Possible role of bacterial and viral infections in miscarriages

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Capsule

With the use of polymerase chain reaction, human papillomavirus and \textit{Chlamydia trachomatis} were detected in 7.4\% and 1\% of placental samples from miscarriages, respectively.
Structured abstract and key words

**Objective:** In order to determine the role of infections in miscarriages, chorionic villi from aborted material were subjected to cytogenetic evaluation and analysed for the presence of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, human cytomegalovirus (HCMV), adeno-associated virus (AAV) and human papillomaviruses (HPV).

**Design:** Retrospective study.

**Setting:** University hospital and academic research institution.

**Main Outcome Measure(s):** Karyotyping and detection of bacterial and viral DNA by means of polymerase chain reaction (PCR) in placenta specimens.

**Result(s):** In 54 (50 %) of 108 samples the karyotype was normal, in 38 (35 %) samples it was abnormal and in 16 (15 %) samples karyotype was undetermined. No *U. urealyticum*, *M. hominis*, HCMV or AAV 2 DNA was detected, while *C. trachomatis* DNA was detected in one (1 %) and HPV DNA in eight (7 %) samples. No significant correlation of HPV positive findings with karyotype status was established.

**Conclusion(s):** Our findings do not support a role of *C. trachomatis*, *U. urealyticum*, *M. hominis*, HCMV and AAV infections in miscarriages during the first trimester of pregnancy. However, further investigation should be made to determine a possible involvement of HPVs in the development of genetic abnormalities of the fetus, and in miscarriages.

**Key Words:** Miscarriage, karyotyping, placenta, PCR, bacterial infections, viral infections
INTRODUCTION

Miscarriages are considered to be the most common complication in pregnancy. The incidence of miscarriages in clinically established pregnancies (over sixth gestational week) is approximately 12 to 15% (1). Recurrent (three or more consecutive) miscarriages represent a special problem resulting with serious physical as well as psychological damage to women. Unfortunately, they occur quite often, in 1 out of 300 pregnancies (2).

Several possible causes of miscarriage have been considered, the major ones being genetic abnormalities of the fetus. It has been established that more than 50% of miscarried fetuses are genetically abnormal (3, 4). Apart from that, other causes of miscarriage could be anatomical anomalies of the mother, endocrine and immunological causes, different environmental factors, such as exposure to various toxic substances (caffeine, nicotine), and infections (5-7). According to Garcia-Enguinados et al. (8), only genetic and anatomical abnormalities have been proven as direct causes of miscarriages, whereas intrauterine infections have been established as a cause of premature rupture of fetal membranes and preterm labor, and their role in miscarriage is still not completely clear. Several studies have confirmed the role of infections as a cause of miscarriage, especially during the second trimester of pregnancy, but the role of infection in first trimester miscarriages is still questionable (9).

Since the main route of infection to placenta and fetus is from vagina and cervix, most studies were oriented on an attempt to find an association between miscarriage and abnormal bacterial flora in the lower genital tract, vagina and cervix, respectively. *Gardnerella vaginalis* and *Mycoplasma hominis* both cause vaginitis and are often found in mixed infections as a cause of amnionitis, whereas *Ureaplasma urealyticum* is found as a sole cause of amnionitis (10, 11). These pathogens are also considered as a cause of preterm labor, beside *Escherichia coli*, enterococci, staphylococci and group B streptococci (12).

A microorganism should have access to the intrauterine surroundings in order to cause inflammatory reaction of endometrium or fetal tissues responsible for a miscarriage. Infectious agents present inside the uterus can cause infections between decidual tissue and fetal membranes (amnion and chorion), inside placenta, in the amniotic fluid (amnionitis) or in the umbilical cord (funisitis). Infections of the placenta are rare and the most common causative agents found are *Treponema pallidum*, *Mycobacterium tuberculosis*, chlamydias, mycoplasmas, *Rubella* virus, human cytomegalovirus (HCMV), herpes simplex virus (HSV), *Toxoplasma gondii* and *Candida albicans* (13, 14). However, it has not been established whether such infections cause miscarriage.

Witkin et al. (15) established that *Chlamydia trachomatis* could provoke miscarriage due to strong immunological reaction to the specific bacterial protein. Moreover, *C. trachomatis* infections of the cervix have been associated with second trimester abortions and premature membrane rupture (16).

It is well established that mycoplasmas are prevalent in the lower genital tract of pregnant women, but they are also quite common in the genitourinary tract of sexually active men and women in general (17). Some studies suggest an association of mycoplasma infection with sporadic and recurrent miscarriages (18, 19).

HCMV is the most common congenital infection in humans, with the incidence of 1% of all live births (20). However, there is no consensus about HCMV involvement in spontaneous abortions (21, 22).
Adeno-associated virus (AAV), which is not a priori associated with diseases in humans (23), has been found in human blood cells, biopsies and cervical epithelial cell brushings, endometrium and material from spontaneous abortion which indicates its possible role in miscarriage induction (24-27).

The role of HPV in the development of cervical carcinoma is well-established (28), however its involvement in the development of miscarriage is also under consideration (29, 30).

Herein, we investigated the association of first trimester miscarriages with the placental presence of suspected pathogens involved in the induction of miscarriages, i.e. *U. urealyticum*, *M. hominis*, *C. trachomatis*, HCMV, AAV and HPV.

**MATERIAL AND METHODS**

**Patients**

Hundred and eight Croatian women attending the Clinic of Gynecology and Obstetrics in University Hospital “Merkur” (Zagreb, Croatia) were involved in this study. Each woman was admitted to the hospital because of the threatening miscarriage. All of them were informed about the following therapeutic and diagnostic procedures and agreed by signed consent. They were 18 to 46 years old (mean age 32 years), and the gestational age at which miscarriage occurred was between 4 and 19 weeks (mean gestational age 10 weeks). Their uterine cervices were normal and chronic or hereditary diseases were excluded.

The Ethical Board of the University Hospital “Merkur” and the Ethical Board of the “Rudjer Boskovic” Institute approved further analysis presented in this study.

**Tissue sampling**

Placental tissue was collected by curettage, cleaned of decidual tissue, rinsed in HAMS F10 medium (Gibco BRL) and dissected under a stereoscopic microscope. Chorionic villi were used to determine the karyotype of the fetuses and DNA was isolated from the placental tissue in order to investigate the presence of bacterial and viral infections by means of polymerase chain reaction (PCR). Karyotyping was performed at the Laboratory for cytology and clinical genetics, University Hospital “Merkur” (Zagreb, Croatia) while the molecular analyses were performed at the Laboratory of Molecular Oncology, Division of Molecular Medicine, “Rudjer Boskovic” Institute (Zagreb, Croatia).

**Cytogenetic analysis**

The direct method for karyotyping chorionic villi was performed according to Singer and Profeta (31). Briefly, chorionic villi were cultivated for 1 to 3 days at 37°C. An hour and 30 minutes before proceeding with slide preparations, 0.01 mg/ml of Colchicine (Fluka) was added to the medium to stop the cells in the metaphase of mitosis. The tissue was then removed from the medium and treated with hypotonic and fixative solution (3:1 methanol and acetic acid) in order to preserve cells in their "swollen" state. After the removal of fixative, a few drops of 60 % acetic acid were added to the tissue to cause cell dissociation. One drop of the cell suspension was placed on each slide and spread across it. The chromosomes were Q- and G-banded, which enabled their identification and analysis. Fifteen metaphases of each sample were analysed under the microscope and at least three metaphases were photographed and subjected to further analysis in order to determine the chromosome number, the structural chromosomal abnormalities and, finally, the karyotype.
DNA isolation

The placenta samples were resuspended in lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 7.9, 0.5 % SDS) and treated overnight at 37°C with proteinase K (100 μg/ml). The DNA was isolated by a phenol and chloroform extraction followed by ethanol precipitation, and dissolved in tridistillated sterile water, according to Sambrook et al. (32).

Polymerase chain reaction (PCR)

Each DNA sample was subjected to several PCR reactions in order to amplify DNA of *U. urealyticum*, *M. hominis*, *C. trachomatis*, HCMV, AAV 2 and HPVs. Prior to PCR analysis of DNA samples for the presence of different microorganisms, primer set PC04/GH20 was used to amplify part of the β-globin gene in order to exclude possible presence of inhibitors of the PCR and to optimise sample DNA concentration (33).

The detection of *U. urealyticum* and *M. hominis* was done by semi-nested PCR according to Abele-Horn et al. (34). Primer pairs U4/U5 and U4/U9 (Table 1) were used to amplify part of the *U. urealyticum* urease gene, while primer pairs M1/M2 and RNAH3/M2, complementary to the part of *M. hominis* rRNA operon of the 16S rRNA gene (Table 1), were used for the detection of *M. hominis*. The reaction mixture (20 μl) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.1 mM of each dNTP, 0.5 μM of each primer, approximately 100 ng of each DNA and 0.4 U of a thermostable AmpliTaq DNA polymerase (Roche). PCR reactions were performed for 40 cycles of denaturation (95°C, 30 s), annealing (46°C, 30 s for *U. urealyticum*; 45°C, 30 s for *M. hominis*) and elongation (72°C, 45 s). DNA extracted from cultured *U. urealyticum* and *M. hominis* (kindly provided by Dr. Z. Persic, Zagreb, Croatia) was used as positive control.

Detection of *C. trachomatis* was done according to Loeffelholz et al. (35). Primer pair CP24/CP27 (Table 1) was used to amplify part of *C. trachomatis* cryptic plasmid. The reaction mixture (20 μl) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.1 mM of each dNTP, 0.5 μM of each primer, approximately 100 ng of each DNA and 0.4 U of a thermostable AmpliTaq DNA polymerase (Roche). PCR reaction was performed for 35 cycles of denaturation (95°C, 50 s), annealing (62°C, 35 s) and elongation (72°C, 35 s). DNA extracted from *C. trachomatis* (ATCC number: VR-878) was used as positive control.

Two sets of primers, complementary to the early regulatory region of HCMV genome (IEA1 gene) CMV-C/CMV-D and CMV-A/CMV-B (Table 1) were used in a nested PCR (36). The reaction mixture (20 μl) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.25 μM of each dNTP, 0.15 μM of primers CMV-C and CMV-D in the first reaction and 0.6 μM of primers CMV-A and CMV-B in the second reaction, approximately 100 ng of each DNA and 0.4 U of a thermostable AmpliTaq DNA polymerase (Roche). The first round of PCR with the primer pair CMV-C/CMV-D was performed for 20 cycles, while the second round of PCR with the primer pair CMV -C/CMV -D was performed for 30 cycles of denaturation (95°C, 30 s), annealing (53°C, 30 s) and elongation (72°C, 45 s). DNA extracted from Wi-38 (ATCC number: CCL-75) cells infected with HCMV (ATCC number: VR-807) was used as positive control.

AAV 2 was detected in a semi-nested PCR. Two sets of primers R78-1/nest2 and R78-1/R78-2 (Table 1) (37, 38), complementary to the regulatory region of AAV genome (REP gene), were used. The reaction mixture (20 μl) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 μM of each dNTP, 0.2 μM of each primer, approximately 100 ng of each DNA and 0.4 U of a thermostable AmpliTaq DNA polymerase (Roche). The first and
second round of PCR were performed for 30 cycles of denaturation (95°C, 30 s), annealing (63°C, 30 s) and elongation (72°C, 45 s). Cloned AAV 2 DNA (kindly provided by Dr. J. R. Schlehofer, Heidelberg, Germany) was used as positive control.

PCR conditions for HPV DNA amplification were as previously described (39). Briefly, for HPV screening MY09/MY11 (40) primers were used, while for HPV typing, type-specific primers for HPV 16 and HPV 18 (41) were used (Table 1). DNA isolated from CaSki (ATCC number: CRL-1550) and HeLa cells (ATCC number: CCL-2), which contain integrated HPV 16 and HPV 18 DNA, respectively, were used as positive controls.

Amplified products were resolved by electrophoresis in a 2% agarose gel stained with ethidium bromide, identified by UV irradiation and photographed (ImageMaster, Pharmacia Biotech).

RESULTS

The majority of the miscarriages, 102 of 108 (94.4 %) occurred during the first trimester of pregnancy, whereas only 6 (5.6 %) occurred in the second trimester. Fifty-five (50.9 %) women miscarried for the first time, 27 (25 %) had one previous miscarriage and 26 (24.1 %) had two or more previous miscarriages, of which only 12 (46.2 %) had more than two consecutive miscarriages, so they can be classified as having recurrent miscarriages (Figure 1).

According to cytogenetic analysis in 16 (14.8 %) of 108 samples karyotype could not be determined due to culture failure (insufficient cell growth in 15 cases and one case of exogenous contamination). In the remaining 92 samples, 54 (50 %) had normal karyotype and 38 (35.2 %) had abnormal karyotype. The most frequently found chromosomal abnormalities were autosomal trisomies in 27 (71 %) of 38 cases (Table 2). One (3 %) monosomy 21 (karyotype 45,XY,-21), two (5 %) cases of monosomy X, three (8 %) triploidies, three (8 %) tetraploidies and two (5 %) mosaicisms were also found (Table 2).

Each DNA sample isolated from placenta of miscarried fetuses was β-globin positive and thus suitable for further analysis, i.e. detection of U. urealyticum, M. hominis, C. trachomatis, HCMV, AAV 2 and HPVs by PCR. The results of bacterial and viral DNA detection, by means of PCR, are summarised in Table 3.

To detect the presence of U. urealyticum and M. hominis, a semi-nested PCR instead of one step PCR was performed in order to increase sensitivity and specificity of the reaction. However, the presence of either bacterium was not detected in any analysed sample.

The presence of C. trachomatis was established in only one (1.0 %) of 108 samples (Table 3). The sample, in which C. trachomatis was detected, had normal karyotype and belonged to a patient without previous miscarriages or births, who was impregnated through in vitro fertilization (IVF).

In order to investigate the presence of HCMV and AAV 2, nested and semi-nested PCR were performed. None of the samples contained either virus.

Although no HPV DNA was detected by consensus MY09/MY11 primers, 8 (7.4 %) of 108 samples were positive with type-specific primers for HPV 16 and HPV 18. Two samples were positive for HPV 16 and HPV 18, respectively, while 4 samples were positive for both HPV types (Table 3).
All HPV positive samples were from women who had miscarriages in the first trimester of pregnancy, 5 of them had 2 or more previous miscarriages, one had 1 previous miscarriage and 2 did not have any previous miscarriages (Table 4). Those with no previous miscarriages were impregnated through IVF procedures. Three of the samples infected with HPV had abnormal, 3 had normal and in 2 cases the karyotype was undetermined.

**DISCUSSION**

Although miscarriage represents the most common complication in pregnancy and as such has been thoroughly investigated, the causes of miscarriages are still unexplained in the majority of cases. There are several indications connected to miscarriage, but only genetic and anatomical abnormalities are mostly accepted. Uterine infections are not generally accepted as a cause of miscarriage, since the results of different studies are inconsistent.

There are contradictory results regarding an association of pathogens with the incidence of miscarriage. A number of studies attempted to find a possible connection between miscarriage and bacterial infections in the vagina, with the assumption that such infections could spread to uterus and induce miscarriage. Robertson et al. (42) found a connection between colonisation of vagina with *U. urealyticum* and premature labor, and Donders et al. (7) found that bacterial vaginosis and presence of *U. urealyticum* and *M. hominis* in the vagina were associated with an increased risk of early miscarriage. *U. urealyticum* has also been found in the placenta, associated to perinatal morbidity and mortality and preterm labor (43, 44). However, colonisation of the uterine cervix with *U. urealyticum* is quite common in women who did not experience any problem during pregnancy (45), so its possible role in induction of miscarriage is questionable.

According to our previous study of 53 pregnant women, *U. urealyticum* was present in the cervix of 20 (38 %) of 53 women, while *M. hominis* was not detected in any of samples (unpublished data). Furthermore, we also investigated the presence of mycoplasmas in the cervix among 18 women with previous miscarriage, and only *U. urealyticum* was found in 5 (28 %) women (unpublished data). These observations do not favour the hypothesis that colonisation of cervix with mycoplasmas could be associated with miscarriages. Furthermore, we did not detect either the presence of *U. urealyticum* nor *M. hominis* in any of 108 samples of the placenta from miscarried fetuses that were analysed herein. There is no doubt in accuracy of these findings, since PCR is a highly sensitive and specific method and it is highly unlikely that an infection remained undetected. Moreover, we amplified the β-globin gene in all the samples, so the presence of inhibitors of PCR in the samples was also excluded.

*C. trachomatis* and *N. gonorrhoeae* are the most frequent cause of cervicitis. They can ascend to the uterus and ovaries and possibly by vertical transmission to placenta and fetus (46). Indications of involvement of *C. trachomatis* in the development of miscarriage do exist, however there is not enough evidence to confirm them. Stokes and Isada (47) established that *C. trachomatis* infects placental trophoblast membrane cells *in vitro*. Study conducted among women undergoing the procedure of IVF implicated a connection between the presence of *C. trachomatis* in cervical scrapings and the failure of implantation and adverse pregnancy outcome (48). In our study we detected *C. trachomatis* in the placenta of a woman that miscarried after IVF, which is concordant with the fact that *C. trachomatis* could infect placental cells *in vivo*. Since, only 1 (1.0 %) of 108 miscarried placenta was positive for *C. trachomatis*, we cannot assume that *C. trachomatis* plays a significant role in the induction of most miscarriages.
Viral infections of the trophoblast, even with viruses that have little or no pathogenicity, are also considered as possible causes of miscarriage, since they can impair trophoblast function and lead to abnormal implantation and placentation (49). For instance, HCMV is the most common congenital infection in humans. Primary infections with HCMV occur in 0.7 to 4.1% pregnancies (50), however, whether they have a role in the induction of miscarriage is questionable. Van Lijnschoten et al. (51) emphasize the possibility of HCMV transplacental transfer – they found the presence of pp65, protein of HCMV, in trophoblastic cells in 6.7% (6/89) cases of spontaneous and induced abortions, but they did not demonstrate that the presence of HCMV is pathogenic for early pregnancy. Transplacental transfer was also confirmed in a mouse model by Li and Tsutsui (52). Fisher et al. (53) showed that HCMV infects placental trophoblasts and diminishes their normal function, which could lead to miscarriage. However, herein, we did not detect HCMV DNA in any of the samples, and our results do not support the hypothesis that HCMV might play a role in the induction of miscarriage.

AAVs are human helper virus-dependent parvoviruses, which require helper viruses for their replication. Adenoviruses, herpes viruses, vaccinia virus and HPVs have been identified as helper viruses for AAV (26, 54). According to several studies AAV DNA has been detected in placental tissue of human origin (25, 27). The virus is widespread in the population, but without any obvious pathogenic effect (55). AAV 2 is the most frequent AAV type infecting the human population (38). Botquin et al. (56) established that infection of pregnant mice with AAV 2 induced fetal death and early abortion. Kiehl et al. (57) detected AAV DNA in 11 (73 %) of 15 samples from spontaneous abortion. However, they did not detect HCMV in any of the samples, while HPV was detected in only one sample of spontaneous abortion. On the other hand, Friedman-Einat et al. (58) analysed a number of clinical samples of different origin, among which 62 samples were from first trimester abortions (38 spontaneous and 24 induced abortions). They did not find AAV DNA in any of aborted material samples, although they found the presence of AAV DNA in 18 % of genital swab samples. Our findings are in concordance with results of the Friedman-Einat et al. study (58), as we did not detect AAV DNA in any of the samples, while HPV was detected in only one sample of spontaneous abortion. Because there is a large discordance in different studies performed so far, further investigations are required. One possible explanation for such discrepancies in detection of AAV in cervical scrapings could be different prevalence of AAV infection in different populations, which was proposed by Venturoli et al. (59).

An increase of HPV during pregnancy has been observed (60) and its transplacental transfer has been proposed by some authors (61), but also disputed by others (62). However, Hermonat et al. (29) found that HPV prevalence in placentas from first trimester miscarriages was as high as 60 % compared to 20 % prevalence in electively aborted fetuses. In their 1998 study, conducted on the subset of paraffin-embedded tissue from their previous study, Hermonat et al. (30) determined that the preferential site of HPV infection was syncytiotrophoblast and since trophoblasts maintain placental contact with maternal tissue, they assumed that altered characteristics of HPV-infected trophoblasts might lead to miscarriage. The results of the Liu et al. (63) study showed that HPV16 carries out its complete life cycle in cultured placental trophoblasts, which is in concordance with the aforementioned study. Contrary to that in the Sifakis et al. (64) study no HPV DNA was detected in spontaneously aborted material.

Malhomme et al. (27) examined 26 paraffin sections from uterine biopsies and early miscarriage that contained AAV for the presence of AAV helper viruses adenovirus, HSV and HCMV. They did not find the presence of these viruses in any of the samples, but they did find HPV DNA in 60 % samples from uterine biopsies and approximately 70 % of early miscarriage.
miscarriage samples. Armbruster-Moraes et al. (65) also detected HPV in 64.9 % (24/37) of amniotic fluid samples taken from pregnant women with cervical lesions indicative for HPV infection. Furthermore, Eppel et al. (66) conducted a study of HPV infection on chorionic villi and placental biopsies taken for the purpose of genetic analysis after the twentieth week of pregnancy. They found 24.6 % (44/179) HPV infection rate in the cervices of women, but did not find HPV in any of 147 placentas and chorionic villi examined. The difference in detection of HPV in the aborted tissue found in different studies is very puzzling, as it varies from 0 to 70 %.

Herein, we found the presence of HPV DNA in 7.4 % of miscarriage specimens, which does not point toward a major role of HPV infection in the induction of miscarriages, but it confirms the possibility of its transplacental transfer. Also, since the infection was found in three samples that had abnormal karyotype and additional two samples with undetermined karyotype, further investigations of a possible association between HPV infection and development of genetic abnormalities during embryogenesis would be in order, at least on animal model, since it is established that HPV is the cause of genetic instabilities which result with the development of various types of complex chromosomal abnormalities in malignant tumor cells (28). It should also be noted that, by using consensus primers (MY09/MY11), which recognise L1 region of HPV genome, we did not detect HPV in any of placental samples, although by using type-specific primers for HPV 16 and HPV 18, HPV infection was detected in 8 samples. This could be due to greater sensitivity of type-specific primers, in case of low copy number of viral genome in the analyzed material (39). Another explanation could be that HPV 16 and HPV 18, which are frequently integrated in the genome of cervical cells, are also integrated into the cell genome of placental cells (not investigated herein), and as such could be involved in the development of genetic abnormalities of the placenta and/or the fetus. Our results show that the number of HPV positive samples is proportionally higher in the group of women with two or more previous miscarriages, but it is not possible to conduct statistical analysis due to small number of samples and low expectancy values.

The possibility of sample contamination with cervical cells is not excluded, since the samples were acquired during curettage and they passed through cervix and vagina. However, in that case we would expect to detect other pathogens that are common inhabitants of the cervix and vagina, i.e. C. trachomatis (detected only in 1 sample herein), U. urealyticum and M. hominis (not detected herein). In this study, only one placental sample was contaminated by exogenous infection during cell cultivation for karyotyping, which can be the consequence of contamination with pathogens commonly found in the surroundings. So, the fact that we did not detect other pathogens by PCR, points to the conclusion that the HPV positive findings in the placenta are not the consequence of the contamination during the passage through cervical canal, but indeed HPV infection of the placenta.

Although the results of our study do not confirm a role of C. trachomatis, U. urealyticum, M. hominis, HCMV and AAV infections as a cause of miscarriages in the first trimester of pregnancy, the role of additional infections could not be excluded. Thus HPV infections, found herein in 7.4 % cases, necessitate further investigation.
REFERENCES

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<td>CGA CAG GAA CGA CTC CAA CGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV 18-R</td>
<td>GCT GGT AAA TGT TGA TTA ACT</td>
<td>201</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2
Types of genetic abnormalities found in the study group (N=38)

<table>
<thead>
<tr>
<th>Type of abnormality</th>
<th>Chromosomes involved / karyotype</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Monosomy 21</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>X</td>
<td>2</td>
</tr>
<tr>
<td>Triploidy</td>
<td>whole set</td>
<td>3</td>
</tr>
<tr>
<td>Tetraploidy</td>
<td>whole set</td>
<td>3</td>
</tr>
<tr>
<td>Mosaicism</td>
<td>46,XX/92XXXX</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>46,XX/69,XXY</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 3
The presence of bacterial or viral DNA in aborted placental tissue (N=108)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Normal karyotype</th>
<th>Abnormal karyotype</th>
<th>Undetermined karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>0</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td><em>M. hominis</em></td>
<td>0</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>1</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>AAV 2</td>
<td>0</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>HCMV</td>
<td>0</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>HPV</td>
<td>3</td>
<td>51</td>
<td>3</td>
</tr>
</tbody>
</table>
### Table 4  
Samples positive for the presence of high risk HPV DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age</th>
<th>Gestational week</th>
<th>Karyotype</th>
<th>Number of live births</th>
<th>Number of miscarriages</th>
<th>Comments</th>
<th>HPV16</th>
<th>HPV18</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>7</td>
<td>46,XX</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>10</td>
<td>46,XX</td>
<td>1</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>9</td>
<td>ND *</td>
<td>1</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>10</td>
<td>92,XXYY</td>
<td>0</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>5</td>
<td>46,XX</td>
<td>0</td>
<td>0</td>
<td>IVF †</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>8</td>
<td>ND *</td>
<td>1</td>
<td>2</td>
<td>CIN III ‡</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>8</td>
<td>47,XY,+22</td>
<td>0</td>
<td>1</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>10</td>
<td>47,XY,+9</td>
<td>1</td>
<td>0</td>
<td>IVF †</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* ND, not determined; † IVF, in vitro fertilisation; ‡ CIN III, cervical intraepithelial neoplasia grade III

![Number of previous miscarriages in the study population (N108)](image)

**Figure 1**  
Number of previous miscarriages in the study population (N108)

0 – no previous miscarriages; 1 – one previous miscarriage; 2 – two or more previous miscarriages