Transplacental transmission of Human Papillomavirus
Renato L Rombaldi*1,3,4, Eduardo P Serafini2,3, Jovana Mandelli1, Edineia Zimmermann1 and Kamille P Losquiavo1

Address: 1Diagnosis – Molecular Laboratory, University of Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil, 2Pathology Medical Laboratory, Department of Health and Biomedical Science, University of Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil, 3Biotechnology Institute, University of Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil and 4Outpatient Clinic of Genital Pathology, Department of Clinical Medicine, University of Caxias do Sul, Caxias do Sul, Rio Grande do Sul, Brazil

Email: Renato L Rombaldi* - rl.rombaldi@gmail.com; Eduardo P Serafini - epserafini@diagnosers.com.br; Jovana Mandelli - jomandelli@terra.com.br; Edineia Zimmermann - edineia@zimmermann-rs.com.br; Kamille P Losquiavo - kamillepl@hotmail.com

* Corresponding author

Abstract
This paper aimed at studying the transplacental transmission of HPV and looking at the epidemiological factors involved in maternal viral infection. The following sampling methods were used: (1) in the pregnant woman, (a) genital; (b) peripheral blood; (2) in the newborn, (a) oral cavity, axillary and inguinal regions; (b) nasopharyngeal aspirate, and (c) cord blood; (3) in the placenta. The HPV DNA was identified using two methods: multiplex PCR of human β-globin and of HPV using the PGMY09 and PGMY11 primers; and nested-PCR, which combines degenerated primers of the E6/E7 regions of the HPV virus, that allowed the identification of genotypes 6/11, 16, 18, 31, 33, 42, 52 and 58. Transplacental transmission was considered when type-specific HPV concordance was found between the mother, the placenta and the newborn or the mother and cord blood. The study included 49 HPV DNA-positive pregnant women at delivery. Twelve placenta (24.5%, n = 12/49) had a positive result for HPV DNA. Eleven newborn were HPV DNA positive in samples from the nasopharyngeal or buccal and body or cord blood. In 5 cases (10.2%, n = 5/49) there was HPV type-specific agreement between genital/placenta/newborn samples. In one case (2%, n = 1/49) there was type specific HPV concordance between genital/cord blood and also suggested transplacental transmission. A positive and significant correlation was observed between transplacental transmission of HPV infection and the maternal variables of immunodepression history (HIV, p = 0.011). In conclusion the study suggests placental infection in 23.3% of the cases studied and transplacental transmission in 12.2%. It is suggested that in future HPV DNA be researched in the normal endometrium of women of reproductive age. The possible consequence of fetal exposure to HPV should be observed.

Background
Human papillomavirus (HPV), the most common sexually transmitted infection, has been recognized as a cause of anogenital warts (HPV type 6 and 11) and cervical cancer (HPV type 16, 18 and others)[1]. In children, HPV-related (type 6 and 11) laryngeal papillomas, conjunctival papillomas and genital warts [2-6].
Although it has been established that HPV is sexually transmitted[7,8], there is growing evidence that non-sexual transmission also occurs[9]. This includes vertical transmission from parents to infants, horizontal transmission from other family members and those in close contact with the child, autoinoculation from one site to another and possibly indirect transmission via phomities[10]. The potential mother-to-child HPV transmission route in the perinatal period has been demonstrated [11-17]. There is evidence of vertical transmission, presumably occurring during passage of the fetus through an infected birth canal[18]. The virus could also be transmitted by ascending infection, especially after premature rupture of the membranes. In utero transmission could be caused either by ascending infection from an infected birth canal, by sperm at fertilization or hematogenously (transplacentally). HPV DNA has been detected in peripheral blood mononuclear cells of pregnant women[19], cord blood specimens of neonates[19], oropharyngeal secretions of neonates[20], amniotic fluid [21-23], fetal membranes[24], placental trophoblastic cells[11], infants born by elective cesarean section delivery[11,13,18,22,24], and in syncytiotrophoblastic cells of spontaneously aborted material[25]. In addition, there are type-discordant cases between mothers and newborns, suggesting that many of these infants did not acquire the HPV from their mothers[26]. These observations could explain the transplacental transmission of HPV from an infected mother to the fetus. However, only a limited number of women have been studied to confirm placental transmission of HPV.

This cross-sectional, prospective study aimed at evaluating transplacental transmission of HPV and enhancing understanding of the maternal epidemiologic features involved.

Methods
Population studied

Between April 2005 and April 2007, a cross-sectional, prospective study was performed on 71 pregnant women (mean age 24.6 ± 7.7 years, 14–41 years) with a prior history of HPV infection (n = 22), or who had abnormal Papanicolaou smear (n = 20) or genital warts (n = 29), due to the high probability that they could have HPV infection. The women were referred from the Obstetrical Service of the University of Caxias do Sul and by the Basic Health Units of the Single Health System in Caxias do Sul. This study was performed with the approval of the Ethics in Research Committee at the University of Caxias do Sul, and of the Editorial and Scientific Board of the General Hospital of Caxias do Sul, and did not present a conflict of interest. The Letter of Free and Informed Consent and the epidemiological evaluation tool were obtained from all the women by individual interviews during the obstetrical examinations. Sixty-three (79.7%) of the 71 pregnant women selected who entered the study underwent delivery and 16 (20.3%) dropped out of the study.

Epidemiological evaluation

The epidemiological study was performed taking the following variables into account: age, race, level of education, smoking, marital status, age at first sexual intercourse, parity, number of sexual partners in lifetime, number of sexual partners in past year, frequency of condom use with sexual partners in lifetime, frequency of condom use with sexual partners in past year, marital stability in years, history of immunodepression (HIV – acquired immunodeficiency syndrome), type of HPV lesion (genital warts, LGSIL – low-grade squamous intraepithelial lesions, HGSIL – high-grade squamous intraepithelial lesions), site of HPV lesion (cervical, vaginal, vulvar and perineal), type of HPV infection (single, double and multiple), gestational age at the time HPV infection was diagnosed (weeks), duration of labor (minutes), time of amniotic membrane rupture (minutes), type of delivery (cesarean section, vaginal and vaginal with forceps) and HPV lesion at delivery (genital warts, LGSIL – low-grade squamous intraepithelial lesions, HGSIL – high-grade squamous intraepithelial lesions).

Sampling methods
Maternal genital

The maternal genital samples were obtained during pregnancy, at the first visit, when the woman was recruited. The sample was obtained using a special brush for cytopathological sampling of the cervix, which was used for genital brushing in the following order: cervix and possible clinical and subclinical lesions of the vagina, vulva and perineal region. The brush was placed in a TE solution (Tris HCl, pH 7.5 – 10 mM; EDTA, 1 mM), and the material collected was kept frozen at -20°C, until the desoxyribonucleic acid (DNA) was extracted.

Peripheral blood maternal

Immediately before delivery (pre-partum period), a sample of peripheral blood was obtained from the woman using a 3 ml disposable syringe (27/5 needle), retrieving about 1 ml of blood which was placed in a KMA type tube with EDTA. The blood collected was kept frozen at -20°C, until DNA was extracted.

In newborns, in the first minutes of the life, buccal, body, nasopharyngeal aspirates and arterial blood from the umbilical cord samples were obtained.

Buccal and body

The swabs were collected in the first minutes of life, using the special brush for cytopathological sampling of the cervix, with which brushing was performed in the following order: buccal cavities, axillary and inguinal regions of the
newborn. The brush was placed in a TE solution (Tris HCl, pH 7.5 – 10 mM; EDTA, 1 mM) and kept frozen at -20°C, until DNA was extracted.

**Nasopharyngeal aspirates**
The distal extremity of the tracheal aspiration catheter (n° 6 or 8, Sondas Descartáveis Mercosul *Linha Sondas Descartáveis Mercosul*, Empresa CPL Medical’s Produtos Médicos LTDA), used to aspirate the upper airways (nasopharyngeal) of the newborn immediately after birth, was removed. The distal extremity of the catheter (about 4 cm long) was cut and placed in TE solution (Tris HCl, pH 7.5 – 10 mM; EDTA, 1 mM), keeping it frozen at -20°C, until DNA was extracted.

**Arterial blood from the umbilical cord**
The sample was collected directly from one of the arteries of the cord using a 3 ml disposable syringe (27/5 needle) to obtain about 1 ml of fetal blood. The collection was performed after clamping the cord and complete delivery of the placenta and fetal membranes. The fetal blood was placed in a KMA type tube with EDTA and frozen at -20°C, until DNA was extracted.

The placental sampling methods were performed immediately after complete delivery and cleaning of the placental disk sides, using surgical compresses.

**Placental swabs**
The swabs were obtained using special brushes for the cytopathological collection from the cervix, by brushing in the following order: initially on the fetal side of the placenta, and later with a new brush, on the maternal side of the placenta. The brushes were placed individually in a TE solution (Tris HCl, pH 7.5 – 10 mM; EDTA, 1 mM), keeping them frozen at -20°C, until DNA was extracted.

**Placental biopsy**
Two biopsies were performed on the sides of the placental disk: one in the more central portion; another in the more peripheral portion (placental border). The biopsies were performed with the help of the rat-tooth forceps, and the curved iris scissors. The fragments collected were placed individually in a TE solution (Tris HCl, pH 7.5 – 10 mM; EDTA, 1 mM) and kept frozen at -20°C, until DNA was extracted.

**DNA extraction**
DNA was extracted from the blood and tissue samples using the Wizard Genomic DNA Purification Kit (Promega), according to the manufacturer’s specifications. In the brush samples, DNA was extracted using 600 μl of NaOH 50 mM stirred in a vortex for 5–10 seconds and later incubated at 95°C for 5 minutes. The solution was then neutralized with 60 μl of Tris HCl pH 8.0 and kept in a freezer at -20°C, until it was submitted to the next stages.

After the DNA extraction methodology, the products were submitted to two different PCR methods to identify and type the HPV DNA: multiplex PCR and type specific nested multiplex-PCR.

**β-globin and HPV amplification**
The DNA samples obtained using the extraction methodology were amplified in multiplex PCR, and this was composed by the PC04 oligonucleotides (CAA CTT CAT CCA CGT TCA CC) e GH20 (GAA GAG CCA AGG ACA GGT AC), which amplified the segment of 268 base pairs (pb) of the human β-globin gene, ensuring the qualification and quantification of DNA for HPV analysis, and by the PGM09 and PGMY11 oligonucleotides, which amplify a segment of 450 pb of a preserved region of gene L1 of *Human Papillomavirus* [27]. The thermocycler, model PTC100 (MJResearch, Watertown, Mass.) was used for amplification; the parameters for denaturation, annealing and lengthening of the ribbons were the following: 95°C for 5 minutes, followed by 40 51°C cycles for 30 seconds, 55°C for 1 minute, 72°C for 1 minute and, finally, 72°C for 5 minutes. Negative and positive controls were included with all amplifications, and the negative control was constituted by all elements except genomic DNA; and the positive control was constituted by HPV DNA type 16, extracted from cells of the SiHa strain (Ludwig Institute for Cancer Research). Four μg of the molecular DNA of the DNA φ X 174RF HaeIII molecular weight marker were used. The presence or absence of HPV DNA fragments and β-globin amplified from the oligonucleotides was analyzed in 1.5% agarose gel, in buffer TBE 0.5× with 0.3% ethidium bromide (0.1 mg/μL solution), under ultraviolet light.

**Viral typing**
The HPV positive samples were submitted to a new type of PCR, specific for viral type identification. For this purpose the RFLP (Restriction Fragment Length Polymorphism) technique was used, according to the methodology described by Bernard et al (1994) [28]. The amplified product was digested by the BamHI, Ddel, HaeII, HinfI, PstI, Rsal and SauAIII enzymes and analyzed by vertical electrophoresis in 4% polyacrylamide gel (20.3% acrylamide, 0.7 bisacrylamide, 0.07% ammonium persulphate, TBE 1X TEMED 0.7 μL/mL – Gibco-BRL). The pGEM (PROMEGA) was used as a molecular weight marker. Later the samples in polyacrylamide gel were stained with silver nitrate and the fragments obtained compared to the prototypes described by Bernard et al. (1994) [28].
Amplification by nested-PCR in region E6/E7 of the HPV

The nested multiplex PCR (NMPCR) assay combines degenerate E6/E7 consensus primers and type-specific primers (MY09/11 and GP5+/6+) for the detection and typing of HPV genotypes 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66 and 68. With regard to sensitivity and performance with clinical samples, the novel NMPCR assay is a potentially useful tool for HPV DNA detection in epidemiologic and clinical follow-up studies, especially when accurate HPV typing and the detection of multiple HPV infections are required.

The samples were amplified during the first PCR reaction using the degenerated primers GP-E6-3F (GGG WKG KAC TGA AAT CCG T), GP-E6-5B (CTG AGC TGT CAR NTA ATT GCT CA) and GP-E6-6B (TCC TCT GAG TYG YCT AAT TGC TC), W being A/T; K, G/T; R, A/G; Y, C/T and N, A/C/G/T. These primers amplify a 630 pb region in the E6/E7 region of the 38 most common types of HPV. The nested-PCR reaction is specific and was performed for the following types: 6/11, 16, 18, 31, 33, 42, 52 and 58, which represent the most prevalent viral types in the region[29]. The primers used and the sizes of the amplified products are shown in table 1. The entire procedure, both the first reaction (PCR) and the second reaction (nested-PCR) occurred according to Sotlar et al., 2004[30].

Transplacental transmission

In the study, the transplacental transmission of HPV was considered when HPV DNA type-specific agreement was observed between the samples: (1) mother (genital or peripheral blood), placental and newborn (buccal, body or cord blood); or (2) mother (genital or peripheral blood) and newborn (cord blood)[19].

Vertical HPV transmission

In the study, vertical HPV transmission was considered when HPV DNA was found in newborns (cord blood or nasopharyngeal aspirates or buccal and body).

Statistical analysis

Statistical analyses were performed with the SPSS computer software package (version 12.0 for Windows). Frequency tables were analyzed by using the chi-square test, with Pearson and likelihood ratio tests for the significance of differences between the categorical variables. The 95% confidence interval (95% CI) was calculated where appropriate. Differences in the means of continuous variables between the groups were analyzed by using nonparametric tests. In all analyses probability values of < 0.05 were regarded as significant.

Results

The study included 49 pairs of mothers and newborns.

HPV DNA in maternal genitalia

HPV DNA was detected in 49 (77.8%) of the 63 pregnant women who underwent delivery. The most frequently detected types of HPV DNA were 6/11 (20.7%), 42 (15.9%), 16 (15.9%), 18 (11%), 58 (6.1%) and 31, 35 and 52 (3.7% each). Of these 54.9%, 1.2%, 40.2% and 3.7% were types considered to present a high carcinogenic risk, possible high risk, low risk and HPV DNA present but not classified for viral type respectively (Table 2). Genital infections produced by a single type of HPV DNA (38.8%), by two types of HPV DNA (30.6%) and more than two types of HPV DNA (30.6%) were identified.

HPV DNA in the placenta

HPV DNA was detected in 12 placentas (24.5%) of the 49 HPV DNA positive pregnant women (HPV DNA+) who

| HPV genotype | Primer sequences | Amplicon (pb) |
|--------------|------------------|--------------|
| 6/11         | TGC AAG AAT GCA CTG ACC AC TGC ATG TTT GCC AGC AGT GT | 334 pb* |
| 16           | CAC AGT TAT GCA CAG AGC TGC CAT ATA TCT ATG CAA TGT AGG TGT A | 457 pb |
| 18           | CAC TTC ACT GCA AGA CAT AGA GTC TGT AAA TCC TGT TTT TTC A | 332 pb |
| 31           | GAA ATT GCA TGA ACT AAG CTC G | 263 pb |
| 33           | ACT ATA CAC AAC ATT GAA CTA GTC TTT ACA CGT CAC AGT GCA | 398 pb |
| 42           | CCC AAA GTA GTG GTC CCA GTT A CAT CTT CTC TAG TGT GCG AGT G | 277 pb |
| 52           | GTA GGC TGC AGT GTG TGC AG | 229 pb |
| 58           | GTA AAG TGT GCT TAC GAT TGC GTC GGT ACA GGG TAC ACT TGT | 274 pb |

* Base pairs.
underwent delivery. The fetal side of the placenta presented HPV DNA+ in 5 cases (41.7%, n = 5/12), the maternal placental side in 2 cases (16.7%, n = 2/12), while in 5 cases (41.7%, n = 5/12) research for HPV DNA was positive on both sides of the placenta. The viral types identified in the placentas were 6/11 (50%, n = 6/12), 16 (25%, n = 3/12), 18 (16.7%, n = 2/12), 52, 58 and 59 (8.3%, n = 1/12 each). The type specific HPV concordance among the genital/placental samples was 91.7% (n = 11/12). Seven placentas (58.3%, n = 7/12) presented viral types considered a high carcinogenic risk (types 16, 18, 52 and 58) and 2 placentas presented two different types of HPV DNA (Table 3).

It was observed that seven (58.2%, n = 7/12) cases presented HPV DNA+ for the genital/placental/newborn samples and five (41.7%, n = 5/12) cases presented HPV DNA+ for the genital/placental samples with negative research for HPV DNA in newborns (NB).

**HPV DNA in newborns**

HPV DNA was identified in eleven NB (22.4%, n = 11/49). Five NB had HPV DNA+ in samples of nasopharyngeal aspirate, six in buccal and body scrapings, and three in arterial cord blood (Table 3). The viral types identified were 6/11 (45.5%, n = 5/11), 42 (18.2%, n = 2/11), 52 (18.2%, n = 2/11), 18 and 59 (9.1%, n = 1/11 each). Four NB (36.4%, n = 4/11) presented viral types considered a high carcinogenic risk (types 18, 52 and 59). In one NB two types of HPV DNA were detected (types 6/11 and 52).

Among the eleven cases of NB HPV DNA+, seven (63.6%, n = 7/11) presented HPV DNA+ for the genital/placental/NB samples. Six of these cases (85.7%, n = 6/7) were in concordance as to the type-specific HPV among the placental/NB samples and five cases (71.4%, n = 5/7) presented concordance as to the type specific HPV among the genital/placental/NB samples, suggesting the transplacental transmission of the virus (10.2%, n = 5/49).

No physical abnormalities or genital warts were observed in the 49 newborns.

Among the 11 cases of NB HPV DNA+ (vertical transmission), four (36.4%, n = 4/11) did not present transplacental infection due to virus (Table 3). Of these, one case presented type specific HPV concordance among the genital/arterial cord blood samples (HPV type 52) suggesting the possibility of transplacental transmission. Among the three other cases, two had type specific HPV concordance among the genital/NB samples (HPV types 11 and 42).

On the other hand, five NB (41.7%, n = 5/12) were negative for HPV DNA research, while in their respective placentas HPV DNA+ was shown (Table 3). The HPV identified were types 16 (40%, n = 2/5), 6/11, 18 and 58 (20%, n = 1/5 each). Four NB (80%, n = 4/5) presented viral types considered a high carcinogenic risk (types 16, 18, 58). The concordance of type specific HPV observed among the genital/placental samples was 100% (n = 5/5).

**HPV DNA in arterial cord blood**

Studying the arterial blood from the umbilical cords of NB (Table 3), 3 cases (6.1%, n = 3/49) HPV DNA+ for viral types 6/11, 18 and 52 were observed. In 2 clinical cases there was concordance of type specific HPV among the genital/placental/arterial cord blood samples, and in the other case, concordance of type specific HPV among the genital/arterial cord blood was observed. The latter case mentioned, which corresponds to the same case mentioned above, was considered transplacental transmission (hematogenic, directly through the placenta, without any infection in the latter). Of the 3 cases studied, two (66.7%) had HPV DNA types 18 and 52 considered a high carcinogenic risk.

**HPV DNA in maternal peripheral blood**

Three (6.1%, n = 3/49) parturients had HPV DNA in their peripheral blood (Table 3). In two cases HPV DNA that
Table 3: Clinical and laboratory history of genital HPV infection during pregnancy and delivery and distribution of HPV types in maternal, newborn and placental samples.

| Case | HPV lesion type | HPV lesion site | Type | HPV lesion | Genital | Peripheral blood | Fetal side | Maternal side | Aspirates nasopharyngeal | Buccal and body | Cord blood |
|------|----------------|----------------|------|------------|---------|------------------|------------|---------------|------------------------|----------------|-----------|
| 1    | Warts          | VV             | V    | No         | 6/11    |                  |            |               |                        |                 |           |
| 2    | HGSIL          | C              | C    | No         | 6/11+6/11 | 6/11 + 6/11     | 6/11       | 6/11          |                        |                 |           |
| 3    | HGSIL          | C              | C    | Yes        | 16+31   |                  |            |               |                        |                 | 16        |
| 4    | Warts          | VV             | V    | No         | 16+42+54 | 16              | 42+16      | 42            |                        |                 |           |
| 5    | LGSIL          | C              | C    | Yes        | 18      |                  |            |               |                        |                 |           |
| 6    | Warts          | VV             | C    | No         | 6/11+16+31 | 6/11+16+31     | 6/11       | 6/11          |                        |                 |           |
| 7    | Warts          | C+VV+VG        | V    | No         | 6/11+42  | 6/11 + 6/11     | 6/11       | 6/11          |                        |                 |           |
| 8    | HGSIL          | C              | C    | Yes        | 42      | 6/11 + 6/11     | 6/11+52    | 6/11          |                        |                 | 6/11      |
| 9    | LGSIL          | C              | V    | Yes        | 52      |                  |            |               |                        |                 |           |
| 10   | Warts          | VV+VG          | V    | No         | 6/11    |                  |            |               |                        |                 |           |
| 11   | LGSIL          | C              | V    | No         | 18      |                  | 18         | 18            |                        |                 | 18        |
| 12   | Warts          | VV             | V+F  | No         | 6/11    |                  |            |               |                        |                 |           |
| 13   | LGSIL          | C              | C    | Yes        | 6/11+42 |                  |            |               |                        |                 |           |
| 14   | LGSIL          | C              | C    | Yes        | 16+42+58 | 6/11 + 6/11     | 6/11       | 6/11          |                        |                 | 59        |
| 15   | Warts          | C+VV+VG        | V    | Yes        | 6/11+42 |                  |            |               |                        |                 |           |
| 16   | Warts          | VV+VG          | C    | No         | 6/11    |                  |            |               |                        |                 |           |
| 17   | HGSIL          | C              | C    | Yes        | 18+51   |                  |            |               |                        |                 |           |
| 18   | Warts          | VV+P           | C    | No         | 42+59   |                  |            |               |                        |                 |           |
| 19   | Warts          | VV             | V    | No         | 6/11    | 6/11 + 6/11     | 6/11       | 6/11          |                        |                 |           |
| 20   | HGSIL          | C              | C    | Yes        | 42+35   |                  |            |               |                        |                 |           |
| 21   | Warts          | VV             | C    | Yes        | 52      |                  |            |               |                        |                 |           |
| 22   | Warts          | VV             | C    | Yes        | 34      |                  |            |               |                        |                 |           |
| 23   | Warts          | VV+VG          | V    | Yes        | 18      |                  |            |               |                        |                 |           |
The HPV types were identified by both multiplex PCR and nested multiplex PCR methods. *NC = HPV DNA positive but could not be classified by type.

Type of delivery = C – cesarean section; V- vaginal; and V+ F – vaginal with forceps.

HPV lesion site = C – cervical; VG – vaginal; VV – vulva; P – perineal.

HPV lesion type = Warts – genital warts; LGSIL – low-grade squamous intraepithelial lesions; HGSIL – high-grade squamous intraepithelial lesions.

| No | Type of Delivery | Type | Site | Presence | HPV Types |
|----|------------------|------|------|----------|-----------|
| 24 | Warts            | V    | V    | No       | 16+73     |
| 25 | Warts            | VV+VG| C    | No       | 68        |
| 26 | Warts            | C    | V    | Yes      | 6/11+16   |
| 27 | Warts            | C+VVVG| V    | No       | 16+58     |
| 28 | Warts            | VV   | C    | Yes      | 6/11+33   |
| 29 | LGSIL            | C    | V    | No       |           |
| 30 | LGSIL            | C    | V    | Yes      | 52+42+58+54 |
| 31 | LGSIL            | C    | C    | No       | 16        |
| 32 | Warts            | VV   | C    | No       | 6/11      |
| 33 | HGSIL            | C    | V    | Yes      | 18        |
| 34 | Warts            | VV   | V    | Yes      | 11        |
| 35 | Warts            | VV+VG| C    | Yes      | 42        |
| 36 | LGSIL            | C    | V    | Yes      | 16        |
| 37 | HGSIL            | C    | C    | Yes      | 58        |
| 38 | HGSIL            | C    | V    | No       | 6/11+18   |
| 39 | HGSIL            | C    | C    | Yes      | 18+31     |
| 40 | Warts            | VV   | V    | No       | 6/11      |
| 41 | Warts            | VV   | C    | No       | 42+35+NC* |
| 42 | LGSIL            | C    | C    | Yes      | 42        |
| 43 | LGSIL            | C    | C    | Yes      | 16+18+42  |
| 44 | LGSIL            | C+VVVG| C    | Yes      | 18+26     |
| 45 | Warts            | VV   | V    | Yes      | 6/11+58+59 |
| 46 | Warts            | VV+VG| C    | Yes      | 6         |
| 47 | Warts            | VV   | V    | Yes      | 35        |
| 48 | Warts            | VV   | V    | No       | 70        |
| 49 | Warts            | VV+VG| V    | No       | 6+45      |

Table 3: Clinical and laboratory history of genital HPV infection during pregnancy and delivery and distribution of HPV types in maternal, newborn and placental samples. (Continued)
was considered a high carcinogenic risk (types 16 and 58) was detected. There was 66.7% (n = 2/3) concordance of type specific HPV among the maternal genital/peripheral blood samples. In all three cases no HPV DNA was identified in the respective placentas and NB.

Statistical analysis showed a significant association between placental HPV infection and the epidemiological variable history of immunodepression (HIV, p = 0.011), as observed in table 4 and 5.

In the group of pregnant women negative for genital HPV DNA (n = 14/63), it was observed that all samples, both of maternal peripheral blood and those of nasopharyngeal aspirate, buccal and bodily scrapings and arterial cord blood and those of placental biopsies and scrapings presented negative results for HPV DNA research.

**HPV detection and typing methods**

Evaluating the HPV DNA detection and typing methods, it was observed that the multiplex PCR methodology identified HPV DNA in 41 pregnant women (83.7%, n =

### Table 4: HPV status of the placenta and maternal factors.

| Maternal variable                  | Placental HPV DNA infection |
|------------------------------------|-----------------------------|
|                                    | Positive (n = 12) | Negative (n = 37) |
| **Age (years)**                    |                |                  |
| ≤ 19                               | 6 (50%)         | 17 (45.9%)       |
| ≥ 20 to ≤ 29                       | 3 (25%)         | 12 (32.4%)       |
| ≥ 30 to ≤ 39                       | 2 (16.7%)       | 7 (18.9%)        |
| ≥ 40 to ≤ 49                       | 1 (8.3%)        | 1 (2.7%)         |
| Mean for placental HPV DNA positive group (24.3 ± 8.3 years) | - | - |
| Mean for placental HPV DNA negative group (23.8 ± 8.2 years) | - | - |
| **Race**                           |                |                  |
| White                              | 11 (91.7%)      | 33 (89.2%)       |
| Non-white                          | 1 (8.3%)        | 4 (10.8%)        |
| **Level of education**             |                |                  |
| Illiterate                         | - | - |
| Elementary (complete or incomplete)| 6 (50%)         | 22 (59.4%)       |
| High school (complete or incomplete)| 5 (41.7%)      | 15 (40.5%)       |
| College (complete or incomplete)   | 1 (8.3%)        | -                |
| **Smoking**                        |                |                  |
| No                                 | 10 (83.3%)      | 24 (64.9%)       |
| < 10 cigarettes per day            | - | - |
| ≥ 10 cigarettes per day            | 2 (16.7%)       | 7 (18.9%)        |
| **Marital status**                 |                |                  |
| Married                            | 3 (25%)         | 8 (21.6%)        |
| Single                             | 2 (16.7%)       | 9 (24.3%)        |
| Cohabiting                         | 6 (50%)         | 19 (51.4%)       |
| Divorced, separated                | 1 (8.3%)        | 1 (2.7%)         |
| **Marital stability (years)**      |                |                  |
| ≤ 2                                | 8 (66.7%)       | 26 (70.3%)       |
| ≥ 3 to ≤ 5                         | 3 (25%)         | 7 (18.9%)        |
| ≥ 6                                | 1 (8.3%)        | 4 (10.8%)        |
| Mean for placental HPV DNA positive group (3.1 ± 4.3 years) | - | - |
| Mean for placental HPV DNA negative group (2.8 ± 4.7 years) | - | - |
| **History of Immunodepression (HIV)** |          |                  |
| No                                 | 10 (83.3%)      | 37 (100%)        |
| Yes                                | 2 (16.7%)       | -                |

Data are reported as number and percentage (in parentheses) of placental positive or negative infection for human papillomavirus. *P < 0.011 indicates a statistically significant difference between the positive and negative groups by Pearson’s chi-square test (HIV – acquired immunodeficiency syndrome).
Table 5: HPV status of the placental and delivery factors.

| Maternal variable                              | Placental HPV DNA infection |
|-----------------------------------------------|----------------------------|
|                                               | Positive (n = 12) | Negative (n = 37) |
| **Type of HPV lesion**                        |                   |                   |
| Genital warts                                 | 5 (41.7%)         | 23 (62.2%)        |
| LGSIL\(^1\)                                   | 3 (25%)           | 9 (24.3%)         |
| HGSIL\(^2\)                                   | 4 (33.3%)         | 5 (13.5%)         |
| **Site of HPV lesion**                        |                   |                   |
| Uterine cervix                                | 8 (66.7%)         | 13 (35.1%)        |
| Vulva                                         | 3 (25%)           | 13 (35.1%)        |
| Vulva + vagina                                | -                 | 7 (18.9%)         |
| Vulva + perineal region                       | -                 | 1 (2.7%)          |
| Uterine cervix + vulva + vagina               | 1 (8.3%)          | 3 (8.1%)          |
| **Type of HPV Infection**                     |                   |                   |
| Single                                        | 3 (25%)           | 16 (43.2%)        |
| Double                                        | 2 (16.7%)         | 13 (35.1%)        |
| Multiple                                      | 7 (58.3%)         | 8 (21.6%)         |
| **Type of delivery**                          |                   |                   |
| Vaginal                                       | 8 (66.7%)         | 15 (40.5%)        |
| Vaginal + forceps                             | -                 | 1 (2.7%)          |
| Cesarean section                              | 4 (33.3%)         | 21 (56.8%)        |
| Mean gestational age of the delivery in the placental HPV DNA positive group (39.7 ± 1.1 weeks) |   |                   |
| Mean gestational age of the delivery in the placental HPV DNA negative group (39.2 ± 2.4 weeks) |   |                   |
| **Gestational age at the time HPV infection was diagnosed (week)** |   |                   |
| ≥ 4 to ≤ 12                                   | 2 (16.7%)         | 17 (45.9%)        |
| ≥ 13 to ≤ 28                                  | 4 (33.3%)         | 8 (21.6%)         |
| ≥ 29 to ≤ 42                                  | 1 (8.3%)          | 6 (18.9%)         |
| Prior to pregnancy                            | 5 (41.7%)         | 5 (13.5%)         |
| Mean in the placental HPV DNA positive group (10.5 ± 13.3 weeks) |   |                   |
| Mean in the placental HPV DNA negative group (14.63 ± 12 weeks) |   |                   |
| **Time of RUPREME\(^3\) (min)**               |                   |                   |
| ≤ 360                                         | 11 (100%)         | 35 (92.1%)        |
| ≥ 361 to ≤ 720                                | -                 | 1 (2.6%)          |
| ≥ 721                                         | -                 | 2 (5.3%)          |
| Mean of placental HPV DNA positive group (37 ± 37 minutes) | -   | -                  |
| Mean of placental HPV DNA negative group (106 ± 244 minutes) | - | -                  |
| **Duration of labor (min)**                   |                   |                   |
| ≤ 240                                         | 6 (54.5%)         | 22 (57.9%)        |
| ≥ 241 to ≤ 360                                | 2 (18.2%)         | 10 (26.3%)        |
| ≥ 361                                         | 3 (27.3%)         | 6 (15.8%)         |
| Mean of placental HPV DNA positive group (236 ± 196 minutes) | - | -                  |
| Mean of placental HPV DNA negative group (185 ± 203 minutes) | - | -                  |
| **HPV lesion at delivery**                    |                   |                   |
| Yes                                           | 7 (58.3%)         | 19 (51.4%)        |
| No                                            | 5 (41.7%)         | 18 (48.6%)        |

Data are reported as number and percentage (in parentheses) of infection placental positive or negative for human papillomavirus. *P < 0.05 indicates a statistically significant difference between the positive and negative groups by Pearson’s chi-square test. \(^1\)Low-grade squamous intraepithelial lesions. \(^2\)High-grade squamous intraepithelial lesions. \(^3\)RUPREME = rupture of membrane amniotic.
41/49), in 31 pregnant women (75.6%, n = 31/41) only a single type of HPV DNA was identified, and two or more types of HPV in 10 pregnant women (24.4%, n = 10/41). The nested multiplex PCR method (although it was used to identify and type 9 types of HPV shown as the most prevalent in the city of Caxias do Sul) identified HPV DNA in 43 pregnant women (87.8%, n = 43/49), only a single type of HPV DNA in 28 pregnant women (83.7%, n = 28/43), and two or more types of HPV in 15 pregnant women (83.7%, n = 15/43). Together the multiplex PCR and nested multiplex PCR methods identified HPV DNA in 49 pregnant women (100%, n = 49/49), only a single type of HPV DNA in 19 pregnant women (38.8%, n = 19/49) and two or more types of HPV in 30 pregnant women (61.2%, n = 30/49).

The multiplex PCR method identified HPV DNA in only two newborns (18.2%, n = 2/11), while the nested multiplex PCR method identified it in 9 newborns (81.8%, n = 9/11).

In the placentas, multiplex PCR identified HPV DNA in only a single one (83.7%, n = 1/12), while the nested multiplex PCR method identified HPV in 12 cases (100%, n = 12/12).

**Discussion**

Human papillomavirus infection is one of the most frequent sexually transmitted diseases [31-33]. Non-sexual transmission [34] of HPV may occur directly by contact with the skin or mucosas (between people or by self-inoculation), or indirectly through contaminated objects, or still during the perinatal period.

Perinatal transmission may occur: (1) directly, during the passage of the fetus through the birth canal and on coming into contact with infected maternal secretions [13,18]; in delivery by cesarean section by ascending infection from the vaginal canal, after a premature rupture of the amniotic membranes [35]; in managing the mother with the baby (changing nappies, bathing) [10]; (2) indirectly, during vaginal delivery from contaminated objects; and (3) intrauterine transmission at the time of fertilization from sperm carrying latent HPV [36]; ascending infection from secretions of the maternal genital tract; and transplacental [11,19].

**HPV DNA in pregnant women**

HPV DNA was detected in 49 pregnant women (77.8%, n = 49/63). The percentage found was considered high compared to the existing literature. However, given the origin of the population studied, from outpatient clinics dealing with prenatal examinations and infectious diseases, these figures were already expected. The data regarding the prevalence of HPV infection in pregnancy are highly discordant: 5.4% reported by Tenti et al. (1997) [37] and 68.8% mentioned by Cason et al. (1995) [15]. The diversity of percentages observed is related to different factors that by themselves could influence the results, such as: diagnostic techniques, the characteristics of the samples and the inclusion criteria. Eppel et al. (2000) [16] observed a 24.6% prevalence of HPV infection in the uterine cervix of pregnant women. Recently, Takakuwa et al. (2006) [38], examining the cervical smears of 1.183 pregnant women for HPV DNA using the PCR-RFLP methods, observed a prevalence of 22.6% in pregnant women aged less than 25 years. This percentage was statistically significant (p < 0.0005) compared to the percentage obtained in pregnant women over the age of 25 years (11.3%), and it was concluded that the prevalence of HPV is considered high in young Japanese pregnant women.

Studying the type of lesion produced by HPV in the maternal genitalia, it was observed that 57.1% had genital warts, 24.5% low grade cervical intraepithelial lesions, and 18.4% high grade cervical intraepithelial lesions, results which could suggest a higher percentage of HPV DNA considered a low carcinogenic risk, which, however, was not observed. Of the HPV DNA types detected 54.9%, 1.2% and 40.2% were viral types considered a high carcinogenic risk, possible high risk and low risk, respectively. Genital infections produced by two or more types of HPV DNA were identified in 61.2% of the cases. Lu et al. (2003) [39] studying the prevalence and viral type in pregnant women with a diagnosis of squamous atypias of the uterine cervix detected HPV DNA in 88.6% of the cases. Of the HPV positive cases, 79.6%, 4.3% and 5.4% were considered a high carcinogenic risk, probable high risk and low risk, respectively. The most frequent viral types detected were 52 (31.2%), 16 (15.1%), 39 (11.8%), 53 (10.8%), and 18 and 58 (9.7% each). Viral infection by multiple types was detected in 43% of the cases. Hernandez-Giron et al. (2005) [40], in a population study in Mexico detected high carcinogenic risk HPV DNA in 37.2% of 274 pregnant women and 14.2% of 1,060 non-pregnant women.

Infections by multiple types of HPV are considered relatively common among the population in general [41]. Thomas et al. (2000) [42] reported that infection by multiple types of HPV are acquired more frequently than expected. These authors suggested that populations with a specific sexual behavior of exposing themselves to an ensemble of different types of HPV, or else the preexistence of a type of HPV could make it easier to acquire a new type of virus through an as yet unknown mechanism. Other authors [43] disagreed with the above statements and suggested that the risk factors are the same, both to acquire a single infection or a multiple one for HPV. A few authors suggested several hypotheses to account for the
high rates of HPV infection observed in pregnant women, such as the immunosuppressive and hormonal states induced by pregnancy[40,44]. These hypotheses could also explain the rate of multiple HPV infection (61.2%) observed in this study.

**HPV DNA in the placenta**

The use of different methods to sample the placentas was determinant for a more accurate identification of HPV DNA in third trimester pregnancy placentas (24.5%, n = 12/49). The results show that the isolated use of scraping methods or biopsies, especially if applied only to one of the sides of the placental disk, would detect a smaller number than the total obtained in this study.

The viral types identified in the placentas were 6/11 (50%, n = 6/12), 16 (25%, n = 3/12), 18 (16.7%, n = 2/12), 42, 52 and 58 (8.3%, n = 1/12 – each). The HPV DNA identified in the placentas were 6/11, 16, 18, 52 and 58. Seven placentas (58.3%, n = 7/12) presented HPV considered a high carcinogenic risk (types 16, 18, 52 and 58) and two (16.7%, n = 2/12) presented two different types of HPV DNA. The presence of HPV DNA in the placenta indicates the possibility of transplacental exposure to viral infection and to the need of considering the possible consequences of this exposure during the period: (1) intrauterine, to miscarriages[45] and possible malformations[16]; (2) postnatal period to genital warts in childhood[46], in adolescence juvenile-onset recurrent papillomatosis[5]; (3) in lifetime, the possible transmission of the carcinogenic agent[47,48].

In addition, the concordance observed in the type specific HPV between the genital/placental samples (91.7%, n = 11/12), strongly suggests that the HPV DNA detected in the placenta comes from maternal viral infection. This placental infection could be the result of an ascending canalicular infection from genital secretions (transamniotic) or hematogenic. The difference found in the types of HPV DNA may be due to different causes, such as contamination of the samples (unlikely), infection from the semen at the time of fertilization, infection due to multiple types, or subtypes and/or variants of HPV. Eppel et al. (2000)[16] in their study on HPV DNA detection in placentas, did not identify them in any of the 147 samples of chorionic vilosity collected by transabdominal amniocentesis. Even so, the authors suggested the possibility of transplacental viral transmission.

**HPV DNA in newborns**

As seen in the evaluation of methods to sample the placenta, the use of different sampling methods in the NB was determinant for a more precise identification of the percentage of vertical transmission of HPV (22.4%, n = 11/49). The results show that the isolated use of oral and bodily cavity scraping methods, or nasopharyngeal aspirates, or arterial cord blood, if applied individually for clinical screening would detect a smaller number of NB HPV DNA+ than the total obtained in this study.

The viral types identified in the NB were 6/11 (45.5%, n = 5/11), 42 (18.2%, n = 2/11), 52 (18.2%, n = 2/11), 18 and 59 (9.1%, n = 1/11 – each). Genital warts, which are caused by HPV types 6 or 11, are considered a frequent complication in pregnancy and clinically important due to the possibility of vertical transmission. Armstrong et al. (2000)[49] considered juvenile recurrent respiratory papillomatosis a consequence of vertical transmission of HPV. However, the risk of developing this complication in a child born to a mother infected with HPV is one to several hundred exposures[50]. Smith et al. (1995)[51] showed a rate of only 1% of vertical transmission of HPV DNA. Other authors reported higher percentages, using the PCR methodology for HPV type 16 and 18 in genital scrapings and oral cavity of mother/NB pairs, respectively, and detected vertical transmission rates between 31% and 73%[13,15,18,52,53].

Four NB (36.4%, n = 4/11) presented viral types considered a high carcinogenic risk (types 18, 52 and 59) and one presented two different types of HPV DNA (types 6/11 and 52). These data are sufficient evidence to confirm the perinatal transmission of HPV, considered a high carcinogenic risk. These findings require future studies to be able to establish: (1) the significance and consequences of infection in the child; (2) their relationship with the infections detected in adults; (3) the risk for the development of cancer in lifetime. The virus infects mainly the epithelial cells, where it may remain latent for a very long time, evolve to the subclinical form, and thus remain, or reactivate, with a resulting accumulation of chromosomal mutations in host cells. The next result after this accumulated latent carcinogenic potential of certain types of HPV during childhood would be the development of a neoplasm in lifetime. The natural history of papilloma infection is characterized by regression in a period that varies from months to years[54].

In 5 cases (41.7%, n = 5/12) concordance of type specific HPV was observed between the genital/placental/NB samples and in 1 case (8.3%, n = 1/12) between the genital/arterial cord blood samples, suggesting that there is often placental transmission (50%, n = 6/12). This was the first study in third trimester placentas to suggest the percentage of transplacental transmission of HPV DNA.

Several authors have focused special attention on the mode of HPV transmission. In 1992, Tseng et al.[19] suggested transplacental transmission of the virus, after detecting the same viral genome (HPV type 16) in cervi-
covaginal smears, in mononuclear cells of peripheral blood of fifteen pregnant women and in the cord blood of seven newborns from these same mothers. Favre et al. (1998)[11] showed the presence of several types of HPV DNA in amniotic liquid, placental cells and cervicovaginal smears of a mother and newborn with epidermodisplasia verruciforme. Hermonat et al. (1997)[25] recorded that the infection of HPV was three times more prevalent in specimens of spontaneous abortion in the first trimester. Later, in 1998, the same authors[55], confirmed the presence of HPV DNA in placental tissue of spontaneous abortions and concluded that the predominant site for HPV DNA type 16 findings were the cells of the syncytiotrophoblast. Thus, they raised the hypotheses of viremia, not yet convincingly documented, and of contamination of placental cells by oocyte infection before or right after implantation, by ascending infection or by infection carried by sperm containing latent HPV.

This study pointed to five cases of NB (41.7%, n = 5/12) negative for the research of HPV DNA, while in their respective placenta HPV DNA was detected. Four of these placenta presented viral types considered a high carcinogenic risk (types 16, 18, 58), and 100% (n = 5/5) of type specific HPV concordance is observed among the genital/placental specimens. The results achieved show that transmission of the virus to the fetus is not a prerogative of every HPV DNA+ woman, or in all cases of HPV DNA+ placenta, pointing to the existence of other as yet unknown factors that could be involved in transplacental transmission. Sedlacek et al. (1989)[56] detected the presence of HPV DNA in the oral cavity of 36.5% of the newborn, delivered vaginally to mothers with a diagnosis of HPV DNA+ for cervical cells. Kaye et al. (1994)[57] showed that the pregnant women who transmitted the virus to their concepts had a higher viral load.

Among the eleven cases of RN HPV DNA+ (vertical transmission), four cases (36.4%, n = 4/11) did not present HPV DNA in their respective placenta. One case out of this total presented type-specific HPV concordance between the genital/arterial cord blood (HPV type 52) and two presented concordance of type specific HPV between the genital/NB samples (HPV types 11 and 42). These results emphasized the possibility that other HPV transmission routes exist during pregnancy (transamniotic ascending infection), or during labor (ascending infection after the amniotic membranes are ruptured), or during delivery (by the fetus passing through the contaminated birth canal).

HPV DNA in arterial cord blood and HPV DNA in maternal peripheral blood
Three cases of NB (6.1%, n = 3/49) who presented HPV DNA+ in arterial cord blood samples were seen. The three cases were considered transplacental transmission due to finding concordance of the type specific HPV among the genital/NB samples. In these three cases, as in all cases of vertical transmission, no HPV DNA was detected in maternal peripheral blood. On the other hand, three cases (6.1%, n = 3/49) were also observed of parturients who had HPV DNA in their peripheral blood, without HPV DNA in the respective placentas and NB. These results suggest that HPV infections in the placentas may have occurred by another route, which was not hematogenic, either by an infection that was already present before pregnancy in the endometrium, or by an ascending infection during the egg implantation period, or at the time of fertilization by sperm contaminated by the virus, or else during pregnancy facilitated by the uterine anatomy. The HPV predilection for tissues, apparently exclusive to the pavement epithelium of the skin and the mucosas, has been challenged in the last few years, since several studies demonstrated the capacity of HPV to infect different sites. The studies of Teseng et al. (1992)[19] may be mentioned, who demonstrated the presence of HPV in the amniotic liquid of pregnant women before labor, and Hermonat et al. (1998)[55] who described the presence of HPV in the syncytiotrophoblast of spontaneous abortions, proving the capacity of HPV to locate also in the uterine cavity. These studies showed the capacity of the virus to infect the uterine cavity, and therefore it is no surprise that the virus appears in the endometrium. Fedrizzi et al. (2004)[58] found HPV DNA (types 16 and 18) in 10% of the women with a normal endometrium. The exception is the work by Lai et al. (1992)[59] who found HPV DNA in 70% of the cases studied that had a normal endometrium or some benign disease. However, O’Leary et al. (1998)[60] did not find HPV DNA in the normal endometrium.

The positive and significant correlation between placental HPV infection and the maternal epidemiological variable “history of immunodepression” (HIV, p = 0.011), may be related to the special characteristics of the gravid cycle, especially the changes in the hormonal and immunological balance prevailing during this period, which might favor placental HPV infection.

**HPV detection and typing methods**
Although the nested multiplex PCR methodology is used to identify and type only 9 types of HPV shown as the most prevalent in the city of Caxias do Sul, it performed very well in identifying maternal HPV DNA, and also considerably increased the number of pregnant women with multiple virus infections. In the newborn and placental samples the nested multiplex PCR method showed its great sensitivity and specificity to identify HPV. The use of that method was also crucial to evaluate the concordance of type specific HPV DNA among the maternal/placental/
newborn samples, thus defining the vertical and transplacental transmission rates of the virus.

Concluding, the HPV DNA detection rate in the placenta was 24.5% (n = 12/49) and the transplacental transmission rate was 12.2% (n = 6/49). A transplacental transmission rate of 54.5% (n = 6/11) was observed when only the cases of vertical transmission were analyzed. These results were achieved in analyses of the placetas and newborns of mothers with genital warts or intraepithelial lesions of the uterine cervix. Thus, different forms of management can be adopted for each of these different stages (pre-gestational, gestation, delivery and the first few months after delivery), both from the diagnostic and therapeutic perspective. The mother and the newborn must be observed clinically and educational preventive measures must be established concerning the forms of HPV transmission, besides effective strategies for specific immunization.

In future, the HPV DNA rates in must be observed in the normal endometrium of women of reproductive age, in order to explain the possible route of infection by continuity and/or contiguity between the endometrium and conception products.

References

1. Sigurdsson K, Taddeo FJ, Benediktsdottrir KR, Olafsdottir K, Sigvaldason H, Oddsson K, Rafnar T: HPV genotypes in CIN 2–3 lesions and cervical cancer: a population-based study. Int J Cancer 2007, 121:2682-2687.
2. Fleming KA, Venning V, Evans M: DNA typing of genital warts and diagnosis of sexual abuse of children. Lancet 1987, 2:454.
3. McDonnell PJ, McDonnell JM, Kessis T, Green WR, Shah KV: Detection of human papillomavirus type 6/11 DNA in conjunctival papillomas by in situ hybridization with radioactive probes. Hum Pathol 1987, 18:1115-1119.
4. Rock B, Naghashfar Z, Barnett N, Buscema J, Woodruff JD, Shah K: HPV genotypes in CIN 2–3 lesions and diagnosis of sexual abuse of children. Lancet 1987, 2:454.
5. Silverberg MJ, Thorsen P, Lindeberg H, Grant LA, Shah KV: Condyloma in Pregnancy Is Strongly Predictive of Juvenile-Onset Recurrent Respiratory Papillomatosis. Obstet Gynecol 2003, 101:645-652.
6. Smith EM, Johnson SR, Cripe TP, Pignatari S, Turek L: Perinatal vertical transmission of human papillomavirus and subsequent development of respiratory tract papillomatosis. Ann Otol Rhinol Laryngol 1991, 100:479-483.
7. Ley C, Bauer HM, Reingold A, Schiffman MH, Chambers JC, Tashiro RJ, Manos MM: ARTICLES: Determinants of Genital Human Papillomavirus Infection in Young Women. J Natl Cancer Inst 1991, 83:997-1003.
8. Wheeler CM, Parmenter CA, Hunt WC, Becker TM, Greer CE, Hildesheim A, Manos MM: Determinants of genital human papillomavirus infection among cytologically normal women attending the University of New Mexico student health center. Sex Transm Dis 1993, 20:286-289.
9. Cason J: Perinatal acquisition of cervical cancer-associated papillomaviruses. Br J Obstet Gynaecol 1996, 103:853-858.
10. Syrjanen S, Puranen M: Human papillomavirus infections in children: the potential role of maternal transmission. Crit Rev Oral Biol Med 2000, 11:259-274.
11. Favre M, Majewski S, De Jesus N, Maleczyk M, Orth G, Jablonska S: A possible vertical transmission of human papillomavirus genotypes associated with epidermodysplasia verruciformis. J Invest Dermatol 1998, 111:333-336.
12. Pakarian F, Kaye J, Cason J, Kell B, Jewers R, Derasi N, Raju K, Best J: Cancer associated human papillomaviruses: perinatal transmission and persistence. Br J Obstet Gynaecol 1994, 101:151-17.7.
13. Puranen MH, Yliskoski MH, Saarikoski SV, Syrjanen KJ, Syrjanen SM: Exposure of an infant to cervical human papillomavirus infection of the mother is common. Am J Obstet Gynecol 1997, 176:1039-1045.
14. Cason J, Kaye JN, Jewers RJ, Kambo PK, Bible JM, Kell B, Shergill B, Pakarian F, Raju KS, Best JM: Perinatal infection and persistence of human papillomavirus types 16 and 18 in infants. J Med Viral 1995, 47:209-218.
15. Eppel W, Worda C, Frigo P, Ulm M, Kucera E, Czerwenka K: Human papillomavirus in the cervix and placenta. Obstet Gynecol 2000, 96:337-341.
16. Moorhead H, Banatvala JE: High risk genital papillomavirus infections are spread vertically. Rev Med Viral 1999, 9:15-21.
17. Cason J, Kaye JN, Jewers RJ, Kambo PK, Bible JM, Kell B, Shergill B, Pakarian F, Raju KS, Best JM: Perinatal infection and persistence of human papillomavirus types 16 and 18 in infants. J Med Viral 1995, 47:209-218.
18. Eppel W, Worda C, Frigo P, Ulm M, Kucera E, Czerwenka K: Human papillomavirus in the cervix and placenta. Obstet Gynecol 2000, 96:337-341.
19. Morroni C, Williamson AL: Early and late human papillomavirus infection among women screened for cervical cancer]. Int J Cancer 2000, 99:9-12.
20. Alberico S, Pinzano R, Comar M, Toffoletti F, Maso G, Ricci G, Guaschino S: [Maternal-fetal transmission of human papillomavirus]. Minerva Ginecol 1996, 48:199-204.
21. Rogo KG, Nyansera PN: Congenital condylomata acuminata with meconium staining of amniotic fluid and fetal hydrocephalus: case report. East Afr Med J 1988, 66:411-413.
22. Xu X, Liu L, Lu S, Ren S: Clinical observation on vertical transmission of human papillomavirus. Chin Med Sci J 1998, 13:29-31.
23. Armbuster-Moraes E, Ioshimoto L, Leao E, Zughib M: Presence of human papillomavirus DNA in amniotic fluids of pregnant women with cervical lesions. Gynecol Oncal 1994, 54:152-158.
24. Wang X, Zhu Q, Rao H: Maternal-fetal transmission of human papillomavirus. Clin Med J (Engl) 1999, 6:722-727.
25. Hermonat PL, Han L, Wendel PJ, Quirk JG, Stern S, Lowrey CL, Rech- tin TM: Human papillomavirus is more prevalent in first trimester spontaneously aborted products of conception compared to elective terminations. J Infect Dis 1995, 171:317-321.
26. Smith EM, Ritchie CJ, Yankowitz J, Swarnavel S, Wang D, Haugen TH, Turek LP: Human papillomavirus prevalence and types in newborns and parents: concordance and modes of transmission. Sex Transm Dis 2004, 31:157-62.
27. Gravitz PE, Peyton CL, Uilke TQ, Wheeler CM, Coutlee F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ: Improved Amplification of Genital Human Papillomaviruses. J Clin Microbiol 2000, 38:357-361.
28. Bernard H, Chan S, Manos M, Ong C, Villa L, Delius H, Peyton C, Bauer H, Wheeler C: Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. J Infect Dis 1994, 170:1077-1085.
29. Rombaldi RL, Serafini EP, Villa LL, Vanni AC, Barbosa F, Frassini R, Xavier M, Paesi S: Infection with human papillomaviruses of sexual partners of women having cervical intraepithelial neoplasia. Braz J Med Biol Res 2006, 39:177-187.
30. Soltar K, Diemer D, Dethlefs M, Hack Y, Stuber N, Vollmer N, Menton S, Menton M, Diez K, Wachter T, Worland R, Bultmann B: Detection and Typing of Human Papillomavirus by E6 Nested Multiplex PCR. J Clin Microbiol 2004, 42:3176-3184.
31. Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz LE: Prevalence of HPV Infection Among Females in the United States. JAMA 2007, 297:813-819.
32. Rama CH, Roteli-Martins CM, Derc hain SF, Longatto-Filho A, Gontijo RC, Sarian LO, Syrjanen K, Alldrighi JM: [Prevalence of genital HPV infection among women screened for cervical cancer]. Rev Saude Publico 2008, 42:123-130.
33. Marais DJ, Constant D, Allan B, Carrara H, Hoffman M, Shapiro C, Morrone C, Williamson AL: Cervical Human Papillomavirus (HPV) Infection and HPV Type 16 Antibodies in South African Women. J Clin Microbiol 2008, 46:732-739.

(page number not for citation purposes)
34. Sinclair KA, Woods CR, Kirse DJ, Sinah SH: Anogenital and Respiratory Tract Human Papillomavirus Infections Among Children, Age, Gender, and Potential Transmission Through Sexual Abuse. Pediatr 2005, 116:815-818.

35. Teni P, Zappatore R, Migliora P, Spinillo A, Belloni C, Carnevali L: Perinatal transmission of human papillomavirus from gravidas with latent infections. Obstet Gynecol 1999, 93:475-479.

36. Lai YM, Yang FP, Pao CC: Human Papillomavirus deoxyribonucleic acid and ribonucleic acid in seminal plasma and sperm cells. Fertil Steril 1996, 65:1026-1030.

37. Teni P, Zappatore R, Migliora P, Spinillo A, Maccarini U, De Benedictis M, Vesentini N, Marchetti G, Silini E, Carnevali L: Latent human papillomavirus infection in pregnant women at term: a case-control study. J Infect Dis 1997, 176:277-280.

38. Takakuki K, Mitsu T, Iwashita M, Kobayashi I, Suzuki A, Oda T, Torii Y, Matsumoto M, Yahata G, Tanaka K: Studies on the prevalence of human papillomavirus in pregnant women in Japan. J Perinatol 2004, 24:77-79.

39. Lu DW, Pirgo EC, Zhi X, Wang HL, Pinto KR: Prevalence and typing of HPV DNA in atypical squamous cells in pregnant women. Acta Cytol 2003, 47:1008-1016.

40. Hernandez-Giron C, Smith JS, Lori ncz A, Lazcano E, Hernandez-Avila M, Salmeron J: High-risk human papillomavirus detection and related risk factors among pregnant and nonpregnant women in Mexico. Sex Transm Dis 2005, 32:613-618.

41. Auvinen E, Zilliacus R, Malm C, Karkkilainen T, Fingersroos R, Pasov nen J: Repeated human papillomavirus DNA findings among female university students. Int J STD AIDS 2007, 18:839-841.

42. Thomas KK, Hughes JP, Kuypers JM, Kwiat NB, Lee SK, Adam DE, Koutsylky LA: Concurrent and sequential acquisition of different genital human papillomavirus types. J Infect Dis 2000, 182:1097-1102.

43. Rousseau M-C, Abrahamowicz M, Villa LL, Costa MC, Rohan TE, Franco EL: Predictors of Cervical Coinfection with Multiple Human Papillomavirus Types. Cancer Epidemiol Biomarkers Prev 2003, 12:1029-1037.

44. Michelin D, Gissmann L, Street D, Porsuk RK, Fisher S, Kaufmann AM, Qiao L, Schreckenberger C: Regulation of human papillomavirus type 18 in vivo: effects of estrogen and progesterone in transgenic mice. Gynecol Oncol 1997, 66:202-208.

45. Gomez LM, Ma Y, Ho C, McGrath CM, Nelson DB, Parry S: Placental infection with human papillomavirus is associated with spontaneous preterm delivery. Hum Reprod 2008, 23:709-715.

46. Marcoux D, Nadeau K, McCusig C, Powell J, Oligny LL: Pediatric Anogenital Warts: A 7-Year Review of Children Referred to a Tertiary-Care Hospital in Montreal, Canada. Pediatr Dermatol 2006, 23:199-207.

47. Pakarian F, Kaye J, Cason J, Kell B, Jewers R, Derias NW, Raju KS, Best JM: Cancer associated human papillomaviruses: perinatal transmission and persistence. Br J Obstet Gynaecol 1994, 101:514-517.

48. Cason J, Kaye JN, Best JM, Raju KS: Cancer associated human papillomaviruses: perinatal transmission and persistence. Br J Obstet Gynaecol 1995, 102:583.

49. Armstrong LR, Preston Ej, Reichert M, Phillips DL, Nisenbaum R, Todd NW, Jacobs IN, Inglis AF, Manning SC, Reeves WC: Incidence and prevalence of recurrent respiratory papillomatosis among children in Atlanta and Seattle. Clin Infect Dis 2000, 31:107-109.

50. Gelinas JF, Manoukian J, Cote A: Lung involvement in juvenile onset recurrent respiratory papillomatosis: A systematic review of the literature. Int J Pediatr Otorhinolaryngol 2008, 72:433-452.

51. Smith EM, Johnson SR, Cripe T, Perlman S, McGuinness G, Jiang D, Cripe L, Turek LP: Perinatal transmission and maternal risks of human papillomavirus infection. Cancer Detect Prev 1995, 19:196-205.

52. Fredericks BD, Balkin A, Daniel HW, Schonrock J, Ward B, Frazer IH: Transmission of human papillomavirus from mother to child. Aust N Z J Obstet Gynaecol 1993, 33:30-32.

53. Puranen M, Ylikoski M, Saarikoski S, Syrjanen K, Syrjanen S: Vertical transmission of human papillomavirus from infected mothers to their newborn babies and persistence of the virus in childhood. Am J Obstet Gynecol 1996, 174:694-699.

54. Sinal SH, Woods CR: Human papillomavirus infections of the genital and respiratory tracts in young children. Semin Pediatr Infect Dis 2005, 16:306-316.

55. Hermonat PL, Kecheleva S, Lowery CL, Korourian S: Tropheoblasts are the preferential target for human papilloma virus infection in spontaneously aborted products of conception. Hum Pathol 1998, 29:170-174.

56. Fedirczak T, Lindheim S, Eder C, Hasty L, Woodland M, Ludomirsky A, Rando R: Mechanism for human papillomavirus transmission at birth. Am J Obstet Gynecol 1989, 161:55-59.

57. Kaye JN, Cason J, Pakarian FB, Jewers R, Kell B, Bible J, Raju KS, Best JM: Viral load as a determinant for transmission of human papillomavirus type 16 from mother to child. J Med Viral 1994, 44:415-421.

58. Fedirrizi EN, Carvalho NS, Villa LL, Souza IV, Sebastiao APM: Search for human papillomavirus in samples of normal endometrial tissue and tissue with carcinoma by the PCR technique. Rev Br GO 2004, 26:277-287.

59. Lai CH, Hsueh S, Lin CY, Huang MY, You GB, Chang HC, Pao CC: Human papillomavirus in benign and malignant ovarian and endometrial tissues. Int J Gynecol Pathol 1992, 11:210-215.

60. O’Leary J, Landers RJ, Crowley M, Healy I, O’Donovan M, Healy V, Kealy WF, Hogan J, Doyle CT: Human papillomavirus and mixed epithelial tumors of the uterus. Hum Pathol 1998, 29:383-389.

61. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJJ, Meijer CJLM, the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group: Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. N Engl J Med 2003, 348:518-527.