA-test described by Krishna et al (1984), using the same microtiter plates and horse-radish peroxidase conjugated antihuman IgG as described for the detection of HSV antibodies.

Epstein-Barr virus: EBV specific IgG antibodies to the virus capsid antigen (VCA) were demonstrated according to standard procedures by incubation of serial two-fold dilutions of sera on P3HR-1 cells in indirect immunofluorescence (Lennette 1988).

Human papilloma virus: By using ELISA tests, serum samples were tested for reactivity with baculovirus-expressed capsids of HPV16 (Kirnbauer et al 1994) and HPV33 (Volpers et al 1994). Disassembled similarly prepared Bovine Papillomavirus capsids were used as negative control, to distinguish between type-specific and cross-reactive HPV antibodies.

4.3 Statistical analysis

Distributions between groups were compared with the Chi-square test or Fisher's exact test. Comparisons of mean values between 2 groups were performed using Student's t-test. Duncan's multiple range test was used to compare mean values between 3 or more groups simultaneously.

5. RESULTS

5.1 Study populations

All 953 invited SQ students answered the questionnaire (I). More than 98% answered at least 17 of the 19 multiple choice questions and more than 90 % answered the remaining 2 multiple-choice questions. Only 2 open-ended questions, concerning where to seek advice and the reasons for controlling STDs caused major default.

Three hundred and twenty three of the women who underwent gynecological examination (96.4%) agreed to participate in the second survey (IV). Coital experience was reported by 298 women and they comprise the study group (FPYC). Thirty two of the 36 CT positive women (CTP) were followed for a mean of 15 months.

One hundred and sixty eight girls started their upper secondary school education in the health care programme course and were invited to participate in a 2 year longitudinal study (II; III; V; VI; VII). Ninety-eight girls (58%) accepted the invitation to participate, 95 came for a second visit and 89 completed the fifth visit of the study. However, complete data is only available for 88 girls. There were no significant differences between girls who dropped out of the study and

the girls who completed the study with respect to age, socioeconomic background, age of coitarche, number of coitus partners and use of contraceptives at the age of 16. In order to respect the integrity of the girls refusing to participate, we did not investigate this group.

5.2 Sexuality

5.2.1 Median age for coitarche was 16 years, both in the cohort of women visiting the family planning clinic or the youth clinic (FPYC) in 1986-87 (IV), and in the cohort of healthy school girls (HSG) in 1989-90 (III). Eighteen women (6%) in the FPYC group reported coitarche before the age of 14, compared to 8 (8%) in the HSG group; 244 (82%) reported coitarche before the age of 18 in the FPYC group compared to 72 (81%) in the HSG group. In the 18-19 years old SQ group from 1988, 79% of the girls and 75% of the boys had coital experience (I). In 1986 more girls in the practical courses compared to girls in the theoretical courses had had their sexual debut ($p<0.01$). Two years later, the difference was less pronounced. In 1988 more girls living in the rural area had experience of coitus compared to girls from the city ($p<0.01$). Healthy school girls (HSG) with coitarche before the age of 15 years had more frequently had their menarche before the age of 13 ($p<0.05$) (III).

5.2.2 Number of partners. In the group of sexually experienced healthy school girls (II; III: V; VI; VII) the mean number of coitus partners at the age of 18 was 3.9 and the median number 3 (range 1-27). Two girls (3%) reported more than 10 life-time partners.

The number of partners was evaluated among the 16-24 years old chlamydia-positive (CTP) women (mean and median ages were 19 years) in the FPYC (IV). Five of 31 responders (17%) reported 1 life-time coital partner, 17 responders (55%) reported 2-4 partners, 6 responders (19%) 5-10 partners and 3 (10%) more than 10 life-time partners.

5.2.3 Steady partner: In the FPYC group, 249 of 298 women (83.5%) reported a steady sex partner according to the woman's own definition (IV). The sexually experienced healthy school girls reported a steady sex partner in 64-77% during 2 years of follow-up and in an increasing degree when getting older (II; III; V; VI; VII). Seven of the 54 girls with more than 2 years of coitus experience reported the same steady partner during the 2 year follow-up period.

5.2.4 Sexual activity: Teenage girls with an early coitarche were sexually more active during the observation period than the girls were who reported a later

coitarche (III). Girls were in general sexually more active at the age of 18 compared to the age of 16. Three of 4 girls, independent of the age for coitarche, had their second coitus within 6 months of their debut but there was a tendency to fewer early debutating girls having their second coitus in less than 1 month after the debut.

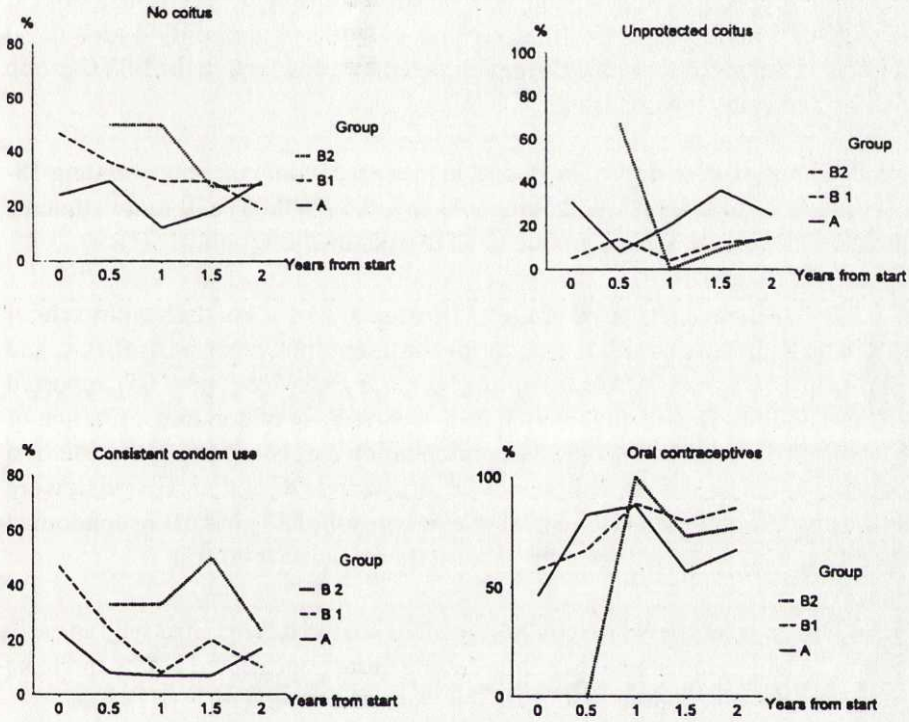

Fig. 1. Results from each visit during the study in the three groups of girls with coitus experience (Group A = sex debut <15 years; Group B1 = sex debut ≥15 years, but before entering the study; Group B2 = sex debut ≥15 years but after entering the study).Upper left: Percentage distribution of girls with no coitus experience last month; Upper right: Percentage distribution of girls with unprotected coitus last month; Lower left: Percentage distribution of consistent condom use during last month; Lower right: Percentage distribution of use of oral contraception last month.

5.3 Contraception

5.3.1 Choice of methods: At the age of 18, 93% of the sexually experienced healthy school girls reported experience of OC, and 41 of 53 girls reporting coitus during the last month had then used OC for contraception at that time (77%) (II). Experience of condom use was reported by 89%, of whom 71% had adopted this method during the last year. Only 7 of 53 girls (13%) reporting coitus the last month, had used a condom at that time. Current condom users often tended to report occasions of unprotected coitus during the last month, both at the age of 16 and 18. At the final visit (at 18 years of age) only 4 girls (6%) reported a latest coitus with OC supplemented by a condom. In the FPYC group 64% of the women were using OC (IV).

5.3.2 Changes over time: The intention to use a condom increased among 18-19 years old students in 1988 compared to 1986 ($p<0.05$) and more students intended to change behaviour due to STD risks in 1988 ($p<0.01$) (I).

5.3.3 Differences correlated to age: At the age of 16, 51% of the healthy school girls who had been sexually active during the last month, reported use of OC and 33% use of condoms (II). At the age of 18, more girls (77%, $p< 0.05$), reported OC use and fewer girls (13%, $p< 0.05$), reported use of condoms. Change of contraceptive method was most common among girls reporting the use of a condom at the age of 16. Thirteen of 18 girls using OC at their first visit were still using OC at their latest visit. However, only 4 of 21 girls using condom at their first visit were still condom users at the last visit ($p<0.05$).

5.3.4 Differences correlated to the age of the sexual debut: Girls with an early coitarche had a tendency to continue having more unprotected coitus and to be less careful with condom or OC use than later debutating girls (III).

5.3.5 Differences in the use of contraception correlated to sexual activity: An unprotected coitus was most common close to the sexual debut, independent of age (II; III). Condom use was most common close to the debut. A steady partner was a reason for testing OC, but compliance tended to be deficient, and unprotected coitus was a common result of interrupting OC use.

5.4 Knowledge and attitudes to sexually transmitted infections

5.4.1 Sources of informations: In 1988, school was most frequently mentioned as the best source of information among 18-19 years old boys and girls (34%) (I). The media were mentioned by 29% and other written material by 16%. In 1989-1991 almost all 16 years old girls had heard about STD in school lessons (II). Most girls emphasized individual reading as the best source of information, and a friend as the most common person for discussion. The sources were as follows: newspapers 93%, friends 91%, boy-friend 84%, parents 41%, and other adult 53%. Nearly half of the girls (47%), considered themselves insufficiently informed about STD; the corresponding figures for birth control methods and for pregnancy and abortion were 10% and 18% respectively.

5.4.2 Changes over time: Significantly more 18-19 year old students in 1988 compared to 1986 had heard about STD in school lessons ($p<0.001$), were aware of veneral warts as sexually transmitted ($p<0.001$) and knew about important aspects of chlamydia infections (prevalence, symptoms, treatment and need of partner follow-up) ($p<0.001$) (I). However in 1988 only slightly more than 50% knew that a chlamydial infection can be asymptomatic and only approximately 25% knew that chlamydia infections at that time were widely spread. Significantly more students in 1988 admitted being concerned about the possibility of infections being transmitted during coitus ($p<0.001$), and their fear of contracting STD was more common. More students had heard about STD in school education ($p<0.001$) and more had spoken to their parents ($p<0.01$) or another adult ($p<0.01$) about STD in 1988. More boys and girls intended to use or let their partner use a condom ($p<0.001$). No more students, however, mentioned the alternative to keep to one partner or to refrain from intercourse.

5.4.3 Differences between boys and girls: The relative differences in knowledge and attitudes between boys and girls were equal in 1986 and in 1988 (I). Girls were better informed about STD than boys ($p<0.001$) and girls had discussed STD with some other person more often than boys ($p<0.01$). Boys were more willing to use a condom ($p<0.05$) but had less intention to keep to one partner ($p<0.001$).

5.4.4 Differences correlated to age: The healthy school girls were better informed when they were 18 years of age than when they were 16, with regard to chlamydia ($p<0.001$), condylomata ($p<0.001$) and gonorrhoea ($p<0.01$) but

with regard to herpes they were still as confused as when they were at the age of 16 (II).

5.4.5 Differences correlated to type of education: No difference in knowledge emerged between students taking part in theoretical or practical courses (I).

5.4.6 Differences correlated to geographic area: There were no differences in knowledge between students from the urban or the rural area (I).

5.4.7 Differences correlated to social background: No differences were seen (II).

5.4.8 Differences correlated to sexual experience: Girls without sexual experience at the age of 18 were less informed about STD and STD protection ($p<0.01$) (II). The groups of healthy school girls (HSG) with more coital partners or an early sexual debut were more or as much informed as others. Girls who recently had coitus with a new partner without using a condom, and consequently ignoring the risk of STD transmission, were as well-informed as others.

5.4.9 Attitudes to risks and risk behaviour: Both in 1986 and in 1988, 4-5% of the boys and 8-9% of the girls with coitus experience had been treated for STD (I). The fear of contracting STD had a tendency to increase between the 2 periods, particularly among boys, but girls were more worried than boys, both in 1986 and 1988. When evaluating the intention to protect one-self against STD most students asserted their intention to keep to one partner. The use of a condom was significantly more often favoured by those who had never had coitus ($p<0.001$), but in 1988 a more positive attitude emerged among all pupils. In 1988 significantly more respondents were worried about contracting STD and stated that this had influenced their behaviour. Nevertheless, in both 1986 and 1988 only about 50% of the boys and 60% of the girls could give adequate reasons why STD must be controlled. Although about one third of the sexually experienced healthy school girls (HSG) acquired an STD during the 2-year follow-up study, only 1 girl feared that she had been at risk of contracting an STD at her latest coitus (III). Two girls thought they might have given their partner an infection. The remaining girls were convinced they had neither acquired nor transmitted an infection at their latest coitus. Among girls with experience of condom use, all mentioned contraception as the purpose of condom use and only half of them mentioned STD protection as a further reason. When the teenage girls were asked how they would try to avoid an STD, 8% mentioned the possibility of monogamy (II).

5.5 Chlamydia trachomatis, gonorrhea and human papillomavirus infections detected in the cervix

5.5.1 Chlamydia trachomatis. In the FPYC group 36 women (12% of the sexually experienced women) were positive for the chlamydial test in 1986-1987 (IV). In the HSG-group 2 girls (4% of girls with coital experience) were positive at the age of 16 (1989-1990) and 1 girl (1% of girls with coital experience) 2 years later at the age of 18 (1991-1992) (V). Another 2 positive tests were seen during the time for follow-up. No virginal girl in the FPYC or in the HSG group had a positive chlamydial test (IV;V).

5.5.2 Gonorrhoea. All of the 36 women from the FPYC with positive chlamydial tests in 1986-1987 (CTP), had negative cultures for gonorrhoea (IV).

5.5.3 Human papillomavirus infection. Virginal girls had neither HPV DNA in the cervix, measured by PCR, nor antibodies against HPV16 or HPV33 in serum, as measured by ELISA tests (V). At the age of 16, 12% of the coitally experienced girls harboured HPV DNA in the cervix compared to 25% at the age of 18. HPV16 and HPV33 were the most common types of virus DNA. Forty five percent of the girls found to harbour HPV DNA in the cervix had clinical signs of an infection, 10% had subclinical changes and 45% had no signs of infection. When following 32 chlamydia positive young women for a mean period of 15 months the cumulative number of patients with changes which could be related to a genitoanal human papillomavirus infection was 27 of 32 (either genital warts, koilocytosis in the PAP smear or colposcopic changes) (IV). At the last visit, 6 patients had cytologic atypia (18.8%). During the same 24-month period the prevalence of cytologic atypia in all smears, taken among women of the same age attending the clinic, was 1.5%. In 1986-1987, 9 of 284 screened FPYC women (3.2%), had koilocytosis in PAP-smears (IV).

5.6 Serological evidence of HPV and herpes virus infections

5.6.1 Human papillomavirus infection: No virginal girl had antibodies against HPV16 or HPV33 in serum (VI). Of the coitally experienced girls, 7 (12%) had antibodies against HPV16 and/or HPV33 in serum at the age of 16 compared to 11 (14%) at the age of 18. Three girls had antibodies against HPV16 at the first visit and another 7 girls seroconverted during the study. Four girls had antibodies against HPV33 at the first visit. Another 4 girls seroconverted for HPV33 before the last visit.

The correlation between serum IgG against HPV16 and HPV33 and the number of coital partners at the age of 18 was calculated and gave an odds ratio (OR) of 16.3 ($p<0.001$) if the girl reported more than one partner in life.

5.6.2 Herpes simplex virus infection. At the first visit, at the age of 16, 41% of the women in the HSG group (VII) had antibodies in serum, due to an HSV-1 infection, 24% of virginal girls compared to 51% of sexually experienced girls ($p<0.01$). One sexually experienced girl was positive for HSV-2 antibodies. Four girls seroconverted for HSV during follow-up, 1 for HSV-2 and 3 for HSV-1. None of the girls had ever had any clinical signs of a genital herpes simplex infection. No virus isolation tests were collected as no girl had any clinical manifestations of a genital herpes infection.

5.6.3 Epstein-Barr virus infection. At the age of 16, 82% of 98 healthy school girls were seropositive for capsid antibodies against Epstein-Barr virus, 65% of the virginal girls and 92% of the sexually experienced girls ($p<0.001$) (VII). Six girls seroconverted during the 2 years of follow-up, 1 of them lacking sexual experience.

5.6.4 Cytomegalovirus infection. No differences were seen between virgins or sexually experienced girls regarding prevalence of serum antibodies against CMV; 45% were seropositive at age 16 and 49% 2 years later (VII). The 3 girls who seroconverted during the time of the study were all sexually active.

5.7 Multiple infections

5.7.1 Dual types of HPV: Of 20 girls, tested positive for HPV-DNA in the cervix either at the first or second examination, 5 (25%) were simultaneously tested positive for dual types, showing combinations as follow: HPV16-6/11, HPV16-18, HPV16-31, HPV18-31, HPV31-33 (V). One girl was seropositive for both HPV16 and HPV33 when the study started and another girl seroconverted for both HPV16 and HPV33 during the study (VI). There is no serological test available, that is type-specific for the other types of HPV.

5.7.2 HPV and CT: In the 32 young women followed up for a chlamydial infection (IV), 15 women had clinical or subclinical changes directly connected to a GPVI: 8 were found to have condyloma acuminata , 8 had morphological changes pathognomic for HPV (koilocytosis), and 6 had vaginal cytological atypias (4 women CIN I, 2 women CIN II). A further 9 women had colposcopic

features or acetowhite areas, non-specific for a HPV infection. Only 5 women were free from all clinical or subclinical signs of a HPV infection. In the group of healthy school girls (V) the incidence and prevalence of chlamydia infections was very low. Of 5 girls treated for a chlamydia infection before the last visit, 3 girls had signs of a GPVI at the last visit: 1 was found to harbour HPV DNA in the cervix, another one had developed antibodies against HPV16 and 33 and she also had an atypical smear. The third girl had an atypical vaginal smear.

5.7.3 There was no covariance seen between the occurence of different herpes viruses, that is between the occurence of **HSV, EBV** and **CMV** (VII).

5.8 Persistence of infections or reinfections

5.8.1 Chlamydia trachomatis: During 15 months of follow-up after treatment of a cervical chlamydia infection, 7 of 32 women were found to be chlamydia positive for a second time (IV). All 32 women were tested negative 1 month after treatment, and these 7 women were considered to have been reinfected. During 24 months of follow up in the group of healthy school girls, none of the 4 teenage girls who had been infected with chlamydia trachomatis once, tested positive again (V).

5.8.2 Human papillomavirus: At the age of 16, 6 of 52 sexually experienced girls were found to harbour HPV-DNA in the cervix (V). Three of 6 girls had became HPV DNA-negative at follow-up. Two girls harboured HPV16 and both girls tested positive to the same HPV type at the next examination 2 years later. A third girl, harbouring HPV31 at the first visit, was still harbouring HPV31 at the last visit. However, we were unable to determine whether these girls had a reinfection or a persistent infection.

5.9 Factors influencing the frequency of infections

5.9.1 Sexual experience: Nobody without coital experience was found to harbour either a chlamydial infection or HPV-DNA in the cervix (IV;V), indicating that cervical chlamydial or human papillomavirus infections in teenage girls are exclusively sexually transmitted. Furthermore none of the virginal girls had serum-IgG against HPV16 or HPV33 (VI). Girls found to harbour HPV DNA in the cervix or being seropositive for HPV16 or HPV33 had been sexually active for a longer time.

Significantly more sexually experienced girls had antibodies in serum against HSV-1 and Epstein-Barr virus (VII). Only 2 girls had antibodies against HSV-2,

both of them were sexually active. No difference due to sexuality was seen regarding the prevalence of serum antibodies against CMV, but all 3 girls seroconverting during the follow-up were sexually active. Significantly more virginal girls were at the same time lacking antibodies to HSV, EBV and CMV ($p<0.01$).

5.9.2 Contraceptive methods: No differences in contraceptive habits were seen between infected girls and others (II; V; VII). Consistent condom use was uncommon and OC:s as well as condoms were temporarily used by the majority of the evaluated girls.

5.9.3 Number of partners: More than 4 coital partners implied a significantly increased risk for harbouring HPV DNA in the cervix (V). A greater number of coital partners was associated with seropositivity to HPV16 and/or HPV33 (VI). Chlamydial infections were too few to illustrate any possible connections. Girls seroconverting for HSV-1, HSV-2, CMV and EBV during the study were all but 1 EBV seroconverting girl sexually active but reported no more coital partners than other girls (VII).

5.9.4 Age of sexual debut: Girls with an early coitarche, defined as a sexual debut before the age of 15, reported more episodes of STD ($p<0.05$) than girls with a later coitarche and they were found to have more cytological atypias than the remaining girls ($p<0.05$) (III). Of 5 girls, positive for the chlamydial tests sometime during the study, 4 had an early coitarche ($p<0.05$) (V). Of 20 girls with HPV DNA in the cervix at the beginning or end of the study, 7 girls had a coitarche before the age of 15 (NS), and of 16 girls with serum-IgG against HPV16 and/or HPV33, 8 girls had an early coitarche according to our definition above ($p<0.02$) (VI). Of 17 girls with an early coitarche, 5 had no signs of a chlamydial or a HPV infection compared to 42 of 55 later debutating girls ($p<0.001$). No differences in seroconversion against the herpes group viruses could be related to age of coitarche. (VII).

5.9.5 Time since coitarche: At the age of 18, 17 girls were HPV-DNA positive in the cervix, 16/52 of the girls with more than 2 years since coitarche and 1/15 with less than 2 years since the debut (NS) (V). All 11 girls seropositive for HPV16 or HPV33 at the age of 18, had been sexually active for more than 2 years (VI).

5.10 Clinical and subclinical appearance

As mentioned above, among the 32 young women followed up for a chlamydial infection (IV), 15 women had at some time clinical or subclinical changes directly connected to a GPVI: 8 were found to have a clinical diagnosis of condyloma acuminatum , 8 women had subclinical morphological changes pathognomic of HPV (koilocytosis), and 6 had cervical cytological atypias (4 women CIN I, 2 women CIN II). Among the healthy school girls (V), 5 girls had genital warts some time during the follow up, and 4 of them harboured HPV DNA in the cervix. It is notable that 4 of 5 girls with a chlamydial infection in the cervix had signs of an infection. Only 39% of the healthy school girls, independent of coital activity or not, never reported symptoms from the genital sphere during the time for follow-up (III). Vaginal discharge was the most common complaint followed by dysmenorrhea, vaginal irritation, abdominal pain and irregular bleeding.

In the group of chlamydia-positive women followed for 15 months, all had a transformation zone accessible for colposcopy (IV). The appearance varied from time to time. All women with cytological or histological signs of koilocytosis or dysplasia had changes in the colposcopic evaluation. In the FPYC group 3% had koilocytosis in the smear.

5.11 Social background and life-style factors influencing reproductive health

The SQ-group was not examined regarding social background factors. Living in an urban or rural district, however, did not influence the level of knowledge or attitudes (I). In 1988 more 18-19 years old girls resident in the rural area than in the city had had their sexual debut (87% vs 74%, $p<0.01$).

In the group of HSG, no significant differences were seen in the level of knowledge, attitudes, or in the time for coitarche, when considering social background factors in general, or the time for coitarche (II). However, girls from large families, or girls leaving their parental home at a younger age, were over-represented in the group of girls having had their coitarche before the age of 15 (III). Life-style factors such as smoking and drinking alcohol also correlated to the time of coitarche (III). Girls with an early coitarche preferred to have their first child at 22 years of age at the earliest, other girls about 1 year later. However, the girls with an early debut seemed to worry less about getting pregnant with passing time ($p<0.05$) and were at age 18 years less worried than the other sexually experienced girls ($p<0.05$). On the contrary, they were more worried about their future fertility than becoming pregnant ($p<0.001$). This was,

however, also the case for the other sexually experienced girls ($p<0.05$). There were no significant differences between groups concerning plans for their future occupation but more of the girls with an early coitarche were planning for a future education ($p<0.05$). No prominent differences were seen according to the girls' appreciation of themselves, regarding mood, wellbeing or relations. However, there was a tendency to less sense of satisfaction among virginal girls at the age of 18.

No evident differences were seen in the prevalences of HPV DNA in the cervix (V) or antibodies to either HPV (VI) or the herpes-group viruses (VII) with regard to socioeconomic background.

6. DISCUSSION

6.1 The studied group – selection, reliability and validity

This thesis is based on studies among teenagers and young women living in Värmland, a county in the mid west of Sweden. Karlstad is the largest town in Värmland (80.000 inhabitants), situated in the more densely populated south part of the county, and Torsby is a small municipality of a rural district (15.000 inhabitants) situated in the more sparsely populated northern part.

The first study (I) describes the conditions among 18-19 year old girls and boys ready to leave upper secondary schools in Karlstad and Torsby. The study compares sexual experience, knowledge and attitudes between boys and girls, between students from practical and theoretical classes and between students living in densely or sparsely populated regions. At the time for the study about 90% of all teenagers completed their upper secondary school and they are represented in this study.

The second study (IV) emanates from young women visiting the Family Planning Clinic or the Youth Clinic in Karlstad. Most of them were sexually experienced, coming for a special purpose, mainly contraceptive advice.

The third study (II; I; V; VI; VII) emanates from 15-17 year old girls, starting their upper secondary school education in a health care program. They were invited in school to take part in a gynecological health control study. It was emphasized that all girls were welcome to participate, girls without sexual experience as well as sexually active girls. Our purpose was to come in contact with girls close to the time of their sexual debut. The study meant a rather substantial undertaking for the committed girls.

The first and second study describes the conditions in 1986-1988 and the third study took place in 1989-1993. In spite of different groups and different time periods, the data about sexuality are very similar in the 3 groups, indicating no

large divergencies from the underlying population. As reported earlier by Lewin (1984) and Traeen & Lewin (1992a), we found only a minority of the girls reporting many partners. On the other hand it is possible, as postulated in paper II, that girls, already sexual experienced at the start of the study, may be slightly overrepresented in the group of healthy school girls. The contraceptive use reported by the healthy school girls may also have been influenced by the fact that the girls had very good access to contraceptive advice. The girls may even have had better knowledge about STD and STD-protection because of their special type of education, and even participation in this study may have contributed to better information. Consequently, the contraceptive habits and knowledge and attitudes are not meant to be compared to other studies but to be correlated to the other examined factors of the group, in order to correlate knowledge and attitudes to actual behaviour. However, the distribution of contraceptive methods, does not differ from figures given by Brännström et al (1991), evaluating contraceptive habits among 15-19 years old girls in a community-based study in another region of Sweden. It is also a distressing observation from our study that the girls in spite of very good access to contraceptive advice had a rather low compliance both for OCs and condoms.

Different techniques have been used to collect data. In the first study (I) the students answered a self administrated questionnaire during a lesson. The questionnaires were all carefully answered and returned without any indecent notes, indicating serious participation. The project was an isolated investigation and not connected to any efforts to improve teaching. In the second study (IV) the women were interviewed by 1 of 3 midwives, and if they were found to be chlamydia-positive, they were later interviewed by the same doctor on 3 occasions. In the third study (II; III; V; VI; VII) the girls were repeatedly interviewed by the same doctor with some recurrent questions mixed with additional questions. The questions concerned social life, sexuality and contraceptive habits. The questioning was performed without access to answers given earlier. Moreover, the girls filled in questionnaires about knowledge and attitudes to sexuality and sexually transmitted diseases and assessed their feelings and self-apprehension using a ten-point visual analogue scale (VAS). Davioli et al (1992) have described the risks of under-reporting if interviewing. We, however, found a very good correspondance between answers given by interviews and by questionnaires and at different times, indicating a good reliability.

The fact that all interviews, gynecological examinations and sampling were performed by the same doctor, probably facilitated homogeneous performance and assessment. A longitudinal study demanding 5 consecutive visits and including discussions about issues making inroads on integrity, necessitates a trustful cooperation. It is our opinion that we attained such a confidence-

inspiring atmosphere. Hornberger et al (1995) reported teenage girls being generally accurate in reporting sexual history information, which is in accordance with our opinion. The intention was to ask questions in a neutral way, not giving the responders the impression that they ought to say the "right" answer. Aral & Cates have in a paper from 1989 discussed the difficulties of obtaining validated sexual behavioural measures. They have pointed out the inconsistency, the complexity and risk of translating separate variables into general indicators of sexual behaviour, and of generalizing to the total sexually active population from data based on specific age groups.

This thesis specifically focuses on teenagers as a special group with regard to sexual behaviour as well as psychological, social and biological conditions. The contraceptive compliance rate was rather low and during the follow up time of 2 years, most teenage girls had experienced use of OC, as well as use of condoms mixed with unprotected coitus. This makes any conclusions of infection rates due to contraception habits impossible.

The gynecological examination was performed in a fixed manner, carefully avoiding the risk of contamination when sampling, i.e. by using brushes, object glasses and examination utilities wrapped and not stored in the examining room. The laboratory process also was done in a careful way, avoiding contamination. The serum samples and the tests for HPV DNA, chlamydia and PAP-smear were all sent to divergent laboratories as described above, and neither the sexual data nor any results from the other analyses were available.

This thesis is based on studies from a limited area of Sweden and can in some respects reflect local circumstances. The very low recognition of the Venereal Disease Clinic, observed in the first study, may reflect such a local fact. However, our main conclusions about knowledge, attitudes and behaviour have been reproduced in several other surveys in Sweden (Andersch & Milsom 1982; Brännström et al 1991; Tydén et al 1991b; Milsom et al 1991; Persson 1992a, 1992b; Klanger et al 1993; Larsson et al 1996) as well as in a neighbouring country (Traeen 1992b; Kraft 1993).

6.2 The diagnostic methods and their restrictions

The only feature specific of HPV infection without dysplasia is condyloma acuminatum (Barasso et al 1992). Colposcopy allows the evaluation of the extent of a lesion and the localization of the squamo-columnar junction. In young women the transformation zone is mostly accessible. As shown by Schneider et al (1988) and Spitzer et al (1990), colposcopy is very sensitive, but has a substantially lower specificity. If colposcopy is routinely performed, it has been reported that about 80% of the atypical transformation zones are found to correspond histologically to a benign acanthotic non-glycogenated squamous

epithelium (Wespi 1986) which represents metaplastic epithelium without any atypical potential (Barrasso 1992). In the light of these facts it should be observed that some of the girls considered to have a GPVI in the follow-up study of the chlamydia positive women (VI) may have been free from an infection. Schneider et al (1988) suggested that all acetowhite epithelia with minor changes showing histological features of mature metaplasia could represent early or discrete HPV-associated lesions. However, Barasso et al (1991) found HPV DNA sequences in only 13% of such epithelia. It is still a thought-provoking fact from our research, that 6 of 32 women (19%), had an atypia 15 months after treatment for a chlamydial infection. The results of the colposcopy in the later longitudinal study, were only used to determine when and where to take a biopsy. The biopsies were taken at the last visit and have subsequently not influenced the results.

One of the girls who tested positive for HPV33 DNA in the cervix developed antibodies to HPV33 first 18 months later, suggesting a slow development of antibodies. Only 1 of the 5 girls, tested positive for HPV33 DNA in the cervix at the last visit had simultaneous antibodies against HPV33, further suggesting a slow antibody response or suggesting a low sensitivity of the assay. HPV16 DNA in the cervix, however, gave a faster and much more frequent occurence of HPV16 antibodies in serum. Seroconversions coinciding with HPV16 DNA acquisition have also been reported before (Wikström et al 1995). Discordance between the type of HPV DNA found in the cervix and the specific antibody response may be due to several factors: multiple infections, cross reacting antibodies, or an "original antigenic sin phenomenon", i.e. the inclination to primarily respond against a similar, earlier familiar type of virus, when being exposed to a new related type of virus (Hoskins 1976).

Samples for PCR (V) were taken using a cytobrush which provides a sample of chiefly superficial cells. Thus the cytological method may be less sensitive than a histological method to obtain cells from a deeper layer of basal cells. Any virus DNA detection method is dependent on a representative sample being taken and we can thus not exclude the possibility that HPV DNA negative women may have had an infection at a site not sampled (V). However, the good correspondance between HPV16 DNA in the cervix and the HPV16 antibody response (VI), indicates that most HPV16 infections were detected.

As nobody during the follow up reported any signs of a genital HSV infection, no girl was evaluated. It remains an interesting field to determine the prevalence of HSV as well as EBV and CMV in the genital tract of healthy teenage girls and to compare their presence to sexual background data.

6.3 Epidemiological comments

Genital chlamydia trachomatis infections and human papillomavirus infections were exclusively present in sexually experienced girls, and in particular the prevalence of HPV in the cervix rose quickly after coitarche. It is in general hazardous to compare prevalences of infections between independent surveys. The population as well as the methods vary. However, in general one can constitute, that the prevalence of chlamydia trachomatis infection among young women decreased in Sweden during the second half of the eighties (Thejls et al 1991; Persson 1993). In Karlstad the reduction was about 75% (Andersson-Ellström 1993). This tendency is reflected in this thesis where the chlamydia screening group in 1986-1987 had 12% chlamydia (IV) and the healthy schoolgirls about 5 years later had a chlamydial prevalence of 4-1% (V). During the second study (IV), this infection was not yet included in the legislation for STD, and some of the reinfected women were reinfected due to their steady partner who had not accepted any treatment.

HPV colonisation, when compared to the colonisation of HSV-2, seems to take place closer to the sexual debut, at least in early debutating girls. This could reflect the actual spread of the organism in the population, the property of the infectious agents, special patterns of sexual behaviour and/or biological age-dependent conditions (Moscicki et al 1989).

Different studies, using the PCR technique, have reported a prevalence of HPV DNA in the cervix of 20-50% in young women (Ley et al 1991, Bauer et al 1991, Wheeler et al 1993). As mentioned before, a major difficulty when analysing the results, is to establish comparisons between studies. The healthy school girls represent a very young group, being about 16 years old at the first examination. Strand (1995) reported a 16% prevalence of HPV DNA in the cervix of sexually experienced 14-19 year old Swedish girls and Evander et al (1995) reported 21% HPV DNA positivity in a cohort of 19-25 year old sexually active Swedish females. In a gynecological health control material from women younger than 30 years, Hagmar et al (1995) reported 16% HPV DNA positivity. In another Swedish routine gynecological health control material in women aged 20-29 years, Hansson et al (1993) reported 13 % HPV DNA positivity. Syrjänen et al (1990) reported a HPV prevalence age peak in women between 20 and 24 years. However, already 16/52 (31%) of our 18 years old girls, with at least a period of 2 years since coitarche, carried HPV DNA in the cervix. This indicates that the peak reflects a short time interval since coitarche. If non-sexual spread of HPV genital infections in teenage girls can be excluded, this makes it possible to determine the incidence of infection close to the sexual debut.

As in other studies (Bauer et al 1991; Hansson et al 1993; Hellberg et al

1995), HPV16 was the most common type of human papillomavirus, found in the cervix. HPV33 was more prevalent than previously published (Moscicki et al 1990; Bauer et al 1991; Hansson et al 1993; Hellberg et al 1995). The presence of HPV6 in the cervix was uncommon in our teenage group, at variance with the theory that the lower share of HPV6 among older women is due to a lower tendency of persistence of this infection (de Sanjose et al 1992).

The time trend data for occurence of a HPV infection has been estimated to describe a viral "survival curve", suggesting a rather steady disappearance of viral infection over the 9-30 months of observation (Schiffman 1994). If the infection persists, it can predispose to CIN and cancer (Morris et al 1995). Among our teenage girls (V), 3 of 6 girls infected at the age of 16, harboured the same type of HPV (HPV16 in 2 cases and HPV31 in 1 case), at the second time of sampling, 18-24 months later. However, a reinfection with the same type of virus cannot be fully excluded, and the figures are small. As the propensity for infection to persist is considered to be a prerequisite for the development of cervical cancer (Morris et al 1995), it will be a continued challenge to follow the natural history of early acquired persistent genital human papillomavirus infections in this population.

We found some dual infections (3/6 at the age of 16, and 2/17 at the age of 18). The PCR technique may allow more dual infections to be detected, due to its higher sensitivity compared to other HPV DNA techniques. Multiple infections have been reported to be associated with an increased risk of cytologically evident low grade CIN (Morrison et al 1991; Schiffman et al 1993). Our population is too small to estimate this. One of 3 girls with multiple HPV types at the first visit had a mild atypia at the last visit.

One of the most important original findings of this thesis are the results concerning the association between cervical HPV DNA and the appearance of serum antibodies against HPV16 and HPV33, and the absence of both in virginal girls. The strength of this study is the longitudinal approach and the participation of women close to their sexual debut. The serological markers support sexual transmission, and the coexistence of HPV DNA in the cervix and antibodies in the serum confirm the sensitivity and the specificity of the tests. However, as mentioned above, the HPV33 antibody reaction had a curious pattern, either due to a low sensitivity of the test, or to a slow or transitory antibody response. Only 2 of 8 girls with an antibody response against HPV 33 were shown to harbour HPV33 in the cervix. A further 3 HPV33-seropositive girls had HPV DNA of other types in the cervix.

Three percent of the young women attending the Family Planning Clinic or the Youth Clinic (IV), had koilocytosis in their PAP smears in 1986. Mitchell et al (1986) reported that cytological evidence of subclinical HPV infection (koilocytosis) in PAP smears in women younger than 25 years, when followed

prospectively over 6 years, revealed a RR of 38.7 for the development of CIN III compared to the general population. Jones et al (1984) found the same distribution of CIN I, CIN II and CIN III in adolescents with abnormal PAP smears as in the adult population except that no invasive carcinoma was found in the adolescents. Recently it has been questioned if there is a continuous development through ASCUS, CIN I, CINII , CIN III and cancer (Kiviat et al 1992). A new Bethesda classification (1989) has included the possibility of low-grade and high-grade squamous intraepithelial lesions (LSIL contra HSIL including CIN II and CIN III) to represent distinct entities with a different potential for progression. So the importance of CIN I or atypical cells of undetermined significance (ASCUS) still has to be questioned. In the teenage group (V), only 1 girl had CIN II, all the others had atypias classified as CIN I.In the chlamydia positive group (IV), 2 of 6 women with an atypia had CIN II.

The association between a chlamydial infection and the appearance of vaginal cytological atypias has to be further evaluated. The high frequency of atypias in the group of young women followed for 15 months after a chlamydial infection (IV), is an important observation. The frequent occurence of clinical or subclinical signs of a HPV infection supports the possibility of chlamydia as an up-regulating factor for HPV replication (von Krogh 1991), or that the cervix becomes more susceptible to infection (Moscicki et al 1989; Wahl 1984). It is still possible, but not yet convincing, that , besides HPV, a concurrent infection may act as a co-factor in the development of a cervical cancer (Piura et al 1985). In the longitudinal 2-year follow-up study the prevalence of chlamydial infections was low, thus inhibiting further investigation of this hypothesis (V).

The increased exposure of cervical columnar epithelium in adolescent girls is believed to increase the probability of Chlamydia trachomatis infection among those exposed to the organism (Stamm & Mårdh 1990). The immature cervix as a *locus minoris resistentiae* has by some authors been proposed to lead to more atypias (Gottardi et al 1984; Robbey et al 1984; Moscicki et al 1989). Girls with an early coitarche had more cervical atypias (III). We even found that teenage girls, with 1 year or less between menarche and coitarche, had more atypical smears compared to other sexually experienced girls (unpublished data, $p=0.05$, Fishers test). Peters et al (1986) have found some evidence that subjects with a short interval between menarche and initiation of sexual intercourse were at an elevated risk for invasive cervical cancer, but Brinton et al (1989) could not confirm this effect.

The link between the prevalence of antibodies against EBV and sexual experience (VII) also has to be further investigated. EBV plays a role in the etiology of nasopharyngeal squamous epithelial carcinoma (Zur Hausen et al 1970; Nonoyama et al 1973). Taylor et al (1994) found, using PCR and cytological samples from the cervix, that 40% of women harboured EBV in the

cervix, but there was no increased prevalence among women with abnormal PAP smears. On the other hand, Landers et al (1993) found EBV with increasing frequency in CIN II, CIN III and carcinoma of the cervix uteri. EBV DNA has been found in 48% of women when cells were sampled from acetowhite koilocytotic vulvar lesions (Voog et al 1994). The role of EBV has to be further evaluated.

The association between an early debut and a tendency to more sexual partners makes the research of the immature cervix as a risk factor more complicated. The further complex coexistence of smoking and STDs (Rahm et al 1991), makes the causal connections difficult to evaluate. Smoking was more common among girls with an early coitarche and there was a tendency to more smoking among girls harbouring HPV DNA in the cervix. Smoking not only corresponds to sexual behaviour but may even increase the susceptibility of the uterine cervix to infectious agents and carcinogens (Holly et al 1992; Hellberg et al 1986 and 1988). Barton et al (1988) demonstrated that HPV16 infection and cigarette smoking each led to a decrease in the cervical epithelium number of Langerhans´cells, an important component of cellular immunity.

In addition it is also necessary to consider the male factor. Are early debutating women exposed to men, more likely to transmit infections? (Opaneye & Willmott 1991). This study has mainly focused on girls and young women and the importance of the male partner has to be further evaluated. Do very young girls to a greater extent have intercourse with older more experienced partners, and is casual sex by these men more common? (Traeen & Lewin 1992a). Our girls with an early coitarche reported more sexual partners than "boyfriends" and reported more often an isolated first intercourse, indicating a casual contact.

Sexual experience does not obviously mean actual sexual activity, and behaviour may be inconsistent. O´Reilly & Aral (1985) pointed out that the use of indicators of sexual experience rather than indicators of sexual activity, will both overestimate the teenage population at risk and simultaneously underestimate the age-specific rates of STD. In our studies an early coitarche coincided with a continued higher sexual activity. The inconsistency of contraceptive use, in spite of good accessibility, has been evidently demonstrated. Decreased use of condoms among girls with many partners and among girls with casual sex has previously been reported by Kraft et al (1990), Tydén et al (1991a), Traeen et al (1992b) and Dickson et al (1993). The primary reason for contraception being birth control and not STD protection has likewise been described by Traeen et al (1992b).

Self-efficacy has been suggested to be an important determinant of contraceptive behaviour. Heinrich (1993) has postulated an association between adequate contraceptive behaviour and factors of self-efficacy. It would be an interesting challenge to incorporate such measurements when further evaluating

the behaviour of Swedish teenagers. This is an obvious challenge, created by our results, where we have reproduced and confirmed the findings reported by Di Clemente et al (1993) and Zimet et al (1993), that a higher level of knowledge not necessarily leads to more adequate behaviour. Knowledge about STD and STD prevention correlated positively to sexual experience, indicating that subjects with experience, were more perceptive and learned more. They were, however, not able to utilise this knowledge practically in their efforts to manage their own situation. The lower level of knowledge in boys may be an effect of them being less sexually experienced and maturing later.

An early sexual debut was in our studies associated with an early menarche (III). Environmental stress has been suggested to trigger an early menarche (Belsky et al 1991), supporting the suggestion that a connection between early menarche and early coitarche in part may be dependent on social and psychological factors. Social background has been postulated by several authors (Hein 1989; Leslie-Harwit & Meheus 1989; Lidegaard & Helm 1990; Forrest 1994; Tyrer 1994) to be an important factor for the determination of age of coital debut and for the consequences of sexual activity. However our studies, as well as studies by Sundet et al (1989, 1992) and Traeen & Lewin (1992a) among Norwegian teenagers have failed to find any association. Those of our own studies focusing on social background factors, however, emanate from a selected group of girls, all students of the same school. This may have tended to erase the differences. In addition, in countries like Sweden, characterized by a wide-spread welfare system, social background factors, only measured by the occupations of the parents, may be too blunt an instrument, and may not accurately indicate the true family situation. In such countries life-style factors have been suggested to have a greater relevance. We found that an early sexual debut and a risky sexual behaviour in teenage girls related to other life-style factors such as smoking and alcohol drinking as reported by Kraft (1991) and Traeen & Lewin (1992a). We thus adhere to the concept proposed by Berg-Kelly (1991), that teenagers perceived as older, may be at a greater risk with regard to their future health.

To summarize, the young age of the studied population, the reliable sexual data, and the 2 years of follow up, has made it feasible to demonstrate some interesting events, related to sexuality. As far as we know, the observation that antibodies against HPV16 and HPV33 in teenage girls are correlated to the occurence of HPV DNA in the cervix, and not found among virginal women, was shown for the first time in the world by us (Andersson-Ellström et al 1994). These results have later been reproduced in other surveys by Dillner et al (unpublished data) and Wideroff et al (unpublished data). The finding that HPV serology was so strictly specific for sexually transmitted HPV has opened new possibilities for elucidating the etiology of cervical cancer by seroepidemiological studies where the dependence of concomitant infections, such as chlamydia

trachomatis as well as the herpes viruses HSV, EBV and CMV, and the role of the immature cervix and of smoking can be evaluated.

7. SUMMARY AND CONCLUSIONS

i. *Young people had reasonable knowledge about STD, at least about chlamydial infections. Knowledge increased during the eighties.*

ii. *Girls were better informed about STD than boys.*

iii. *Knowledge about STD and STD protection among teenage girls correlated to sexual experience.*

iv. *Risk behaviour was independent of the level of knowledge.*

v. *Among teenage girls, an early sexual debut was associated with continued risk behaviour regarding reproductive health as well as the consumption of tobacco and alcohol.*

vi. *An early sexual debut occurred more often in girls with an early menarche or with several sisters and brothers, and it tended to be more frequent in girls with older friends, indicating that girls who were perceived to be older than their biological age were at a greater risk.*

vii. *There were indications that a genital chlamydia trachomatis infection increased the risk for a HPV manifestation and hence cervical intraepithelial neoplasia.*

viii. *HPV-DNA in the cervix detected by PCR, occurred only in girls having had penetrating sex, but was common already a couple of years after the sexual debut, and the frequency correlated to the number of coital partners.*

ix. *HPV16 DNA acquisition in the cervix uteri was followed by a seroconversion of antibodies against HPV16 in most cases.*

x. *Antibodies against HPV16 and HPV33 in serum occurred only in girls who had had penetrating sex and was correlated to the number of sexual partners, indicating that non-sexual acquisition of these viruses is rare, if it at all exists in teenage girls.*

xi. *Antibodies to HSV-1 and EBV correlated to the sexual debut, probably in part due to the close association between sexual activity and kisses; Further studies are necessary to evaluate the frequency and mode of transmission of HSV-1 and EBV to the cervix.*

xii. *During the first years after the sexual debut, HPV was the most common sexually transmitted agent of those tested.*

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9. REFERENCES

Andersch B, Milsom I. Contraception and pregnancy among young women in an urban Swedish population. Contraception 1982;26:211-219.

Andersson-Ellström Agneta. Klamydiainfektioner i Värmland. Research Reports 1:1993. Centre for Public Health Research. Landstinget i Värmland.

Andersson-Ellström A, Dillner J, Hagmar B, Forssman L. No serological evidence for non-sexual spread of HPV16. Lancet 1994;344:1435.

Aral SO, Cates W: The multiple dimensions of sexual behavior as risk factor for sexually transmitted disease: The sexually experienced are neccessarly sexually active. Sex Transm Dis 1989;16:173-177.

de Barbeyrac B, Pellet J, Dutilh B et al: Evaluation of the Amplicor Chlamydia trachomatis test versus culture in the genital samples in various prevalence populations. Genitourin Med 1994;70:162-166.

Barrasso R, Guillemotonia A, Huynh B. The future of colposcopy: routine colposcopy. (In French) Gynécologie 1991;42:52-58.

Barrasso R. Colposcopic diagnosis of HPV cervical lesions. In The Epidemiology of Cervical Cancer and Human Papillomavirus. Ed Munoz et al. Lyon, IARC 1992.

Barton SE, Jenkins D, Cuzick J et al. Effect of cigarette smoking on cervical epithelial immunity: a mechanism for neoplastic change? Lancet 1988;II:652-654.

Baselski VS, Mc Neeley SG, Ryan G, Robison M. A comparison of nonculture-dependent methods for detection of chlamydia trachomatis infections in pregnant women. Obstet Gynecol 1987;70: 47-52.

Bauer H, Ting Y, Greer JC et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 1991;265:472-477.

Bauer HM, Hildesheim A, Schiffman MH et al. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. Sex Transm Dis 1993;20:274-278.

Bell TA, Holmes KK. Age-specific risks of syphilis, gonorrhea, and hospitalized pelvic inflammatory disease in sexually experienced US women. Sex Transm Dis 1984;11:291-295.

Belsky J, Steinberg L, Draper P. Childhood experience, interpersonal development, and reproductive strategy: An evolutionary theory on socialization. Child Development 1991;62:647-670.

Berg-Kelly K. Self-reported health status and use of medical care by 3500 adolescents in western Sweden. II. Acta Paediatr Scand 1991;80:844-851.

Bergström S. Svek mot kvinnorna. Dagens Nyheter 28th Febr. 1993, p 4.

Bethesda System. The 1988 Bethesda system for reporting cervical/vaginal cytological diagnosis. J Am Med Assoc 1989;262:931-934.

Biro FM, Rosenthal SL. Psychological sequelae of sexually transmitted diseases in adolescents. Obstetrics and Gynecologiy Clinics of North America 1992;19:209-218.

Bosch FX, Manos MM, Munoz N et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995;87:796-802.

Brinton LA, Herrero R, Fraumeni JF. Response. J Clin Epidemiol 1989;42:927-928.

Brorsson B, Herlitz C. The AIDS epidemic in Sweden: Changes in awareness, attitudes and behaviors. Scand J Soc Med 1988;16:67-71.

Brännström M, Josefsson GB, Liljestrand J. Contraception and sexuality in an area-specific group of Swedish women 15-34 years of age. Contraception 1991;44:445-452.

Cates W. Teenagers and sexual risk taking: The best of times and the worst of times. J Adolesc Health 1991;12:84-94.

Davioli M, Perucci CA, Sangalli M et al. Reliability of sexual behavior data among high school students in Rome. Epidemiology 1992;3(6):531-535.

Dickson N, Paul C, Herbison P. Adolescents, sexual behavior and implications for an epidemic of HIV/AIDS among the young. Genitourin Med 1993;69:133-140.

Di Clemente R, Brown LK, Beausoleil NI, Lodico M. Comparison of AIDS knowledge and HIV-related sexual risk behaviors among adolescents in low and high AIDS prevalence communities. J Adolesc Health 1993;14:231-236.

Dillner J. Serology of human papillomavirus. Cancer J 1995;8:264-269.

Editorial. Femidom - a condom for women. Drug Ther Bull. 1993;31:15-16.

Erlich HA, Bugawan TL. HLA class II gene polymorphism: DNA typing evolution and relationship to disease susceptibility: PCR technology, principles and applications for DNA amplification. New York Stockton press 1989:193-208.

Elkind D. Understanding the young adolescent. Adolescence 1978;13:127-134.

Evans AS, Niederman JC. Epstein-Barr virus. In: Evans AS, ed. Viral infections in human 3rd edn. New York Plenum Press 1989;393:265-292.

Evander M, Edlund K, Gustafsson Å et al. Human papillomavirus infection is transient in young women: A population based cohort study. J Infect Dis 1995;171:1026-1030.

Fairley CK, Chen S, Tabrizi SN et al. The absence of genital human papillomavirus DNA in virginal girls. Int J STD&AIDS 1992;3:414-417.

Forrest JD. Epidemiology of unintended pregnancy: the role of hormonal contraceptives. Am J Obstet Gynecol 1994;170:1485-1489.

Gibson JJ, Hornung CA, Alexander GR et al. A cross-sectional study of herpes simplex virus type 1 and 2 in collage students: occurence and determinants of infection. J Infect Dis 1990; 162:306-312.

Gisecke J, Scalia-Tomba G, Göthberg M Tull P. Sexual behaviour related to the spread of sexually transmitted diseases - a popultion based survey. Int J STD&AIDS 1992;3:255-260.

Gottardi G, Gritti P, Marzi MM, Sideri M. Colposcopic findings in virgin and sexually active teenagers. Obstet Gynecol 1984;63:613-615.

Greenberg J, Magder L, Aral S. Age at first coitus. A marker for risky sexual behavior in women. Sex Transm Dis 1992;19:331-334.

Gästrin B, Kallings LO. Improved methods for gonococcal sampling and examination on a large scale. Acta Path Microbiol Scand 1968;74:362-370.

Hagmar B, Kalantari M, Skyldberg B et al. Human Papillomavirus in cell samples from Stockholm gynecologic health screening. Acta Cytol 1995;39:741-745.

Hansson BG. Human papillomavirus types in routine cytological screening and at colposcopic examinations. Eur J Ob Gyn Repr Biol 1993;52:49-55.

Hein K. AIDS in adolescence. Exploring the challenge. J Adolesc Health Care 1989;10:10S-35S.

Heinrich LB. Contraceptive self-efficacy in college women. J Adolesc Health 1993;14:269-276.

Hellberg D, Valentin J, Nilsson S. Smoking and cervical intraepithelial neoplasia. An association independent of sexual and other risk factors? Acta Obstet Gynecol Scand 1986;65:625-631.

Hellberg D, Nilsson S, Haley NJ et al. Smoking and cervical intraepithelial neoplasia: nicotine and cotinine in serum and cervical mucus in smokers and nonsmokers. Am J Obstet Gynecol 1988;158:910-913.

Hellberg D, Borendal N, Sikström B et al. Comparison of women with cervical human papillomavirus infection and genital warts. I. Some behavioural factors and clinical findings. Genitourin Med 1995;71:88-91.

Hildesheim A, Gawitt P, Schiffman MH et al. Determinants of genital human papillomavirus infection in low-income women in Washington DC. Sex Transm Dis 1993;20:279-285.

Ho M. Epidemiology of cytomegalovirus infections. Rev Inf Dis 1990;12(suppl 17):701-710.

Holly EA, Cress RD, Ahn DK, Aston DA, Kristiansen JJ, Felton JS: Characteristics of women by smoking status in the San Fransisco bay area. Cancer Epidemiology, Biomakers & Prevention 1992;1:491-497.

Hornberger LL, Rosenthal SL, Biro FM, Stanberry LR: Sexual histories of adolescent girls: Comparison between interview and chart. J Adolesc Health 1995;16:235-239.

Hoskins TW, Davies JR, Smith AJ et al. Assessment of inactivated influenza – A vaccine after three outbreaks of influenza A at Christ's Hospital. Lancet 1979;I:33-35.

Irwin CE. The theoretical concept of at-risk adolescents. Adolescent Med. State of the Art Reviews 1990;1:1

Jeansson S, Forsgren M, Svennerholm B. Evaluation of solubilized herpes simplex virus membrane antigen by enzyme-linked immunosorbent assay. J Clin Microbiol 1983;18:1160-1166.

Jenison SA, Yu XP, Valentine JM et al. Evidence of prevalent genital-type human papillomavirus infections in adults and children. J Infect Dis 1990;162:60-69.

Jones EF, Forrest DF, Goldman N et al: Teenage pregnancy in developed countries: Determinants and policy implications. Fam Plan Perspect 1985;17:53-63.

Jones D, Russo JF, Dombroski RA, Lentz SS. Cervical intraepithelial neoplasia in adolescents. J Adolesc Health Care 1984;5:243-247.

Kegeles S, Adler N, Irwin C. Sexually active adolescents and condoms: Changes over one year in knowledge, attitudes and use. Am J Public Health 1988;78:460-461.

Kellog JA. Clinical and laboratory considerations of cultures vs antigen assays for detection of Chlamydia trachomatis from genital specimens. Arch Pathol Lab Med 1989;113:453-460.

Kirnbauer R, Hubbert NL, Wheeler CM, et al. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with

human papillomavirus type 16. J Natl Cancer Inst 1994;86:494-498.

Kiviat NB, Critchlow CW, Kurman RJ. Reassessment of the morpholgical continuum of cervical intraepithelial lesions: does it reflect different stages in the progression to cervical carcinoma? In The Epidemiology of Cervical Cancer and Human Papillomavirus. Ed Munoz et al. Lyon IARC 1992.

Klanger B, Tydén T, Ruusuvaara L: Sexual behavior among adolescents in Uppsala, Sweden. J Adolesc Health 1993;14:468-474.

Kontula O, Haavio-Mannila E. Sexual behavior changes in Finland during the last 20 years. Nordisk Sexologi 1994;12:196-214.

Kraft P, Traeen B, Rise J. Adolescents and prevention - use of contraception by Norwegian adolescents during the most recent intercourse and during casual sex. (In Norwegian). Tidsskr Nor Laegeforen 1990;110:1486-1489.

Kraft P. Age at first experience of intercourse among Norwegian adolescents: a life-style perspective. Soc Sci Med 1991;33:207-213.

Kraft P. Sexual knowledge among Norwegian adolescents. J Adolesc 1993;16:3-21.

Krishna RV, Meurman OH, Zigler T, Kresh UH. Solid-phase enzyme immunoassay for determination of antibodies to cytomegalovirus. J Clin Microbiol 1984;12:46-51.

von Krogh G. Genitoanal papillomavirus infection: diagnostic and terapeuthic objectives in the light of current epidemiological observations. Int J STD&AIDS 1991;2:391-404.

Landers RJ, O´Leary JJ, Crowley M et al. Epstein-Barr virus in normal, premalignant, and malignant lesions of the uterine cervix. J Clin Pathol 1993;46:931-935.

Larsson G, Milsom I, Andersch B, Blohm F. A comparison of contraception and pregnancy outcome at 19 years of age in two cohorts of Swedish women born 1962 and 1972. Contraception (In Press 1996).

Lefebvre J, Laperrière H, Rousseau H, Massé R. Comparison of three techniques for detection of chlamydia trachomatis in endocervical specimens from asymptomatic women. J Clin Microbiol 1988;26:726-731.

Lennette E: Laboratory diagnosis of infectious diseases. In: Lenette E, Halonen P, Murphy FA (eds) Viral, rickettsial and chlamydial diseases. Berlin, New York. Springer-Verlag 1988:230-266.

Leslie-Harwit M, Meheus A. Sexually transmitted disease in young people: The importance of health education. Sex Transm Dis 1989;16:15-20.

Lewin B. Sex and family planning: How to teach the young. Uppsala, Department of Sociology, University of Uppsala, Sweden, 1984 (Public Health in Europe 23, World

Health Organisation, Regional Office for Europe, Copenhagen).

Ley C, Bauer HM, Reingold A et al. Determinants of genital human papillomavirus infection in young women. J Natl Cancer Inst 1991;83:997-1003.

Lidegaard Ö, Helm P, Pelvic inflammatory disease: the influence of contraceptive , sexual and social life events. Contraception 1990;41:475-483.

Lörincz A. Detection of human papillomavirus DNA without amplification: prospects for clinical utility. In The epidemiology of cervical cancer and human papillomavirus. Ed Munos N et al. IARC 1992.

Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR: Use of polymerase chain reaction amplification for detection of human papillomaviruses. Cancer Cells 1989;7:209-221.

Meisels A. The story of a cell. Acta Cytol 1983;27:584-596.

Milsom I, Sundell G, Andersch B. A longitudinal study of contraception and pregnancy outcome in a representative sample of young Swedish women. Contraception 1991;43:111-119.

Mitchell H, Drake M, Medley G. Prospective evaluation of risk of cervical cancer after cytological evidence of human papillomavirus infection. Lancet 1986;I:573-575.

Morris JDH, Eddleston ALWF, Crook T. Viral infection and cancer. Lancet 1995;346:754-758.

Morrison EAB, Ho GYF, Vermund SH et al. Human papillomavirus infection and other risk factors for cervical neoplasia: a case-control study. Int J Cancer 1991;49:6-10.

Moscicki AB, Winkler B, Irwin C, Schachter J. Differences in biological maturation, sexual behavior, and sexually transmitted disease between adolescents with and without cervical intraepithelial neoplasia. J Pediatr 1989;115:487-493.

Moscicki AB, Palefsky J, Gonzales J, Schoolnik GK. Human Papillomavirus infection in sexually active adolescent females: Prevalence and risk factors. Pediatr Res 1990;28:507-513.

Munoz N, Bosch FX, Shah KV, Meheus A, eds. The epidemiology of human papillomavirus and cervical cancer. Oxford: Oxford University Press, 1992. (IARC Scientific Publications no 119).

Nahmias AJ, Lee FK, Beckman-Nahmias S. Sero-epidemiological and -sociological patterns of herpes simplex virus infection in the world. Scand J Infect Dis Suppl 1990;69:19-36.

Nonoyama M, Huang CH, Pagona JS et al. DNA of Epstein-Barr virus detected in

tissues of Burkitt's lymphoma and nasopharyngeal carcinoma. Proc Natl Acad Sci USA 1973;70:3265-3268.

Näher H, Gissman L, Freese UK et al. Subclinical Epstein-Barr virus infection of both the male and female genital tract - indication for sexual transmission. J Invest Dermatol 1992;98:791-793.

Opaneye AA, Willmott C. The role of genito-urinary medicine in adolescent sexuality. Genitourin Med 1991;67:44-46.

O'Reilly KR, Aral SO. Adolescence and sexual behavior. Trends and implications for STD. J Adolesc Health Care 1985;6:262-270.

Pao CC, Tsai PL, Chang YL et al. Possible non-sexual transmission of gential human papillomavirus infections in young women. Eur J Clin Microbiol Infect Dis 1993;12:221-222.

Persson E, Sandström B, Jarlbro G. Sources of information, experiences and opinions on sexuality, contraception and STD protection among young Swedish students. Advances in Contraception 1992;8:41-49. (1992a)

Persson E, Jarlbro G. Sexual behavior among young clinic visitors in Sweden: Knowledge and experiences in an HIV perspective. Genitourin Med 1992;68:26-31. (1992b)

Persson E. The sexual behaviour of young people. Br J Obstet Gynecol 1993;100:1074-1076.

Peters RK, Thomas D, Hagan DG et al. Risk factors for invasive cervical cancer among latinas and non-latinas in Los Angeles County. J Natl Cancer Inst 1986;77:1063-1077.

Piot P, Islam MQ. Sexually transmitted diseases in the 1990s. Global epidemiology and challenges for control. Sex Transm Dis 1994;21(2suppl):7-13.

Piura B, Sarov B, Sarov I. Genital chlamydial infection and cervical cancer. Am J Obstet Gynecol 1985:152:363-364.

RahmVA, Gnarpe H, Odlind V. Chlamydia trachomatis among sexually active teenage girls. Lack of correlation between chlamydial infection, history of the patient and clinical signs of infection. Br J Obstet Gynecol 1988;95:916-919.

Rahm VA, Odlind V, Pettersson R. Chlamydia trachomatis in sexually active teenage girls. Factors related to genital chlamydial infection; Aprospective study. Genitourin Med 1991;67:317-321.

Ramstedt K, Hallhagen GJ, Bygdeman SM et al. Serologic classification and contact-

tracing in the control of microepidemics of beta-lactamase-producing Neisseria gonorrhoeae. Sex Transm Dis 1985;12:209-214.

Ramstedt K, Forssman L, Gisecke J, Granath F. Risk factors for chlamydia trachomatis infection in 6810 young women attending family planning clinics. Int J STD&AIDS 1992;3:117-122.

Rapp F. Sexually transmitted viruses. The Yale J Biology Med 1989;62:173-185.

Rawls WE, Clarke A, Smith KO et al. Specific antibodies to herpes simplex virus type 2 and women with cervical cancer. CSH Conferences on cell proliferation 1980;7:117-133.

Riphagen FE, von Schoultz B. Contraception in Sweden. Contraception 1989;39:633-642.

Robbey SJ, Noller KL, O'Brien B et al. Increased incidence of cervical and vaginal dysplasia in 3980 diethylstilbestrolexposed young women. JAMA 1984;252:2979-2983.

Rylander E, Ruusuvaara L, Wiksten Almströmer M et al. The absence of vaginal human papillomavirus 16 DNA in women who have not experienced sexual intercourse. Obstet Gynecol 1994;83:735-737.

de Sanjose S, Santamaria M, de Ruiz A et al. HPV types in women with normal cervical cytology. In The Epidemiology of Cervical Cancer and Human Papillomavirus. Ed. Munoz et al. Lyon. IARC 1992.

Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993;85:958-964.

Schiffman MH. Epidemiology of cervical human papillomavirus infections. In Zur Hausen (ed) Human Pathogenic Papillomaviruses. Springer Verlag, Berlin, Heidelberg 1994.

Schneider A, Sterzik K, Buck G, De Villiers EM. Colposcopy is superior to cytology for the detection of early genital human papillomavirus infection. Obstet Gynecol 1988;71:236-241.

Schneider A, Koutsky LA. Natural history and epidemiological features of genital HPV infection. In The epidemiology of cervical cancer and human papillomavirus. Ed. Munoz et al. Lyon IARC 1992.

Sedlacek TV, Lindheim S, Eder C et al. Mechanism for human papillomavirus transmission at birth. Am J Obstet Gynecol 1989;161:55-59.

Seidman SN, Mosher WD, Aral SO. Predictors of high-risk behavior in unmarried American women: Adolescent environment as risk factor. J Adolesc Health 1994;15:126-132.

Sixbey JW, Lemon SM, Pagano JS. A second site for Epstein-Barr virus shedding: the uterine cervix. Lancet 1986;ii:1122-1124

Skyldberg B, Kalantari M, Karki M, Johansson B, Hagmar B, Walaas L. Detection of human papillomavirus infection in tissue blocks by in situ hybridization as compared with a polymerase chain reaction procedure. Hum Pathol 1991;22:578-582.

Skyldberg B, Hagmar B, Johansson B, et al. HPV detection in cytological cases with condylomatous or dysplastic changes. A study with PCR and in situ hybridization on cytological material. Diagnost Cytopath 1995;13:8-14.

Spitzer M, Brandsma JL, Chernys AE, Krumholz BA. Detection of conditions related to human papillomavirus. Comparison of cytology, colposcopy, histology and hybridization. J Reprod Med 1990;35:697-703.

Stamm WE, Mårdh PA. Chlamydia trachomatis. In: Holmes KK, Mårdh PA, Sparling PF, Weisner PJ, eds. AIDS and other sexually transmitted diseaes. 2nd ed New York: McGraw-Hill Information Services 1990:917-202.

Strand A. Genital Human Papillomavirus Infection. Epidemiological and morphological aspects. Acta Universitatis Upsaliensis, Comprehensive Summeries of Upsala Dissertations from the faculty of Medicine, 581.44p. Uppsala 1995. ISBN 91-554-3647-1.

Sun XW, Ferency A, Johnson BA et al. Evaluation of the hybrid capture human papillomavirus deoxyribonucleic acid detection test, Am J Obstet Gynecol 1995;173:1432-1437.

Sundet JM, Magnus P, Lundin Kvalem I et al. Number of sexual partners and use of condoms in the heterosexaul popultion of Norway - relevance to HIV-infection. Health Policy 1989;13:159-167.

Sundet JM, Magnus P, Lundin Kvalem I et al. Secular trends and sociodemographic regularities of coital debut age in Norway. Arch Sex Behavior 1992;21:241-252.

Sundström-Feigenberg K. Reproductive health and reproductive freedom at stake. In: Society and HIV/AIDS: Selected Knowledge base for research and action. (G Sterky&I Krantz, eds), Karolinska Institute. David Broberg. Stockholm 1988:117-122.

Svennerholm B, Olofsson S, Jeansson A, Vahlne A, Lycke E. Herpes simplex virus type-selective enzyme-linked immunosorbent assay with helix pomatia lecitin-purified antigens. J Clin Microbiol 1984;19:235-239.

Svensson LO, Mares I, Olsson SE, Nordström ML. Screening for Chlamydia trachomatis infection in women and aspects of the laboratory diagnostics. Acta Obstet Gynecol Scand 1991;70:587-590.

Syrjänen K, Hakama M, Saarikoski S et al. Prevalence, incidence and estimated life-time risk of cervical human papillomavirus infections in a nonselected finnish female population. Sex Transm Dis 1990;17:15-19.

Taylor Y, Melvin WT, Sewell HF et al. Prevalence of Epstein-Barr virus in the cervix. J Clin Pathol 1994;47:92-93.

Thejls H, Gnarpe J, Gnarpe H, Larsson G. Age-related decrease in prevalence of chlamydia trachomatis among pregnant women. Sex Transm Dis 1991;18:137.

Traeen B, Lewin B. Casual sex among Norwegian adolescents. Arch Sex Behav 1992;21:253-269. (1992a)

Traeen B, Lewin B, Sundet JM. Use of birth control pills and condoms among 17-19-year-old adolescents in Norway: contraceptive versus protective behaviour? AIDS Care 1992;4:371-380. (1992b)

Traeen B. Norwegian adolescents sexuality in the era of Aids. Acta Obstet Gynecol Scand 1994;73:439-440.

Treharne J, Darougar S, Jones BR. Modification of the immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. J Clin Path 1977;30:510-517.

Tydén T, Björkelund C, Olsson SE. Sexual behavior and sexually transmitted diseases among Swedish university students. Acta Obstet Gynecol Scand 1991;70:219-224. (1991a)

Tydén T, Norden L, Ruusuvaara L. Swedish adolescents'knowledge of sexually transmitted diseases and their attitudes to the condom. Midwifery 1991;7:25-30. (1991b)

Tydén T, Björklund C, Odlind V, Olsson S-E. Increased use of condoms among female university students: a 5 year follow-up of sexual behaviour. Acta Obstet Gynecol Scand. (In Press 1996).

Tyrer LB. Obstacles to use of hormonal contraception. Am J Obstet Gynecol 1994;170:1495-1498.

Wahl RW. Chlamydia, repair and intraepithelial neoplasia. Acta Cytol 1984;28:89.

Wespi HJ. Colposcopic-histologic correlations in the benign acanthotic non glycogenated squamous epithelium. Colposc Gyn Laser Surg 1986;2:147-158.

Weström L. Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am J Obstet Gynecol 1980;138:880-892.

Wheeler CM, Parmenter CA, Hunt WC et al. Determinants of genital human papillomavirus infection among cytologically normal women attending the University of New Mexico student health center. Sex Transm Dis 1993;20:286-290.

Wielandt H, Boldsen J. Age at first intercourse. J Biosoc Sci 1989;21:169-177.

Wikström A, van Doornum GJJ, Quint WGV et al. Identification of human papillomavirus seroconversions. J General Virol 1995;76:529-539.

Wilkinson AE. Culture methods for diagnosis of gonorrhoea. In: Gonorrhoea, epidemiology and pathogenesis (Eds F a Skinner et al). Academic Press, London 1977;17-25.

Volpers C, Schirmacher P, Streeck RE, Sapp M. Assembly of the major and the minor capsid protein of human papillomavirus type 33 into virus-like particles and tubular structures in insect cells. Virology 1994;200:504-512.

Voog E, Ricksten A, Löwhagen GB, Ternesten A. Demonstration of Epstein-Barr virus DNA in acetowhite lesions of the vulva. Int J STD&AIDS 1994;5:25-28.

Zetterberg HL. Om sexuallivet i Sverige. Stockholm. Allmänna förlaget. 1969 (SOU 1969:2).

Zimet GD, Di Lemente RJ, Lazebnik R et al. Changes in adolescents'knowledge and attitudes about AIDS over the course of the AIDS epidemic. J Adolesc Health 1993;14:85-90.

Zur Hausen H, Schulte-Holthauzen H, Klein G et al. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. Nature 1970;228:1956-1958.

Örtqvist Å, Grillner L, Olofsson I, Nordin A: Chlamydiainfektioner på en ungdoms-mottagning i Stockholm. Läkartidningen 1984;81:3929-3931.

APPENDIX

The questionnaires are too extensive to be published, but can be ordered from Agneta Andersson-Ellström, Gyn.mottagningen, VC Gripen, Box 547, S-65112 Karlstad, Sweden.

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